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IMPACTO DA MELATONINA NA FUNÇÃO COGNITIVA E DO SISTEMA SOMATO-SENSORIAL DE PACIENTES SOB O PRIMEIRO CICLO DE QUIMIOTERAPIA PARA CÂNCER DE MAMA: UM ENSAIO CLÍNICO, RANDOMIZADO, DUPLO-CEGO, CONTROLADO COM PLACEBO

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Ana Cláudia de Souza

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Orientador: Prof. Wolnei Caumo, M.D., PhD.

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5-MIAA	Ácido 5-metoxindole acético
5-MTOL	5-metoxitroptofol
6-BH4	6-tetrahidropterina
α-MSH	Hormônio alfa-melanócito estimulante
AAD	Aminoácido aromático descarboxilase
AANAT	Enzima arilalquilamina *N*-acetiltransferase
ACBC	Quimioterapia adjuvante para câncer de mama
AFMK	N1-acetil-N2-formil-5-metoxicinuramina
BDNF	Fator neurotrófico derivado do cérebro
CaM	Calmodulina
CPM	Teste de modulação condicionada da dor
COWAT	Teste cognitivo de associação de palavras controladas
Cry1	Gene-relógio
CYP	Citocromo P450
CYP1A	Gene do citocromo P450, família 1, subfamília A
CYP1A1	Gene do citocromo P450, família 1, subfamília A, polipéptido 1
CYP1A2	Gene do citocromo P450, família 1, subfamília A, polipéptido 2
CYP1B1	Gene do citocromo P450, família 1, subfamília B, polipéptido 1
CYP2C19	Gene do citocromo P450, família 2, subfamília C, polipéptido 19
DPMS	Sistema modulador descendente da dor
E2	Estradiol
ER	Receptor de estrogênio

ERα	Receptor de estrogênio alfa
ERK	Quinase reguladora extracelular
FEC	Agente quimioterápico [fluorouracil (5-FU) + epirrubicina + ciclofosfamida]
FXR	Receptor farnesóide X
GABA	Ácido gama-aminobutírico
GnRH	Hormônio liberador de gonadotropina
GR	Glicocorticóide
HPT	Limiar de dor ao calor
HPTo	Tolerância à dor pelo calor
HPA	Eixo hipotálamo-pituitária-adrenal
HIOMT	Hidroxiindole-O-metil transferase
IL-2	Interleucina 2
INFγ	Interferon-gama
IL-10	Interleucina 10
IL-1	Interleucina 1
JNK	c-Jun-N-terminal quinase
Kir3	Canal de potássio
MSH	Hormônio estimulante de melanócito
MT1	Receptor de melatonina tipo 1
MT2	Receptor de melatonina tipo 2
MT3	Receptor de melatonina MT3
MCF-7	Linhagens celulares de câncer de mama humano
NR	Receptor nuclear
PAG	Substância cinzenta periaquedutal

PPARγ	Receptores ativado por proliferador de peroxissoma do tipo gama
PPARs	Receptores ativados por proliferadores de peroxissoma
pRb2	Proteína rica em prolina BstNI, subfamília 2
Pyk2	Proteínas cinases não-receptoras
RZR1	Receptor nuclear
RAR	Receptor de ácido retinóide
RAVLT	Teste cognitivo de aprendizagem auditivo-verbal de Rey
Rev-ErbA	Receptor nuclear do hormônio tireóide
RNAm	Ácido ribonucleico mensageiro
ROR	Receptor nuclear órfão do ácido retinóico
RVM	Medula rostral ventromedial
RXR	Receptores retinóides X
S100B	Proteína ligante de cálcio
SCN	Núcleo supraquiasmático
SNC	Sistema nervoso central
TMT-A-B	Teste cognitivo de Trilhas Partes A e B
TNFα	Fator de necrose tumoral alfa
TPH	Enzima triptofano hidroxilase
TR	Receptor do hormônio da tireóide
TrkB	Receptor tropomiosina quinase
Tyr402	Tirosina 402
TRPH	Enzima triptofano hidroxilase
VEGF	Fator de crescimento vascular endotelial
VDR	Receptor de vitamina D

RESUMO

O tratamento sistêmico do câncer de mama é complexo e concorre com efeitos colaterais que podem comprometer a qualidade de vida. Dentre efeitos prevalentes como náuseas e vômitos, o comprometimento da função cognitiva, dor, alterações de humor e a qualidade do sono são frequentemente prejudicados. Atualmente não existe terapêutica disponível com a finalidade de auxiliar como adjuvante profilático na redução deste conjunto de sintomas sistêmicos. Evidências apontam a melatonina como um agente protetivo para os efeitos adversos dos quimioterápicos. O objetivo do estudo foi avaliar o efeito da melatonina em pacientes com câncer de mama submetidos ao primeiro ciclo de quimioterapia nos seguintes desfechos: (i) Função cognitiva relacionada à flexibilidade mental, memória episódica e atenção; (ii) Relação da performace cognitiva e níveis séricos de BDNF e TrkB; (iii) Sintomas depressivos; (iv) Qualidade de sono; (v) função do sistema modulador descendente da dor (DPMS), avaliado pela modulação da dor condicionada (CPM); (vi) efeito no estado neuroplástico por meio dos níveis séricos de BDNF, TrkB e S100B. Neste ensaio clínico randomizado, duplo-cego, controlado com placebo, foram incluídas 36 mulheres, com diagnóstico de câncer de mama e com indicação de quimioterapia adjuvante. Elas foram randomizadas para receber 20 mg de melatonina diária, ou placebo, antes de dormir, tendo início três dias antes do primeiro ciclo de quimioterapia e se mantendo até o sétimo dias após a quimioterapia. O efeito do tratamento foi avaliado por meio de mudanças (valores pós menos pré tratamento) nos seguintes desfechos: Função cognitiva por meio do teste de trilhas partes A e B (TMT-A-B), teste de aprendizagem auditivo-verbal de Rey (RAVLT), teste de associação de palavras controladas (COWAT) e uma tarefa de ação inibitória do tipo *Go / No-Go*; a qualidade sono, por meio do Índice de Qualidade de Sono de Pittsburgh; os níveis de sintomas depressivos por meio do Inventário de Depressão de Beck II; DPMS, limiar de dor ao calor (HPT), e tolerância à dor pelo calor (HPTo), pelo teste sensorial quantitativo da dor (QST) e modulação da dor condicionada (CPM). Os resultados desse estudo demonstraram que a melatonina melhorou função executiva no TMT-A-B, aumentou pontuação na memória episódica (imediata e tardia) e o reconhecimento no RAVLT e aumentou a fluência verbal no COWAT ortográfico. TMT-A-B foram negativamente correlacionados com os níveis basais de TrkB e BDNF, respectivamente. No final do tratamento, as alterações no TrkB e BDNF foram inversamente associadas com sintomas depressivos e qualidade do sono, mas não com o TMT-A-B. As alterações na NPS durante a tarefa de CPM no grupo placebo e melatonina foram de [1,70 (1,45) vs. -1,35 (1,11), $P < 0,001$; $\eta^2 = 0,60$], respectivamente. A potência do DPMS aumentou em 43,5% no grupo melatonina e diminuiu em 93% no grupo placebo. A melatonina aumentou o HPT e a HPTo enquanto reduziu os níveis séricos de BDNF, TrkB e proteína S100-B. De acordo com os resultados deste estudo, sugere-se que o uso da melatonina pode contra-regular os efeitos adversos da quimioterapia adjuvante para câncer de mama na função cognitiva e do sistema somata-sensorial, com benéficios na qualidade de vida e bem estar das pacientes durante o tratamento.

Palavras-chave: câncer de mama; quimioterapia adjuvante; cognição; dor; depressão; distúrbios do sono; fator neurotrófico derivado do cérebro; receptor TrkB.

Registro dos estudos: WebGPPG 14-0701 e *clinical trials.gov*: NCT03205033.

ABSTRACT

The systemic treatment for breast cancer is complex and competes with side effects that may compromise quality of life. Among the prevalent side effects include nausea and vomiting, impairment of cognitive function, pain, mood changes and poor sleep quality. There is currently no available therapy with prophylactic adjuvant action to reduce this set of systemic symptoms. Evidence points to melatonin as a protective agent for the adverse effects of chemotherapy. This randomized, double-blinded, placebo-controlled trial included 36 women with the diagnosis of breast cancer and with a prescription for adjuvant chemotherapy. They were randomized to receive 20 mg of melatonin or placebo every night before bedtime, starting three days prior to their first cycle of chemotherapy and continuing until the seventh day after. To evaluate the effect of melatonin in breast cancer patients submitted to the first cycle of chemotherapy in the following outcomes: (i) Cognitive function related to mental flexibility, work memory and attention; (ii) Relation of cognitive performace and serum levels of BDNF and TrkB; (iii) Depressive symptoms; (iv) Sleep quality; (v) Pain modulating system (DPMS) evaluated by 0-10 changes on the Numerical Pain Scale (NPS) during the conditioned pain modulation test (CPM); (vi) Effect on the neuroplastic state through the serum levels of BDNF, TrkB and S100B, and (vii) the effects of melatonin on pain and if it's relationship with the neuroplastic state is due to the impact on sleep quality. The treatment effect was evaluated by means of changes (post-pre-treatment values) in the following outcomes: Cognitive function by the Trail Making Test parts A and B (TMT-A-B), Rey's auditory-verbal learning test (RAVLT), Controlled word association test (COWAT) and an inhibitory action task type Go / No-Go; quality of sleep by the Pittsburgh Sleep Quality Index; levels of depressive symptoms by the Depression Inventory of Beck II; DPMS, heat pain threshold (PTH), heat pain tolerance (HPTo), and serum levels of BDNF, TrkB and S100B. Melatonin improved executive function in TMT-A-B, increased scores of episodic memory (immediate and late) and recognition in RAVLT, and increased verbal fluency in COWAT. TMT-A-B were negatively correlated with the basal levels of TrkB and BDNF, respectively. At the end of treatment, changes in TrkB and BDNF were inversely associated with depressive symptoms and sleep quality, but not with TMT-A-B. Changes in NPS during the CPM task in placebo and melatonin groups were [1.70 (1.45) vs. -1.35 (1.11), $P < 0.001$; $\eta^2 = 0.60$], respectively. The power of DPMS increased by 43.5% in the melatonin group and decreased by 93% in the placebo group. Melatonin increased HPT and HPTo while reducing serum levels of BDNF, TrkB and S100-B protein. According to the results, it is suggested that the use of melatonin may counteract the adverse effects of adjuvant chemotherapy for breast cancer on cognitive and somato-sensory system function with benefits to quality of life and well-being of patients during treatment.

Keywords: breast neoplasms, adjuvant chemotherapy, cognition, pain, depression, sleep disorders, brain-derived neurotrophic factor (BDNF), TrkB receptor.

1 INTRODUÇÃO

O câncer de mama é a neoplasia mais prevalente e a maior causa de morte por câncer em mulheres (GLOBOCAN, 2012). No Brasil, é o mais incidente em mulheres, principalmente após os 50 anos de idade (Cecilio et al., 2015). Segundo o Instituto Nacional do Câncer (INCA), a expectativa de novos casos de câncer de mama no país era de 57.960 para o ano de 2016. Mais da metade dos casos de câncer de mama (51,3%) estimados para este ano ocorreu na região Sul, sendo 5.210 novos casos no Rio Grande do Sul (130,99/100 mil), e, destes, 1.040 na capital (INCA 2016). Apesar dos esforços para melhorar o diagnóstico e o tratamento, essa neoplasia representa um dos principais desafios enfrentados pelo governo brasileiro (Celico et al., 2015).

O tratamento do câncer de mama envolve uma abordagem multidisciplinar e inclui cirurgia (tumorectomia ou mastectomia), radioterapia e terapia sistêmica (Kesson et al., 2012). A quimioterapia é responsável por grande parte da redução na mortalidade e resulta em uma melhoria tanto na sobrevida livre de doença como na sobrevida geral (EBCTCG, 2018). Porém, os efeitos do câncer *per se* e do tratamento comprometem a qualidade de vida das pacientes (Debess et al., 2009) e podem se manisfestar em diferentes dimensões. Os efeitos adversos incluem naúseas e vomitos (Grunberg et al., 2004), fadiga (Bower et al., 2000), dor (Ripamonti et al., 2012), distúrbios do sono (Savard et al., 2001), prejuízo cognitivo (Wefel et al., 2004), sintomas depressivos (Hansen et al, 2014). Sabe-se que estas manifestações devem-se a processos complexos mediados pela desregulação de várias cascatas de sinalização ao nível de Sistema Nervoso Central (SNC). Embora tenha ocorrido avanços terapêuticos com o objetivo de atenuar estes efeitos, ainda existe a carência de intervenções que possam prevenir ou diminuir este conjunto de sintomas que, com frequência, dificultam o seguimento do tratamento.

Dentre terapêuticas disponíveis e com evidência a partir de estudos experimentais (Borin et al., 2016) e clínicos (Lissoni et al., 1999), existe a melatonina. Estudos *in vitro* e *in vivo* demonstraram efeitos oncostáticos da melatonina em vários tipos de câncer (Goradel et al., 2017), incluindo células de câncer de mama (Alvarez-García et al., 2013; Yeh et al., 2017). A melatonina possui efeitos multifacetados que envolvem múltiplos mecanismos neuromodulatórios, avaliados por meio de diversas dimensões de funcionamento, tais como o efeito cronobiótico, que sincroniza o ritmo circadiano (Laste, et al., 2013), propriedades imunomodulatórias (Najafi et al., 2017), coanalgésicas (Stefani et al., 2013; de Zanette et al.,

2014), antidepressivas (Hansen et al., 2014) e cognitivas (Liet et al., 2015).

No contexto da quimioterapia, os sintomas neuropsiquiátricos e a dessincronizacão do ritmo sono-vigília envolvem a ação de citocinas pró-inflamatórias e consequente alteração dos fatores de crescimento neural, como o fator neurotrófico derivado do cérebro (BDNF) (Lévi, 2006). No contexto da dor e inflamação, a melatonina possui efeitos co-análgesicos por meio de ações em diversas vias neurobiológicas, tais como a opioidérgica, GABA-érgica, glutamatérgica, dentre outras. Parte do seu efeito analgésico está relacionada a alterações neuroplásticas, como sugerido pela redução dos níveis de BDNF no seu uso em condições de dor crônica, como na endometriose (Schwertner et al., 2013) e na fibromialagia (Zanette et al., 2014).

O BDNF regula o crescimento e a sobrevida neuronal, e os níveis desta neurotrofina estão correlacionados de maneira direta com o estado de excitabilidade / inibição do SNC, conforme mensurado por meio de medidas de excitabilidade das vias de comunicação cortico-espinhal através do potencial evocado motor (MEP) (da Silva et al., 2015) e da potência do sistema inibitório descendente de dor (Botelho, 2016). No processamento da memória, o BDNF é um mediador chave por meio de seus efeitos em diferentes níveis moleculares: regula canais iônicos, incluindo canais de Na^{+} e K^{+}; aumenta positivamente a expressão dos receptores AMPA, induzindo sua rápida translocação de superfície para aumentar a transmissão excitatória (Cunha et al., 2010); também modula a transmissão excitatória e inibitória por meio da ativação dos receptores NMDA glutamatérgicos e dos receptores GABA inibitórios (Whitehead; Rose; Jenner, 2004).

O receptor primário do BDNF, receptor tropomiosina quinase B (TrkB), tem sido associado à metástase tumoral (Descamps et al., 2001, Blasco-Gutiérrez et al., 2007). A expressão excessiva de TrkB em pacientes com câncer de mama pode ser um marcador preditivo de fraco prognóstico clinicopatológico (Zhang et al., 2017). A expressão da via BDNF/TrkB é frequentemente aumentada em pacientes com câncer de mama (Zhang et al., 2010), e um estudo recente demonstrou que a regulação dessa via estaria associada com a melhora do aprendizado e da memória (Chen et al., 2017).

A melatonina é um hormônio indólico produzido principalmente pela glândula pineal, distribuída de forma ubíqua, com diversas funções biológicas (Reiter et al., 2010). A maioria das ações da melatonina ocorre pela ligação aos seus receptores acoplados à proteína G, MT1

e MT2, que desencadeiam vias de sinalização celular (Hill et al., 2015). O declínio na produção de melatonina com a idade contribui para a imunossenescência e potencial desenvolvimento de doenças neoplásicas (Asayama et al., 2003). Em metanálise, Seely et al. (2012) incluiu 21 ensaios clínicos com pacientes de tumores sólidos. Nos ensaios que combinavam melatonina com quimioterapia adjuvante, houve diminuição da mortalidade em um ano e melhora nos resultados gerais. A melatonina pode beneficiar pacientes com câncer em quimioterapia e radioterapia, melhorando a sobrevida e os efeitos colaterais da quimioterapia, como demonstrado na metanálise de Wang (2012), que inclui oito ensaios clínicos randomizados, com o uso de melatonina adjuvante à terapia empregada em pacientes com câncer de tumores sólidos. Os ensaios clínicos utilizaram a dose de 20 mg de melatonina demonstraram remissão do tumor, aumento da sobrevida de um ano e redução dos efeitos colaterais relacionados à quimioterapia.

Diante dos potenciais benefícios da melatonina para contrarregular os efeitos adversos da quimioterapia, neste estudo testou-se a hipótese de que, em pacientes submetidas à quimioterapia por câncer de mama, o uso de melatonina na dose de 20 mg/diária, por via oral, iniciado previamente à realização da primeira sessão de quimioterapia adjuvante em câncer de mama e mantido por até sete dias após, seria superior ao placebo, nos seguintes desfechos: (i) função cognitiva (flexibilidade mental, memória de trabalho e atenção) e sincronizadora do ritmo circadiano (desfechos primários); (ii) Qualidade de vida, sono, sintomas depressivos e níveis séricos do BDNF e TrkB (desfechos secundários).

2 REVISÃO SISTEMÁTICA DA LITERATURA

2.1 ESTRATÉGIAS PARA LOCALIZAR E SELECIONAR AS INFORMAÇÕES

Na revisão da literatura foram abordados função cognitiva e tratamento com melatonina, em pacientes submetidos à quimioterapia adjuvante para câncer de mama. Foi elaborada a pergunta: Existe melhora no ritmo sono-vigília e função cognitiva em pacientes submetidos à quimioterapia, tratados com melatonina? Utilizou-se a estratégia PICO – **P**opulação: Pacientes submetidos à quimioterapia adjuvante para câncer de mama, **I**ntervenção: Melatonina, **C**omparação: Placebo e "**O**utcomes" (desfecho): Melhora da função cognitiva e da percepção à dor. A estratégia de busca envolveu as seguintes bases de dados: MEDLINE (PubMed), EMBASE e Lilacs, sem período delimitado. Foram realizadas buscas através dos descritores [MeSH (MEDLINE/PubMed) e EMTREE (EMBASE)]: (1) *Breast cancer*; (2) *Chemotherapy;* (3) *Melatonin;* (4) *Cognition;* e (5) *Pain*. Para acessar os marcadores relacionados à plasticidade na quimioterapia por câncer de mama, foi realizada uma busca com os descritores (6) *Brain-Derived Neurotrophic Factor (BDNF);* (7) *TrkB receptor* and (8); e *Protein S-100 B*. A estratégia de busca envolveu as seguintes bases de dados: MEDLINE (PubMed) e EMBASE, sem período delimitado. Foram realizadas buscas através dos descritores [MeSH (MEDLINE/PubMed) e EMTREE (EMBASE)]. A seguinte pergunta foi abordada: Ocorre modulação dos níveis séricos de BDNF e TrkB na quimioterapia adjuvante por câncer de mama com uso concomintante de melatonina? A síntese da estratégia de busca das referências bibliográficas usadas, com base nos aspectos que estruturam os objetivos do estudo, está listada na **Tabela 1**.

Tabela 1 – Estratégia de Busca de Referências Bibliográficas

Palavras-chave	**PubMed**	**EMBASE**	**Lilacs**
"Breast cancer" e "Chemotherapy"	34.518	69.564	115.359
"Breast cancer" e "Chemotherapy" e "Melatonin"	25	60	240
"Breast cancer" e "Chemotherapy" e "Cognition"	121	840	584
"Breast cancer" e "Chemotherapy" e "Pain"	374	6.077	3.070

“Breast cancer” e “Chemotherapy” e “Melatonin” e “Cognition”	0	3	1
“Breast cancer” e “Chemotherapy” e “Melatonin” e “Pain”	0	14	5
“Breast cancer” e “Chemotherapy” e “BDNF”	1	31	31
“Breast cancer” e “Chemotherapy” e “TrkB receptor”	0	4	0
“Breast cancer” e “Chemotherapy” e “BDNF” e “TrkB receptor”	0	2	0
“Breast cancer” e “Chemotherapy” e “Protein S-100 B”	0	0	0
“Breast cancer” e “Chemotherapy” e “Melatonina” e “BDNF”	0	0	0
“Melatonin” e “Cognition”	115	2.480	302
“Melatonin” e “Pain”	171	1.937	483
“Melatonin” e “BDNF”	32	210	84
“Melatonin” e “Protein S-100 B”	4	0	19
“Melatonina” e “BDNF” e “TrkB receptor”	6	8	11
“Breast cancer” e “Chemotherapy” e “Melatonina” e “BDNF” e “TrkB receptor”	0	0	0
“Breast cancer” e “Chemotherapy” e “Melatonina” e “Protein S-100 B”	0	0	0
“Breast cancer” e “Chemotherapy” e “Melatonina” e “Protein S-100 B” e “Pain”	0	0	0
“Breast cancer” e “Chemotherapy” e “Melatonina” “Cognition” e “BDNF” e “TrkB receptor”	0	0	0
“Breast cancer” e “Chemotherapy” e “Melatonina” e “Pain” e “BDNF” e “TrkB receptor”	0	0	0

Fonte: da autora, 2019.

2.2 CÂNCER DE MAMA

2.2.1 Epidemiologia

O câncer de mama é a neoplasia não cutânea mais diagnosticada em todo o mundo, incluindo países de baixa e média renda (Ghoncheh et al., 2016). Prevê-se que, até 2020, o câncer de mama seja diagnosticado em mais de 1,97 milhões de mulheres em todo o mundo, e 622 mil morrerão desta doença (GLOBOCAN, 2012). As taxas de incidência mais altas são na América do Norte, na Austrália / Nova Zelândia e no Oeste e Norte da Europa. As taxas mais baixas são Ásia e África (Torre et al., 2015). Essas diferenças internacionais provavelmente estão relacionadas a mudanças societárias, como resultado da industrialização.

De acordo com um relatório da Organização Mundial da Saúde (OMS), o aumento da renda e as melhorias nos padrões de vida nos países em desenvolvimento são acompanhados por aumento na incidência de câncer de mama (WHO, 2013). Isso pode ser reflexo da vida mais longa, maior exposição a fatores de risco, maior consumo de gordura, aumento de obesidade e menores taxas de gravidez (Fitzmaurice et al., 2013; Rocha-Brischiliari et al., 2017). No Brasil, é o câncer que ocorre com maior incidência em mulheres, além de ser a maior causa de mortalidade. Segundo o Instituto Nacional do Câncer (INCA), a taxa de incidência no país é 56,20/100 mil habitantes, sendo que a região Sul tem as maiores taxas de incidência (74,30 / 100 mil), que aumenta para 90,20/100 mil no Rio Grande do Sul e 130,99/100 mil para a capital deste estado (INCA, Estimativas para o ano de 2016).

Como resultado da disponibilidade de serviços de detecção precoce em combinação com o tratamento, as taxas de sobrevida de câncer de mama no Brasil melhoraram acentuadamente nos últimos 10 anos (Allemani et al., 2015). No entanto, as desigualdades persistem no acesso ao rastreio do câncer, que geralmente é associado com disparidades geográficas e socioeconômicas (De Castro et al., 2013). Como não existe um programa nacional organizado de triagem de câncer de mama, as mulheres acessam os serviços de prevenção por meio dos sistemas públicos ou privados (Marchi; Gurgel, 2010). Além disso, é importante considerar que, no Brasil, a distribuição da incidência e da mortalidade por câncer de mama pode variar de acordo com a região, sendo maiores nas regiões mais desenvolvidas (Sul e

Sudeste) em comparação com as regiões mais carentes (Norte, Nordeste e Central) (Figueiredo et al., 2017).

2.2.2 Diagnóstico e tratamento

Importantes avanços aconteceram no tratamento e na abordagem terapêutica do câncer de mama nos últimos anos. O tratamento cirúrgico é mais conservador, a radioterapia utiliza doses de radiação mais altas e localizadas, e a quimioterapia passou a utilizar moléculas específicas, como antagonistas hormonais (Xing et al., 2016), anticorpos monoclonais (Mendes et al., 2017) e modalidades da terapia com drogas-alvo dirigidas (Nicolini et al., 2017). Após o diagnóstico de câncer de mama, os desafios imediatos no manejo do paciente são a determinação do prognóstico e a identificação da terapia sistêmica adjuvante mais apropriada. O uso de quimioterapia após a cirurgia é administrada com o objetivo de erradicar focos microscópicos de células cancerígenas. No entanto, o tratamento está associado a complicações à curto e longo prazo para o sobrevivente de câncer de mama. Em geral, pacientes com câncer de mama em estágio inicial são submetidos à cirurgia primária (tumorectomia ou mastectomia) aos seios mamários e regionais com ou sem radioterapia (RT). Após o tratamento local definitivo, a terapia sistêmica adjuvante pode ser oferecida com base nas características primárias do tumor, como tamanho, grau, número de linfonodos envolvidos, status de receptores de estrogênio (ER) e progesterona (PR) e expressão do receptor do fator de crescimento epidérmico humano (HER2).

Os compostos quimioterápicos são geralmente aplicados por infusão intravenosa, visando células com alta taxa de renovação. A quimioterapia é recomendada após a cirurgia (quimioterapia adjuvante). Os tratamentos mais utilizados após a cirurgia são radiação, quimioterapia, terapia direcionada e terapia hormonal (Maughan et al., 2010).

A terapia neoadjuvante refere-se a tratamentos que são administrados antes da cirurgia. O benefício da quimioterapia neoadjuvante é que pode causar uma diminuição no tamanho do tumor, facilitando a remoção, com uma cirurgia menos invasiva (Mamounas, 2015). Além disso, a administração de quimioterapia antes da remoção do tumor, pode auxiliar o monitoramento subsequente da doença, pois, no caso de o primeiro coquetel de drogas não diminuir o tamanho do tumor, outros compostos podem ser considerados (Masood, 2016).

2.2.2.1 Quimioterapia Adjuvante

A quimioterapia consiste em tratamento farmacológico, administrado por via intravenosa ou oral, com a finalidade de matar as células cancerígenas. Geralmente são empregados combinações de fármacos para tratar tumores mamários em estágios iniciais da carcinogênese, enquanto o câncer avançado é tratado com uma única molécula quimioterápica (Suter; Marcum, 2007). A quimioterapia é geralmente administrada em sessões alternadas com períodos de repouso, para permitir a recuperação do paciente e minimizar os efeitos colaterais da terapia. Para pacientes com câncer de mama em estágio inicial, o tratamento baseia-se nas características do tumor, no estado do paciente e nas preferências dos pacientes. O tratamento geralmente inclui quimioterapia combinada com diferentes agentes antineoplásicos. As recomendações baseadas em evidências para o tratamento são fornecidas pelo guia do *National Comprehensive Cancer Network* (NCCN) e estão apresentados no **Anexo 1**.

2.2.2.1.1 Efeitos negativos associados à quimioterapia

Partridge e colaboradores (2001) caracterizaram a frequência e a gravidade dos efeitos adversos agudos mais comuns na quimioterapia adjuvante para o câncer de mama, usando os efeitos tóxicos relatados em 12 experimentos que avaliaram a qualidade de vida. Os efeitos agudos mais observados na análise foram náuseas, vômitos, diarreia, estomatites, alopecia, neutropenia, infecções, trombocitopenia, neuropatia e mialgias. Estes efeitos podem ser observados já no primeiro ciclo de quimioterapia e variam de intensidade conforme o tipo de droga utilizada. Outros efeitos observados incluem dor (Wang et al., 2016; Eversley et al., 2005), depressão (Badger et al., 2005), distúrbios do sono (Berger; Farr, 1999), alterações cognitivas (McDonald et al., 2012) e disrupção do ritmo circadiano (Liu et al., 2013). Alguns sintomas podem ser observados antes do terapia sistêmica, em consequência da doença, e magnificados durante o tratamento.

2.2.2.1.1.1 Dor

A dor é um sintoma prevalente entre as pacientes em tratamento e reduz, de maneira significativa, a qualidade de vida (Eversley et al., 2005; Given et al., 2001). Várias etiologias para a dor pós-tratamento do câncer foram identificadas, incluindo dor pós-cirúrgica, neuropatias, mucosite, linfedema, metástase e neuralgias pós-herpética (Longman et al., 1999; Shapiro; Recht, 2001). A sensibilização central, presente em pacientes com dor, está associada ao aumento do campo receptivo e é consequência da lenta somação temporal nos neurônios dorsais da medula, por impulsos repetidos a partir dos nociceptores primários (fibras C), fenômeno também conhecido como somação temporal ou *windup* (Price, 2005). Cabe ressaltar que a informação primária transmitida pela via nociceptiva ascendente é modulada por várias vias descendentes, as quais se originam de diferentes áreas no cérebro, incluindo a substância cinzenta periaquedutal (PAG), o lócus cerúleos e o eixo hipotálamo-pituitária-adrenal (HPA) (Abeles, 2007), ativando comportamentos reflexos e protetivos subsequentes e influenciando a percepção da dor (Price, 2005).

Além do controle inibitório, o processamento da dor pode ser facilitado por circuitos no SNC. Esse processo contribui para o desenvolvimento da dor crônica ou persistente (Vanegas; Schaible, 2004; Gebhart, 2004). Além disso, a dor anormal após a lesão está ligada ao aprimoramento neuronal envolvido na modulação da dor (Ren; Dubner, 2007). O aumento da excitabilidade nos circuitos moduladores da dor é dinâmico e envolve a ativação de mediadores químicos, incluindo aminoácidos excitatórios e seus receptores, recrutamento de cascatas de transdução de sinalização intracelular e mudanças sinápticas duradouras (Ren; Dubner, 2002). A plasticidade dependente da atividade dos circuitos moduladores da dor é complementar aos mecanismos de hiperexcitabilidade. Esse mecanismo é semelhante a plasticidade observada nas sinapses do hipocampo envolvidas na potenciação de longa duração (LTP, do inglês *long-term potenciation*) e na depressão de longa duração (LTD, do inglês *long-term* depression), que parece imitar estados funcionais de aprendizagem e memória (Malenka; Bear, 2004).

2.2.2.1.1.2 Declínio Cognitivo

O declínico cognitivo é comumente observado em pacientes com câncer de mama e tem sido amplamente estudado desde o final da década de 1990 (Ganz, 1998). O termo amplamente utilizado no inglês *chemo brain* ou *brain fog* descreve pacientes que experimentaram os efeitos neurotóxicos da quimioterapia e apresentam comprometimento cognitivo na capacidade verbal,

habilidade visuo-espacial, resolução de problemas, concentração e memória (Selamat et al, 2014). Entre todos os pacientes submetidos à quimioterapia, 10-40% apresenta déficits cognitivos. No entanto, a neurotoxicidade induzida por quimioterapia que leva à disfunção cognitiva em pacientes com câncer tem sido questionada, uma vez que a disfunção cognitiva também foi encontrada em pacientes cujo tratamento sistêmico ainda não havia começado (Cox; Reid-Arndt, 2012; Ahles et al ., 2008). Essa evidência é sustentada por Jansen e colaboradores (2011), os quais demonstram que, antes da quimioterapia, 23% das mulheres com câncer de mama apresentaram comprometimento cognitivo, no entanto, esse número aumentou para 52% durante a quimioterapia.

Na maioria dos estudos, as queixas subjetivas são constituídas principalmente por sintomas associados à depressão e ansiedade (Castellon et al., 2004; Ahles et al., 2002), embora o desempenho cognitivo também possa ser influenciado pela dor, insônia e fadiga (Asher, 2016). Em um estudo de Castellon et al. (2004), os domínios mais afetados, em pacientes com câncer de mama, são a memória visual, a função visuoespacial e a aprendizagem verbal, com tamanhos de efeito moderado e grande, observados em comparações entre pacientes expostos e não expostos ao tratamento adjuvante. Uma meta-análise realizada por Jim et al. (2012), confirma que o período de pós-tratamento, com quimioterapia de dose padrão, pode causar déficits estatisticamente significativos na capacidade verbal e visuoespacial.

Os comprometimento cognitivo associado ao tratamento do câncer pode ter um efeito dramático sobre a qualidade de vida das pacientes. Dam e colaboradores (1998) demonstraram que o risco de déficit cognitivo, nas pacientes que receberam quimioterapia em altas doses, foi 8,2 vezes maior do que o risco do grupo controle e 3,5 vezes maior do que no grupo de pacientes que receberam quimioterapia em dose-padrão. Estudos de imagem cerebral começaram a fornecer mais informações sobre a biologia da função cognitiva após exposições ao tratamento do câncer. Em estudo prospectivo, controlado, em que foram aplicados teste de desempenho neuropsicológico e ressonância magnética antes e após a quimioterapia, foram encontradas mudanças na massa cinzenta associadas ao tratamento com quimioterapia, o que sugere uma explicação fisiológica para o distúrbio cognitivo (McDonald et al., 2012). Dois estudos de imagem adicionais demonstraram que as pacientes com câncer de mama expostas à quimioterapia relataram queixas cognitivas (memória e função executiva) que se alinhavam

com os domínios de ensaio pertinentes (neuropsicológicos), bem como com as regiões cerebrais anatômicas associadas (Kesler, 2011).

2.2.2.1.1.3 Sintomas Depressivos

Sintomas depressivos são frequentemente observados em paciente com câncer de mama. Depressão ou ansiedade ocorrem em até 50% das mulheres com a doença (Burgess et al., 2005). Porém, a depressão é subdiagnosticada e subtratada em muitos pacientes (Fann et al., 2008). Os sintomas depressivos têm efeitos adversos na qualidade de vida, podem afetar o tratamento, reduzindo a aderência (Mausbach et al., 2015), e estão associados à menor satisfação do paciente (Bui et al., 2005), maior morbidade e mortalidade (Hjerl et al., 2002).

2.2.2.1.1.4 Distúrbios do Sono

Distúrbios no sono são uma queixa comum entre pacientes em tratamento adjuvante para o câncer de mama. Os problemas relacionados ao sono têm um impacto negativo sobre as funções fisiológicas e psicológicas, incluindo função imune (Savard et al., 1999), declínio cognitivo (Caplette-Gingras et al., 2013), depressão (Hsiao et al., 2013) e fadiga (Berger et al., 2007), e também é um forte preditor de qualidade de vida (Rand et al., 2011).

Em uma metanálise de Costa et al. (2014), foi demonstrada uma tendência de distúrbios do sono em mulheres submetidas à quimioterapia, e quando comparado à radioterapia, a quimioterapia também foi associada a maiores níveis de distúrbios do sono. Quando comparado os diferentes tipos de quimioterapia, os regimes de doxorrubicina e FEC [fluorouracil (5-FU) + epirrubicina + ciclofosfamida] sugerem maior prevalência de distúrbios do sono. As mulheres tratadas com agentes taxanos apresentaram menor prevalência de distúrbios do sono do que as tratadas com quimioterapia sem agentes taxanos.

O tratamento adjuvante quimioterápico para câncer de mama tem vários efeitos colaterais associados. O efeito cumulativo de agentes tóxicos nas funções corporais, o impacto físico de sintomas angustiantes (náuseas, vômitos, diarreias), alteração da imagem corporal e hospitalização, além de outras condições associadas, como dor, fadiga, depressão, ansiedade e estresse, que são fatores agravantes da qualidade de sono (Vena et al., 2004). A alta proporção de sintomatologia vasomotora, como ondas de calor e suores noturnos, como efeito colateral da

interrupção do ciclo mestrual induzido pela quimioterapia, é outro fator relevante para piora do sono (Savard et al., 2009).

Para o diagnóstico clínico, essas dificuldades são tipicamente associadas à incapacidade diurna, como fadiga ou sonolência excessiva pelo menos 3 vezes por semana, durante 30 minutos ou mais, durante pelo menos 1 mês (Palesh et al., 2013). Quase 80% das mulheres submetidas à quimioterapia para câncer de mama sofrerá sintomas de insônia, e aproximadamente metade atenderá critérios clínicos completos para insônia (Palesh et al., 2010). Antes da cirurgia, 69% das mulheres com câncer de mama não metastático relatou ter sintomas de insônia e, embora essas taxas tenham diminuído ao longo do tempo, 42% das mulheres ainda estava com sintomas de insônia 18 meses após a cirurgia (Savard et al., 2011).

Os distúrbios e a privação de sono podem levar a supressão da função imune e desequilíbrio na produção de citocinas, com predomínio de citocinas tipo 1, incluindo citocinas anticancerígenas, como a IL-2 e INFγ, e citocinas estimuladoras de câncer tipo 2, como a IL-10 (Dimitrov et al., 2004). Como parte do mecanismo humoral da regulação do sono, citocinas como IL-1, IL-2 e TNFα pode induzir sintomas associados à distúrbios do sono, como sonolência, fadiga e déficit cognitivo (Jewett & Krueger, 2014). Sugere-se que os distúrbios do sono exerçam influências estimulantes no câncer por alterações diretas no equilíbrio de citocinas e/ou pela ruptura circadiana da melatonina.

2.2.2.1.1.5 Disrupção do ritmo circadiano e câncer de mama

Evidências demonstram que pacientes oncológicos exibem uma disrupção do ritmo sono-vigília (Lévi et al., 2014; Parganiha et al., 2014) e o nível de ruptura piora durante as infusões de quimioterapia (Lévi et al., 2010; Roscoe et al., 2002). Esse padrão é também observado em pacientes com câncer de mama durante a quimioterapia (Ancoli-Israel et al., 2014; Cash et al., 2015; Hansen, 2006). Em estudo, Savard e colaboradores (2009) sugerem que a primeira administração de quimioterapia está associada à interrupção transitória do ritmo sono-vigília, enquanto a administração repetida resulta em pioras progressivas e mais duradouras nos ritmos de atividade-repouso. Além da interrupção dos ritmos circadianos poder acelerar a progressão da doença (Escobar et al., 2011; Fu; Lee, 2003), é também sugerido que o desacoplamento dos sistemas circadianos e de sono-vigília resulta em uma maior incidência de patologias, como envelhecimento acelerado (Kondratova; Kondratov, 2012), síndrome

metabólica (Garaulet; Madrid, 2010), deficiências afetivas e cognitivas (Cochrane; Robertson; Coogan; 2012) e também maior incidência de câncer (Innominato et al., 2010). A disrupção circadiana e a qualidade de vida são consideradas preditores importantes da sobrevida em pacientes com câncer (Innominato et al., 2009; 2012).

Considerando os danos negativos associados à quimioterapia adjuvante e diante de poucos recursos terapêuticos a fim de atenuar esses efeitos, emerge a melatonina, uma molécula anticancerígena que demonstrou afetar as células malignas por múltiplos mecanismos, porém, ainda não foi demonstrado como ela atua em benefício dos pacientes em tratamento quimioterápico.

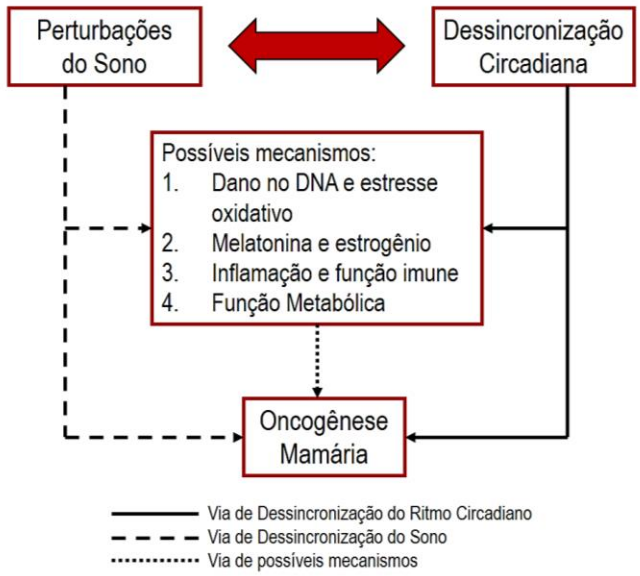
2.2.2.1.1.6 Sistema Circadiano no Câncer de Mama

A primeira hipótese sobre uma possível relação entre a função pineal e a carcinogênese mamária foi publicada em 1978, por Cohen e colaboradores. Esta hipótese foi examinada em vários estudos clínicos e experimentais. Os primeiros estudos clínicos demonstram uma diminuição no pico plasmático noturno de melatonina em pacientes com câncer de mama ER positivo (Tamarkin et al., 1982). Em mulheres cegas, o risco de câncer de mama era mais baixo (Coleman; Reiter, 1992; Kliukiene et al., 2001), e foi demostrada uma correlação inversa entre incidência de câncer de mama e grau de deficiência visual (Verkasalo et al., 1999). Em indivíduos com deficiência visual, supõe-se que a supressão total ou parcial da entrada de luz resulte em níveis aumentados de melatonina circulante, que poderiam explicar a baixa incidência de tumores. Algum tempo depois, foi demonstrado, em um estudo prospectivo de casos e controles, em enfermeiros, que níveis mais altos de melatonina estavam associados a um menor risco de câncer de mama (Schernhammer; Hankinson, 2005). E, ao contrário, foi observada a maior incidência de câncer de mama entre as mulheres expostas à luz durante a noite, como as que trabalham em turnos norturnos (Kheifets; Matkin, 1999). Esse aumento da incidência de câncer de mama pode ser explicado pela síntese reduzida de melatonina sob essas condições ambientais. Em estudos experimentais, essa evidência ficou comprovada com o aumento do crescimento de tumores mamários em ratos expostos à luz de baixa intensidade durante a escuridão noturna, com subsequente redução da síntese e secreção de melatonina (Cos et al., 2006).

Os estudos experimentais realizados com diferentes modelos animais de câncer de mama e linhagens celulares confirmaram que a melatonina, *in vivo*, reduz a incidência e o

crescimento de tumores mamários induzidos quimicamente ou espontaneamente em roedores. Enquanto que, *in vitro*, em concentrações correspondentes aos níveis fisiológicos no sangue humano (1 nM), durante à noite, a melatonina inibe a proliferação, aumenta a expressão de p53 e reduz a invasividade das células de câncer de mama do tipo MCF-7 (Linhagem celular responsiva ao estrogênio Blask) (Blask et al., 2002).

Figura 1 – Modelo biopsicossocial de dessincronizações circadianas e de sono na oncogênese mamária



Fonte: Adaptado de Samuelsson et al., 2018.

O sistema circadiano é uma organização hierárquica de relógios endógenos e oscilantes em todo o corpo que são regulados pelo núcleo supraquiasmático (SCN), localizado no hipotálamo (Fuller et al., 2006). O SCN recebe sinal dos *zeitgebers* ou ZTs (do alemão, *Zeit* = tempo; *geber* = dar), que são pistas externas que sincronizam o sistema temporal endógeno. O *zeitgeber* mais potente é a percepção da luz por meio de células localizadas na retina (Altimus et al., 2010). Por diversas projeções, o SCN coordena inúmeros processos autonômicos, neuroendócrinos e reprodutivos, que estão envolvidos na oncogênese mamária (Kalsbeek; Buijs, 2002). O SCN também está envolvido no controle do sistema de hormônio liberador de gonadotropina (GnRH) no hipotálamo e, assim, comanda a atividade circadiana do eixo hipotálamo-hipófise-gonadal (HHG) (Tonsfeldt; Chappell, 2012). Por conta da diversidade de projeções, a ruptura do SCN resulta na interrupção de numerosos sistemas

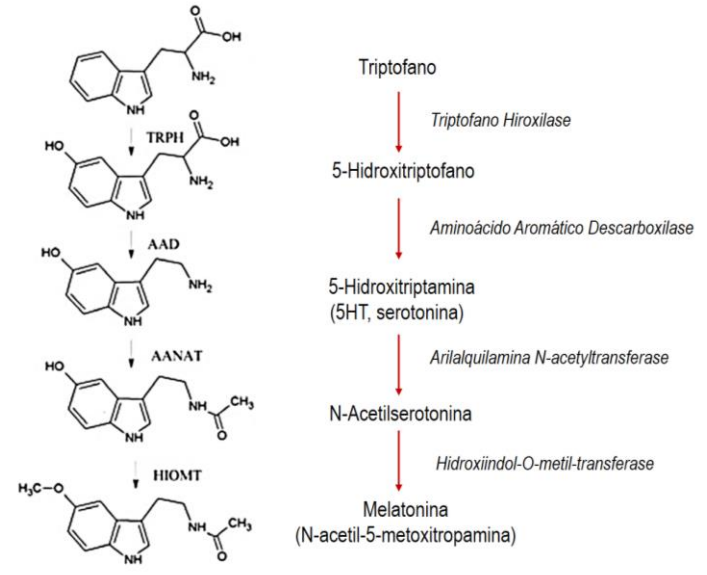
coordenados, que é o argumento fundamental subjacente à hipótese de ruptura circadiana no câncer de mama.

A melatonina foi o primeiro sinal anticâncer identificado em seres humanos, que faz conexão entre o sistema circadiano e o câncer de mama (Blask, 2009). A liberação de melatonina depende da glândula pineal e do SCN, e ocorre à noite pelo SCN (Pevet; Challet, 2011b, Shah et al., 1984). Rupturas no SCN, seja por meio de perturbações do sono e/ou dessincronização circadiana, podem alterar a liberação de melatonina (**Figura 1**).

2.3 MELATONINA

A melatonina (*N*-acetil-5-metoxitropamina) é uma indolamina, caracterizada e isolada por Lerner há mais de 50 anos (Lerner et al., 1959). Lerner e colaboradores (1960) definiram a estrutura química e mostraram a ação antagonista do hormônio estimulante dos alfa-melanócitos (α-MSH). Logo após, foi estabelecida a via biossintética da melatonina (**Figura 2**).

Figura 2 – Via Biossintética da Melatonina



TRPH, do inglês, *tryptophan hydroxylase*; AAD, do inglês, *aromatic amino acid decarboxylase*; AANAT, do inglês, *arylalkylamine N-acetyltransferase*; HIOMT, do inglês, *hydroxyindole-O-methyl transferase*. Fonte: Adaptado de Slominski et al., 2012.

2.3.1 Biossíntese e Metabolismo

A síntese de melatonina é um processo de múltiplas etapas que se inicia com a

hidroxilação do aminoácido aromático L-triptofano em 5-hidroxitriptofano, catalisado pela enzima triptofano hidroxilase (TPH, EC 1.14.16.4). A 6-tetrahidropterina (6-BH4) é um co-fator essencial para esta reação (McIsaac; Page, 1959; Schallreuter et al., 1994). O 5-Hidroxitriptofano é então convertido em serotonina (5-hidroxitriptamina) pelo aminoácido aromático descarboxilase (AAD, EC 4.1.1.28) (Lovenberg et al., 1962). A serotonina é subsequentemente convertida em *N*-acetilserotonina pela enzima arilalquilamina *N*-acetiltransferase (AANAT, EC 2.3.1.87) (Lovenberg et al., 1967). Por fim, ocorre a conversão de *N*-acetilserotonina em melatonina pela hidroxiindole-O-metil transferase (HIOMT, EC 2.1.1.4) (Weissbach, 1960).

Existem três vias de degradação da melatonina: (1) a via hepática, que gera 6-hidroximelatonina (Reiter, 1991c); (2) a via indólica, que produz ácido 5-metoxindole acético (5-MIAA) ou 5-metoxitroptofol (5-MTOL) (Grace et al., 1991); e (3) a via da quinurenina, que produz N1-acetil-N2-formil-5-metoxicinuramina (AFMK) (Hardeland et al., 1993; Tan et al., 2007). Na via hepática clássica, as enzimas CYP1A1, CYP1A2 e CYP1B1, do CYP P450, do fígado, metabolizam a melatonina em 6-hidroximelatonina (Ma et al., 2005). Este produto é então conjugado com sulfato ou glucuronido e depois secretado na urina (Arendt, 1988). No fígado, a melatonina pode ser desmetilada em *N*-acetilserotonina pela CYP2C19 ou CYP1A (Facciola et al., 2001).

A melatonina é sintetizada na glândula pineal (Reiter, 1991c). Quando entra na circulação, atua como fator endócrino e mensageiro químico de claro e escuro (marca-passo circadiano) (Reiter, 1993). Há evidências de que a melatonina também é produzida em vários órgãos extra-pineais, incluindo cérebro, retina, epitélio pigmentar da retina, trato gastrointestinal, medula óssea, linfócitos e pele (Bubenik, 2002; Pandi-Perumal et al., 2008; Slominski et al., 2008). Nesses órgãos, a melatonina pode sinalizar em modos autócrinos ou parácrinos, incluindo a possibilidade de esses modos atuarem como receptores intracelulares expressos pelas mesmas células que produzem as moléculas. Além disso, a melatonina produzida localmente poderia proteger a célula de danos mediados por radicais livres (Slominski et al., 2008).

2.3.2 Efeitos da melatonina

A melatonina é amplamente distribuída em plantas, organismos unicelulares, algas,

bactérias, invertebrados e vertebrados (Stehle et al., 2011). Sua ampla distribuição permite que ela desempenhe funções pleiotrópicas. Nos vertebrados, a melatonina pode atuar em diversas funções, como na regulação do ritmo circadiano (Bonmati-Carrion et al., 2014), na modificação da resposta neuronal (Tan et al., 2011), hormonal e inflamatória (Reiter et al., 2000). Também pode atuar nas funções imunes (Slominski, et al., 2012), gastrointestinais (Chojnacki et al., 2011), cardiovasculares (Rodella et al., 2013), renais (Stacchiotti et al., 2014) e ósseas (Slominski et al., 2014), além de como uma molécula oncostática (Reiter et al., 2017) e anti-envelhecimento (Jung-Hynes; Reiter; Ahmad; 2010). Muitas das ações da melatonina são mediadas pela interação com receptores de membrana específicos, MT1 e MT2. Os exemplos incluem a atividade anticonvulsivante e vasoconstritora, pela ativação de receptores MT1, e vasodilatação, via ativação de receptores MT2 (Masana et al., 2002). A melatonina também mostrou ter um efeito protetor contra o infarto do miocárdio, limitação do ganho de peso e inibição dos efeitos do estrogênio (Boutin et al., 2005; Tengattini et al., 2008).

A melatonina também pode atuar por mecanismos não mediados por receptor, por exemplo, como eliminadora de espécies reativas de oxigênio e nitrogênio (Gómez-Moreno et al., 2010). As espécies reativas com ação da melatonina incluem: o radical hidroxila (HO^-), peróxido de hidrogênio (H_2O_2), óxido nítrico (NO^-), entre outros (Galano, 2011; Tan et al., 2001). Tan e colaboradores (2001) descreveram a melatonina como "antioxidante suicida", pela capacidade de reagir com esses agentes e formar produtos não reciclados novamente à melatonina. Além de ser um potente antioxidante, a melatonina também pode ativar enzimas citoprotetoras (Rodriguez et al., 2004). Nas concentrações fisiológicas e farmacológicas, a melatonina atenua ou neutraliza o estresse oxidativo e regula o metabolismo celular (Korkmaz et al., 2009; Slominski et al., 2008; Tan et al., 2007).

2.3.2.1 Sinalização dos receptores MT1 e MT2 no câncer de mama

É sabido que os receptores de melatonina ligados à membrana estão acoplados à proteína G, no entanto, a melatonina também mostrou inibir a ação do hormônio estimulante de melanócitos (MSH) e prevenir a formação de AMPc (Abe et al., 1969). Os receptores acoplados à proteína G incluem domínios transmembranares helicoidais 7α e ativam vias de sinalização da proteína G . O receptor MT1 é acoplado às subunidades de proteína G_{i2}, G_{i3} e $G_{q/11}$ (Brydon et al., 1999). O receptor MT1 acoplado a proteína G_i reduz os níveis de AMPc (Capsoni et al.,

1994).

Os receptores de melatonina nos tecidos mamários modulam a ligação ao receptor de estrogênio (Danforth et al., 1983). O receptor MT1 é expresso em MCF-7 e MDA-MB-231 (linhagens celulares de câncer de mama humano) e nos tecidos de câncer de mama (Ram et al., 2002; Rogelsperger et al., 2011). Nas células MCF-7, a melatonina inibiu reversivelmente a proliferação e invasão celular (Mao et al., 2010). Confirmado por imuno-histoquímica e microscopia imunofluorescente, o sinal do receptor MT1 foi localizado em caveolina, uma proteína da membrana plasmática, por Lai e colaboradores (Lai et al., 2008, 2009). Existe uma interferência entre o receptor MT1 e as vias dos receptores de estrogênio no câncer de mama. Em células MCF-7A, a expressão de MT1 foi infrarregulada por estradiol exógeno e melatonina. Além disso, a expressão de MT1 é suprarregulada em células negativas dos receptores de estrogênio (MDA-MB-231) e infrarregulada nas células positivas dos receptores de estrogênio (MCF-7) (Girgert et al., 2009).

2.3.2.2 Receptores Nucleares

A melatonina também pode mediar suas ações por meio do grupo ROR/RZR (receptores retinoides órfãos / receptores retinoides Z) (Smirnov, 2001). As subfamílias que, de acordo com alguns autores, ligam-se à melatonina incluem: RZRα, RORα, RORα2 e RZRβ (Becker-Andre et al., 1994; Carrillo-Vico et al., 2005). Os grupos de receptores nucleares (NR) são distribuídos de acordo com seu subtipo, sendo que o RZRβ é encontrado em tecidos neuronais, e o RZRα, em tecido adiposo, pele, testículos, cartilagem e fígado (Smirnov, 2001). Ainda não foi estabelecido como a melatonina interage na sinalização desses receptores, mas alguns pesquisadores propõem que ocorreria ativação indireta por meio de receptores MT1 (Dai et al., 2001; Ram et al., 2002).

A metade dos NR são os chamados receptores "órfãos" porque a identidade do seu ligante, se houver, é desconhecida. O termo "receptor" implica que deva existir um ligante fisiológico, embora não haja consenso sobre isso ser verdade para todos os NR órfãos. Como a ausência de prova não é a prova de ausência, é extremamente difícil demonstrar que um determinado NR órfão realmente não tenha um ligante endógeno (Korkmaz et al., 2009). Dois exemplos principais são os PPARs e RXRs, que foram descobertos como NR órfãos, mas que, agora, são claramente considerados receptores (Lu et al., 2006).

2.3.2.2.1 *Receptores Nucleares no Câncer de Mama*

Os NR, também chamados fatores de transcrição dependente, desempenham um papel essencial no desenvolvimento, na homeostase, na reprodução e na função imune (Mangelsdorf et al., 1995). Eles regulam a transcrição e podem ativar e inibir a expressão gênica (Glass; Rosenfeld, 2000). Os NR incluem os fatores da transcrição esteroidal, tais como o glicocorticoide (GR), estrogênio (ER), receptor do hormônio da tireoide (TR), receptor do fígado X (LXR), receptor farnesoide X (FXR), receptor de vitamina D (VDR), receptor de ácido retinoide (RAR), receptores retinoides X (RXR) e receptores ativados por proliferadores de peroxissoma (PPARs) (Lu et al., 2006; Benoit et al., 2006).

Considerando o envolvimento nuclear e epigenético do câncer de mama no contexto da melatonina, é notável que a atividade transcricional dos receptores ERα, GR e RAR, que estão envolvidos na regulação do crescimento celular do câncer de mama, são moduladas pela melatonina (Schneider et al., 1999; Kiefer et al., 2005). No mesmo contexto, os agonistas de PPARγ inibem significativamente o crescimento do câncer de mama (Jarrar; Baranova, 2007), e os agonistas de RXR potencializam os efeitos antiproliferativos e apoptóticos dos agonistas de PPARγ (Crowe; Chandraratna, 2004).

2.3.2.2.1.1 *Receptor Nuclear de Estrogênio e melatonina*

O estrogênio desempenha um papel importante no desenvolvimento do câncer de mama, atua em receptores nucleares de estrogênio (ER) e induz a expressão de genes estrogênio-dependentes. Além disso, a exposição prolongada ao estradiol (E2), que exerce efeito direto nas células epiteliais ou por meio de células estromais, permite que as células propaguem mudanças hereditárias, incluindo a metilação de DNA (Szyf et al., 2004). Assim, por mais de um século, a supressão das ações estrogênicas tem sido uma ferramenta terapêutica no tratamento do câncer de mama. A melatonina neutraliza os efeitos do estradiol e xenoestrógenos na proliferação de células de câncer de mama, na invasividade e atividade de telomerase (Leon-Blanco et al., 2003; Martinez-Campa et al., 2006), aumenta a sensibilidade de células MCF-7 a outros anti-estrogênios, como o tamoxifeno, e regula a expressão de proteínas, fatores de crescimento e proto-oncogenes regulados por estrogênios (Molis et al., 1995). A melatonina diminui a

expressão do ERα e inibe a ligação do complexo E2-ER ao elemento de resposta do estrogênio no DNA (Molis et al., 1994; Rato et al., 1999). Esses efeitos dependem da ligação da melatonina aos receptores específicos de membrana MT1, também encontrados no tecido mamário humano, ambos normais e tumorais (Dillon et al., 2002).

A calmodulina (CaM) exerce ação mediadora nas interações da melatonina (Garcia et al., 2002). A melatonina atua como antagonista de calmodulina, induzindo alterações conformacionais no complexo ERα-CaM, prejudicando a ligação do complexo E2-ERα-CaM ao DNA e impedindo a transcrição de Erα; enquanto que a transativação mediada por ERβ não é inibida ou ativada, por isso não interage com a calmodulina. Curiosamente, a melatonina não afeta a ligação de coativadores ao ERα, indicando que a ação da melatonina é diferente da dos anti-estrogênios atuais utilizados na terapia do câncer de mama (del Río et al., 2004).

A perda de expressão do ER indica um mau prognóstico para um número significativo de pacientes com câncer de mama (Giacinti et al., 2006). Aproximadamente um terço dos tipos de câncer de mama não expressam ERα. Estes tumores apresentam uma maior agressividade e não respondem à terapia endócrina com estrogênios (Korkmaz et al., 2009). A perda da expressão de estrogênio é resultado da hipermetilação das ilhas de dinucleotídeo CpG no ERα promotor (Giacinti et al., 2006). Alguns estudos demonstraram que o p53 suprarregula a expressão de genes, e ERα e pRb2/p130 também têm um papel importante na regulação transcricional dos promotores do ERα (Macaluso et al., 2003; Martin et al., 2004). Esses achados sugerem que tanto p53 como pRb2/p130 podem ser alvos para o desenvolvimento de novas estratégias terapêuticas no tratamento de câncer de mama ER-negativo, ao restabelecer a expressão de ER (Giacinti et al., 2006).

2.3.5 Efeitos da melatonina na secreção de citocinas

A melatonina exerce seu efeito modulador no câncer controlando a produção e a secreção de várias citocinas. Essas citocinas são produzidas por células do câncer de mama e regulam a diferenciação dos fibroblastos localizados nas proximidades das células epiteliais malignas (Martínez-Campa et al., 2017). Demonstrou-se que as citocinas produzidas por células cancerígenas estimulam a expressão e atividade da aromatase nesses fibroblastos e nas células endoteliais proximais (Alvarez-García et al., 2012, 2013). O fator de crescimento vascular endotelial (VEGF) é um fator de crescimento essencial na angiogênese. Ele é produzido e

secretado por células epiteliais malignas e é responsável pelo reconhecimento dos seus receptores localizados na superfície celular das células endoteliais. A ligação do VEGF ao seu receptor desencadeia uma cascata de eventos intracelulares que estimulam as células endoteliais à proliferação e migração (Zarychta et al, 2019; Gingis-Velitski et al., 2004).

A melatonina é capaz de regular os mecanismos parácrinos que conectam as células epiteliais tumorais e as células endoteliais circundantes (Martínez-Campa et al., 2017). Um dos principais resultados no tratamento com melatonina é a redução na expressão do VEGF em células de câncer de mama ER-positivo. Os níveis reduzidos de estrogênio e a menor capacidade de formação de novos vasos, como resultado da presença de melatonina, diminuem a capacidade tumoral de se espalhar e crescer (Alvarez-García et al., 2013).

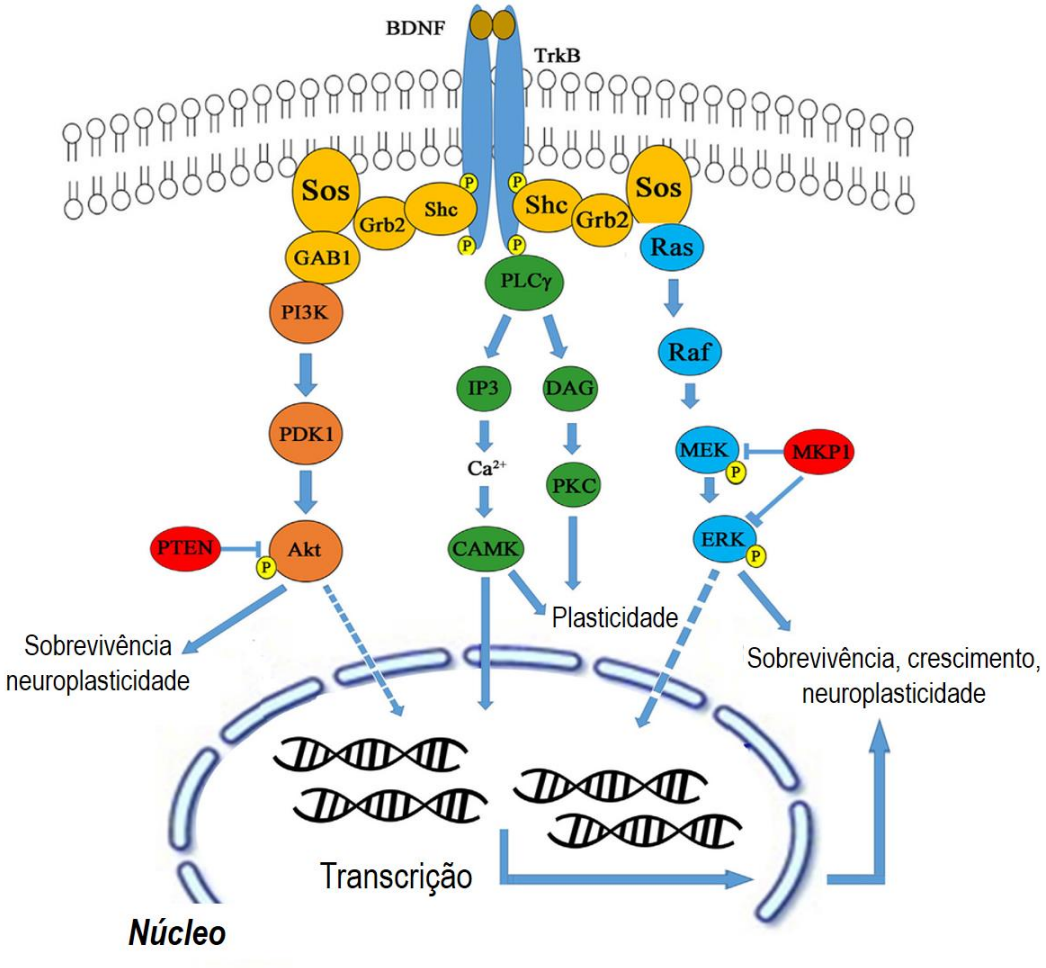
2.3.6 BDNF / TrkB no Câncer de Mama

Entre todas as neurotrofinas, o fator neurotrófico derivado do cérebro (BDNF) se destaca pelo alto nível de expressão no cerébro e seus potentes efeitos nas sinapses. Esses efeitos provêm de complexas cascatas de sinalizaçao e por efeitos em receptores distintos, como o TrkB (**Figura 3**).

A ativação de TrkB pelo BDNF pode promover mecanismos de resistência à quimioterapia. BDNF e TrkB são preferencialmente expressos em tumores agressivos e o aumento da sinalização BDNF/TrkB em células de neuroblastoma pode representar um sistema autócrino para promover o crescimento, invasão e metástase do tumor (Roesler et al., 2011). O TrkB, quando ativado pelo BDNF, pode induzir a fosforilação de Pyk2 em Tyr402, o que leva à ativação ERK e promove invasão celular (Zhang et al., 2010).

Evidências recentes enfatizaram a importância da via de sinalização BDNF/TrkB na regulação da carcinogênese e metástase (**Figura 4**) (Lawn et al., 2015). Além disso, demonstrou-se que a expressão aumentada de BDNF e TrkB é um preditor de desfecho clínico adverso e pior sobrevida em pacientes com câncer de mama (Tajbakhsh et al., 2017). A sinalização BDNF/TrkB desempenha um papel importante na metástase tumoral e é reconhecida como um alvo terapêutico no tratamento do câncer de mama, porém, os mecanismos envolvidos ainda não estão claros.

Figura 3 – Via de sinalização BDNF/TrkB



Fonte: Adaptado de Alam et al., 2016.

A expressão de BDNF é maior em amostras de câncer de mama em comparação com tecido normal, e níveis elevados de BDNF foram significativamente associados a parâmetros patológicos desfavoráveis e desfechos clínicos adversos, incluindo pior prognóstico e morte por câncer de mama (Patani et al., 2011). O BDNF induz resistência à apoptose em células de câncer de mama (Vanhecke et al., 2011), e o bloqueio de BDNF reduz o crescimento tumoral (Huang et al., 2010).

Alguns estudos sugerem que o tratamento com melatonina, em condições dolorosas, podem reduzir os níveis séricos de BDNF (Schwertner et al., 2013; de Zanette et al., 2014). Considerando as possíveis ações da melatonina na regulação de BDNF, uma associação entre declínio cognitivo e supraexpressão da via BDNF/TrkB tem sido especulada. Os mecanismos envolvidos no câncer de mama e o envolvimento da melatonina em vários desses processos,

apontam como sendo uma molécula promissora para atenuar os efeitos negativos da quimioterapia no câncer de mama.

Figura 4 – Sinalização BDNF / TrkB no desenvolvimento de câncer de mama

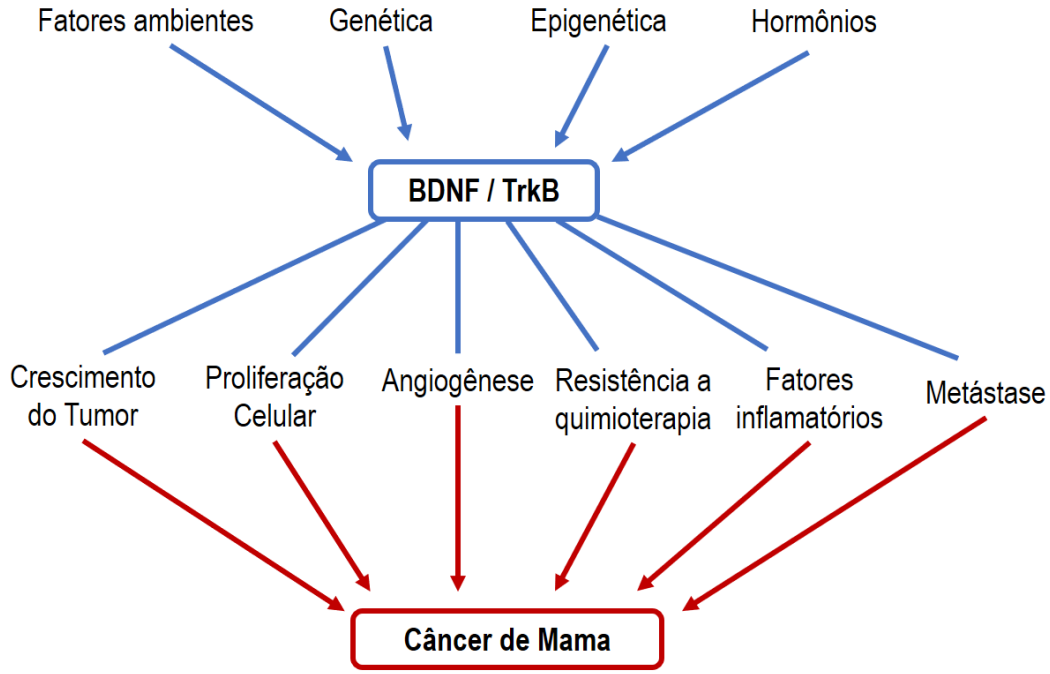


Ilustração esquemática de vários fatores que influenciam a sinalização BDNF/TrkB, que consequentemente levam ao câncer de mama. Fonte: Adaptado de Tajbakhsh et al., 2017.

2.3.7 Efeitos da melatonina na qualidade de sono e regulação do ritmo

Os distúrbios do sono são comuns em pacientes de câncer de mama (Fiorentino; Ancoli-Israel, 2006). A melatonina foi amplamente avaliada como tratamento para jet lag e insônia (Buscemi, 2006; Herxheimer; Petrie, 2002). O tempo do ciclo sono-vigília, que é adequadamente sincronizado com a noite, na maioria dos indivíduos (Roenneberg; Merrow, 2007), é regulado pelo sistema de tempo circadiano. A Classificação Internacional de Distúrbios do Sono (2014) define os distúrbios do sono-vigília do ritmo circadiano como padrões persistentes ou recorrentes de distúrbios do sono por conta, principalmente, das seguintes possibilidades: alterações do sistema de ritmo circadiano ou desalinhamento entre o ritmo circadiano endógeno; e fatores exógenos que afetam o tempo ou a duração de sono. Os distúrbios de sono relacionados ao ritmo circadiano levam à insônia, sonolência diurna excessiva, ou ambos.

A melatonina, comercializada como suplemento alimentar dietético nos Estados Unidos, está disponível em formulações e doses variadas. Porém, como suplemento dietético, não foi avaliado ou aprovado pela *Food and Drug Administration* (FDA) para prevenir ou tratar qualquer doença ou condição. Com base na revisão da literatura disponível, as diretrizes clínicas da *American Academy of Sleep Medicine* (AASM) determinaram uma série de recomendações para o uso de melatonina em distúrbios do sono (Auger et al., 2015). É importante levar em consideração a variabilidade substancial em potência, pureza, dissolução e segurança entre diferentes marcas de melatonina comercializadas. Já em estudos clínicos, geralmente, é utilizada uma composição de melatonina com declaração de pureza comprovada, concedida pelo fornecedor, o que se aplica ao presente estudo. O desenvolvimento farmacêutico dos agonistas dos receptores de melatonina produziu uma coleção de compostos que, em virtude do processo de aprovação, possuem o benefício de perfis farmacológicos bem caracterizados e com dados de segurança. Dois desses compostos, ramelteona e tasimelteona, são aprovados pela FDA e disponíveis comercialmente nos Estados Unidos, e dois compostos - melatonina de liberação prolongada e tasimelteona - são aprovados pela Agência Europeia de Medicamentos e estão comercialmente disponíveis na Europa. No Brasil, a ramelteona foi aprovada para a comercialização no Brasil em junho de 2017, pela ANVISA, e é indicada para o tratamento de insônia, com ação agonista de receptores MT1 e MT2.

O benefício da melatonina, para reduzir distúrbios do sono, foi demonstrado em uma meta-análise que investigou a latência, a qualidade geral e o tempo total de sono em pacientes com distúrbios do sono primário (Ferracioli-Oda; Qawasmi; Bloch; 2013). A análise incluiu 19 estudos aleatorizados controlados com placebo (n = 1683), no quais os pacientes receberam melatonina em doses que variaram de 0.1 mg - 5 mg e 0.05 mg/kg – 0.15 mg/kg. A melatonina reduziu a latência, melhorou a qualidade geral e aumentou o tempo de sono. Mais tarde, um estudo conduzido com pacientes com câncer de mama, submetidos à cirurgia oncológica, o tratamento com melatonina 6 mg aumentou a eficiência do sono e o tempo de total de sono (Hansen et al., 2014).

Dessa forma, destaca-se a importância de estudos que avaliem o potencial efeito da melatonina como sincronizadora do ritmo sono-vigília, na redução de distúrbios do sono em diferentes tipos de doença e em tratamentos que causam a disrupção circadiana com consequentes efeitos na qualidade do sono, como na quimioterapia para câncer de mama.

2.3.8 Efeitos da melatonina na função cognitiva

Além dos potenciais efeitos benéficos da melatonina nos distúrbios do sono, tem sido considerado que a melatonina pode ser benéfica para a cognição. Porém, não foi encontrado na literatura nenhum estudo que avaliou o tratamento com melatonina na cognição em pacientes submetidos à quimioterapia para câncer de mama. Apenas um estudo (Hansen et al., 2014) avaliou função cognitiva no tratamento com melatonina oral 6 mg, em pacientes com câncer de mama submetidos à cirurgia oncológica, porém, não foram encontrados resultados significativos.

O sono perturbado e a função cognitiva podem surgir em torno do tempo do diagnóstico, do tempo de cirurgia, no curso ou após a terapia adjuvante (Bower et al., 2008), tornando a etiologia difícil de estabelecer diante de tantos fatores que podem contribuir para o desenvolvimento. A disfunção cognitiva pós-operatória tipicamente apresenta prejuízo de memória ou concentração a curto prazo (Krenk; Rasmussen; Kehlet; 2010). É sabido que a fragmentação e a perda geral do sono podem ter consequências cognitivas negativas (Walker, 2008; Durmer; Dinges, 2005). Foi demonstrado que os distúrbios do sono podem surgir no pós-operatório, após a cirurgia de câncer de mama (Hansen et al., 2013) e podem persistir por meses ou até mesmo anos (Savard et al., 2001).

Alguns estudos mostraram que a melatonina pode influenciar a cognição positivamente em homens saudáveis expostos a um teste de estresse e em adultos com comprometimento cognitivo leve (Furio et al., 2007; Rimmele et al., 2009). Portanto, pode-se considerar que a melatonina teria um efeito benéfico sobre a função cognitiva em pacientes submetidos à quimioterapia para câncer de mama.

2.3.9 Efeitos da melatonina na modulação da dor

Evidências demonstram que a elevação endógena da melatonina melhora a tolerância à dor, e a variação diurna da tolerância à dor pode ser atribuída à variação de melatonina (Aviram et al., 2015). O BDNF é um importante mediador e um modulador central da dor (Pezet; McMahon, 2006; Ren; Dubner, 2007; Guo et al., 2006). Na dor neuropática, a liberação de BDNF foi observada na medula espinal e está relacionada à dor crônica (Ulmann et al., 2008). Outro estudo demonstrou que o BDNF contribuiu para a hiperpatia por meio da inibição pré-

sináptica GABAérgica (Chen et al., 2014). Em pacientes com síndrome do intestino irritável, o aumento da expressão de BDNF foi correlacionado com hiperalgesia visceral e aumento da dor abdominal (Yu et al., 2012). Os mecanismos para essas observações podem estar envolvidos na elevação da expressão de BDNF e do seu receptor TrkB (Yu et al., 2012). O BDNF é um importante regulador da dor, e os mecanismos antinociceptivos da melatonina podem ser atribuídos à diminuição dos níveis de BDNF (de Zanette et al., 2014).

Um estudo randomizado, duplo-cego, controlado com placebo, conduzido pelo grupo de pesquisa de Dor e Neuromodulação do HCPA, investigou o efeito analgésico da melatonina exógena em humanos (Stefani et al., 2013). O estudo incluiu 60 voluntários saudáveis e empregou testes sensoriais quantitativos (QST) para medir limiar/tolerância de dor à pressão e limiar/tolerância de dor ao calor. Melatonina sublingual (0,05 mg/kg; 0,15 mg/kg; 0,25 mg / kg) ou placebo foram administrados 30 min antes dos testes. As variáveis do QST foram significativamente aumentadas de acordo com a dose de melatonina exógena. Além disso, a melatonina e os níveis plasmáticos correlacionaram-se aos efeitos analgésicos. Da mesma forma, em outro estudo randomizado controlado com placebo, incluindo 33 pacientes, indicadas para histerectomia, foi administrado 5 mg de melatonina oral antes da cirurgia (Caumo et al., 2007). A melatonina reduziu o consumo de analgésicos pós-operatório e reduziu a intensidade da dor no pós-operatório em comparação com o placebo.

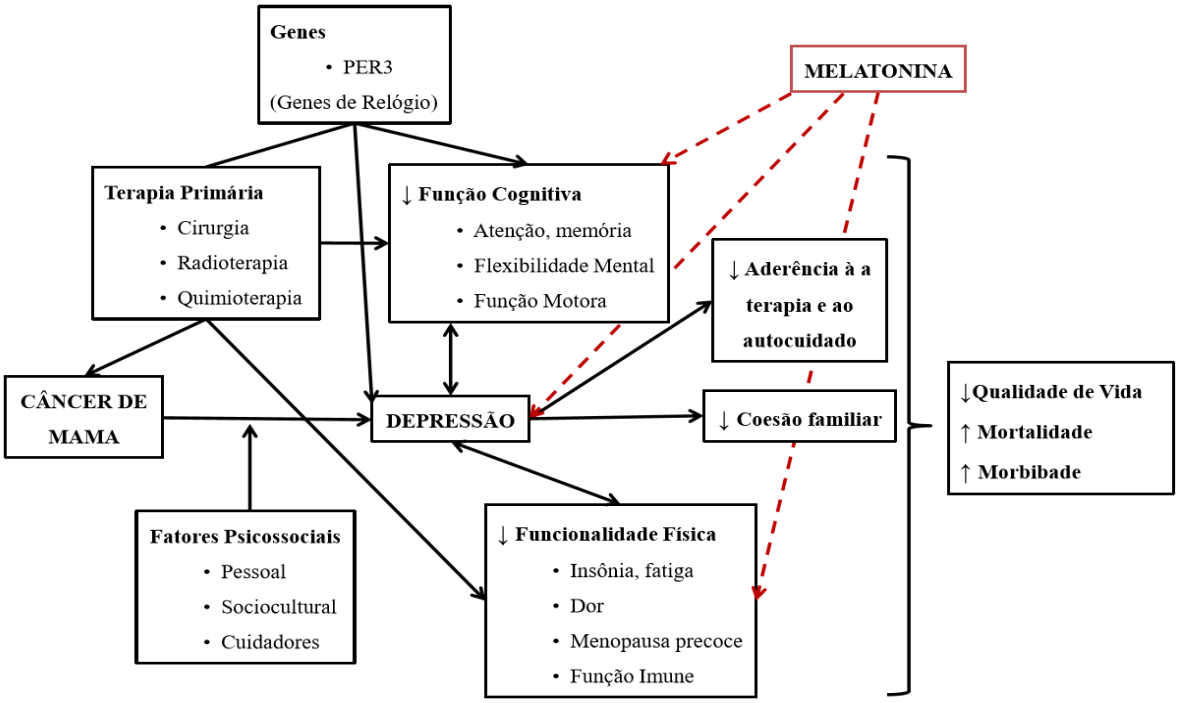
Estes achados sugerem que a melatonina produz efeitos analgésicos clinicamente relevantes, bem como destacam a importância de estudos que avaliem as propriedades analgésicas da melatonina em diferentes condições patológicas.

2.3.10 Efeitos da melatonina na depressão

A melatonina exógena demonstrou ações antidepressivas em modelos animais (Detanico et al., 2009). Em estudos clínicos, a melatonina diminuiu significativamente a incidência de sintomas depressivos em pacientes com câncer de mama submetidos à cirurgia oncológica (Hansen et al., 2014), provavelmente por seu efeito na regulação circadiana central (Srinivasan et al., 2009) e melhora da função cognitiva (Furio et al., 2007). Foi demonstrado que a depressão é um fator de risco para o não cumprimento do tratamento médico, as chances de que os pacientes deprimidos não cumprirão as recomendações de tratamento médico são três vezes maiores (DiMatteo; Lepper; Croghan; 2000). Especificamente em pacientes com câncer

de mama, demonstrou-se que a depressão representa um fator crucial para a aceitação de quimioterapia adjuvante (Colleoni et al., 2000). A **Figura 5** mostra a complexidade da relação entre o câncer de mama e a depressão, e os possíveis alvos da melatonina.

Figura 5 – Relação entre câncer de mama e depressão



Fonte: Adaptado de Hansen et al., 2014.

Após a cirurgia oncológica, a secreção de melatonina pode estar agudamente perturbada (Ram et al., 2005), com um atraso na secreção e amplitude reduzida (Kärkelä et al., 2002; Gögenur et al., 2007). Os pacientes deprimidos também podem apresentar distúrbios tanto na amplitude quanto na forma do ritmo de secreção de melatonina (Srinivasan et al., 2009). Além disso, observou-se que os níveis séricos de melatonina diminuem com a idade (Toffol et al., 2013) e estão significativamente diminuídos em pacientes com câncer de mama (Toffol et al., 2014). Considerando os baixos níveis de melatonina em pacientes com câncer de mama e que os efeitos antidepressivos da melatonina podem estar associados à baixa melatonina endógena, o uso adjuvante na quimioterapia para câncer de mama é promissor.

2.3.11 Possíveis efeitos adversos do tratamento com melatonina

Na revisão sistemática de Mills et al. (2005), observa-se que a melatonina tem sido amplamente avaliada em pacientes com câncer e não parece ter nenhum efeito adverso significativo em uma ampla gama de doses e períodos de uso. Um estudo mais recente, randomizado, duplo-cego, controlado com placebo, em pacientes com câncer de mama, submetidos à cirurgia oncológica, demonstrou que os efeitos secundários mais comuns, encontrados no grupo da melatonina, foram tonturas (14%), dor de cabeça (10%) e parestesia na região da boca, braços ou pernas (10%). No grupo placebo, foi relatado dor de cabeça (27%), dificuldade em adormecer (13%) e náuseas (13%). Dos pacientes do grupo da melatonina, 56% (15/27) experimentou pelo menos um efeito colateral, enquanto que isso se aplicou a 50% (12/24) no grupo placebo (P = 0,78) (Hansen et al., 2014). Assim, pode-se concluir que a melatonina não parece ter nenhum efeito adverso significativo.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Este estudo avaliou as respostas da melatonina como adjuvante ao primeiro ciclo de quimioterapia na função cognitiva e do sistema soma-sensorial, assim como seus efeitos nos marcadores de neuroplasticidade (BDNF, TrKB e proteina S100B) em mulheres submetidas ao primeiro ciclo de quimioterapia por câncer de mama.

3.2 OBJETIVOS ESPECÍFICOS

Este estudo avaliou o efeito de 20mg/dia de melatonina, por via oral, comparados ao placebo, ambos iniciados três dias prévios ao primeiro ciclo de quimioterapia, e contínuos por sete dias após, em pacientes com câncer de mama, para responder as seguintes questões, hipotetizando que fosse superior ao placebo, para melhorar os aspectos mensurados por meio dos seguintes desfechos:

(i) Função cognitiva relacionada à flexibilidade mental, memória de trabalho e atenção;

(ii) Relação da performance cognitiva e níveis séricos de BDNF e TrkB;

(iii) Limiar de dor e função do sistema modulador descendente da dor;

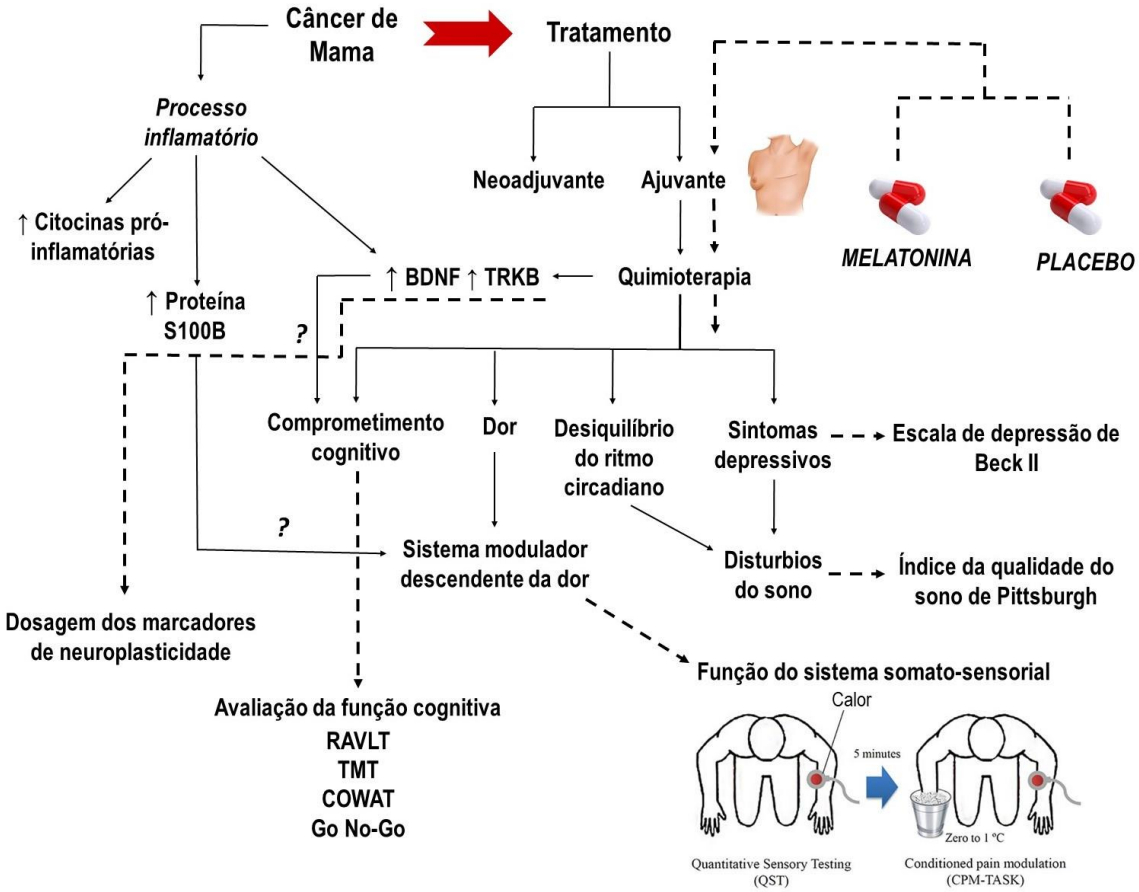
(iv) Efeito nos processos de plasticidade avaliados por meio dos níveis séricos do BDNF, TrkB e S100B;

(v) Sintomas depressivos;

(vi) Qualidade de sono.

4 MAPA CONCEITURAL

Figura 6 – Mapa conceitual



Em pontilhado as intervenções e as avaliações que foram utilizadas nesse estudo. Fonte: da autora, 2019.

5 ARTIGO 1 – ORIGINAL EM INGLÊS

ADJUVANT MELATONIN BLUNTS NEUROTOXIC EFFECTS OF CHEMOTHERAPY IN BREAST CANCER PATIENTS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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ADJUVANT MELATONIN BLUNTS NEUROTOXIC EFFECTS OF CHEMOTHERAPY IN BREAST CANCER PATIENTS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Abstract

This randomized, double-blinded, placebo-controlled trial tested the hypothesis that 20 mg of melatonin before and during the first cycle of adjuvant chemotherapy for breast cancer (ACBC) reduced the neurotoxicity associated with the chemotherapy. We evaluated the effects of melatonin on cognition, depressive symptoms and sleep quality, and if these effects were related to serum levels of Brain Derived Neurotrophic Factor (BDNF) and its receptor tropomyosin kinase B (TrkB). Thirty-six women were randomly assigned to receive melatonin or placebo for 10 days. To evaluate cognitive performance, we used the Trail-Making-Test A-B, Rey Auditory-Verbal Learning Test (RAVLT), Controlled Oral Word Association Test (COWAT) and an inhibitory task type Go / No-Go. Our results revealed that melatonin improved executive function on TMT-A-B, enhanced episodic memory (immediate and delayed) and recognition on RAVLT, and increased verbal fluency in the orthographic COWAT. TMT-A-B were negatively correlated with baseline levels of TrkB and BDNF, respectively. At the end of treatment, changes in TrkB and BDNF were inversely associated with depressive symptoms and sleep quality, but not with TMT-A-B. These results suggest a neuroprotective effect of melatonin to counteract the adverse effects of ACBC on cognitive function, sleep quantity and depressive symptoms.

Key words: breast cancer, chemotherapy, melatonin, cognition, Brain-Derived Neurotrophic Factor, Tropomyosin Receptor Kinase B, depression, sleep quality.

Introduction

Cognitive impairment in patients receiving chemotherapy for breast cancer can manifest with acute and/or delayed complications[1,2]. According to Jansen et al.[3], 23% of women with breast cancer had experienced cognitive impairment before chemotherapy. However, this increased to 52% during chemotherapy[3]. The most impacted domains in breast cancer patients are related to visual memory, visuospatial function and verbal learning, with a moderate to large size effect[4,5]. The neurotoxicity associated with chemotherapy for breast cancer is also substantiated by the persistent cognitive deficits related to volume reduction in the hippocampal gray matter one year after treatment completion[6]. Also, there is evidence that the reduction in hippocampal volume is associated with a decrease in cognitive function in patients with major depression[7]. The mechanisms underpinning these symptoms need to be further investigated.

Current evidence points to a central role of inflammatory cascades activated by cancer or chemotherapy on disruptive cognitive and behavioral changes[8]. According to pre-clinical studies, these effects involve an interplay between neuro-inflammation and neuroplasticity states, especially on neurogenesis processes mediated by Brain-Derived Neurotrophic Factor (BDNF)[9]. This neurotrophic factor is essential to long-term potentiation, learning and memory[10], also it serves as a critical regulator of synaptic plasticity, neuronal survival and neurogenesis[11]. In fact, BDNF expression in the brain activates many biological functions via cell surface tropomyosin receptor kinase B (TrkB). Also, the BDNF / TrkB signaling pathway may act as a regulator of carcinogenesis and metastasis[12] and its overexpression may predict a poor clinical outcome and a worse prognosis in patients with breast cancer[13].

Among multiple mechanisms involving neuroplasticity processes and neurotoxicity effects, salient candidates are the pro-inflammatory cytokines. They mediate neuro-inflammatory processes that disrupt the blood-brain barrier with consequent neuronal

dysfunction and activation of astrocyte activity, myotoxicity and eventual apoptosis[14]. Increased serum levels of IL-6 and TNF-alfa were found in breast cancer survivors treated with chemotherapy. This finding was correlated with persistent changes in hippocampal structural[15] and reduction in verbal memory processing during chemotherapy infusions[16]. A gap persists in understanding the mechanisms involved in cognitive dysfunction, depressive symptoms and poor sleep quality in breast cancer patients and during the adjuvant chemotherapy for breast cancer (ACBC). In the same way, there is limited evidence regarding neuroprotective treatments to counteract the neurotoxic effects on neuroplasticity processes involving cognitive and emotional dysfunctions.

According to several studies, exogenous melatonin has demonstrated a positive influence on depressive symptoms and sleep quality in breast cancer patients[17–19], however, its neuroprotective effect to contra-regulate the neurotoxicity induced by ACBC on cognitive function needs more exploration. All together, we hypothesize a central mechanism of ACBC involved in the dysfunction of neural plasticity and that melatonin has neuroprotective qualities. Thus, we evaluated the effect of melatonin prior to the first cycle and during ACBC on cognitive function related to mental flexibility, episodic memory (immediate and delayed), verbal fluency and inhibitory control, and if patient performance is related to baseline the neuroplasticity state assessed by BDNF and TrkB. Also, we evaluated the effect of melatonin on mental flexibility along with the changes on pre- and post-treatment assessed by the delta-value (Δ) of the Trail Making Test (Δ-TMT-A-B (primary outcome), Δ-sleep quality and Δ-depressive symptoms and its relationship with Δ-BDNF and Δ-TrkB.

Results

Socio-demographic and clinical characteristics, blinding and side effects

We selected one hundred and ten women for eligibility, and we excluded 74 of them because they did not meet the inclusion criteria. The sample comprised of 36 women scheduled for adjuvant chemotherapy. The characteristics of the participants is presented in **Table 1**. Randomization produced balance groups for most of the characteristics, except in years of school.

In the melatonin and placebo group, 13 (54.2%) vs. 11 (45.8%) assumed to have received melatonin, respectively. In the melatonin and placebo group, 4 (44.4%) vs. 5 (55.6%) assumed that they received placebo. Two in the melatonin and 1 in the placebo group assumed to not know their treatment (P=0.69). Regarding the side effects, the severity of the side effect scores was compared using the Wilcoxon- Mann-Whitney test. The median and interquartile (Q25-75) at baseline was 10 (Q_{25-75} = 2; 20) vs. 9 (Q_{25-75} = 0; 24), P=0.35, in the melatonin and placebo group, respectively. We observed that melatonin treatment reduced the severity of side effects and the median and interquartile (Q25-75) was 7 (Q_{25-75}= 2; 19) vs. 12.5 (Q_{25-75} = 3; 25), *P*=0.01, in the melatonin and placebo group, respectively.

----------Insert Table I here----------

Univariate analysis of the according to treatment group and their correlations

Mean and standard deviation at baseline and end of treatment of the cognitive tests according to the treatment group as well as their Δ-value are presented in **Table 2**. The t-test for independent samples was used to compare the Δ-value of cognitive measures.

----------Insert table 2----------

Primary and secondary outcomes: Multivariate analysis to compare the treatment group effect on the measurement of cognition according to the neuroplasticity state at baseline assessed by serum TrkB and BDNF

MANCOVA analyses with the Δ-value of cognitive measurements as dependent variables, the factor was the treatment group and the baseline serum level of BDNF and TrkB as covariates are presented in **Table 3**. This analysis revealed significant effects of treatment, Pillai's Trace's F (6, 23) =7.98; p<0.001; η^2partial=0.68. Linear regression analysis demonstrated that the TrkB level at baseline was negatively correlated with the Δ-TMT-B-time (Standardized Beta=-0.19; t=-.68, P=0.001, η^2partial=0.32). The serum BDNF level at baseline was negatively correlated with the Δ-TMT-A-time (Standardized Beta=-0.005, t=-2.92 P=0.007, η^2partial=0.24).

----------Insert Table 3 here----------

Exploratory analysis: Effects of treatment on the neuroplasticity state assessed by Δ-BDNF, Δ-TrkB and measures of cognitive flexibility (TMT-A-B), depressive symptoms (BDI II) and sleep quality (PSQI)

Univariate analysis to compare between treatment groups the Δ-value of depressive symptoms, sleep quality and serum levels of BDNF and TrkB

The score on BDI-II at baseline and end of treatment and respective Δ-value presented as mean and SD was [11.41(7.73) vs. 6.71(4.57), Δ-value=-4.70 (5.83)] and [10.83 (5.11) vs. 14.56 (7.76), Δ-value=3.72 (5.21)] (t=-3.62, P<0.001), in the melatonin and placebo group, respectively. The score on the PQSI at baseline and end of treatment and respective Δ-value presented as mean and SD was [8.24 (3.98) vs. 5.06 (3.34), Δ-value=-3.18 (2.00)] and [8.44 (2.83) vs. 11.06 (3.35) (Δ-value=2.61 (2.06)] (t=-8.40, P<0.001), in melatonin and placebo group, respectively. Serum levels of BDNF at baseline and end of treatment and respective Δ-value presented as mean and SD was [41.65 (17.72) vs. 21.32 (7.190), Δ-value= -0.43 (0.22)]

and [40.88 (23.78) vs. 43.76 (17.74), Δ-value=0.12 (0.20)] (t=-.76, P<0.001), in melatonin and placebo group, respectively. Serum levels of TrkB at baseline and end of treatment and respective Δ-value presented as mean and SD was 0.56 (0.40) vs. 0.41(0.37), Δ-value= -0.19 (0.33) and 0.47 (0.50) vs. 0.52 (0.46), Δ-value= 0.42 (0.65) (t=-.3.42, P=0.002), in melatonin and placebo group, respectively.

Multivariate analysis to compare Δ-value of cognitive flexibility (TMT-A-B), depressive symptoms and sleep quality considering the effect of treatment on the neuroplasticity biomarkers

The MANCOVA model was used to compare the effect of treatment between groups and its relationship with the neuroplasticity state assessed by Δ-values of BDNF and TrkB. Dependent variables were Δ-value (pre- to post-treatment) on the cognitive flexibility (TMT-A-B), depressive symptoms (BDI II), and the sleep quality (PSQI). The factor was the treatment group, and covariates were Δ-BDNF and Δ-TrkB. To assess if the effect of treatment in the outcomes (TMT-A-B, BDI II and PSQI) was associated with changes in the neuroplasticity state (Δ-BDNF, Δ-TrkB), we analyzed the interaction between groups and Δ-values of the biomarkers of neuroplasticity state. These results are presented in **Table 4**. This analysis revealed significant effects of treatment, Pillai's Trace's F (4, 26)=12.67; p<0.001; η^2partial=0.66.

The beta coefficients of MANCOVA analyses with Δ-TrkB as covariate demonstrated that the interaction between the Δ-TrkB and the treatment group was related to higher changes in Δ-TrkB compared to placebo, and the Δ-TrkB was correlated with a higher reduction in depressive symptoms (Standardized Beta=-9.31; t=-2.13, P=0.04, confidence interval (CI 95%= -18.26 to -0.37, η^2partial=0.14). In the same way, the interaction between the Δ-TrkB and the treatment group was related with higher changes in Δ-TrkB compared to placebo and was also

correlated with a higher reduction in the sleep index quality scores (Standardized Beta=-4.50; t=-2.98, P=0.006, confidence interval (CI 95%= -7.59 to -1.42, η^2partial=0.24). However, neither the Δ-BDNF nor its interaction with the treatment group was associated with the effect of treatment on depressive symptoms, sleep and cognitive flexibility assessed by the TMT-A-B.

----------Table 4----------

Scatter plots of the raw Δ-changes on Δ-BDI-II and Δ-PSQI with Δ-TrkB according to placebo and melatonin group is presented in Figure 3 (A, B, C, D). The Δ-Changes on Δ-TrkB in the melatonin showed a statically significant negative Pearson correlation (r) with Δ-BDI-II and Δ-PSQI. However, in the placebo group we not observed correlation of Δ-TrkB neither PSQI nor with BDI-II.

----------Figure 3----------

Discussion

These findings confirm the benefits of melatonin use compared to placebo prior to ACBC in reducing performance time on the TMT-A-B, increasing the score in immediate and delayed recall, and improving recognition in the RAVLT and increasing words recited during the orthographic COWAT. TMT-B and TMT-A were negatively correlated with baseline levels of TrkB and BDNF, respectively. At the end of treatment, TrkB changes were inversely associated with depressive symptoms and sleep quality, but not with TMT-A-B. However, melatonin did not change the capacity for sustained attention and control of responses assessed by the inhibitory control (a Go/No-Go task).

This study extended data that 20mg of melatonin use prior to the first cycle of ACBC has a neuroprotective effect on cognitive functions evaluated by a set of tests that measures several dimensions of cognitive flexibility allied to attention. However, a study that used 6mg

of melatonin prior and post ACBC did not find a similar benefit in cognitive function[17]. In fact, our results are in the sense of most clinical studies that demonstrated a benefit of 20mg of melatonin use in cancer patients on clinical outcomes (i.e. mortality, tumor remission, etc.)[20–22]. Accordingly, two meta-analyses demonstrated benefits of melatonin as an adjuvant to ameliorating radio chemotherapy-related side effects (i.e., asthenia, nausea and vomiting, hypotension, and thrombocytopenia), and improve tumor remission and survival[23,24]. Although some of these previous studies reported neuroprotective effects of melatonin on neurotoxicity, all studies that used melatonin in a dosage of 20mg or higher did not design to evaluate performance on cognition, depressive symptoms, and sleep quality. In this way, the novelty of this study reveals the benefits of melatonin prior to ACBC on different dimensions of cognition (cognitive flexibility, attention, immediate and delayed episodic memory, executive control and verbal fluency), depressive symptoms and sleep quality allied to the baseline neuroplasticity state and changes in serum BDNF and TrkB induced by melatonin. Indeed, these results showed a statistical difference in these outcome measures, and they have potential clinical relevance to highlight evidence of the neuroprotective effect of melatonin. Additional reasons for the importance of these findings is the scarcity of treatments available to contra-regulate neurotoxicity induced by chemotherapy, as well as the lack of proof of complementary therapies with potential neuroprotective benefit. Thus, melatonin`s properties present an attractive option, since it can blunt the most prevalent complaints related to breast cancer chemotherapy, which are comprised of memory deficits, depressive symptoms, and sleep disorders.

Although our results revealed the benefits of melatonin as neuroprotective during ACBC, the underlying mechanism of this is unknown. In fact, this is the first study that investigates the interplay between the neuroplasticity state, neuroprotective effect of melatonin and performance in cognitive flexibility and attention (i.e., TMT-A-B). The trail making test

evaluates mainly personal differences in speed and fluid cognitive abilities and both abilities vary according to a particular context[25]. Indeed, in the present study this test was chosen to assert a specific paradigm of executive functioning according to a specific contextual analysis, in this case, the chemotherapy for breast cancer[25]. Although studies generally indicate that large-scale brain networks including prefrontal and parietal structures mediate the trail making test performance, according to Cole and colleagues[26], higher scores on measures of cognitive control (i.e. standard fluid intelligence tests) may be related to a higher degree of global functional connectivity in the lateral prefrontal cortex. Importantly, fluid intelligence tests and the TMT are all based on visual information, for which patients receiving ACBC are found to have an impaired processing[4]. Over this set of the findings related to chemotherapy neurotoxicity, preclinical studies have elicited that melatonin effects can modulate the neuroplastic processes implied in cognitive impairment[27].

Overall, this set of evidence supports our finding that the neuroplasticity state may be considered as a marker to explain the substantial differences between cognitive capacity and impairment in breast cancer patients. Also, it can help to comprehend the susceptibility to neurotoxicity attributed to additive or synergistic mechanisms of anti-cancer drugs. In fact, these results open an avenue to embrace and to investigate variations of cognitive tests in breast cancer patients on chemotherapy, as well, they emphasize the importance to account for the baseline neuroplasticity state as a measure to explore the impact of future melatonin neuroprotective treatment effects. In this way, the relationship between the baseline BDNF and TrkB with cognitive flexibility may be explained by the influence of pro-inflammatory cytokines on BDNF secretion. According to Dietrich et al.[28], the inflammatory cascade drives many processes of neuroinflammation, such as oxidative stress, direct cellular toxicity, and inflammation contributing to altered cellular kinetics in the hippocampus as well as

neurovascular blood barrier brain disruption. Even though melatonin´s action is not fully elucidated, previous studies have reported that melatonin increased activity of natural killer cells (T and B) and cytokine production[29,30]. Also, it demonstrated antiestrogenic effects through the termed MT1 high-affinity G protein-coupled receptors[30–32]. Thus, melatonin´s effect on neuroplasticity processes may be explained partially by its multifaceted anti-inflammatory properties. Besides, it could modulate astrocyte reactivity or death through upregulation of astrocytic anti-oxidative defenses[33]. Overall, we see consistency in existing literature regarding the anti-inflammatory effects of melatonin.

Given our findings, the effects of melatonin on cognitive flexibility are not concurrent with changes in the biomarkers of the neuroplasticity state (**Table 5**). However, the mechanisms involved in the neuroprotective effect are complex and need to be further elucidated. In the same way, the current results need to be interpreted with parsimony due to the fact that BDNF represents a bridge between inflammation and neuroplasticity. This does not permit us to consider that the effect of melatonin on cognition could be a consequence of its anti-inflammatory properties as there was a previous pre-clinical study in rodents which demonstrated that melatonin elicits all stages of neuroplasticity (i.e., neurogenesis, axogenesis, dendritogenesis, and synaptogenesis)[27]. Indeed, in the present study, the neuroprotective effect of melatonin on neuroplasticity processes is confirmed by a reduction in serum levels of BDNF and TrkB. Also, the relationship between BDNF secretion and the effect of melatonin is further validated in earlier studies of conditions that concur with excessive activation of the stress system associated with inflammation[34] such as in fibromyalgia[35], endometriosis[36].

Also, this study produces evidence that the effect of melatonin on depressive symptoms and sleep quality involves interplay of neuroplasticity processes as demonstrated by changes in Δ-values of BDNF and TrkB. The leading role of melatonin is to regulate sleep and circadian

rhythm, and this counteracting circadian misalignment has been proved to be beneficial for the clinical management of mood disorders37. It is important to emphasize that in this study, the effects were observed in a sample of women living under intense stressful conditions (i.e. undergoing their first cycle of ACBC), hence, the improvement in these symptoms does not allow definite conclusions regarding melatonin effects as an antidepressant in humans. However, this beneficial effect of melatonin on depressive symptoms and sleep quality are supported by its anti-stress and antidepressant actions. The effects of melatonin involve an interaction with several neurotransmitter systems, including the GABAergic, serotoninergic38, glutamatergic and nitrergic39, as well as the modulation of the hypothalamic-pituitary-adrenal axis40. As aforementioned, its effect also involves the neuroplasticity mechanism, which can explain the concurrent changes in neuroplasticity markers and the improvement of depressive symptoms and sleep quality in the current study. This hypothesis is supported by pre-clinical evidence demonstrating that the antidepressant effects of melatonin stimulate dendritogenesis and synaptogenesis in the hippocampus, which is disrupted by chronic psychosocial stress as seen in depression41. In the same way, the effects of melatonin on neuroplasticity in the hippocampus attenuated cognitive and memory deficiencies caused by sleep deprivation42.

We did not observe a significant difference between groups in accuracy or response time on the Go/No-Go task. Several details should be considered in the interpretation of these findings such as the differences observed between groups in regards to task performance and neuropsychological testing, as this does not necessarily suggest that the underlying neurocircuitry is normal. In the same way, these findings could reflect factors related to the nature and difficulty level of the task as well as specific sample characteristics. Consistent with this idea, a review of functional magnetic resonance imaging (fMRI) studies in breast cancer demonstrated a pattern of hyperactivation or recruitment of additional neural resources at low

difficulty tasks[43]. However, other studies have shown that when the task difficulty increases, breast cancer patients may be unable to maintain this compensatory response, resulting in decreased neural activation or connectivity[44]. As far as we can discern, the neurobiological process as well as the function of neural networks underpinning the results of these psychometric tests are complex. However, one should realize that the aim of this study was to assess the effects of melatonin on neural networks involved in the inhibitory control mechanism utilizing the psychometric Go/No-Go test. Hence, we cannot associate its pharmacological effects as a response related to a specific biological system, although pre-clinical studies showed that melatonin's effect involve candidates of the neurobiological systems leading to the response on the Go/No-Go task[45] (i.e., dopaminergic, serotonin, and noradrenaline systems). Overall, our results suggest that the effects of melatonin, according to the protocol of this study, was not sufficient to influence a typical Go/No-Go decision task of sustained attention linked to inhibitory control.

We addressed several limitations concerning our study: First, we agree that our research has an exploratory nature and the results of secondary outcomes should be interpreted as explanatory by increased type I and type II error. Second, although our sample is homogeneous and this is methodologically advantageous, the issue of external validity arises. Third, we assessed serum levels of BDNF and TrKB as measures of the neuroplasticity state, which are less prone to suffer some influences of evaluators. Fourth, a potential limitation is the short-term treatment with melatonin. It would have been difficult to justify a prolonged treatment period in patients undergoing ACBC if they had a high incidence of severe side effects. However, our results are in line with previous studies revealing the benefits of melatonin at improving side effects induced by chemotherapy[30]. Therefore, future studies are required with more substantial number of patients before defining conclusions regarding melatonin`s impact

on the neurotoxicity due to chemotherapy. Finally, all analyses were conducted comparing the Δ-values to adjust for changes within each individual subject, and to promote a better control for personality-related variability in self-reported measures[46].

In conclusion, these findings show the benefit of melatonin use prior and concurrent with the first cycle of ACBC when compared to placebo in improving cognitive flexibility and attention. These cognitive benefits are modulated by the baseline neuroplasticity state as well its neuroprotective effect on depressive symptoms and sleep quality and are allied to changes in neuroplasticity biomarkers assessed by serum BDNF and TrkB.

Materials and Methods

This trial was structured according to the Consolidated Standards of Reporting Trials (CONSORT) 2010 guideline and was carried out in accordance with the Declaration of Helsinki[47]. The flow of the study is presented in **Figure 1**.

----------Insert Figure 1----------

Study Design and Eligibility

This randomized, double-blinded, placebo-controlled trial was approved by the Institutional Review Board of Hospital de Clínicas of Porto Alegre (IRB HCPA/Approval number: 14-0701). The study was registered on http://www.clinicaltrials.gov/ (No NCT03205033 Study start: January 1, 2016 End date: January 1, 2017). We obtained oral and written informed consent from all patients before participating in this study.

Inclusion and Exclusion Criteria

The patients were selected from the Mastology and Oncology Service at HCPA, which is a public tertiary teaching Medical School located in the South of Brazil. Initially, they were

invited to answer a questionnaire to check inclusion and exclusion criteria. All evaluations were performed at the HCPA Clinical Research Center.

We included 36 females, age range 18 to 75 years. *Inclusion criteria:* Patients scheduled for the first cycle of ACBC one month after lumpectomy or mastectomy and with a read and write capacity *Exclusion criteria:* We excluded patients with previous chemotherapy treatment, those who planned for neoadjuvant chemotherapy, and those with another current or prior cancer. We also excluded patients with a history of allergic reaction to melatonin, sleep apnea, diabetes, autoimmune disease (i.e. systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, etc.), decompensated liver cirrhosis, severe kidney disease, rotor or Dubin–Johnson syndrome, epilepsy, multiple sclerosis, cerebrovascular stroke, Body Mass Index (BMI) above 35 kg / m^2, pregnant and breastfeeding.

Sample Size

We estimated the sample size based on previous studies that used the TMT-A-B to assess cognitive flexibility in breast cancer patients[48]. Accordingly, for two dependent variables (TMT-A with a standard deviation equal to 9.64) and (TMT-B with a standard deviation equal to 11.85) with a moderate effect size (f^2=0.3) to compare melatonin and placebo by MACONVA, with two predictors in a 1:1 ratio, the estimate indicated a sample size of 32 for a power of 90% and an α of 0.01. Considering possible dropouts, we increased the sample by 12.5% so the final sample size comprised of 36 patients (18 per group). The G-Power 3.0.10 software was used to estimate sample size.

Randomization and Masking

Randomization was generated using a computer software. To solve the problem of some

researchers or evaluators predicting what the next patients will be assigned for treatment, we used a randomly different block size of 8 and 6. Thirty-six women were allocated to receive melatonin or placebo, an allocation of 1:1. Before the recruitment phase, envelopes containing the protocol materials were prepared by two investigators who made the randomization and they were not involved in the patient's assessments. Each envelope was sealed and numbered sequentially and included an allocated treatment. The envelope was opened following the sequence of numbers registered in the envelope after the participant consented to participate in the trial. During the entire protocol timeline, the participants, health care providers, research staff, and investigators assessing the outcomes were all blinded to allocation and sequence by receiving numbered sealed envelopes. Further, to assess whether blinding was adequate, at the end of treatment we asked patients to guess the treatment received, with three answer options: melatonin, placebo or unknown.

Interventions

Patients were randomly assigned to receive melatonin or placebo for ten days, beginning three days prior to the first session of adjuvant chemotherapy. The intervention group received 20 mg of oral melatonin daily approximately 1 hour before bedtime. The placebo group received placebo capsules within the same time. Melatonin capsules were produced using crystalline melatonin with a certificate of purity (M-5250, Sigma Chemical, Saint Louis, MO, USA) from a compounding pharmacy. Placebo capsules contained only cellulose, an indigestible fiber. The tablets and packages were physically identical. The pharmacy packed the melatonin or placebo sequentially numbered in sealed containers. We employed the following strategies to measure adherence to medication use: i) At the end of treatment a researcher counted the number of tablets consumed during the study period. ii) The patients

were asked to record a diary entry if they failed to use the medication. iii) Eligible patients were strongly encouraged to remain on the drug throughout the ten days of treatment, and they visited the clinical center at the end of treatment. Regardless of the patients' decision to continue or discontinue melatonin after randomization, the patients continued to be assessed during the study period.

Assessments and Instruments

The baseline evaluations were performed up to 4 days prior to the first cycle of chemotherapy. Melatonin was initiated three days prior to first cycle of adjuvant chemotherapy and continued during and seven days following chemotherapy. Day eight following chemotherapy, the subjects returned for the final evaluation. All assessments were conducted by two independently trained researchers to apply memory and psychological tests. They were monitored by a supervisor psychologist from the Pain and Neuromodulation Research Group at HCPA. The timeline of assessments is presented in **Figure 2**.

----------Insert figure 2----------

Outcomes

The primary outcome was the total time to accomplish the Trail Making Test parts A and B (TMT-A-B). Secondary outcomes were the scores from the Rey Auditory-Verbal Learning Test (RAVLT), Controlled Oral Word Association Test (COWAT) and an inhibitory task type Go / No-Go. Additionally, other secondary outcomes were evaluation of depressive symptoms and sleep quality. All tests used to measure cognitive function were selected according to recommendations described by Wefel et al.[49] to harmonize studies in patients with

cancer.

Primary Outcomes Assessment

Trail Making Test (TMT A-B)

The trail-making test is a brief two-part test that evaluates processing speed, divided attention, and cognitive flexibility[50]. The test consists of two parts (A and B). Each part has 25 points on a sheet of paper, which participants connect with a pencil. Part A contains only sequential numbers 1 to 25. Part B consists of numbers and letters alternately mixed: 1 to A, A to 2, 2 to B, and so on. The test results were analyzed as total time to accomplish each part, as well as the proportion and individual differences. Scoring is based on time required to complete the task and number of errors. It has been hypothesized to reflect a wide variety of cognitive processes including attention, visual search and scanning, sequencing and shifting, psychomotor speed, abstraction, flexibility, ability to execute and modify a plan of action, and ability to maintain two trains of thought simultaneously[51].

Secondary outcomes assessment

Cognitive Tests

Rey Auditory-Verbal Learning Test (RAVLT)

This measures episodic memory, verbal learning, susceptibility to interference (proactive and retroactive), information retention after a certain period of time following performance of other activities, and memory recognition[52]. It is a rapid and direct test, and its use has been widely recognized in the neuropsychological literature. In this study, the adapted test by Malloy-Diniz et al.[53] for the Brazilian population was utilized. In the RAVLT, a list of 15 nouns (list A) is read aloud five consecutive times. Each trial is followed by a spontaneous

recovery test. Following the fifth attempt, a list of interferences, which also includes 15 nouns (list B), is read to the patient, followed by recovery (attempt B1). After trial B1, the investigator requested that the patient recall the words from list A without reading it again (attempt A6). To evaluate the learning curve of words during attempts A1 to A5, the learning rate during the attempts is used and are incorporated into the following formula: sum total of A1 to A5. After an interval of 20 to 30 minutes, the patient has to remember the words from list A (tentative A7), without the list being read again. Following the A7 trial, the patient underwent a memory recognition test comprised by reading a list with 15 words from list A, 15 words from list B, and 20 words of distraction (similar to words in list A and B in phonological or semantic terms). With each word read aloud, the patient was asked to indicate whether she belonged to list A or not.

Controlled Oral Word Association Test (COWAT)

This test assess lexical knowledge, lexical retrieval ability and executive control abilities[54] and involves word fluency organized into two categories: orthographic and semantic. In orthographic fluency, patients were asked to name as many words as possible, beginning with a certain letter, that is, F, A, and S. Sixty seconds were given for each letter. Patients could not use proper names or words with different tense or suffixes, since the root word was given. In semantic fluency, the patients had to name as many animals as possible in sixty seconds[55].

Go / No-Go Task

It is a simple and sensitive test of frontal lobe dysfunction, developed to evaluate response inhibition (language and motor function) in a computerized evaluation format. The frequency of Go stimuli relative to No-go is 80%, which maintains a bias and tendency to respond at each trial. The Go / No-Go test was used to measure the capacity for sustained

attention and control of responses[56]. On the center of the computer screen were shown a fixation cross (1000 ms) followed by a go letter (e.g., “A”, “G”, “T”, etc.) or a no-go letter (e.g. “H”) for 500 ms. Subjects were instructed to press the “space” key as fast as possible for the go letters and do not press any key for no-go letters (“H”, “X” and “K”). Total task time was 17 minutes. Test instructions were translated to Brazilian Portuguese, but task stimuli and procedures were according to the English version.

Clinical measurements: depressive symptoms and sleep quality

Beck Depression Inventory (BDI-II)

Is a questionnaire composed of 21 multiple-choice questions with four options each (0 - 3). The total BDI score ranges from 0 - 63, with a higher score indicating a higher degree of depressive symptoms [57].

Pittsburgh Sleep Quality Index (PSQI)

PSQI is a self-reporting questionnaire that comprises 19-items to assess quality of sleep and identifies sleep disorders. Results are reported with a score ranging from 0 to 21 and is composed of seven domains: (1) subjective quality of sleep; (2) sleep latency, (3) sleep duration; (4) usual sleep efficiency; (5) sleep disorders; (6) use of sleeping pills; and (7) daytime dysfunction. Each item has a response scale ranging from 0 to 3, and lower scores indicate better sleep quality. A total score of 5 or more on the PSQI suggests high sensitivity and specificity of sleep deficiency (Cole et al., 2006). For cancer patients, a cut-off score of 8 was recommended (Carpenter et al., 1998), and a cutoff score of 10 needed to diagnose clinical insomnia[58]. The PQSI reliability and internal validity have been tested in cancer patients[57,59].

Biomarkers of neuroplasticity state measured by BDNF and TrkB

Serum levels of BDNF and TrkB

Blood samples were collected at the HCPA Clinical Research Center. The samples were centrifuged in plastic tubes for 10 minutes at 4,500 rpm, 4°C and stored at the Unit of Molecular and Protein Analyzes in the HCPA in a −80°C freezer for further BDNF and TrkB assays. Serum-mediator concentrations were determined using BDNF (Chemicon CYT306, lower detection limit 7.8 pg/mL; EMD Millipore, Billerica, MA, USA) and TrkB (MYBI – MBS9346917, lower detection limit 0.25 ng/ml; MyBiosource, San Diego, CA, USA) enzyme-linked immunosorbent-assay kits, according to the manufacturer's instructions. These serum markers were measured at baseline and after treatment with melatonin.

Other Instruments and Assessments

We used demographic questionnaires to collect data such as age, weight, height, years of study, medications, use of cigarettes, alcohol and other drugs. Also, medical comorbidities were assessed using a standardized questionnaire. To determine side effects related to chemotherapy we applied questionnaires from the European Organization for Cancer Research and Treatment validated for the Brazilian population (EORTC QLQ-C30 and QLQ-BR 23) before and after treatment[60]. The symptoms analyzed in this study composed of dry mouth, sick feeling, hot flushes, headaches, weakness, lack of appetite, nausea, vomit, constipation, diarrhea, tiredness, difficulty concentrating, worriedness, irritability and memory difficulty. The score for each question varies from absent, mild, moderate and severe.

Statistics analysis

Descriptive analysis were performed using mean, standard deviation and frequency.

Inferential tests for demographic and clinical measures, as well as for cognitive outcomes, were based on independent sample t-Tests for continuous variables and the Mann-Whitney non-parametric test was used. To control for core cognitive trait of the individual and some imbalance between groups at baseline differences, we assessed change in cognitive, depression, sleep quality scores and BDNF and TrkB levels based on the mean differences [deltas (Δ-value), mean at treatment end *minus* mean prior treatment]. To analyze the treatment effect on all primary and secondary outcomes, we conducted multivariate analyses of covariance (MANCOVA). The MANCOVA model was used to examine the influence of BDNF and TrkB levels as modulators of the treatment's effectiveness in the Δ-value of the cognitive measurements. The dependent variables were the Δ-value of cognitive tests; the treatment group was the factor, and BDNF and TrkB were covariates. Linear regression analyses to examine the relationship between cognitive flexibility and BDNF and TrkB biomarkers were run when appropriate. A MANCOVA model was also used to examine if the treatment effect on the cognitive flexibility scores, depressive symptoms, and sleep quality was mediated by its effect on neuroplasticity state. The dependent variables of the MANCOVA model were the Δ-Trail Making-Test (TMT-A-B), Δ-BDI-II and Δ-PSQI; the factor was the treatment group, and Δ-BDNF and Δ-TrkB were covariates (see **Table 4**). Bonferroni's Multiple Comparison adjusted all analyses. We considered all of the randomized patients as part of the analysis using the intention-to-treat (ITT) method, with the worst-case observation carried forward in the respective treatment group (melatonin or placebo). For all analyses, we considered a Type I two-sided error (bicaudal) $\alpha < 0.05$. For statistical analyses, the IBM SPSS Statistics for Windows Version 20.0 was used (IBM Corp., Armonk, NY, U).

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Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial and non-financial relationships that could be construed as a potential competing interest.

Author Contributions Statement

A.C.S.P. and **M.Z.** planned the assessments, carried out the cognitive tests and data collection, **A.C.S.P.;** **M.Z.** and **W.C.** designed and implemented the study. **M.Z.** and **W.C.** conducted all statistical analyses and interpretation of the results. **M.Z.** monitored the cognitive tests. **A.S.** contributed to randomization. **A.C.S.P.** and **A.S.** did the sample preparation and biochemical analysis. **A.C.S.P.; M.Z.; V.S.** and **W.C.** interpreted the results and contributed to the writing of the manuscript. **J.V.B**. helped assessing patients from the oncology and mastology service. **I.L.S.T.** and **F.F.** provided critical feedback and helped shape the research and the manuscript. **W.C.** conceived of the presented idea, was involved in planning and supervising the study.

REFERENCES

1. Selamat, M. H., Loh, S. Y., Mackenzie, L. & Vardy, J. Chemobrain experienced by breast cancer survivors: A meta-ethnography study investigating research and care implications. *PLoS One* **9,** (2014).

2. Schagen, S. B. & Wefel, J. S. Chemotherapy-related changes in cognitive functioning. *Eur. J. Cancer, Suppl.* **11,** 225–232 (2013).

3. Jansen, Lina; Michael, Hoffmeister; Jenny Chang-Claude, Moritz Koch, Hermann Brenner, V. A. Age-Specific Administration of Chemotherapy and Long-Term Quality of Life in Stage II and III Colorectal Cancer Patients: A Population-Based Prospective Cohort. *Oncol.* 384–387 (2011). doi:10.1634/theoncologist.12-4-418

4. Castellon, S. A. *et al.* Neurocognitive performance in breast cancer survivors exposed to adjuvant chemotherapy and tamoxifen. *J. Clin. Exp. Neuropsychol.* **26,** 955–969 (2004).

5. Jim, H. S. L. *et al.* Meta-analysis of cognitive functioning in breast cancer survivors previously treated with standard-dose chemotherapy. *J. Clin. Oncol.* **30,** 3578–3587 (2012).

6. Wardill, H. R. *et al.* Cytokine-mediated blood brain barrier disruption as a conduit for cancer/chemotherapy-associated neurotoxicity and cognitive dysfunction. *Int. J. Cancer* **139,** 2635–2645 (2016).

7. Drevets, W. C., Price, J. L. & Furey, M. L. Brain structural and functional abnormalities in mood disorders: Implications for neurocircuitry models of depression. *Brain Struct. Funct.* **213,** 93–118 (2008).

8. Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W. & Kelley, K. W. From inflammation to sickness and depression: When the immune system subjugates the brain. *Nat. Rev. Neurosci.* **9,** 46–56 (2008).

9. Calabrese, F. *et al.* Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity. *Front. Cell. Neurosci.* **8,** 1–7 (2014).

10. Zhang, J.-C., Yao, W. & Hashimoto, K. Brain-derived neurotrophic factor (BDNF) - TrkB signaling in inflammation-related depression and potential therapeutic targets. *Curr. Neuropharmacol.* 721–731 (2016). doi:10.2174/1570159X14666160119094

11. Nijs, J. *et al.* Brain-derived neurotrophic factor as a driving force behind neuroplasticity in neuropathic and central sensitization pain: a new therapeutic target? *Expert Opin. Ther. Targets* **19,** 565–576 (2015).

12. Lawn, S. *et al.* Neurotrophin Signaling via TrkB and TrkC Receptors Promotes the Growth of Brain Tumor-initiating Cells. *J. Biol. Chem.* **290,** 3814–3824 (2015).

13. Tajbakhsh, A. *et al. Therapeutic Potentials of BDNF/TrkB in Breast Cancer; Current Status and Perspectives*. *Journal of Cellular Biochemistry* **118,** (2017).

14. Cheung, Y. T. *et al.* Association of pro-inflammatory cytokines and chemotherapy-

associated cognitive impairment in breast cancer patients: A multi-centered, prospective, cohort study. *Ann. Oncol. Adv.* (2015).

15. Bower, J. E. *et al.* Inflammatory responses to psychological stress in fatigued breast cancer survivors: Relationship to glucocorticoids. *Brain. Behav. Immun.* **21,** 251–258 (2007).

16. Collado-Hidalgo, A., Bower, J. E., Ganz, P. A., Cole, S. W. & Irwin, M. R. Inflammatory biomarkers for persistent fatigue in breast cancer survivors. *Clin. Cancer Res.* **12,** 2759–2766 (2006).

17. Hansen, M. V *et al.* Effect of Melatonin on Cognitive Function and Sleep in relation to Breast Cancer Surgery: A Randomized, Double-Blind, Placebo-Controlled Trial. *Int J Breast Cancer* **2014:41653,** (2014).

18. Chen, W. Y. *et al.* A randomized, placebo-controlled trial of melatonin on breast cancer survivors: Impact on sleep, mood, and hot flashes. *Breast Cancer Res. Treat.* **145,** 381–388 (2014).

19. Innominato, P. F. *et al.* The effect of melatonin on sleep and quality of life in patients with advanced breast cancer. *Support. Care Cancer* **24,** 1097–1105 (2016).

20. Lissoni, P. Biochemotherapy with standard chemotherapies plus the pineal hormone melatonin in the treatment of advanced solid neoplasms. *Pathol. Biol.* **55,** 201–204 (2007).

21. Sookprasert, A. *et al.* Melatonin in patients with cancer receiving chemotherapy: a randomized, double-blind, placebo-controlled trial. *Anticancer Res.* **34,** 7327–7337 (2014).

22. Rasmussen, C. L. *et al.* Effects of melatonin on physical fatigue and other symptoms in patients with advanced cancer receiving palliative care: A double-blind placebo-controlled crossover trial. *Cancer* **121,** 3727–3736 (2015).

23. Wang, Y. *et al.* The efficacy and safety of melatonin in concurrent chemotherapy or radiotherapy for solid tumors: a meta-analysis of randomized controlled trials. *Cancer Chemother. Pharmacol.* **69,** 1213–1220 (2012).

24. Seely, D. *et al.* Melatonin as adjuvant cancer care with and without chemotherapy: A systematic review and meta-analysis of randomized trials. *Integr. Cancer Ther.* **11,** 293–303 (2012).

25. Salthouse, T. A. What cognitive abilities are involved in trail-making performance? *Intelligence* **39,** 222–232 (2011).

26. Cole, M. W., Yarkoni, T., Repovs, G., Anticevic, A. & Braver, T. S. Global Connectivity of Prefrontal Cortex Predicts Cognitive Control and Intelligence. *J. Neurosci.* **32,** 8988–8999 (2012).

27. Valdés-Tovar, M. *et al.* Circadian modulation of neuroplasticity by melatonin: A target in the treatment of depression. *Br. J. Pharmacol.* (2018). doi:10.1111/bph.14197

28. Dietrich, J., Monje, M., Wefel, J. & Meyers, C. Clinical Patterns and Biological Correlates of Cognitive Dysfunction Associated with Cancer Therapy. *Oncologist* **13,**

1285–1295 (2008).

29. Srinivasan, V., Spence, D. W., Pandi-Perumal, S. R., Trakht, I. & Cardinali, D. P. Therapeutic actions of melatonin in cancer: Possible mechanisms. *Integrative Cancer Therapies* **7,** 189–203 (2008).

30. Sanchez-Barcelo, E. J., Mediavilla, M. D., Alonso-Gonzalez, C. & Reiter, R. J. Melatonin uses in oncology: breast cancer prevention and reduction of the side effects of chemotherapy and radiation. *Expert Opin. Investig. Drugs* **21,** 819–831 (2012).

31. Pandi-Perumal, S. R. *et al.* Physiological effects of melatonin: Role of melatonin receptors and signal transduction pathways. *Prog. Neurobiol.* **85,** 335–353 (2008).

32. Hill, S. M. *et al.* Molecular mechanisms of melatonin anticancer effects. *Integr. Cancer Ther.* **8,** 337–346 (2009).

33. Esposito, E. & Cuzzocrea, S. Antiinflammatory Activity of Melatonin in Central Nervous System. *Curr. Neuropharmacol.* **8,** 228–242 (2010).

34. Liu, Y.-Z., Wang, Y.-X. & Jiang, C.-L. Inflammation: The Common Pathway of Stress-Related Diseases. *Front. Hum. Neurosci.* **11,** 1–11 (2017).

35. Zanette, S. A. *et al.* Higher serum S100B and BDNF levels are correlated with a lower pressure-pain threshold in fibromyalgia. *Mol. Pain* **10,** 1–9 (2014).

36. Schwertner, A. *et al.* Efficacy of melatonin in the treatment of endometriosis: A phase II, randomized, double-blind, placebo-controlled trial. *Pain* **154,** 874–881 (2013).

37. Dmitrzak-Weglarz, M. & Reszka, E. Pathophysiology of Depression: Molecular Regulation of Melatonin Homeostasis – Current Status. *Neuropsychobiology* 1–13 (2018). doi:10.1159/000489470

38. Micale, V., Arezzi, A., Rampello, L. & Drago, F. Melatonin affects the immobility time of rats in the forced swim test: The role of serotonin neurotransmission. *Eur. Neuropsychopharmacol.* **16,** 538–545 (2006).

39. Mantovani, M., Pértile, R., Calixto, J. B., Santos, A. R. S. & Rodrigues, A. L. S. Melatonin exerts an antidepressant-like effect in the tail suspension test in mice: Evidence for involvement of N-methyl-D-aspartate receptors and the L-arginine-nitric oxide pathway. *Neurosci. Lett.* **343,** 1–4 (2003).

40. Detanico, B. C. *et al.* Antidepressant-like effects of melatonin in the mouse chronic mild stress model. *Eur. J. Pharmacol.* **607,** 121–125 (2009).

41. Kendler, K. S., Karkowski, L. M. & Prescott, C. A. Causal Relationship Between Stressful Life Events and the Onset of Major Depression. *Psychiatry Interpers. Biol. Process.* **156,** 837–841 (1999).

42. Zhang, L. *et al.* Melatonin ameliorates cognitive impairment induced by sleep deprivation in rats: Role of oxidative stress, BDNF and CaMKII. *Behav. Brain Res.* **256,** 72–81 (2013).

43. Reuter-Lorenz, P. A. & Cimprich, B. Cognitive function and breast cancer: Promise and potential insights from functional brain imaging. *Breast Cancer Res. Treat.* **137,**

33–43 (2013).

44. Bahuguna, J., Aertsen, A. & Kumar, A. Controlling the Go / No-Go decision threshold in the striatum. *BMC Neurosci.* **14,** P228 (2013).

45. Dillon, D. G. & Pizzagalli, D. A. Inhibition of action, thought, and emotion: A selective neurobiological review. *Appl. Prev. Psychol.* **12,** 99–114 (2007).

46. Hidalgo, M. P. L. *et al.* The clinical effect of small oral clonidine doses on perioperative outcomes in patients undergoing abdominal hysterectomy. *Anesth. Analg.* **100,** 795–802 (2005).

47. Turner, L. *et al.* Consolidated standards of reporting trials (CONSORT) and the completeness of reporting of randomised controlled trials (RCTs) published in medical journals. *Cochrane Database Syst. Rev.* (2012). doi:10.1002/14651858.MR000030.pub2

48. Jung, M. S. & Cimprich, B. Cognitive deficits in Korean women treated with chemotherapy for breast cancer. *Cancer Nurs.* **37,** 31–42 (2014).

49. Wefel, J. S., Vardy, J., Ahles, T. & Schagen, S. B. International Cognition and Cancer Task Force recommendations to harmonise studies of cognitive function in patients with cancer. *Lancet Oncol.* **12,** 703–708 (2011).

50. Hamdan, A. C. & Hamdan, E. M. L. R. Effects of age and education level on the Trail Making Test in a healthy Brazilian sample. *Psychol. Neurosci.* **2,** 199–203 (2009).

51. Hagenaars, S. P. *et al.* Genetic contributions to Trail Making Test performance in UK Biobank. *Mol. Psychiatry* 1–9 (2017). doi:10.1038/mp.2017.189

52. Van der Elst, W., Van Boxtel, M. P. J., Van Breukelen, G. J. P. & Jolles, J. Rey's verbal learning test: Normative data for 1855 healthy participants aged 24-81 years and the influence of age, sex, education, and mode of presentation. *J. Int. Neuropsychol. Soc.* **11,** 290–302 (2005).

53. Malloy-Diniz, L. F., Lasmar, V. A. P., Gazinelli, L. D. S. R., Fuentes, D. & Salgado, J. V. The Rey Auditory-Verbal Learning Test: Applicability for the Brazilian elderly population. *Rev. Bras. Psiquiatr.* **29,** 324–329 (2007).

54. Shao, Z., Janse, E., Visser, K. & Meyer, A. S. What do verbal fluency tasks measure? Predictors of verbal fluency performance in older adults. *Front. Psychol.* **5,** 1–10 (2014).

55. Lezak, M. D. Neuropsychological assessment in behavioral toxicology - Developing techniques and interpretative issues. *Scand. J. Work. Environ. Health* **10,** 25–29 (1984).

56. Garavan, H., Ross, T. J. & Stein, E. a. Right hemispheric dominance of inhibitory control: an event-related functional MRI study. *Proc. Natl. Acad. Sci. U. S. A.* **96,** 8301–8306 (1999).

57. Warmenhoven, F. *et al.* The Beck Depression Inventory (BDI-II) and a single screening question as screening tools for depressive disorder in Dutch advanced cancer patients. *Support. Care Cancer* **20,** 319–324 (2012).

58. Okun, M. L. *et al.* Psychometric evaluation of the insomnia symptom questionnaire: A self-report measure to identify chronic insomia. *J. Clin. Sleep Med.* **5,** 41–51 (2009).

59. Akman, T., Yavuzsen, T., Sevgen, Z., Ellidokuz, H. & Yilmaz, A. U. Evaluation of sleep disorders in cancer patients based on Pittsburgh Sleep Quality Index. *Eur. J. Cancer Care (Engl).* **24,** 553–559 (2015).

60. Makluf, A. S. D., Barra, A. D. A., Dias, R. C. & Barros, C. P. De. Quality of life among Brazilian women having undergone surgery for breast cancer: Validity and reliability of the Quality of life Questionnaire (EORTC QLQ-C30) and Breast Cancer Module (QLQ BR-23). *Integr. Cancer Sci. Ther.* **2,** 107–111 (2015).

Legends of Figures

Figure 1. Flowchart of the study.

Figure 2. Timeline of assessments and chemotherapy schemes used.

Figure 3. Scatter plots of the Pearson correlation between Δ-TrkB with both Δ-BDI-II (A melatonin, B placebo) and with Δ-PSQI (C melatonin, D placebo).

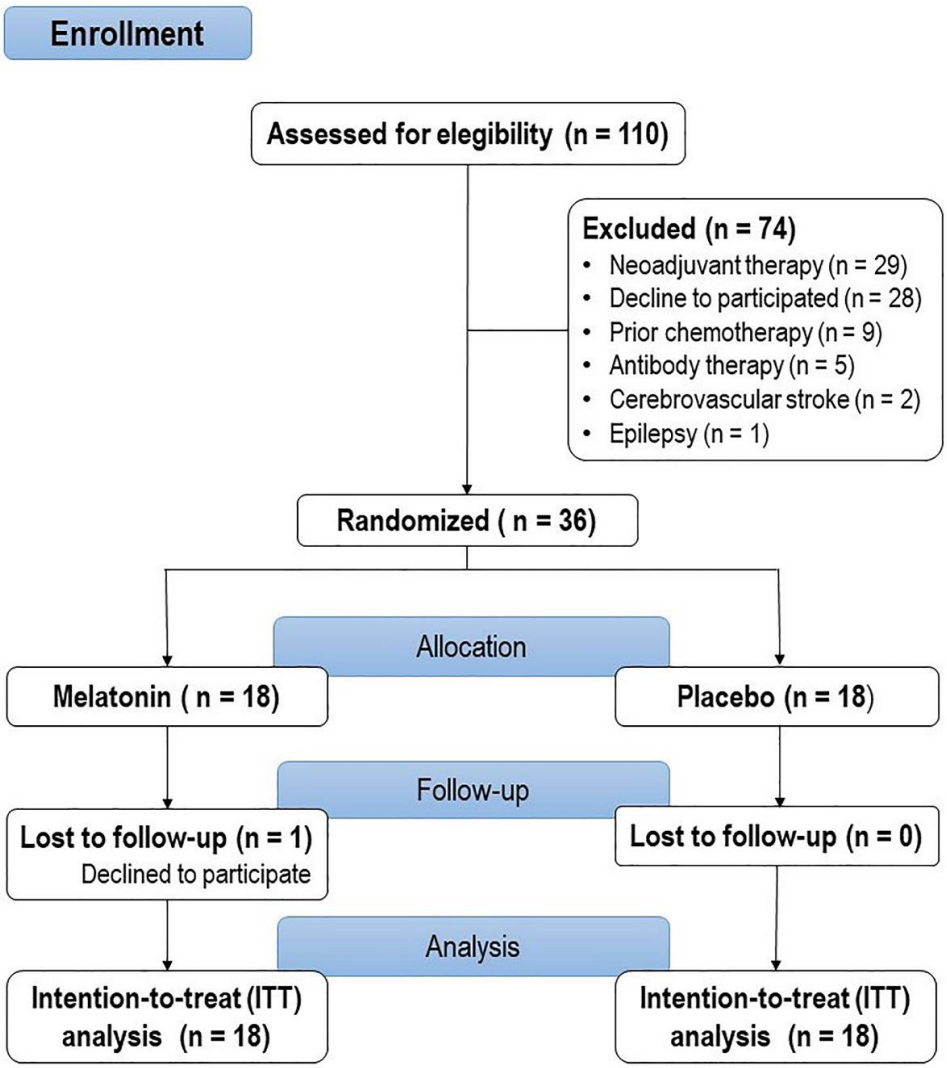
Enrollment
Assessed for elegibility (n = 110)
Excluded (n = 74)
• Neoadjuvant therapy (n = 29)
• Decline to participated (n = 28)
• Prior chemotherapy (n = 9)
• Antibody therapy (n = 5)
• Cerebrovascular stroke (n = 2)
• Epilepsy (n = 1)
Randomized (n = 36)
Allocation
Melatonin (n = 18)
Placebo (n = 18)
Follow-up
Lost to follow-up (n = 1)
Declined to participate
Lost to follow-up (n = 0)
Analysis
Intention-to-treat (ITT) analysis (n = 18)
Intention-to-treat (ITT) analysis (n = 18)

Figure 1.

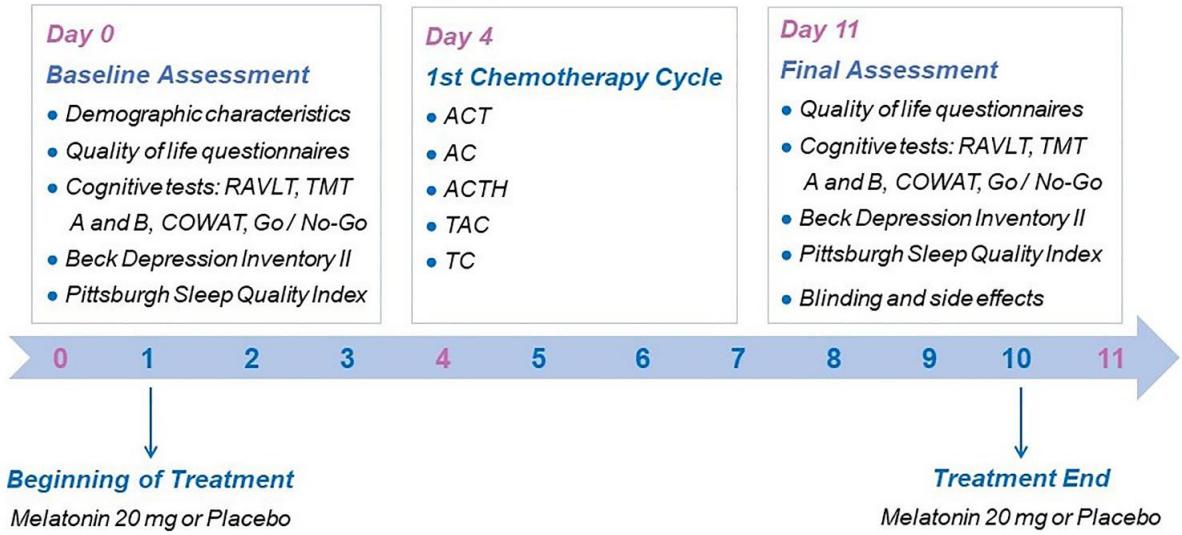
Day 0
Baseline Assessment
• Demographic characteristics
• Quality of life questionnaires
• Cognitive tests: RAVLT, TMT A and B, COWAT, Go / No-Go
• Beck Depression Inventory II
• Pittsburgh Sleep Quality Index
Day 4
1st Chemotherapy Cycle
• ACT
• AC
• ACTH
• TAC
• TC
Day 11
Final Assessment
• Quality of life questionnaires
• Cognitive tests: RAVLT, TMT A and B, COWAT, Go / No-Go
• Beck Depression Inventory II
• Pittsburgh Sleep Quality Index
• Blinding and side effects
0 1 2 3 4 5 6 7 8 9 10 11
Beginning of Treatment
Melatonin 20 mg or Placebo
Treatment End
Melatonin 20 mg or Placebo

Figure 2.

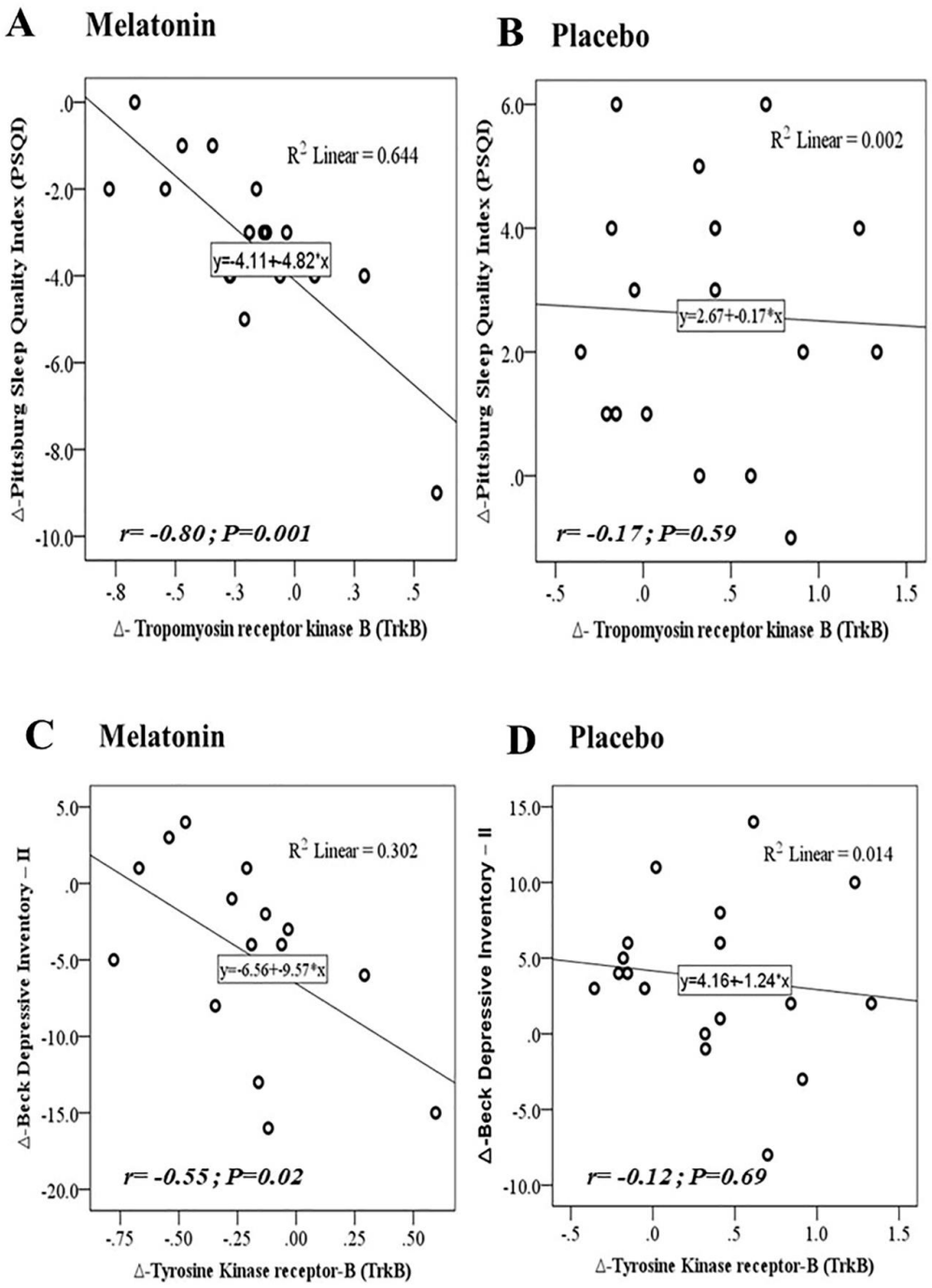
A Melatonin
B Placebo
Δ-Pittsburg Sleep Quality Index (PSQI)
R² Linear = 0.644
y=-4.11+4.82*x
r= -0.80 ; P=0.001
Δ- Tropomyosin receptor kinase B (TrkB)
R² Linear = 0.002
y=2.67+-0.17*x
r= -0.17 ; P=0.59
C Melatonin
D Placebo
Δ-Beck Depressive Inventory – II
R² Linear = 0.302
y=-6.56+-9.57*x
r= -0.55 ; P=0.02
Δ-Tyrosine Kinase receptor-B (TrkB)
R² Linear = 0.014
y=4.16+-1.24*x
r= -0.12 ; P=0.69

Figure 3.

Table 1. Baseline demographic and clinical characteristics according to treatment group. Data are presented as mean standard deviation (SD) (n=36).

Variables	***Melatonin (n = 18)***	***Placebo (n = 18)***	***P-value***
Age (years)	54.24 (10.59)	54.11 (9.15)	0.97
Formal education (years)	9.29 (4.04)	6.94 (2.57)	0.08†
Body Mass Index (kg/m^2)	28.0 (6.14)	29.94 (5.70)	0.25†
Visual Analogue Scale (0-100)	50 (20.00)	50 (16.48)	0.80
Brain-Derived Neurotrophic Factor (ng / mL)	42.92 (17.54)	42.24 (23.95)	0.92
Tropomyosin receptor kinase B (ng / mL)	0.48 (0.25)	0.47 (0.50)	0.49
Protein S100 Beta (pg / mL)	38.16 (12.42)	32.37 (8.93)	0.21
Pittsburgh Sleep Quality Index	8.24 (3.97)	8.44 (2.83)	0.86
Beck Depression Inventory II	11.41 (7.73)	10.83 (5.11)	0.79
Chronic disease			
Hypertension	7 (38.9%) / 11 (61.1%)	8 (44.4%) / 10 (55.6%)	
Hypothyroidism	3 (16.7%) / 15 (83.3%)	1 (5.6%) / 17 (94.4%)	
Diabetes mellitus	1 (5.6%) / 17 (94.4%)	1 (5.6%) / 17 (94.4%)	
Asthma	1 (5.6%) / 17 (94.4%)	1 (5.6%) / 17 (94.4%)	
Psychotropic medication (yes / no) *			
Selective serotonin reuptake inhibitors	3 (16.7%) / 15 (83.3%)	3 (16.7%) / 15 (83.3%)	
Tricyclics	1 (5.6%) / 17 (94.4%)	2 (11.1%) / 16 (88.9%)	
Benzodiazepines	3 (16.7%) / 15 (83.3%)	4 (22.2%) / 14 (77.8%)	
Antipsychotics	1 (5.6%) / 17 (94.4%)	_____	
Chemotherapy regiments (yes / no)			
ACT (doxorubicin plus cyclophosphamide followed by weekly paclitaxel) [1]	9 (50%) / 9 (50%)	9 (50%) / 9 (50%)	
AC (doxorubicin plus cyclophosphamide) [1]	5 (27.8%) / 13 (72.2%)	2 (11.1%) / 16 (88.9%)	
ACTH (doxorubicin plus cyclophosphamide followed by paclitaxel plus trastuzumab) [1]	2 (11.1%) / 16 (88.9%)	3 (16.7%) / (83.3%)	
TAC (docetaxel, doxorubicin, and cyclophosphamide) [2]	1 (5.6%) / 17 (94.4%)	2 (11.1%) / 16 (88.9%)	
TC (docetaxel plus cyclophosphamide) [2]	1 (5.6%) / 17 (94.4%)	2 (11.1%) / 16 (88.9%)	

† Mann-Whitney non-parametric test was used. Independent t-tests were applied to all other measures.

** Three patients use more than one psychotropic medication.*

Prophylaxis for infusion reactions:

[1] Dexamethasone 20 mg IV 30 minutes before drug administration.

[2] Dexamethasone 8 mg orally every 12 hours starting one day prior to docetaxel administration.

Table 2. Cognitive measures at baseline and end of treatment according to melatonin or placebo groups. Data are presented as the mean and standard deviation (SD) (n=36).

	Placebo (n=18)		***Melatonin (n=17)***		
	Mean (SD)	**Δ-value**	***Mean (SD)***	**Δ-value**	***P-value****
Primary outcome					
Trail Making-Test (TMT-A)					
Baseline	48.06 (29.27)	0.02 (0.29)	44.71 (26.52)	-0.19 (0.13)	0.009
End treatment	49.72 (29.46)		35.14 (18.57)		
Trail Making-Test (TMT-B)					
Baseline	109.50 (55.69)	0.12 (0.19)	122.58 (68.21)	-0.23 (0.16)	<0.001
End treatment	123.72 (60.59)		92.29 (48.25)		
Secondary outcomes					
Rey Auditory-Verbal Learning Test *List A1-A5*					
Baseline	42.33 (10.84	-0.04 (0.27)	39.10 (9.64)	0.25 (0.24)	0.002
End treatment	39.44 (11.99)		49.47 (8.65)		
Rey Auditory-Verbal Learning Test *List A7*					
Baseline	8.33 (4.10)	-0.14 (0.32)	7.82 (3.78)	0.17 (0.22)	0.002
End treatment	6.83 (3.40)		9.62 (4.82)		
Rey Auditory-Verbal Learning Test *Recognition*					
Baseline	42.94 (6.67)	-0.05 (0.10)	44.47 (4.91)	0.06 (0.06)	<0.001
End treatment	40.50 (6.48)		46.89 (3.70)		
Controlled Oral Word Association Test *Orthographic*					
Baseline	30.22 (9.05)	-0.04 (0.27)	32.88 (9.47)	0.24 (0.13)	0.001
End treatment	28.17 (8.62)		39.92 (9.40)		
Controlled Oral Word Association Test *Semantic*					
Baseline	14.44 (3.54)	-0.02 (0.20)	16.89 (4.02)	0.02 (0.21)	0.563
End treatment	14.06 (4.12)		17.05 (4.54)		
Go / No-Go *Hit*					
Baseline	0.84 (0.11)	0.02 (0.14)	0.85 (0.11)	0.03 (0.16)	0.884
End treatment	0.85 (0.12)		0.86 (0.11)		
Go / No-Go *False alarm*					
Baseline	0.16 (0.12)	-1.90 (4.15)	0.16 (0.10)	-0.28 (3.70)	0.286
End treatment	0.14 (0.13)		0.15 (0.14)		
Go / No-Go – *D`*					
Baseline	2.27 (0.91)	0.15 (0.41)	2.28 (0.95)	0.17 (0.49)	0.894
End treatment	2.49 (1.10)		2.46 (1.03)		

* Correspond to comparisons of Δ-value by the t-test for independent sample.

Table 3. MANCOVA model to compare the treatment effect in the Δ-value of memory measures according to the baseline neuroplasticity state evaluated by the serum BDNF and TrkB (n=36).

Corrected Model	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*P value*	$\eta^2_{partial}$
Dependent Variables						
Δ-Trail Making-Test (TMT-A)	0.89[a]	3	0.30	8.07	<0.01	0.47
Δ-Trail Making-Test (TMT-B)	1.19[b]	3	0.40	22.99	<0.01	0.72
Δ-Rey Auditory-Verbal Learning Test *List A1-A5*	0.44[c]	3	0.15	3.2	0.04	0.26
Δ-Rey Auditory-Verbal Learning Test *List A7*	0.89[d]	3	0.30	3.31	0.04	0.27
Δ-Controlled Oral Word Association Test *Semantic*	0.02[e]	3	<0.01	0.17	0.92	0.02
Δ-Controlled Oral Word Association Test *Orthographic*	0.60[f]	3	0.20	3.87	0.02	0.30
Intercept						
Δ-Trail Making-Test (TMT-A)	0.13	1	0.13	3.42	0.08	0.11
Δ-Trail Making-Test (TMT-B)	0.04	1	0.04	2.12	0.16	0.07
Δ-Rey Auditory-Verbal Learning Test *List A1-A5*	0.07	1	0.08	1.54	0.23	0.05
Δ-Rey Auditory-Verbal Learning Test *List A7*	0.02	1	0.02	0.19	0.66	<0.01
Δ-Controlled Oral Word Association Test *Orthographic*	<0.01	1	<0.01	0.02	0.88	<0.01
Δ-Controlled Oral Word Association Test *Semantic*	0.11	1	0.11	2.05	0.16	0.07
Treatment group						
Δ-Trail Making-Test (TMT-A)	0.54	1	0.54	14.55	<0.01	0.35
Δ-Trail Making-Test (TMT-B)	0.84	1	0.84	48.60	<0.01	0.64
Δ-Rey Auditory-Verbal Learning Test *List A1-A5*	0.43	1	0.43	9.25	<0.01	0.26
Δ-Rey Auditory-Verbal Learning Test *List A7*	0.85	1	0.85	9.45	<0.01	0.26
Δ-Controlled Oral Word Association Test *Orthographic*	<0.01	1	<0.01	0.10	0.75	<0.01
Δ-Controlled Oral Word Association Test *Semantic*	0.56	1	0.56	10.81	<0.01	0.29
Baseline tropomyosin receptor kinase B (TrkB)						
Δ-Trail Making-Test (TMT-A)	0.01	1	0.01	0.27	0.60	0.01
Δ-Trail Making-Test (TMT-B)	0.22	1	0.22	12.78	<0.01	0.32
Δ-Rey Auditory-Verbal Learning Test *List A1-A5*	0.04	1	0.04	0.81	0.38	0.03
Δ-Rey Auditory-Verbal Learning Test *List A7*	2.05E	1	2.05E	<0.01	0.99	<0.01
Δ-Controlled Oral Word Association Test *Orthographic*	0.02	1	0.02	0.35	0.56	0.01
Δ-Controlled Oral Word Association Test *Semantic*	0.08	1	0.08	1.52	0.23	0.05
Baseline Brain Derivate Neurotrophic Factor (BDNF)						
Δ-Trail Making-Test (TMT-A)	0.32	1	0.32	8.55	<0.01	0.24
Δ-Trail Making-Test (TMT-B)	0.01	1	0.01	0.78	0.39	0.03
Δ-Rey Auditory-Verbal Learning Test *List A1-A5*	<0.01	1	<0.01	0.01	0.92	<0.01
Δ-Rey Auditory-Verbal Learning Test *List A7*	0.04	1	0.04	0.44	0.51	0.02
Δ-Controlled Oral Word Association Test *Orthographic*	<0.01	1	<0.01	0.04	0.84	<0.01
Δ-Controlled Oral Word Association Test *Semantic*	<0.01	1	<0.01	0.05	0.83	<0.01

To perform the analysis, we calculated the Δ-value before and after treatment for all outcomes measures.

R Squared = 0.473 (Adjusted R Squared =0.414) [a]
R Squared = 0.719 (Adjusted R Squared = 0.687) [b]
R Squared = 0.262 (Adjusted R Squared = 0.180) [c]
R Squared = 0.269 (Adjusted R Squared = 0.188) [d]
R Squared = 0.019 (Adjusted R Squared = 0.091) [e]
R Squared = 0.301 (Adjusted R Squared = 0.223) [f]

Table 4. MANCOVA to compare the effect of treatment between groups and its relationship with Δ-values of BDNF and TrkB and cognitive flexibility (TMT-A-B), depressive symptoms and sleep quality (n=36).

Corrected Model	*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*P value*	$\eta^2_{partial}$
Dependent variables						
Δ-Trail Making-Test (TMT-A)	0.61[a]	5	0.12	2.45	0.06	0.30
Δ-Trail Making-Test (TMT-B)	1.12[b]	5	0.23	7.35	<0.01	0.56
Δ-Pittsburg Sleep Quality Index (PSQI)	336.3[c]	5	67.26	20.9	<0.01	0.78
Δ-Beck Depressive Inventory – II (BDI II)	841.7[d]	5	168.33	6.22	<0.01	0.52
Intercept						
Δ-Trail Making-Test (TMT-A)	0.13	1	0.13	2.56	0.12	0.08
Δ-Trail Making-Test (TMT-B)	0.09	1	0.09	3.04	0.09	0.10
Δ-Pittsburg Sleep Quality Index (PSQI)	9.62	1	9.61	2.99	0.10	0.09
Δ-Beck Depressive Inventory – II (BDI II)	2.48	1	2.48	0.09	0.76	<0.01
Treatment group						
Δ-Trail Making-Test (TMT-A)	0.03	1	0.03	0.55	0.46	0.02
Δ-Trail Making-Test (TMT-B)	0.24	1	0.24	7.70	0.01	0.21
Δ-Pittsburg Sleep Quality Index (PSQI)	126.09	1	126.09	39.19	<0.01	0.58
Δ-Beck Depressive Inventory – II (BDI II)	157.70	1	157.70	5.83	0.02	0.17
Δ- Tropomyosin receptor kinase B (TrkB)						
Δ-Trail Making-Test (TMT-A)	<0.01	1	<0.01	0.015	0.91	<0.01
Δ-Trail Making-Test (TMT-B)	0.03	1	0.03	0.85	0.37	0.03
Δ-Pittsburg Sleep Quality Index (PSQI)	35.05	1	35.05	10.89	<0.01	0.27
Δ-Beck Depressive Inventory – II (BDI II)	169.57	1	169.56	6.27	0.02	0.18
Δ-Brain derivate neurotrophic factor (BDNF)						
Δ-Trail Making-Test (TMT-A)	0.05	1	0.05	0.99	0.33	0.03
Δ-Trail Making-Test (TMT-B)	<0.01	1	<0.01	0.23	0.63	0.01
Δ-Pittsburg Sleep Quality Index (PSQI)	<0.01	1	<0.01	<0.01	0.97	<0.01
Δ-Beck Depressive Inventory – II (BDI II)	6.03	1	6.03	0.22	0.64	0.01
Interaction						
Treatment group * Δ-Tyrosine Kinase receptor-B (TrkB)						
Δ-Trail Making-Test (TMT-A)	0.07	1	0.08	1.48	0.23	0.05
Δ-Trail Making-Test (TMT-B)	<0.01	1	<0.01	0.0	0.91	<0.01
Δ-Pittsburg Sleep Quality Index (PSQI)	28.63	1	28.63	8.90	0.01	0.24
Δ-Beck Depressive Inventory – II (BDI II)	122.7	1	122.69	4.54	0.05	0.14
Treatment group * Δ-Brain derivate neurotrophic factor (BDNF)						
Δ-Trail Making-Test (TMT-A)	0.01	1	0.01	0.23	0.64	0.01
Δ-Trail Making-Test (TMT-B)	0.03	1	0.03	1.07	0.31	0.04
Δ-Pittsburg Sleep Quality Index (PSQI)	1.66	1	1.66	0.56	0.48	0.02
Δ-Beck Depressive Inventory – II (BDI II)	41.43	1	41.43	1.53	0.23	0.05

R Squared =0.297 (Adjusted R Squared =0.175) [a]
R Squared =0.559 (Adjusted R Squared =0.483) [b]
R Squared =0.783 (Adjusted R Squared =0.745) [c]
R Squared =0.518 (Adjusted R Squared =0.434) [d]

6 ARTIGO 2 – ORIGINAL EM INGLÊS

THE EFFECTS OF MELATONIN ON THE DESCENDING PAIN INHIBITORY SYSTEM AND NEURAL PLASTICITY MARKERS IN BREAST CANCER PATIENTS RECEIVING CHEMOTHERAPY: RANDOMIZED, DOUBLE-BLINDED, PLACEBO-CONTROLLED TRIAL

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The Effects of Melatonin on the Descending Pain Inhibitory System and Neural Plasticity Markers in Breast Cancer Patients Receiving Chemotherapy: Randomized, Double-Blinded, Placebo-Controlled Trial

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Abstract

Background: Adjuvant chemotherapy for breast cancer (ACBC) has been associated with fatigue, pain, depressive symptoms, and disturbed sleep. Accordingly, previous studies in non-cancer patients showed that the melatonin could improve the descending pain modulatory system (DPMS). We tested the hypothesis that melatonin use before and during the first cycle of ACBC is better than placebo to improve the DPMS function assessed by changes on the 0-10 Numerical Pain Scale (NPS) during the conditioned pain modulating (CPM-task) (primary outcome). The effects of melatonin were evaluated in the following secondary endpoints: heat pain threshold (HPT), heat pain tolerance (HPTo) and the neuroplasticity state assessed by serum brain-derived neurotrophic factor (BDNF), Tropomyosin Kinase Receptor B (TrkB), and S100B-protein also, whether melatonin`s effects on pain and the neuroplasticity are due more so to its impact on sleep quality.

Methods: Thirty-six women, age 18 to 75 years old, scheduled for their first cycle of ACBC were randomized to receive 20mg of oral melatonin (n=18) or placebo (n=18). The effect of treatment was assessed by changes delta [Δ-values (prior minus treatment end)] in psychophysical pain measures, serum BDNF, TrkB, and S100B.

Results: MANCOVA revealed that the changes in the NPS (0-10) during the CPM-task evaluated by Δ-means of each group, mean difference (md) between two groups with their respective confidence interval (CI, 95%) was (-1.07 vs. 2.76); md=–3.83, (-5.66 to -2.00)], respectively. The η^2partial=0.60 indicates that the melatonin effect explains 60% of the variance in the CPM-task. The melatonin reduced the serum levels of neuroplasticity markers: BDNF [Δ-means (-30.64 vs. 1.29); md=-32.93; (-50.19 to -13.67)]; TrKB [Δ-means (-0.33 vs. 0.09), md=-0.43; (-0.66 to - 0.25)] and S00B-protein [Δ-means (-14.85 vs. 2.22); md=-16.87; (-31.30 to -2.45)]. However, melatonin`s effect increased in the HPTo and HPT, and its effects on pain and the neuroplastic state are not due to its impact on sleep quality.

Conclusions: These results suggest that oral melatonin together with first ACBC counteracts the dysfunction in the inhibitory DPMS and improves pain perception measures. Also, it shows that changes in the neuroplasticity state mediate the impact of melatonin in on pain.

Key words: breast cancer, chemotherapy, melatonin, BDNF, S100-B protein, sleep quality.

Register on clinicaltrials.gov: NCT03205033.

1. Introduction

Chemotherapy treatment for breast cancer has been associated with fatigue, pain, depressive symptoms, and disturbed sleep (1–4). Even in healthy women, sleep deprivation produces a significant decline in descending pain-inhibitory functions [i.e. a loss of Diffuse Noxious Inhibitory Controls (DNIC)], and an increase in spontaneous painful symptoms (5). Indeed, these previous findings affirm that poor sleep quality is a risk factor for exacerbation of chronic pain (6,7). Accordingly, previous studies showed that melatonin can improve both sleep quality and pain measures (i.e., endometriosis and fibromyalgia) (8,9). Also, it optimizes the descending pain modulatory system (DPMS) (10).

Additionally, experimental models show that the anti-inflammatory properties of melatonin reduced nuclear factor-κB (NF-κB) activity, a transcription factor found within neurons and glial cells (11–13). NF-κB regulates cellular processes such as migration, maturation, plasticity and synaptic communication and it is constitutively activated in glutamatergic neurons (14,15). In vitro studies revealed that melatonin resists microglial cytotoxicity by suppressing apoptosis and inhibiting the activity of NF-κB (16). Also, such activated cytokines may induce the secretion of neurotrophins such as BDNF and S100-β-protein (1,17).

BDNF has been positively correlated with the potency of the DPMS (18). Also, it modulates excitatory and inhibitory transmission through the activation of glutamatergic NMDA receptors and inhibitory GABA receptors (19). The primary BDNF receptor, tropomyosin kinase B (TrkB), can be a predictive marker of poor clinicopathological prognosis in breast cancer patients (20), while preclinical studies have shown that inhibiting TrkB leads to favorable effects in neuropathic pain (21). A positive correlation between BDNF and central sensitization (CS) has been shown in humans and carries a central role in the pathophysiology of chronic pain (22).

Overall, this set of evidence suggests that the benefits of neuroprotective effects of melatonin can counteract the neurotoxic effects induced by ACBC on neuroplastic mechanisms involved in the pathophysiology of pain associated with chemotherapy. Thus, we tested the hypothesis that supplementing patients with melatonin before and during the first cycle of ACBC is better than placebo. We tested the hypothesis that melatonin use before and during the first cycle of ACBC is better than placebo to improve the DPMS function assessed

by changes on the 0-10 Numerical Pain Scale (NPS) during the conditioned pain modulating (CPM) task (primary outcome). The melatonin`s effects were evaluated in the following secondary endpoints: heat pain threshold (HPT), heat pain tolerance (HPTo) and the neuroplasticity state assessed by serum BDNF, TrkB, and S100B-protein also, whether melatonin`s effects on pain and the neuroplasticity are due more so to its impact on sleep quality.

2. Materials and Methods

2.1. Study Design and Eligibility

This randomized, double-blinded, placebo-controlled trial was approved by the Institutional Review Board of Hospital de Clínicas of Porto Alegre (IRB HCPA/Approval number: 14-0701) and it was registered on http://www.clinicaltrials.gov/ (No NCT03205033 Study start: January 2016, End date: April, 2017) before inclusion of the first patient and the Good Clinical Practice Unit at HCPA monitored the trial. We obtained oral and written informed consent from all patients before participating in this study. The identified data related to interventions and primary outcomes will be available upon request to interested to Caumo W (wcaumo@hcpa.edu.br) with no time restriction. Flow of this study is presented in **Figure 1**.

---------- Figure 1----------

2.2. Participants

Patients were selected from the Mastology and Oncology Service at HCPA, a public tertiary teaching Medical School. Females aged 18 to 75 years with the capacity to read and write were selected. *Inclusion criteria*: females scheduled for their first cycle of ACBC one month following lumpectomy or mastectomy. *Exclusion criteria*: patients with previous chemotherapy, patients planned for neoadjuvant chemotherapy, or those with prior or other concurrent malignancies. Also excluded were patients with a history of melatonin allergy, sleep apnea, diabetes, autoimmune disease (i.e. systemic lupus erythematosus, type I diabetes, rheumatoid arthritis, inflammatory bowel disease, etc.), decompensated liver cirrhosis, severe kidney disease, epilepsy, cerebrovascular stroke, Body Mass Index (BMI) above 35 kg / m2, pregnant or breastfeeding, and a predictable likelihood of poor compliance.

2.3. Sample Size Considerations

We estimated the sample size based on previous studies that assessed melatonin`s effect on the DPMS measured by the change on the NPS during the CPM-task (9). Accordingly, with six dependent variables and a large effect size (f^2=0.35) to compare melatonin and placebo by MACONVA, with two predictors in a 1:1 ratio, the estimate indicated a sample size of 32 for a power of 80% and an α of 0.05. Considering possible dropouts, we increased the sample by 12%, and the final sample size comprised of 36 patients (18 per group).

2.4. Randomization and Masking

We used a randomly different block size of 8 and 6. Thirty-six women were allocated to receive melatonin or placebo, an allocation of 1:1. Before the recruitment phase, randomization was computer generated by two investigators uninvolved in the patients' assessments. Envelopes containing the allocated treatment were prepared, sealed and numbered sequentially. The envelope was opened following the sequence of numbers registered in the envelope after the participant consented to participate in the trial. Following the conclusion of treatment, we assessed the effectiveness of the blinding protocol by asking patients to guess which treatment they each received (i.e. melatonin, placebo or unknown).

2.5. Interventions

Patients were instructed to take 20 mg of oral melatonin or placebo daily approximately 1 hour before bedtime. Melatonin capsules were produced using crystalline melatonin with a certificate of purity (M-5250, Sigma Chemical, Saint Louis, MO, USA) by a compounding pharmacy. The tablets of melatonin and placebo were physically identical. Assessments to confirm adherence to treatment included: i) Pill counting during the study period. ii) Patient diaries were kept in order to record if they failed to use the medication. iii) Patients were encouraged to remain on melatonin throughout the ten days of treatment.

2.6. Assessments and instruments

All assessments were conducted by two independently trained research personnel to apply psychophysical pain measurements. The timeline of assessments is presented in Figure 2.

----------Figure 2----------

2.6.1. Outcomes

The treatment effect on primary and secondary outcomes were evaluated by the Δ-value, defined by measurements at treatment end minus values at baseline. Changes in the NPS during the CPM-task assessed the function of the DPMS (primary outcome). The secondary outcomes were the changes produced by treatment in the following measures: HTP, HPTo, BDNF, TrkB, S-100B-protein.

2.6.2. Assessment of primary and secondary outcomes

a) QST was the method utilized in the assessment of heat pain thresholds using the method of limits with a computer Peltier-based device thermode (30 × 30 mm) [26] that was attached to the skin surface of the ventral portion of the mid-forearm. The initial temperature of the QST is set at 32°C and it increases at a rate of 1°C/s to a maximum of 52°C. The average temperature in °C of three consecutive assessments sufficient to induce pain comprises the HPT.

b) HPTo is the temperature induced by the QST to induce the maximum pain tolerated, with a ceiling of 52°C.

c) DPMS was evaluated by the changes on the NPS ranging from 0 (no pain) to 10 (worst pain imaginable). The CPM was induced by a heterotopic noxious stimulus administered concurrently with a QST sufficient to produce a pain score of 6/10. The conditioned *pain* modulation (CPM) test was provoked by immersions nondominant hand into cold water (0^0C) for one minute. During the CPM-task, subjects were asked to rate the pain induced by a thermal stimulus pre-defined to produce a score of 6/10 on the NPS, then 30 seconds later the heterotopic stimulus with cold-water hand immersion was performed. The CPM was defined as the difference between the average pain rating on the NPS before and after cold water immersion.

d) Neuroplasticity state biomarkers were evaluated using serum levels of BDNF, TrkB and S100B collected in plastic tubes and centrifuged for 10 minutes at 4,500 rpm at 4°C in a −80°C freezer for further BDNF and TrkB assays. Serum-mediator concentrations were determined using BDNF (Chemicon CYT306, lower detection limit 7.8 pg/mL; EMD Millipore, Billerica, MA, USA), TrkB (MYBI – MBS9346917, lower detection limit 0.25 ng/ml; MyBiosource, San Diego, CA, USA) S100B (EZHS100B-33 K, Millipore, Missouri, USA, lower detection limit 2.7 pg/mL), and enzyme-linked immunosorbent-assay kits in accordance with the manufacturer's instructions.

2.6.3. Clinical Measurements: Depressive Symptoms and Sleep Quality

Beck Depression Inventory (BDI-II) is a questionnaire composed of 21 multiple-choice questions with four options each (0 - 3). The total BDI score ranges from 0-63; higher scores indicate a higher degree of depressive symptoms (23).

Pittsburgh Sleep Quality Index (PSQI). The PSQI is a self-reporting questionnaire that comprises 19-items to assess the quality of sleep and identifies sleep disorders. The score ranging from 0 to 21.

2.6.4. Other Instruments and Assessments

The patients' demographic data were assessed using standardized demographic questionnaires. The side effects related to chemotherapy were assessed by the questionnaire of the European Organization for Cancer Research and Treatment validated for the Brazilian population (EORTC QLQ-C30) before and after treatment.

2.7. Statistical Analysis

Inferential tests for demographic and clinical measures, as well as for the psychophysical pain measures and biomarkers of neuroplasticiity (i.e. BDNF, TrkB, S100-B), were based on independent sample t-Tests for continuous variables and utilization of the Mann-Whitney non-parametric test. To control the individual variability, existing imbalances between groups, and baseline differences of the descending pain modulatory system function assessed using the NPS, HPT, HPTo, BDNF, TrkB and S100-B protein, we used the mean differences [delta(Δ)-values of mean difference averages of measures at treatment end minus baseline means]. To analyze the treatment effect on all primary and secondary outcomes, we conducted multivariate analyses of covariance (MANCOVA). The dependent variables were the Δ-values of outcome measures (Change on NPS (0-10) during CPM-task, HPT, HPTo, BDNF, TrkB, S-100B); the treatment group was the factor and the Δ-values of sleep quality and depressive symptoms were covariates. Regression analyses to examine the relationship between primary and secondary outcomes were run when appropriate. Bonferroni's Multiple Comparison adjusted all analyses. Using the intention-to-treat (ITT) method, we considered all of the

randomized patients as part of the analysis with the worst-case observation carried forward in the respective treatment group (melatonin or placebo). For all analyses, we considered a Type I two-sided error (bicaudal) $\alpha = 0.025$. For statistical analyses, the IBM SPSS Statistics for Windows, Version 20.0 was used (IBM Corp., Armonk, NY, U).

3. Results

3.1. Socio-demographic and Clinical Characteristics

The characteristics of the participants are presented in Table 1. Randomization produced balanced groups for most of the characteristics, except in years of school. In the melatonin and placebo group, 13 (54.2%) vs. 11 (45.8%) assumed to have received melatonin, respectively. In the melatonin and placebo group, 4 (44.4%) vs. 5 (55.6%) assumed that they received placebo, respectively. Two in the melatonin group and 1 in the placebo group assumed that their treatment was unknown (P=0.69). Regarding the severity of the adverse effect scores, according to EORTC the median and interquartile (Q25-75) were observed to be at 10 ($Q_{25\text{-}75}$ = 2; 20) vs. 9 ($Q_{25\text{-}75}$ = 0; 24), P=0.35, in the melatonin and placebo group, respectively. We observed that melatonin treatment reduced the severity of adverse effects as the median and interquartile (Q25-75) was 7 ($Q_{25\text{-}75}$= 2; 19) vs. 12.5 ($Q_{25\text{-}75}$ = 3; 25), *P*=0.01, in the melatonin and placebo group, respectively.

----------Table I ----------

3.2. Primary and Secondary Outcomes

3.2.1. Univariate analysis of the primary outcome to compare the treatment group effect on the NPS (0-10) during the CPM-task

The efficiency of the DPMS assessed by the change on the NPS during the CPM-task increased 43.5% from T0 to T1 in the melatonin group, whereas it decreased 93% in the placebo group [t = -4.14, df = 33.57; P< 0.001]. The mean on the NPS during the CPM-task at T0, T1 and the Δ-value is presented in **Figure 3**.

----------***figure 3*** ----------

3.2.2. Multivariate analysis of primary and secondary outcomes to compare the treatment group effect on the psychophysical pain measures considering melatonin's effect on the neuroplasticity state and sleep quality

The MANCOVA analysis to compare between groups of mean differences [delta(Δ)-

values of mean difference averages of measures (at treatment end minus baseline means)]with the adjustment for multiple comparisons is presented in Table 3. The MANCOVA analysis revealed a significant main effect of treatment; Pillai's Trace's F (6, 24) =5.11; $p<0.001$; η^2partial=0.56 (Table 3A).

The change on the NPS(0-10) during CPM-task in the melatonin vs. placebo group presented as mean Δ-values of each group, the mean difference (md) between two groups with their respective confidence interval (CI, 95%) was (-1.07 vs. 2.76); md= –3.83, CI 95% (-5.66 to -2.00)]. It confirmed that melatonin`s effect optimizes the DPMS supported by the change on the NPS during the CPM-task. Also, it increased the HPT and HPTo, while reducing the serum levels of BDNF, TrkB, and S-100B. In Table 3B, the coefficients of the linear regression analysis of MANCOVA are presented. was shown in Table 3B the regression coefficient. The result showed that the Δ-Pittsburgh Sleep Quality Index was negatively correlated with the Δ-value of Changes on NPS (0-10) during the CPM-task (Standardized Beta=-0.37; t=-2.20, P=0.03, η^2partial=0.13). It is important to remember that a higher change on the Δ-PSQI means a better effect of melatonin on sleep quality, while a larger change on the NPS during the CPM-task indicates that the heterotopic stimulus was more effective. Hence, the difference in the NPS (PPT1 minus PPT0) produced a higher negative value. Thus, this explains the coherence of this negative correlation. The interaction analysis showed that melatonin`s effect on the DPMS was not related to its impact on the improvement of sleep quality. (Standardized Beta=0.20, t=0.78 P=0.44).

The Δ-values of means (Δ-means) of each group (mean at treatment end minus mean before treatment) and the mean difference between the melatonin vs. placebo group, with their respective confidence interval (CI; 95%) compared the MANCOVA and adjusted for multiple comparisons showed that the melatonin reduced the serum levels of neuroplasticity markers BDNF [Δ-means (-30.64 vs. 1.29); md= -32.93;CI 95%, -50.19 to -13.67)]; TrKB [Δ-means (-0.33 vs. 0.09), md= -0.43; CI 95% (-0.66 to - 0.25)] and S00B-protein [Δ-means (-14.85 vs. 2.22); md= -16.87; CI 95% (-31.30 to -2.45)]. However, it was observed that melatonin compared to placebo increased in the HPTo [Δ-means (1.94 vs. -3.06); md= 5.00, CI95% (2.34 to 7.65)] and HTP [(3.50 vs. -4.31); md=7.81, CI95% (4.68 to 10.94)], respectively.

----------*Table 3A-B* ----------

4. Discussion

These findings confirm the benefits of melatonin compared to placebo prior to and during the first cycle of ACBC by counteracting the neurotoxic effects on the inhibitory function of the DPMS evaluated by the change on the NPS during the CPM-task. Melatonin also increased the HPT and HPTo, while reducing the serum levels of the neuronal and astrocyte neuroplastic markers (i.e., BDNF, TrkB, and S100-B-protein). The analysis showed that the effect of melatonin on the DPMS and the neuroplasticity state was not related to its impact on sleep quality.

The novelty of this study was to reveal that melatonin may counteract processes related to ACBC that produces dysfunction in the inhibitory DPMS and in the neuroplastic state. These findings corroborate our previous results of melatonin's effect on the DPMS in fibromyalgia patients (9) and provides mechanistic support to explain the high prevalence of pain claims in patients receiving ACBC. Also, these findings extend evidence as to how melatonin's effects on improving the inhibitory DPMS and changes in the neuroplastic state are independent of its impact related to improving sleep quality. Thereby, these findings show that the influence of melatonin on the neural plasticity field can induce improvement in clinical outcomes related to pain and sleep quality. Even though the effect of melatonin on neuroplasticity in pre-clinical studies has been vastly studied (24–27) persists a gap to confirm the pre-clinical potential benefits in clinical settings.

Although extensive literature support the relationship between poor sleep quality and chronic pain, this relationship has been comprehended as a vicious cycle (5,28). However, according to pre-clinical studies, sleep deprivation induces a synaptic instability in spinal cord neurons (29), which may encompass the imbalance between the excitability and inhibitory mechanisms. In the same way, this study indicates that melatonin`s effect on sleep quality concurs with an improvement of the DPMS function, as well as reducing serum levels of BDNF. These results are in line with evidence from previous studies on chronic pain using melatonin, as serum BDNF was found to be reduced in patients with endometriosis (8), while in fibromyalgia the DPMS was improved (9). Accordingly, mechanistic studies indicate that melatonin up-regulates gene expression of serum BDNF while pharmacological studies showed a direct role of TrkB signaling in the development of neuropathic pain (30). Thus, these results support the notion that melatonin contra-regulates the disruption in the BDNF–TrkB signaling induced by ACBC, which is crucial for the development, plasticity, and remodeling of neuronal

circuits (31). In this way, preclinical studies have demonstrated that TrkB inhibition has significant favorable effects in animal models regarding neuropathic pain, depression, cancer and addictive behavior (32–34).

Other additional mechanisms to explain the neuroprotective impacts of melatonin's ability to counteract the neurotoxicity of ACBC include anti-apoptotic, anti-inflammatory, and antioxidant effects (35,36). Indeed, it is possible that the multiplicity of melatonin`s properties reduces transient apoptosis in the brain induced by chemotherapy, as well as reducing neurotoxicity by reversing the microvasculature changes or cytokine activity responsible for diminishing neurogenesis and neuroplasticity. Additionally, a decrease of serum S100B concentration in our results supports that melatonin provides a modulatory effect on astrocytic activity (37). In this way, it is also congruent with the function of serum S100B, a neurotrophic factor that may increase neural survival, neurite extension, and suppression of glial reactivity (38). However, S100B-protein is toxic at very high concentrations (39). Present data further supports, due to its ability to surpass biological barriers such as the blood-brain barrier (BBB) (40), that S100B inhibits inflammatory pathways that would cause brain damage. According to most pre-clinical studies on neuronal damage, melatonin is often given within a 1–20 mg/kg dose range (24–26), while at doses higher than 5 mg/kg it provides maximum neuroprotection in ischemic stroke models (27). However, we cannot transpose the doses used in pre-clinical studies to humans without evaluating its effects on humans due to pharmacokinetic differences.

Similarly, BDNF can cross the BBB bidirectionally, therefore a substantial portion of its serum levels originate from neuronal and glial cells, reflecting its neuronal concentration. Thus, BDNF concentrations found in the brain have been correlated with its serum concentrations (41), suggesting that the neuroprotective effects of melatonin may involve a reduced release of BDNF. Similarly, in a study examining subjects with depression, melatonin safeguarded hippocampal neurons from damage via BDNF or glial cell-derived neurotrophic factor activation (42). Contrarily, the S100B astroglial protein released in response to neuronal injury, exerts neurotrophic effects on neurons and glial cells (39). As an astrocytic marker, S100B can be easily detected in the serum (37), and an increase or decrease in levels has been observed in multiple known brain disorders. Although serum S100B may be elevated in acute neuronal damage, S100B levels were decreased in patients with underlying neurodegenerative disease (43) and also observed in patients who carry lower pain thresholds diagnosed with fibromyalgia (10).

Several concerns related to our study must be addressed: Firstly, the homogeneity of our sample gives rise to the issue of external validity as it is methodologically advantageous to answer the question of this study. Secondly, the awareness of group allocation assessment (either active or placebo) demonstrated that blinding was guaranteed, since the rate of patients of the melatonin group believed to have received placebo, or vice-versa, was very similar. Thirdly, our objective surrogate biomarkers and psychological measurements are less susceptible to bias, hence the unblinding issue is unlikely to have affected our conclusions. A positive effect of melatonin was to reduce the adverse effects due to ACBC.

In conclusion, these results suggest that oral melatonin together with first ACBC counteracts the dysfunction in the inhibitory DPMS and improves pain perception measures. Also, it shows that changes in the neuroplasticity state mediate the impact of melatonin on pain.

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Author Contributions Statement

ACS: Conceived the study and participated in its design, coordination, sequence alignment and drafting of the manuscript. AS, AEZ: Participated in the design of the study, completion of the statistical analysis and drafting of the manuscript. ILST: Participated in the sequence alignment and drafting of the manuscript. JAC, FS: Participated in the sequence alignment. VSS: Participated in the design of the study and completion of the statistical analysis. FF: Participated in the design of the study and coordination, as well as with help drafting the manuscript. WC: Conceived the study and participated in its design, coordination, sequence alignment and drafting of the manuscript.

Conflict of Interest Statement

The authors declare that this research was carried out in the absence of commercial or financial relationships that could be interpreted as a potential conflict of interest.

References

1. Lévi F. Chronotherapeutics: The relevance of timing in cancer therapy. Cancer Causes Control. 2006;17(4):611–21.
2. Given CW, Given B, Azzouz F, Kozachik S, Stommel M. Predictors of pain and fatigue in the year following diagnosis among elderly cancer patients. J Pain Symptom Manage. 2001;21(6):456–66.
3. Rief W, Bardwell WA, Dimsdale JE, Natarajan L, Flatt SW, Pierce JP. Long-term course of pain in breast cancer survivors: A 4-year longitudinal study. Breast Cancer Res Treat. 2011;130(2):579–86.
4. Eversley R, Estrin D, Dibble S, Wardlaw L, Pedrosa M, Favila-Penney W. Post-Treatment Symptoms Among Ethnic Minority Breast Cancer Survivors. Oncol Nurs Forum [Internet]. 2005;32(2):250–6. Available from: http://onf.ons.org/onf/32/2/post-treatment-symptoms-among-ethnic-minority-breast-cancer-survivors
5. Smith M, Edwards R, McCann U, Haythornthwaite J. The Effects of Sleep Deprivation on Pain Inhibition and Spontaneous Pain in Women. Sleep Deprivation Pain Woman [Internet]. 2007;30(4):494–505. Available from: http://www.hindawi.com/journals/prm/2004/949187/abs/
6. L. K-K, M. K, M. H, H. R, R. L, J. K, et al. Sleep disturbances as predictors of hospitalization for back disorders-A 28-year follow-up of industrial employees. Spine (Phila Pa 1976) [Internet]. 2006;31(1):51–6. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed7&NEWS=N&AN=2006113934
7. Affleck G, Urrows S, Tennen H, Higgins P, Abeles M. Sequential daily relations of sleep, pain intensity, and attention to pain among women with fibromyalgia. Pain. 1996;68(2–3):363–8.
8. Schwertner A, Conceição Dos Santos CC, Costa GD, Deitos A, De Souza A, De Souza ICC, et al. Efficacy of melatonin in the treatment of endometriosis: A phase II, randomized, double-blind, placebo-controlled trial. Pain [Internet]. 2013;154(6):874–81. Available from: http://dx.doi.org/10.1016/j.pain.2013.02.025
9. de Zanette SA, Vercelino R, Laste G, Rozisky JR, Schwertner A, Machado CB, et al. Melatonin analgesia is associated with improvement of the descending endogenous pain-modulating system in fibromyalgia: a phase II, randomized, double-dummy, controlled trial. BMC Pharmacol Toxicol [Internet]. 2014 Jul 23;15:40. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4119581/
10. Zanette SA, Dussan-Sarria JA, Souza A, Deitos A, Torres ILS, Caumo W. Higher serum S100B and BDNF levels are correlated with a lower pressure-pain threshold in fibromyalgia. Mol Pain. 2014;10(1):1–9.
11. Jumnongprakhon P, Govitrapong P, Tocharus C, Pinkaew D, Tocharus J. Melatonin Protects Methamphetamine-Induced Neuroinflammation Through NF-κB and Nrf2 Pathways in Glioma Cell Line. Neurochem Res. 2015;40(7):1448–56.
12. Lezoualc F, Sparapani M, N-acetj BC. N-acetyl-serotonin (normelatonin) and melatonin protect neurons against oxidative challenges and suppress the activity of the transcription factor NF-kappaB. J Pineal Res. 1998;168–78.
13. Kaltschmidt B, Widera D, Kaltschmidt C. Signaling via NF-κB in the nervous system. Biochim Biophys Acta - Mol Cell Res. 2005;1745(3):287–99.
14. Malek R, Borowicz KK, Jargiello M, Czuczwar SJ. Role of nuclear factor kappaB in the central nervous system. Pharmacol Rep. 2007;59(1):25–33.

15. Grilli M, Memo M. Nuclear factor-κB/Rel proteins: A point of convergence of signalling pathways relevant in neuronal function and dysfunction. Biochem Pharmacol. 1999;57(1):1–7.
16. Jang MH, Jung S Bin, Lee MH, Kim CJ, Oh YT, Kang I, et al. Melatonin attenuates amyloid beta25-35-induced apoptosis in mouse microglial BV2 cells. Neurosci Lett. 2005;380(1–2):26–31.
17. Bower JE, Ganz PA, Irwin MR, Kwan L, Breen EC, Cole SW. Inflammation and behavioral symptoms after breast cancer treatment: Do fatigue, depression, and sleep disturbance share a common underlying mechanism? J Clin Oncol. 2011;29(26):3517–22.
18. Botelho LM, Morales-Quezada L, Rozisky JR, Brietzke AP, Torres ILS, Deitos A, et al. A Framework for Understanding the Relationship between Descending Pain Modulation, Motor Corticospinal, and Neuroplasticity Regulation Systems in Chronic Myofascial Pain. Front Hum Neurosci [Internet]. 2016;10(June):1–12. Available from: http://journal.frontiersin.org/Article/10.3389/fnhum.2016.00308/abstract
19. Whitehead KJ, Rose S, Jenner P. Halothane Anesthesia Affects NMDA-Stimulated Cholinergic and GABAergic Modulation of Striatal Dopamine Efflux and Metabolism in the Rat In Vivo. Neurochem Res [Internet]. 2004 Apr;29(4):835–42. Available from: https://doi.org/10.1023/B:NERE.0000018858.64265.e9
20. Zhang J, Yao W, Hashimoto K. Brain-derived Neurotrophic Factor (BDNF)-TrkB Signaling in Inflammation-related Depression and Potential Therapeutic Targets. Curr Neuropharmacol [Internet]. 2016 Oct;14(7):721–31. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5050398/
21. Wang X, Ratnam J, Zou B, England PM, Basbaum AI. TrkB signaling is required for both the induction and maitenance of tissue and nerve injury-induced persistent pain. J Neurosci. 2009;29(17):5508–15.
22. Caumo W, Antunes LC, Elkfury JL, Herbstrith EG, Sipmann RB, Souza A, et al. The central sensitization inventory validated and adapted for a Brazilian population: Psychometric properties and its relationship with brain-derived neurotrophic factor. J Pain Res. 2017;10:2109–22.
23. Warmenhoven F, Van Rijswijk E, Engels Y, Kan C, Prins J, Van Weel C, et al. The Beck Depression Inventory (BDI-II) and a single screening question as screening tools for depressive disorder in Dutch advanced cancer patients. Support Care Cancer. 2012;20(2):319–24.
24. Serikov VS, Lyashev YD. Effects of Melatonin on Stress-Induced Changes in the Liver of Rats with Different Resistance to Stress. Bull Exp Biol Med. 2015;159(3):314–7.
25. Jing YL, Wu Q Bin, Yuan XC, Li BW, Liu MM, Zhang XY, et al. Microvascular protective role of pericytes in melatonin-treated spinal cord injury in the C57BL/6 mice. Chin Med J (Engl). 2014;127(15):2808–13.
26. Villapol S, Fau S, Renolleau S, Biran V, Charriaut-Marlangue C, Baud O. Melatonin promotes myelination by decreasing white matter inflammation after neonatal stroke. Pediatr Res. 2011;69(1):51–5.
27. Macleod MR, O'Collins T, Horky LL, Howells DW, Donnan GA. Systematic review and meta-analysis of the efficacy of melatonin in experimental stroke. J Pineal Res. 2005;38(1):35–41.
28. Roehrs T, Roth T. Sleep and pain: Interaction of two vital functions. Semin Neurol. 2005;25(1):106–16.
29. Trošt Bobić T. The Impact of Sleep Deprivation on the Brain. Acta Clin Croat [Internet].

2016;469–73. Available from: http://hrcak.srce.hr/index.php?show=clanak&id_clanak_jezik=254705
30. Ren K, Dubner R. Pain facilitation and activity-dependent plasticity in pain modulatory circuitry: Role of BDNF-TrkB signaling and NMDA receptors. Mol Neurobiol. 2007;35(3):224–35.
31. Yu H, Chen ZY. The role of BDNF in depression on the basis of its location in the neural circuitry. Acta Pharmacol Sin [Internet]. 2011;32(1):3–11. Available from: http://dx.doi.org/10.1038/aps.2010.184
32. Zhou D, Zhang Z, Liu L, Li C, Li M, Yu H, et al. The antidepressant-like effects of biperiden may involve BDNF/TrkB signaling-mediated BICC1 expression in the hippocampus and prefrontal cortex of mice. Pharmacol Biochem Behav [Internet]. 2017;157:47–57. Available from: http://dx.doi.org/10.1016/j.pbb.2017.02.004
33. Ghilardi JR, Freeman KT, Jimenez-Andrade JM, Mantyh WG, Bloom AP, Kuskowski MA, et al. Administration of a tropomyosin receptor kinase inhibitor attenuates sarcoma-induced nerve sprouting, neuroma formation and bone cancer pain. Mol Pain. 2010;6(December):1–12.
34. Sharma R, McMillan CR, Tenn CC, Niles LP. Physiological neuroprotection by melatonin in a 6-hydroxydopamine model of Parkinson's disease. Brain Res. 2006;1068(1):230–6.
35. Zhang HM, Zhang Y. Melatonin: A well-documented antioxidant with conditional pro-oxidant actions. J Pineal Res. 2014;57(2):131–46.
36. Manchester LC, Coto-Montes A, Boga JA, Andersen LPH, Zhou Z, Galano A, et al. Melatonin: An ancient molecule that makes oxygen metabolically tolerable. J Pineal Res. 2015;59(4):403–19.
37. Gonçalves CA, Concli Leite M, Nardin P. Biological and methodological features of the measurement of S100B, a putative marker of brain injury. Clin Biochem. 2008;41(10–11):755–63.
38. Donato R, R. Cannon B, Sorci G, Riuzzi F, Hsu K, J. Weber D, et al. Functions of S100 Proteins. Curr Mol Med [Internet]. 2013;13(1):24–57. Available from: http://openurl.ingenta.com/content/xref?genre=article&issn=1566-5240&volume=13&issue=1&spage=24
39. Donato R. S100: A multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol. 2001;33(7):637–68.
40. Reiter RJ, Tan DX, Kim SJ, Cruz MHC. Delivery of pineal melatonin to the brain and SCN: role of canaliculi, cerebrospinal fluid, tanycytes and Virchow–Robin perivascular spaces. Brain Struct Funct. 2014;219(6):1873–87.
41. Karege F, Schwald M, Cisse M. Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. Neurosci Lett. 2002;328(3):261–4.
42. Ruksee N, Tongjaroenbuangam W, Mahanam T, Govitrapong P. Melatonin pretreatment prevented the effect of dexamethasone negative alterations on behavior and hippocampal neurogenesis in the mouse brain. J Steroid Biochem Mol Biol [Internet]. 2014;143:72–80. Available from: http://dx.doi.org/10.1016/j.jsbmb.2014.02.011
43. Chaves ML, Camozzato AL, Ferreira ED, Piazenski I, Kochhann R, Dall'igna O, et al. Serum levels of S100B and NSE in Alzheimer's disease patients. J Neuroinflammation [Internet]. 2010;7(1):6. Available from: http://dx.doi.org/10.1186/1742-2094-7-6

Legends

Figure 1. Flowchart of the study.

Figure 2. (A) Timeline of study. (B) Conditioned pain modulation – CPM-task.

Figure 3. Change on NPS (0-10) during CPM-task at baseline (T0), treatment end (T1) and the Δ=values (T1 minus T0). Error bars indicate standard error of the mean (SEM). Asterisks (*) positioned above symbols indicate significant differences ($p<0.01$) at those time points using Bonferroni's test. B0 = baseline.

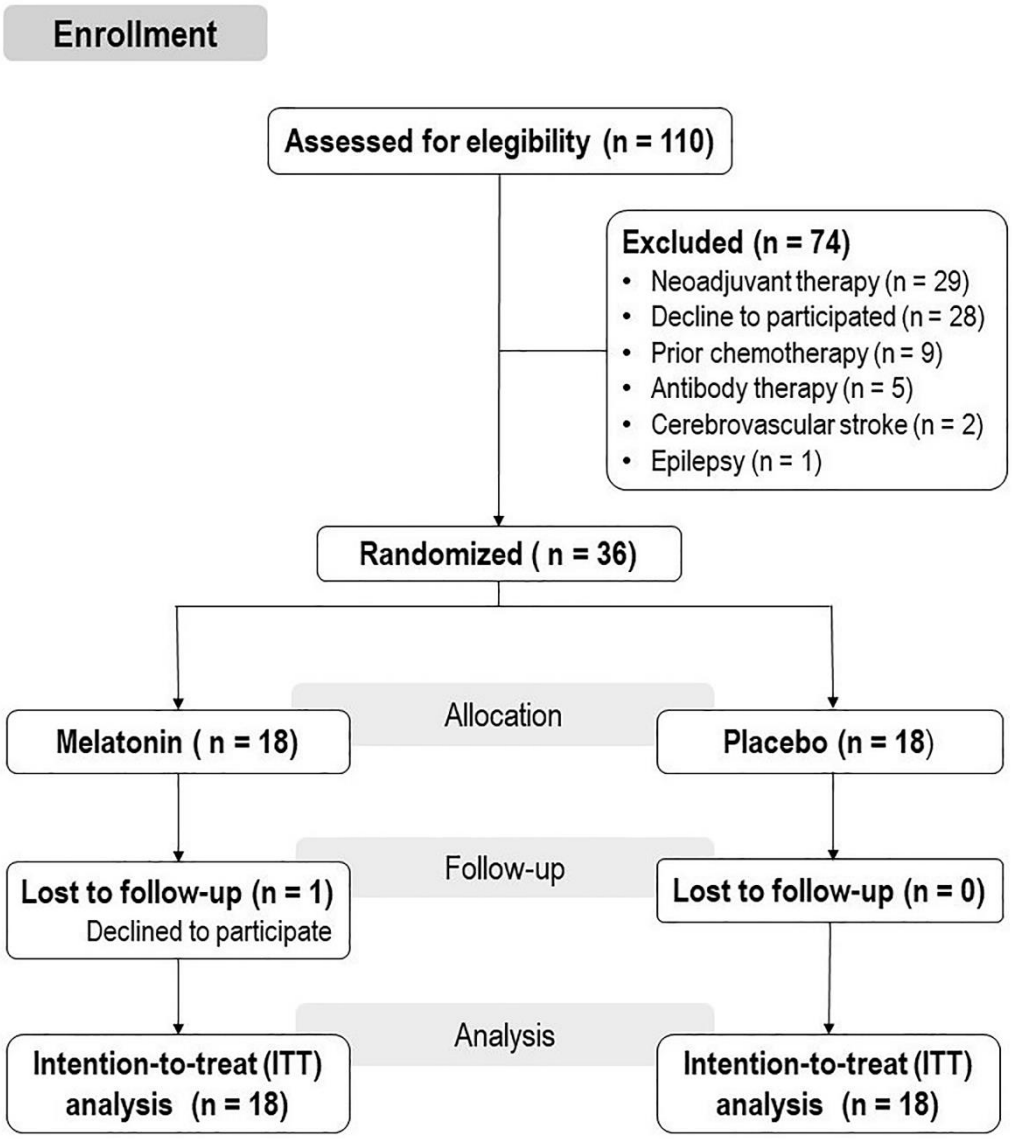
Enrollment
Assessed for elegibility (n = 110)
Excluded (n = 74)
• Neoadjuvant therapy (n = 29)
• Decline to participated (n = 28)
• Prior chemotherapy (n = 9)
• Antibody therapy (n = 5)
• Cerebrovascular stroke (n = 2)
• Epilepsy (n = 1)
Randomized (n = 36)
Allocation
Melatonin (n = 18)
Placebo (n = 18)
Follow-up
Lost to follow-up (n = 1)
Declined to participate
Lost to follow-up (n = 0)
Analysis
Intention-to-treat (ITT) analysis (n = 18)
Intention-to-treat (ITT) analysis (n = 18)

Figure 1.

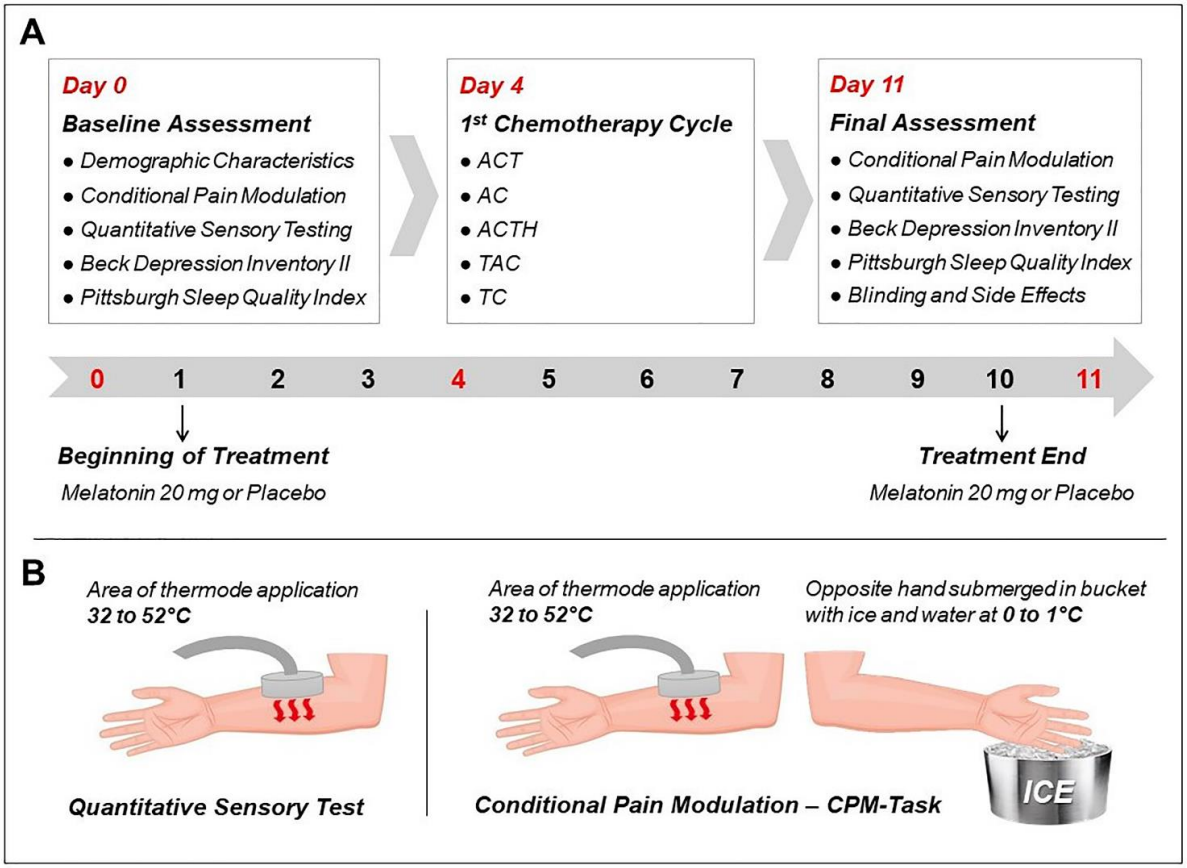
A
Day 0
Baseline Assessment
• Demographic Characteristics
• Conditional Pain Modulation
• Quantitative Sensory Testing
• Beck Depression Inventory II
• Pittsburgh Sleep Quality Index
Day 4
1st Chemotherapy Cycle
• ACT
• AC
• ACTH
• TAC
• TC
Day 11
Final Assessment
• Conditional Pain Modulation
• Quantitative Sensory Testing
• Beck Depression Inventory II
• Pittsburgh Sleep Quality Index
• Blinding and Side Effects
0 1 2 3 4 5 6 7 8 9 10 11
Beginning of Treatment
Melatonin 20 mg or Placebo
Treatment End
Melatonin 20 mg or Placebo
B
Area of thermode application
32 to 52°C
Quantitative Sensory Test
Area of thermode application
32 to 52°C
Opposite hand submerged in bucket
with ice and water at 0 to 1°C
ICE
Conditional Pain Modulation – CPM-Task

Figure 2.

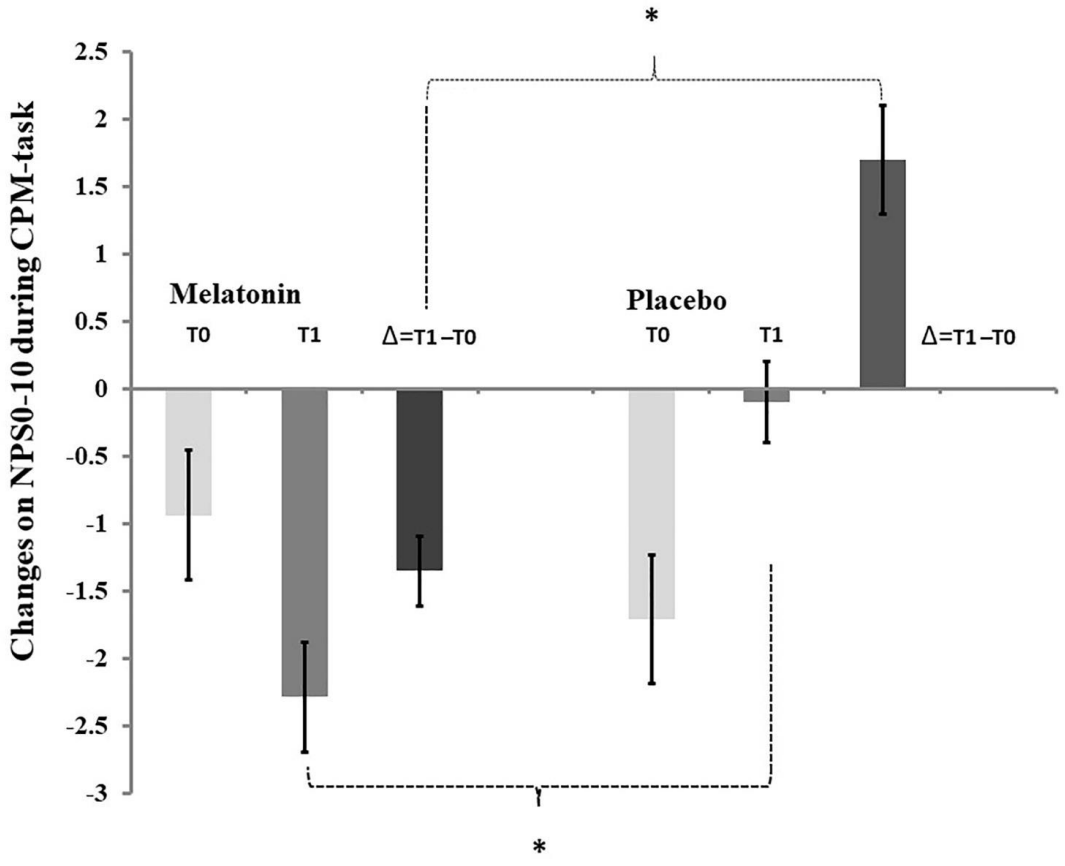
*
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
Changes on NPS0-10 during CPM-task
Melatonin
T0
T1
Δ=T1 –T0
Placebo
T0
T1
Δ=T1 –T0
*

Figure 3.

Table 1. Baseline demographic and clinical characteristics according to treatment group. Data are presented as mean standard deviation (SD) (n=36).

Variables	*Melatonin (n = 18)*	*Placebo (n = 18)*	*P-value*
Age (years)	54.24 (10.59)	54.11 (9.15)	0.97
Formal education (years)	9.29 (4.04)	6.94 (2.57)	0.08†
Body Mass Index (kg/m^2)	28.0 (6.14)	29.94 (5.70)	0.25†
Visual Analogue Scale (0-100)	50 (20.00)	50 (16.48)	0.80
Brain-Derived Neurotrophic Factor (ng / mL)	42.92 (17.54)	42.24 (23.95)	0.92
Tropomyosin receptor kinase B (ng / mL)	0.48 (0.25)	0.47 (0.50)	0.49
Protein S100 Beta (pg / mL)	38.16 (12.42)	32.37 (8.93)	0.21
Pittsburgh Sleep Quality Index	8.24 (3.97)	8.44 (2.83)	0.86
Beck Depression Inventory II	11.41 (7.73)	10.83 (5.11)	0.79
Chronic disease			
Hypertension	7 (38.9%) / 11 (61.1%)	8 (44.4%) / 10 (55.6%)	
Hypothyroidism	3 (16.7%) / 15 (83.3%)	1 (5.6%) / 17 (94.4%)	
Diabetes mellitus	1 (5.6%) / 17 (94.4%)	1 (5.6%) / 17 (94.4%)	
Asthma	1 (5.6%) / 17 (94.4%)	1 (5.6%) / 17 (94.4%)	
Psychotropic medication (yes / no) *			
Selective serotonin reuptake inhibitors	3 (16.7%) / 15 (83.3%)	3 (16.7%) / 15 (83.3%)	
Tricyclics	1 (5.6%) / 17 (94.4%)	2 (11.1%) / 16 (88.9%)	
Benzodiazepines	3 (16.7%) / 15 (83.3%)	4 (22.2%) / 14 (77.8%)	
Antipsychotics	1 (5.6%) / 17 (94.4%)	_____	
Chemotherapy regiments (yes / no)			
ACT (doxorubicin plus cyclophosphamide followed by weekly paclitaxel) [1]	9 (50%) / 9 (50%)	9 (50%) / 9 (50%)	
AC (doxorubicin plus cyclophosphamide) [1]	5 (27.8%) / 13 (72.2%)	2 (11.1%) / 16 (88.9%)	
ACTH (doxorubicin plus cyclophosphamide followed by paclitaxel plus trastuzumab) [1]	2 (11.1%) / 16 (88.9%)	3 (16.7%) / (83.3%)	
TAC (docetaxel, doxorubicin, and cyclophosphamide) [2]	1 (5.6%) / 17 (94.4%)	2 (11.1%) / 16 (88.9%)	
TC (docetaxel plus cyclophosphamide) [2]	1 (5.6%) / 17 (94.4%)	2 (11.1%) / 16 (88.9%)	

† Mann-Whitney non-parametric test was used. Independent t-tests were applied to all other measures.
** Three patients use more than one psychotropic medication.*
Prophylaxis for infusion reactions:
[1] Dexamethasone 20 mg IV 30 minutes before drug administration.
[2] Dexamethasone 8 mg orally every 12 hours starting one day prior to docetaxel administration.

Table 2. Pain psychophysical measures and serum markers of neuroplasticity state at treatment end according to melatonin or placebo groups. Data are presented as the mean prior, post-treatment and Δ-value (post-minus prior) and standard deviation (SD) (n=36).

	Placebo (n=18)		***Melatonin (n=18)***		
	Mean (SD)	**Δ-value**	***Mean (SD)***	**Δ-value**	***P-value****
Primary outcome					
Conditional Pain Modulation: change on NPS (0-10) during CPM-task					
Baseline	-1.81 (1.67)	1.70 (1.45)	- 0.94 (1.61)	-1.35 (1.11)	<0.001
End treatment	-0.10 (1.52)		-2.29 (1.61)		
Secondary outcomes					
Heat Pain Threshold					
Baseline	40.99 (2.52)	-3.05 (2.74)	38.47 (2.58)	2.46 (1.98)	<0.001
End treatment	37.94 (2.99)		40.99 (1.92)		
Heat Pain Tolerance					
Baseline	49.78 (2.62)	1.18 (1.95)	49.01 (2.66)	1.32 (2.07)	0.001
End treatment	48.59 (2.96)		50.33 (1.79)		
Brain-derived neurotrophic factor (BDNF)					
Baseline	40.88 (23.78)	1.87 (7.17)	41.65 (17.72)	-20.44 (17.17)	<0.001
End treatment	42.76 (17.75)		21.31 (7.18)		
Tropomyosin Kinase Receptor B (TrkB)					
Baseline	0.47 (0.50)	0.46 (0.17)	0.56 (0.39)	-0.15 (0.18)	0.003
End treatment	0.52 (0.46)		0.41 (0.37)		
S100 calcium binding protein B protein (S100B)					
Baseline	33.21 (9.25)	2.89 (11.18)	38.17 (12.42)	-11.16 (9.75)	<0.001
End treatment	36.11 (12.19)		26.96 (8.45)		
Pittsburgh Sleep Quality Assessment					
Baseline	8.44 (2.83)	2.83 (2.31)	8.24 (3.98)	-3.18 (2.01)	<0.001
End treatment	11.06 (3.35)		5.06 (3.34)		
Beck Depression Inventory II					
Baseline	10.83 (5.11)	3.72 (5.21)	11.41 (7.73)	-4.71 (5.83);	<0.001
End treatment	14.56 (7.76)		6.41 (4.57)		

** Correspond to comparisons of Δ-value by the t-test for independent sample.*

Table 3. MANCOVA model to compare the treatment effect in the Δ-value of psychophysical pain measures and the neuroplasticity state considering melatonin's effect, depressive symptoms and sleep quality (n=36).

(A) Main effects						
Corrected Model		***Type III Sum of Squares***	***df***	***Mean Square***	***F***	***P-value***
Dependent Variables						
Δ- Changes on NPS0-10 during CPM-task		102.47[a]	4	25.62	10.99	<0.01
Δ- Heat pain threshold		285.03[b]	4	71.26	13.18	<0.01
Δ-Heat pain tolerance		79.17[c]	4	19.79	5.56	<0.01
Δ-Brain-derived neurotrophic factor (BDNF)		5176.77[d]	4	1294.19	7.89	<0.01
Δ- Tropomyosin receptor kinase B (TrkB)		0.62[e]	4	0.16	6.13	<0.01
Δ- S100 calcium binding protein B (S-100B)		1871.64[f]	4	467.91	3.88	0.01
Intercept						
Δ- Changes on NPS0-10 during CPM-task		4.40	1	4.40	1.89	0.18
Δ- Heat pain threshold		1.18	1	1.18	0.22	0.65
Δ-Heat pain tolerance		1.47	1	1.47	0.41	0.53
Δ-Brain-derived neurotrophic factor (BDNF)		2133.52	1	2133.52	13.01	<0.01
Δ- Tropomyosin receptor kinase B (TrkB)		0.16	1	0.16	6.37	0.02
Δ- S100 calcium binding protein B (S-100B)		405.62	1	405.61	3.36	0.08
Treatment group						
Δ- Changes on NPS0-10 during CPM-task		59.85	1	59.85	25.69	<0.01
Δ- Heat pain threshold		154.14	1	154.14	28.50	<0.01
Δ-Heat pain tolerance		60.56	1	60.56	17.01	<0.01
Δ-Brain-derived neurotrophic factor (BDNF)		2375.01	1	2375.01	14.48	<0.01
Δ- Tropomyosin receptor kinase B (TrkB)		0.45	1	0.46	18.01	<0.01
Δ- S100 calcium binding protein B (S-100B)		703.52	1	703.52	5.83	0.02
Δ-Pittsburgh Sleep Quality Index						
Δ- Changes on NPS0-10 during CPM-task		9.95	1	9.95	4.27	0.048
Δ- Heat pain threshold		24.57	1	24.57	4.54	0.042
Δ-Heat pain tolerance		14.37	1	14.37	4.04	0.054
Δ-Brain-derived neurotrophic factor (BDNF)		208.11	1	208.11	1.27	0.269
Δ- Tropomyosin receptor kinase B (TrkB)		0.11	1	0.11	4.32	0.047
Δ- S100 calcium binding protein B (S-100B)		8.18	1	8.175	0.08	0.796
Δ-Beck Depression Inventory-II						
Δ- Changes on NPS0-10 during		0.32	1	0.32	0.14	0.71
Δ- Heat pain threshold		0.03	1	0.03	0.01	0.94
Δ-Heat pain tolerance		4.79	1	4.79	1.35	0.26
Δ-Brain-derived neurotrophic factor (BDNF)		2.47	1	2.47	0.06	0.90
Δ- Tropomyosin receptor kinase B (TrkB)		0.04	1	0.04	1.59	0.22
Δ- S100 calcium binding protein B (S-100B)		14.71	1	14.71	0.12	0.73
Grupo * Δ-Pittsburgh Sleep Quality Index						
Δ- Changes on NPS0-10 during CPM-task		1.42	1	1.41	0.61	0.44
Δ- Heat pain threshold		0.13	1	0.11	0.02	0.89
Δ-Heat pain tolerance		3.86	1	3.86	1.09	0.31
Δ-Brain-derived neurotrophic factor (BDNF)		419.34	1	419.34	2.56	0.12
Δ- Tropomyosin receptor kinase B (TrkB)		0.08	1	0.08	3.00	0.09
Δ- S100 calcium binding protein B (S-100B)		71.13	1	71.13	0.59	0.45
(B) Coefficients						
		Beta	***SEM***	***t***	***P-value***	***CI 95***
Dependent variable: Δ- Changes on NPS (0-10) during the CPM-task						
Treatment group	Melatonin	-4.97	0.98	-5.07	0.00*	(-6.98 to
	Placebo	0[reference]				

Δ-Pittsburgh Sleep Quality Index		-0.37	0.17	-2.20	0.03*	(-0.70 t
Δ-Beck Depression Inventory-II		-0.02	0.05	-0.37	0.71	(-0.12 t
Interaction						
Melatonin* Δ-Pittsburgh Sleep Quality Index		0.20	0.27	0.78	0.44	(-0.33 t
Placebo* Δ-Pittsburgh Sleep Quality Index		0[reference]				
Dependent variable: Δ- Heat pain threshold						
Treatment group	Melatonin	7.981	1.49	5.34	0.00*	(4.92 to
	Placebo	0[reference]				
Δ-Pittsburgh Sleep Quality Index		0.44	0.25	1.75	0.09	(-0.07 t
Δ-Beck Depression Inventory-II		-0.006	0.08	-0.08	0.94	(-0.16 t
Interaction						
Melatonin* Δ-Pittsburgh Sleep Quality Index		-0.06	0.40	-0.14	0.88	(-0.88 t
Placebo* Δ-Pittsburgh Sleep Quality Index		0[reference]				
Dependent variable: Δ-Heat pain tolerance						
Treatment group	Melatonin	5.00	1.21	4.12	0.00*	(2.52 t
	Placebo	0[reference]				
Δ-Pittsburgh Sleep Quality Index		0.49	0.20	2.38	0.02*	(0.06 t
Δ-Beck Depression Inventory-II		0.07	0.06	1.16	0.25	(-.006 t
Interaction						
Melatonin* Δ-Pittsburgh Sleep Quality Index		-0.34	0.33	-1.04	0.30	(-1.0 t
Placebo * Δ-Pittsburgh Sleep Quality Index		0[reference]				
Dependent variable: Δ-Brain-derived neurotrophic factor (BDNF)						
Treatment group	Melatonin	-31.33	8.23	-3.80	0.00*	(-48.16 t
	Placebo	0[reference]				
Δ-Pittsburgh Sleep Quality Index		0.58	1.39	0.42	0.68	(-2.26 t
Δ-Beck Depression Inventory-II		-0.05	0.42	-0.12	0.90	(-0.93 t
Interaction						
Melatonin* Δ-Pittsburgh Sleep Quality Index		-3.55	2.22	-1.56	0.12	(-8.09 t
Placebo* Δ-Pittsburgh Sleep Quality Index		0[reference]				
Dependent variable: Δ- Tropomyosin receptor kinase B (TrkB)						
Treatment group	Melatonin	-0.43	.102	-4.24	0.00*	(-0.64 t
	Placebo	0[reference]				
Δ-Pittsburgh Sleep Quality Index		-0.004	.017	0.20	0.84	(-0.04 t
Δ-Beck Depression Inventory-II		-0.007	.005	-1.26	0.21	(-0.02 t
Interaction						
Melatonin* Δ-Pittsburgh Sleep Quality Index		-0.05	0.03	-1.73	0.09	(-0.10 t
Placebo* Δ-Pittsburgh Sleep Quality Index		0[reference]				
Dependent variable: Δ- S100 calcium binding protein B (S-100B)						
Treatment group	Melatonin	-17.05	7.06	-2.41	0.02*	(-31.49 t
	Placebo	0[reference]				
Δ-Pittsburgh Sleep Quality Index		0.49	1.18	0.41	0.68	(-1.94 t
Δ-Beck Depression Inventory-II		-0.13	0.36	-0.35	0.73	(-0.88 t
Interaction						
Melatonin* Δ-Pittsburgh Sleep Quality Index		-1.46	1.90	-0.77	0.45	(-5.35 t
Placebo* Δ-Pittsburgh Sleep Quality Index		0[reference]				

R Squared = 0.603 (Adjusted R Squared = 0.548) [a]

R Squared = 0.645 (Adjusted R Squared = 0.596) [b]

R Squared = 0.434 (Adjusted R Squared = 0.356) [c]

R Squared = 0.521 (Adjusted R Squared = 0.455) [d]

R Squared = 0.458 (Adjusted R Squared = 0.384) [e]

7 DISCUSSÃO

Os resultados deste estudo confirmam os benefícios do uso da melatonina em comparação com o placebo antes do primeiro ciclo de quimioterapia para câncer de mama (ACBC), na redução do tempo de execução no teste de trilhas (TMT-A-B), aumento da pontuação na recordação imediata e tardia e melhora do reconhecimento de palavras no teste de memória de Rey (RAVLT) e aumento das palavras recitadas durante o teste de fluência verbal ortográfico (COWAT). O teste de trilhas foi negativamente correlacionado com os níveis basais de TrkB e BDNF, respectivamente. No final do tratamento, as alterações de TrkB foram inversamente associadas com sintomas depressivos e qualidade do sono, mas não com o teste de trilhas. No entanto, a melatonina não alterou a capacidade de atenção sustentada e controle das respostas avaliadas pelo controle inibitório (Go / No-Go). Quando avaliada a função do sistema somato-sensorial, a melatonina neutralizou os efeitos neurotóxicos na função inibitória do sistema modulador descendente da dor (DPMS) avaliado pela mudança na escala numérica da dor durante a tarefa modulação condicionada da dor. A melatonina também aumentou o limiar de dor ao calor e a tolerância à dor pelo calor, enquanto reduziu os níveis séricos dos marcadores neuronais e astrocíticos (BDNF, TrkB e proteína S100-B). A análise mostrou que o efeito da melatonina no DPMS e no estado de neuroplasticidade não foi relacionado à qualidade do sono.

No geral, estes resultados sugerem que o estado de neuroplasticidade pode ser um marcador para explicar as diferenças substanciais do comprometimento da capacidade cognitiva de pacientes com câncer de mama. Embora o efeito da melatonina parece contrapor os efeitos neurotoxicos relacionados aos quimioterápicos, os níveis basais do BDNF e do seu receptor TrkB predisseram o efeito da melatonina na flexibilidade cognitiva. Hipotetiza-se que este efeito esteja relacionado ao efeito da melatonina na neuroinflamação com redução da síntese de citocinas pró-inflamatórias e de BDNF. De acordo com Dietrich et al. (2008), este efeito da melatonina na cascata inflamatória atenua o efeito do estresse oxidativo, a toxicidade celular direta e inflamação, contribuindo para a alteração da cinética celular no hipocampo, bem como ruptura neurovascular da barreira hematoencefálica. Embora a redução nos marcadores séricos relacionados a neurolpasticidade esteja alinhada ao efeito clínico que mostrou benefício da melatonina, os mecanismos subjecentes ainda não são conhecidos.

Outros efeitos obeservados nesta pesquisa, foi a capacidade da melatonina em neutralizar

os efeitos adversos da ACBC relacionados à disfunção no DPMS inibitório e o estado neuroplástico. Também observamos redução do BDNF enquanto melhora do DPMS, o que corrobora estudos anteriores em dor crônica em pacientes com endometriose (Schwertner et al., 2013) e fibromialgia (de Zanette et al., 2014). Assim, estes resultados suportam que a melatonina contra-regula a disrupção na sinalização BDNF-TrkB induzida pela ACBC, que é crucial para o desenvolvimento, plasticidade e remodelação de circuitos neuronais (Yu & Chen, 2011). Além disso, observamos uma diminuição da concentração sérica de S100B, o que sustenta que a melatonina fornece um efeito modulador na atividade astrocítica (Gonçalves et al., 2008).

Nossos resultados estão de acordo com estudos anteriores e revelam os benefícios da melatonina na melhora dos efeitos colaterais induzidos pela quimioterapia. Porém, estudos futuros são necessários com um número mais substancial de pacientes antes de definir conclusões sobre o impacto da melatonina na neurotoxicidade devido à quimioterapia.

8 CONSIDERAÇÕES FINAIS

Em conclusão, os resultados desta tese demostram os benefícios do uso de melatonina antes e durante com o primeiro ciclo de quimioterapia adjuvante para câncer de mama quando comparado ao placebo na melhoria da flexibilidade e atenção cognitiva, e proteção da disfunção do sistema modulador descendente da dor. Os benefícios cognitivos são modulados pelo estado de neuroplasticidade, bem como pelo efeito neuroprotetor dos sintomas depressivos e a qualidade do sono, e estão alinhados à alterações nos biomarcadores de neuroplasticidade. Além disso, aponta que as mudanças no estado de neuroplasticidade mediam o impacto da melatonina na dor. Dessa forma, demonstramos com este estudo que o uso da melatonina pode contra-regular os efeitos adversos da quimioterapia adjuvante para câncer de mama na função cognitiva e do sistema somata-sensorial, com benéficios na qualidade de vida e bem estar de pacientes durante o tratamento.

9 PERSPECTIVAS FUTURAS

O estudo dos efeitos e mecanismos da melatonina na adjuvância quimioterápica constitui um importante passo no campo científico. O aumento da amostragem do estudo, com o objetivo de alargar os resultados e a análise dos marcadores relativos à resposta ao tratamento e monitorização de pacientes com câncer de mama, constituem objetivos futuros importantes para este trabalho. O estudo poderá também ser expandido a outros modelos tumorais, de forma a avaliar o papel da melatonina em diferentes tipos de tumor.

Neste estudo, avaliou-se os efeitos da melatonina adjuvante ao tratamento quimioterápico na primeira sessão de quimioterapia adjuvante e diante dos resultados faz-se importante estudos que extendam o tempo de tratamento e o acompanhamento a longo prazo destas pacientes. A continuação deste estudo é, então, importante, de forma a melhorar a compreensão dos mecanismos da melatonina a longo prazo.

10 REFERÊNCIAS

Abe K, Robison GA, Liddle GW, Butcher RW, Nicholson WE, Baird CE. Role of cyclic AMP in mediating the effects of MSH, norepinephrine, and melatonin on frog skin color. Endocrinology. 1969 Oct;85(4):674-82.

Abeles AM, Pillinger MH, Solitar BM, Abeles M. Narrative review: the pathophysiology of fibromyalgia. Ann Intern Med. 2007 May 15;146(10):726-34.

Ahles, Tim A.; Andrew J. Saykin; Brenna C. McDonald, Charlotte T. Furstenberg, Bernard F. Cole, Brett S. Hanscom, Tamsin J. Mulrooney, Gary N. Schwartz, Peter A. Kaufman

Alam MA1, Subramanyam Rallabandi VP1, Roy PK1. Systems Biology of Immunomodulation for Post-Stroke Neuroplasticity: Multimodal Implications of Pharmacotherapy and Neurorehabilitation. Front Neurol. 2016 Jun 28;7:94. doi: 10.3389/fneur.2016.00094. eCollection 2016.

Allemani C, Weir HK, Carreira H, Harewood R, Spika D, Wang X-S, Bannon F, Ahn JV, Johnson CJ, Bonaventure A (2015) Global surveillance of cancer survival 1995–2009: analysis of individual data for 25 676 887 patients from 279 population-based registries in 67 countries (CONCORD-2). Lancet 385(9972):977–1010

Altimus CM, Guler AD, Alam NM, Arman AC, Prusky GT, et al. Rod photoreceptors drive circadian photoentrainment across a wide range of light intensities. Nature neuroscience. 2010;13:1107–12.

Alvarez-García V, González A, Alonso-González C, Martínez-Campa C, Cos S.Melatonin interferes in the desmoplastic reaction in breast cancer by regulating cytokine production. J Pineal Res. 2012 Apr;52(3):282-90. doi: 10.1111/j.1600-079X.2011.00940.x. Epub 2011 Dec 12.

Alvarez-García V, González A, Alonso-González C, Martínez-Campa C, Cos S. Regulation of vascular endothelial growth factor by melatonin in human breast cancer cells. J Pineal Res. 2013 May; 54(4):373-80.

Ancoli-Israel S, Liu L, Rissling M, et al. (2014). Sleep, fatigue, depression, and circadian activity rhythms in women with breast cancer before and after treatment: A 1-year longitudinal study. Support Care Cancer. 22:2535–45.

Andersen BL, Yang H-C, Farrar WB, et al. Psychologic Intervention Improves Survival for Breast Cancer Patients: A Randomized Clinical Trial. Cancer. 2008;113(12):3450-3458. doi:10.1002/cncr.23969.

Asayama K, Yamadera H, Ito T, Suzuki H, Kudo Y, Endo S. Double blind study of melatonin effects on the sleep-wake rhythm, cognitive and non-cognitive functions in Alzheimer type dementia. J Nippon Med Sch 2003 70(4):334-41.

Asher A, Fu JB, Bailey C, Hughes JK. Fatigue among patients with brain tumors. CNS Oncol. 2016;5(2):91–100. doi:10.2217/cns-2015-0008

Auger RR, Burgess HJ, Emens JS, Deriy LV, Thomas SM, Sharkey KM.Clinical Practice Guideline for the Treatment of Intrinsic Circadian Rhythm Sleep-Wake Disorders: Advanced Sleep-Wake Phase Disorder (ASWPD), Delayed Sleep-Wake Phase Disorder (DSWPD), Non-24-Hour Sleep-Wake Rhythm Disorder (N24SWD), and Irregular Sleep-Wake Rhythm Disorder (ISWRD). An Update for 2015: An American Academy of Sleep Medicine Clinical Practice Guideline. J Clin Sleep Med. 2015 Oct 15;11(10):1199-236. doi: 10.5664/jcsm.5100.

Aviram J1, Shochat T1, Pud D1. Pain perception in healthy young men is modified by time-of-day and is modality dependent. Pain Med. 2015 Jun;16(6):1137-44. doi: 10.1111/pme.12665. Epub 2014 Dec 28.

Badger T, Segrin C, Meek P, Lopez AM, Bonham E. Profiles of women with breast cancer: who responds to a telephone interpersonal counseling intervention. J Psychosoc Oncol. 2005;23(2-3):79-99.

Bahuguna, J., Aertsen, A. & Kumar, A. Controlling the Go / No-Go decision threshold in the striatum. BMC Neurosci. 14, P228 (2013).

Becker-André M1, Wiesenberg I, Schaeren-Wiemers N, André E, Missbach M, Saurat JH, Carlberg C. Pineal gland hormone melatonin binds and activates an orphan of the nuclear receptor superfamily. J Biol Chem. 1994 Nov 18;269(46):28531-4.

Benoit G1, Cooney A, Giguere V, Ingraham H, Lazar M, Muscat G, Perlmann T, Renaud JP, Schwabe J, Sladek F, Tsai MJ, Laudet V. International Union of Pharmacology. LXVI. Orphan nuclear receptors. Pharmacol Rev. 2006 Dec;58(4):798-836.

Berger AM1, Farr L. The influence of daytime inactivity and nighttime restlessness on cancer-related fatigue. Oncol Nurs Forum. 1999 Nov-Dec;26(10):1663-71

Berger, L.A. Farr, B.R. Kuhn, P. Fischer, S. AgrawalValues of sleep/wake, activity/rest, circadian rhythms, and fatigue prior to adjuvant breast cancer chemotherapy. J Pain Symptom Manage, 33 (4) (2007), pp. 398-409.

Blasco-Gutiérrez MJ, José-Crespo IJ, Zozaya-Alvarez E, Ramos-Sánchez R, García-Atarés N. TrkC: a new predictive marker in breast cancer? Cancer Invest. 2007 Sep;25(6):405-10.

Blask DE, Sauer LA, Dauchy RT. Melatonin as a chronobiotic/anticancer agent: cellular, biochemical, and molecular mechanisms of action and their implications for circadian-based cancer therapy. Current Topics in Medicinal Chemistry. 2002;2:113–132

Blask DE, 2009. Melatonin, sleep disturbance and cancer risk. Sleep Med. Rev. 13 (August), 257–264.

Borin TF1, Arbab AS2, Gelaleti GB1,3, Ferreira LC1,3, Moschetta MG1, Jardim-Perassi BV1, Iskander AS2, Varma NR4, Shankar A2, Coimbra VB1, Fabri VA1, de Oliveira JG5, Zuccari DA1,3. Melatonin decreases breast cancer metastasis by modulating Rho-associated kinase protein-1 expression. J Pineal Res. 2016 Jan;60(1):3-15. doi: 10.1111/jpi.12270. Epub 2015 Oct 20.

Botelho, Leonardo M., Leon Morales-Quezada, Joanna R. Rozisky, Aline P. Brietzke, Iraci L. S. Torres, Alicia Deitos, Felipe Fregni, Wolnei Caumo. A Framework for Understanding the Relationship between Descending Pain Modulation, Motor Corticospinal, and Neuroplasticity Regulation Systems in Chronic Myofascial Pain. Front Hum Neurosci. 2016; 10: 308. Published online 2016 Jun 27. doi: 10.3389/fnhum.2016.00308

Boutin JA, Chatelain-Egger F, Vella F, Delagrange P, Ferry G. Quinone reductase 2 substrate specificity and inhibition pharmacology. Chem Biol Interact. 2005 Feb 10;151(3):213-28.

Bower JE, Ganz PA, Desmond KA, Rowland JH, Meyerowitz BE, Belin TR. Fatigue in breast cancer survivors: occurrence, correlates, and impact on quality of life. J Clin Oncol. 2000;18(4):743.

Bower JE. Behavioral symptoms in patients with breast cancer and survivors. J Clin Oncol. 2008;26(5):768–777. doi:10.1200/JCO.2007.14.3248

Bower JE, Ganz PA, Irwin MR, Kwan L, Breen EC, Cole SW. Inflammation and behavioral symptoms after breast cancer treatment: do fatigue, depression, and sleep disturbance share a common underlying mechanism? J Clin Oncol. 2011 Sep 10;29(26):3517-22.

Brydon L, Roka F, Petit L, de Coppet P, Tissot M, Barrett P, Morgan PJ, Nanoff C, Strosberg AD, Jockers R. Dual signaling of human Mel1a melatonin receptors via G(i2), G(i3), and G(q/11) proteins. Mol Endocrinol. 1999 Dec;13(12):2025-38.

Bubenik GA. Gastrointestinal melatonin: localization, function, and clinical relevance. Dig Dis Sci. 2002;47:2336–2348.

Bui QU, Ostir GV, Kuo YF, Freeman J, Goodwin JS (2005) Relationship of depression to patient satisfaction: findings from the barriers to breast cancer study. Breast Cancer Res Treat 89:23–28

Burgess C, Cornelius V, Love S, Graham J, Richards M, Ramirez A (2005) Depression and anxiety in women with early breast cancer: five year observational cohort study. BMJ 330:702

Buscemi N, Vandermeer B, Hooton N, Pandya R, Tjosvold L, Hartling L, Vohra S, Klassen TP, Baker G. Efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction: meta-analysis. BMJ. 2006 Feb 18;332(7538):385-93. Epub 2006 Feb 10. Review.

Caplette-Gingras, J. Savard, M.H. Savard, H. IversIs insomnia associated with cognitive impairments in breast cancer patients? Behav Sleep Med, 11 (4) (2013), pp. 239-257

Capsoni S, Tongiorgi E, Cattaneo A, Domenici L. Differential regulation of brain-derived neurotrophic factor messenger RNA cellular expression in the adult rat visual cortex. Neuroscience. 1999;93(3):1033-40.

Cash E, Sephton SE, Chagpar AB, et al. (2015). Circadian disruption and biomarkers of tumor progression in breast cancer patients awaiting surgery. Brain Behav Immun. 48:102–14.

Castellon, Steven A.; Patricia A. Ganz; Julienne E. Bower, Laura Petersen, Laura Abraham & Gail A. Greendale. Neurocognitive Performance in Breast Cancer Survivors Exposed to Adjuvant Chemotherapy and Tamoxifen. Journal of Clinical and Experimental Neuropsychology Vol. 26, Iss. 7, 2004.

Caumo W1, Torres F, Moreira NL Jr, Auzani JA, Monteiro CA, Londero G, Ribeiro DF, Hidalgo MP. The clinical impact of preoperative melatonin on postoperative outcomes in patients undergoing abdominal hysterectomy. Anesth Analg. 2007 Nov;105(5):1263-71, table of contents.

Cecilio AP1, Takakura ET1, Jumes JJ1, Dos Santos JW1, Herrera AC2, Victorino VJ3, Panis C1. Breast Cancer (Dove Med Press). 2015 Jan 29;7:43-9.Breast cancer in Brazil: epidemiology and treatment challenges. doi: 10.2147/BCTT.S50361. eCollection 2015.

Chaves ML, Camozzato AL, Ferreira ED, Piazenski I, Kochhann R, Dall'igna O, et al. Serum levels of S100B and NSE in Alzheimer's disease patients. J Neuroinflammation [Internet]. 2010;7(1):6.

Chen X1,2, Wang X1,2, Tang L1, Wang J2, Shen C1,2, Liu J1,2, Lu S1, Zhang H1, Kuang Y2, Fei J2, Wang Z1,2,3. Nhe5 deficiency enhances learning and memory via upregulating Bdnf/TrkB signaling in mice. Am J Med Genet B Neuropsychiatr Genet. 2017 Dec;174(8):828-838. doi: 10.1002/ajmg.b.32600. Epub 2017 Oct 5.

Chojnacki C, Wisniewska-Jarosinska M, Walecka-Kapica E, Klupinska G, Jaworek J, Chojnacki J. Evaluation of melatonin effectiveness in the adjuvant treatment of ulcerative colitis. J Physiol Pharmacol. 2011 Jun;62(3):327-34.

Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Breast Cancer V.2.2017. Disponível em: http://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Acessado em dezembro, 2017.

Cochrane A, Robertson IH, Coogan AN. Association between circadian rhythms, sleep and cognitive impairment in healthy older adults: an actigraphic study. J Neural Transm 2012;119:1233–1239.

Cohen M, Lippman M & Chabner B 1978 Role of the pineal gland in the aetiology and treatment of breast cancer. Lancet 2 814–816.

Coleman MP & Reiter RJ. Breast cancer, blindness and melatonin. European Journal of Cancer 28 501–503, 1992.

Colleoni M, Mandala M, Peruzzotti G, Robertson C, Bredart A, Goldhirsch A. Depression and degree of acceptance of adjuvant cytotoxic drugs. Lancet. 2000 Oct 14;356(9238):1326-7.

Cos S, et al. Melatonin inhibits the growth of DMBA-induced mammary tumors by decreasing the local biosynthesis of estrogens through the modulation of aromatase activity. International Journal of Cancer. 2006;118:274–278.

Costa, AR; Fontes F, Pereira S; et al. Impact of breast cancer treatments on sleep disturbances

– A systematic review, The Breast, Volume 23, Issue 6, 2014, Pages 697-709, ISSN 0960-9776, https://doi.org/10.1016/j.breast.2014.09.003.

Cox, Cathy R.; Reid-Arndt, Stephanie A.; Jamie Arndt, Richard P. Moser. Considering the Unspoken: The Role of Death Cognition in Quality of Life among Women with and without Breast Cancer. J Psychosoc Oncol. Author manuscript; Published in final edited form as: J Psychosoc Oncol. 2012 Jan; 30(1): 128–139. doi: 10.1080/07347332.2011.633980

Crowe DL1, Chandraratna RA. A retinoid X receptor (RXR)-selective retinoid reveals that RXR-alpha is potentially a therapeutic target in breast cancer cell lines, and that it potentiates antiproliferative and apoptotic responses to peroxisome proliferator-activated receptor ligands. Breast Cancer Res. 2004;6(5):R546-55. Epub 2004 Jul 23.

Cunha, R. Brambilla, K.L. ThomasA simple role for BDNF in learning and memory? Front. Mol. Neurosci., 3 (2010), pp. 1-14

da Silva, Nádia Regina Jardim; Gabriela Laste, Alícia Deitos, Luciana Cadore Stefani, Gustavo Cambraia-Canto, Iraci L. S. Torres, Andre R. Brunoni, Felipe Fregni, Wolnei Caumo. Combined neuromodulatory interventions in acute experimental pain: assessment of melatonin and non-invasive brain stimulation. Front Behav Neurosci. 2015; 9: 77. Published online 2015 Mar 31. doi: 10.3389/fnbeh.2015.00077

Dam Fv, Schagen S, Muller M, et al. Impairment of cognitive function in women receiving adjuvant treatment for high-risk breast cancer: high-dose versus standard-dose chemotherapy. J Natl Cancer Inst 1998;90:210–8

Danforth DN Jr, Tamarkin L, Lippman ME. Melatonin increases oestrogen receptor binding activity of human breast cancer cells. Nature. 1983 Sep 22-28;305(5932):323-5.

De Castro Mattos JS, Caleffi M, Da Costa Vieira RE (2013) Rastraemento mamográfico no Brasil: resultados preliminares. Rev Brasil Mastol 23(1):22–27

de Zanette SA, Vercelino R, Laste G, Rozisky JR, Schwertner A, Machado CB, Xavier F, de Souza IC, Deitos A, Torres IL, Caumo W. Melatonin analgesia is associated with improvement of the descending endogenous pain-modulating system in fibromyalgia: a phase II, randomized, double-dummy, controlled trial. BMC Pharmacol Toxicol. 2014;15:40.

Debess J1, Riis JØ, Pedersen L, Ewertz M. Cognitive function and quality of life after surgery for early breast cancer in North Jutland, Denmark. Acta Oncol. 2009;48(4):532-40. doi: 10.1080/02841860802600755.

del Río B1, García Pedrero JM, Martínez-Campa C, Zuazua P, Lazo PS, Ramos S. Melatonin, an endogenous-specific inhibitor of estrogen receptor alpha via calmodulin. J Biol Chem. 2004 Sep 10;279(37):38294-302. Epub 2004 Jun 30.

Descamps S, Toillon RA, Adriaenssens E, Pawlowski V, Cool SM, Nurcombe V, Le Bourhis X, Boilly B, Peyrat JP, Hondermarck H. Nerve growth factor stimulates proliferation and survival of human breast cancer cells through two distinct signaling pathways. J Biol Chem. 2001;276(21):17864–17870. doi: 10.1074/jbc.M010499200.

Detanico, B. C. et al. Antidepressant-like effects of melatonin in the mouse chronic mild stress model. Eur. J. Pharmacol. 607, 121–125 (2009).

Dietrich, J., Monje, M., Wefel, J. & Meyers, C. Clinical Patterns and Biological Correlates of Cognitive Dysfunction Associated with Cancer Therapy. Oncologist 13, 1285–1295 (2008).Dimitrov S, Lang T, Tieken S, Fehm HL, Born J. Sleep associated regulation of T helper 1/T helper 2 cytokine balance in humans. Brain Behav Immun 2004;18:341–8

Dillon DC1, Easley SE, Asch BB, Cheney RT, Brydon L, Jockers R, Winston JS, Brooks JS, Hurd T, Asch HL. Differential expression of high-affinity melatonin receptors (MT1) in normal and malignant human breast tissue. Am J Clin Pathol. 2002 Sep;118(3):451-8.

Dillon, D. G. & Pizzagalli, D. A. Inhibition of action, thought, and emotion: A selective neurobiological review. Appl. Prev. Psychol. 12, 99–114 (2007).

Donato R. S100: A multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol. 2001;33(7):637–68.

Donato R, R. Cannon B, Sorci G, Riuzzi F, Hsu K, J. Weber D, et al. Functions of S100 Proteins. Curr Mol Med [Internet]. 2013;13(1):24–57.

Durmer JS1, Dinges DF. Neurocognitive consequences of sleep deprivation. Semin Neurol. 2005 Mar;25(1):117-29.

Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Long-term outcomes for neoadjuvant versus adjuvant chemotherapy in early breast cancer: meta-analysis of individual patient data from ten randomised trials. Lancet Oncol. 2018;19(1):27–39. doi:10.1016/S1470-2045(17)30777-5

Escobar, R. Salgado-Delgado, Gonzalez-Guerra, A. Tapia Osorio, M.Angeles-Castellanos, R.M. BuijsCircadian disruption leads to loss of homeostasis and disease. Sleep Disord., 2011 (2012), p. e964510

Eversley R, Estrin D, Dibble S, Wardlaw L, Pedrosa M, Favila-Penney W. Post-treatment symptoms among ethnic minority breast cancer survivors. Oncol Nurs Forum. 2005 Mar 5;32(2):250-6

Fann JR, Thomas-Rich AM, Katon WJ, Cowley D, Pepping M, McGregor BA, Gralow J (2008) Major depression after breast cancer: a review of epidemiology and treatment. Gen Hosp Psychiatry 30:112–126.

Ferracioli-Oda E1, Qawasmi A, Bloch MH. Meta-analysis: melatonin for the treatment of primary sleep disorders. PLoS One. 2013 May 17;8(5):e63773. doi: 10.1371/journal.pone.0063773. Print 2013.

Figueiredo FW dos S, Almeida TC do C, Cardial DT, Maciel É da S, Fonseca FLA, Adami F. The role of health policy in the burden of breast cancer in Brazil. BMC Women's Health. 2017;17:121. doi:10.1186/s12905-017-0477-9.

Fiorentino L, Ancoli-Israel S. Insomnia and its treatment in women with breast cancer. Sleep Med Rev. 2006 Dec;10(6):419-29. Epub 2006 Sep 11. Review.

Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, et al. The Global Burden of Cancer 2013. Global Burden of Disease Cancer Collaboration. JAMA Oncol. 2015;1(4):505–27

Fitzmaurice J. "How does it feel?" Mastectomy, radiotherapy, and adjuvant chemotherapy.N Z Nurs J. 1981 Oct;74(10):9-11, 33. No abstract available.

Fu; Lee, 2003. The circadian clock: pacemaker and tumour suppressor. Nat. Rev. Cancer, 3 (2003), pp. 350-361.

Fuller, P. M., Gooley, J. J., & Saper, C. B. (2006). Neurobiology of the Sleep-Wake Cycle: Sleep Architecture, Circadian Regulation, and Regulatory Feedback. Journal of Biological Rhythms, 21(6), 482–493.

Furio AM1, Brusco LI, Cardinali DP. Possible therapeutic value of melatonin in mild cognitive impairment: a retrospective study. J Pineal Res. 2007 Nov;43(4):404-9.

Galano A. On the direct scavenging activity of melatonin towards hydroxyl and a series of peroxyl radicals. Phys Chem Chem Phys. 2011 Apr 21;13(15):7178-88. doi: 10.1039/c0cp02801k. Epub 2011 Mar 15.

Ganz PA. Cognitive dysfunction following adjuvant treatment of breast cancer: a new dose-limiting toxic effect? J Natl Cancer Inst. 1998; 90(3):182–183.

Garaulet M1, Madrid JA. Chronobiological aspects of nutrition, metabolic syndrome and obesity. Adv Drug Deliv Rev. 2010 Jul 31;62(9-10):967-78. doi: 10.1016/j.addr.2010.05.005. Epub 2010 May 24.

Gebhart GF (2004) Descending modulation of pain. Neurosci Behav Rev 27:729–737.
Asian Pac J Cancer Prev. 2016;17(S3):43-6.

Ghoncheh M, Pournamdar Z, Salehiniya H. Incidence and Mortality and Epidemiology of Breast Cancer in the World. Asian Pac J Cancer Prev. 2016;17(S3):43-6.

Girgert R1, Hanf V, Emons G, Gründker C. Membrane-bound melatonin receptor MT1 down-regulates estrogen responsive genes in breast cancer cells. J Pineal Res. 2009 Aug;47(1):23-31. doi: 10.1111/j.1600-079X.2009.00684.x. Epub 2009 Jun 10.

Given CW, Given B, Azzouz F, Kozachik S, Stommel M. Predictors of pain and fatigue in the year following diagnosis among elderly cancer patients. J Pain Symptom Manage. 2001 Jun;21(6):456-66.

Ghilardi JR, Freeman KT, Jimenez-Andrade JM, Mantyh WG, Bloom AP, Kuskowski MA, et al. Administration of a tropomyosin receptor kinase inhibitor attenuates sarcoma-induced nerve sprouting, neuroma formation and bone cancer pain. Mol Pain. 2010;6(December):1–12.

Gingis-Velitski S, Zetser A, Flugelman MY, Vlodavsky I, Ilan N. Heparanase induces

endothelial cell migration via protein kinase B/Akt activation. J Biol Chem. 2004 May 28;279(22):23536-41. Epub 2004 Mar 24.

Glass CK1, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev. 2000 Jan 15;14(2):121-41.

Gögenur I, Middleton B, Kristiansen VB, Skene DJ, Rosenberg J. Disturbances in melatonin and core body temperature circadian rhythms after minimal invasive surgery. Acta Anaesthesiol Scand. 2007 Sep; 51(8):1099-106.

Gómez-Moreno G, Guardia J, Ferrera MJ, Cutando A, Reiter RJ. Melatonin in diseases of the oral cavity. Oral Dis. 2010 Apr;16(3):242-7. doi: 10.1111/j.1601-0825.2009.01610.x. Epub 2009 Aug 4. Review.

Gonçalves CA, Concli Leite M, Nardin P. Biological and methodological features of the measurement of S100B, a putative marker of brain injury. Clin Biochem. 2008;41(10–11):755–63.

Goradel NH, Asghari MH, Moloudizargari M, Negahdari B, Haghi-Aminjan H, Abdollahi M. Melatonin as an angiogenesis inhibitor to combat cancer: Mechanistic evidence. Toxicol Appl Pharmacol. 2017 Nov 15;335:56-63. doi: 10.1016/j.taap.2017.09.022. Review.

Grunberg, R.R. Deuson, P. Mavros, O.Geling, M. Hansen, G. Cruciani, B. Daniele, G. De Pouvourville, E.B. Rubenstein, G.DaugaardIncidence of chemotherapy-induced nausea and emesis after modern antiemetics. Cancer, 100 (2004), pp. 2261-2268

Giacinti L, Claudio PP, Lopez M, Giordano A. Epigenetic information and estrogen receptor alpha expression in breast cancer. Oncologist. 2006 Jan;11(1):1-8. Review.

Hansen J. Risk of breast cancer after night- and shift work: Current evidence and ongoing studies in Denmark. Cancer Causes Control. (2006). 17:531–7.

Hansen MV1, Andersen LT, Madsen MT, Hageman I, Rasmussen LS, Bokmand S, Rosenberg J, Gögenur I. Effect of melatonin on depressive symptoms and anxiety in patients undergoing breast cancer surgery: a randomized, double-blind, placebo-controlled trial. Breast Cancer Res Treat. 2014 Jun;145(3):683-95. doi: 10.1007/s10549-014-2962-2. Epub 2014 Apr 23.

Hansen, Melissa Voigt; Michael Tvilling Madsen, Lærke Toftegård Andersen, Ida Hageman, Lars Simon Rasmussen, Susanne Bokmand, Jacob Rosenberg, Ismail Gögenur. Effect of Melatonin on Cognitive Function and Sleep in relation to Breast Cancer Surgery: A Randomized, Double-Blind, Placebo-Controlled Trial. Int J Breast Cancer. 2014; 2014: 416531. Published online 2014 Aug 27. doi: 10.1155/2014/416531

Hansen MV, Madsen MT, Wildschiødtz G, Rosenberg J, Gögenur I. Sleep disturbances and changes in urinary 6-sulphatoxymelatonin levels in patients with breast cancer undergoing lumpectomy. Acta Anaesthesiol Scand. 2013 Oct;57(9):1146-53. doi: 10.1111/aas.12157. Epub 2013 Jul 12

Herxheimer A1, Petrie KJ. Melatonin for the prevention and treatment of jet lag. Cochrane

Database Syst Rev. 2002;(2):CD001520.

Hill, S. M. et al. Molecular mechanisms of melatonin anticancer effects. Integr. Cancer Ther. 8, 337–346 (2009).

Hjerl K, Andersen EW, Keiding N, Mortensen PB, Jorgensen T (2002) Increased incidence of affective disorders, anxiety disorders, and non-natural mortality in women after breast cancer diagnosis: a nation-wide cohort study in Denmark. Acta Psychiatr Scand 105:258–264

Hsiao, K. Chang, W. Kuo, C. Huang, Y. Liu, Y. Lai, et al. A longitudinal study of cortisol responses, sleep problems, and psychological well-being as the predictors of changes in depressive symptoms among breast cancer survivors. Psycho neuroendocrinology, 38 (3) (2013), pp. 356-366

Huang YT, Lai PC, Wu CC, Hsu SH, Cheng CC, Lan YF, Chiu TH. BDNF mediated TrkB activation is a survival signal for transitional cell carcinoma cells. Int J Oncol. 2010 Jun;36(6):1469-76.

Innominato PF, Levi FA, Bjarnason GA. Chronotherapy and the molecular clock: clinical implications in oncology. Adv Drug Deliv Rev 2010; 62:979–1001

Innominato PF, Giacchetti S, Bjarnason GA, et al. (2012). Prediction of overall survival through circadian rest-activity monitoring during chemotherapy for metastatic colorectal cancer. Int J Cancer. 131:2684–92.

Innominato PF et al. The effect of melatonin on sleep aglond quality of life in patients with advanced breast cancer. Support Care Cancer. (2016)

International Agency of Research Cancer, World Health Organization. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. 2012. http://globocan.iarc.fr. Accessado em novembro de 2016.

Jansen, Lina; Michael, Hoffmeister; Jenny Chang-Claude, Moritz Koch, Hermann Brenner, Volker Arndt. Age-Specific Administration of Chemotherapy and Long-Term Quality of Life in Stage II and III Colorectal Cancer Patients: A Population-Based Prospective Cohort. Oncologist. 2011 Dec; 16(12): 1741–1751. doi: 10.1634/theoncologist.2011-0124.

Jarrar MH1, Baranova A. PPARgamma activation by thiazolidinediones (TZDs) may modulate breast carcinoma outcome: the importance of interplay with TGFbeta signalling. J Cell Mol Med. 2007 Jan-Feb;11(1):71-87.

Jim, Heather S.L.; Kristin M. Phillips; Sari Chait, Leigh Anne Faul, Mihaela A. Popa, Yun-Hsiang Lee, Mallory G. Hussin, Paul B. Jacobsen, and Brent J. Small. Meta-Analysis of Cognitive Functioning in Breast Cancer Survivors Previously Treated With Standard-Dose Chemotherapy. Journal of Clinical Oncology 2012 30:29, 3578-3587

Jing YL, Wu Q Bin, Yuan XC, Li BW, Liu MM, Zhang XY, et al. Microvascular protective role of pericytes in melatonin-treated spinal cord injury in the C57BL/6 mice. Chin Med J (Engl). 2014;127(15):2808–13.

Jung-Hynes B, Reiter RJ, Ahmad N. Sirtuins, melatonin and circadian rhythms: building a bridge between aging and cancer. J Pineal Res. 2010 Jan;48(1):9-19. doi: 10.1111/j.1600-079X.2009.00729.x. Review.

Kalsbeek A, Buijs RM, 2002. Output pathways of the mammalian suprachiasmatic nucleus: coding circadian time by transmitter selection and specific targeting. Cell Tissue Res. 309 (July), 109–118.

Karege F, Schwald M, Cisse M. Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. Neurosci Lett. 2002;328(3):261–4.

Karege F, Schwald M, Cisse M. Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. Neurosci Lett. 2002;328(3):261–4. Kesler SR, Kent JS, O'Hara R. Prefrontal cortex and executive function impairments in primary breast cancer. Arch Neurol. 2011; 68(11):1447–1453.

Kärkelä J, Vakkuri O, Kaukinen S, Huang WQ, Pasanen M. The influence of anaesthesia and surgery on the circadian rhythm of melatonin. Acta Anaesthesiol Scand. 2002 Jan; 46(1):30-6.

Kesson EM, Allardice GM, George WD, Burns HJ, Morrison DS. Effects of multidisciplinary team working on breast cancer survival: retrospective, comparative, interventional cohort study of 13 722 women. BMJ. 2012;344:e2718. Epub 2012 Apr 26.

Kheifets LI, Matkin CC. Industrialization, electromagnetic fields, and breast cancer risk. Environ Health Perspect. 1999 Feb;107 Suppl 1:145-54. Review.

Kiefer TL1, Lai L, Yuan L, Dong C, Burow ME, Hill SM. Differential regulation of estrogen receptor alpha, glucocorticoid receptor and retinoic acid receptor alpha transcriptional activity by melatonin is mediated via different G proteins. J Pineal Res. 2005 May;38(4):231-9.

Kliukiene J, Tynes T & Andersen A. Risk of breast cancer among Norwegian women with visual impairment. British Journal of Cancer 84 397–399, 2001.

Kondratova AA, Kondratov RV. The circadian clock and pathology of the ageing brain. Nat Rev Neurosci 2012;13:325– 335.

Korkmaz A, Sanchez-Barcelo EJ, Tan DX, Reiter RJ. Role of melatonin in the epigenetic regulation of breast cancer. Breast Cancer Res Treat. 2009 May;115(1):13-27. doi: 10.1007/s10549-008-0103-5. Epub 2008 Jul 1. Review.

Krenk L1, Rasmussen LS, Kehlet H. New insights into the pathophysiology of postoperative cognitive dysfunction. Acta Anaesthesiol Scand. 2010 Sep;54(8):951-6. doi: 10.1111/j.1399-6576.2010.02268.x. Epub 2010 Jul 12.

Krueger JM, Fang J, Hansen MK, Zhang J, Obal Jr F. Humoral regulation of sleep. News Physiol Sci 1998;13:189–94

Lai L, Yuan L, Chen Q, Dong C, Mao L, Rowan B, Frasch T, Hill SM. The Galphai and Galphaq proteins mediate the effects of melatonin on steroid/thyroid hormone receptor transcriptional

activity and breast cancer cell proliferation. J Pineal Res. 2008 Nov;45(4):476-88. doi: 10.1111/j.1600-079X.2008.00620.x. Epub 2008 Aug 13.

Lai L, Yuan L, Cheng Q, Dong C, Mao L, Hill SM. Alteration of the MT1 melatonin receptor gene and its expression in primary human breast tumors and breast cancer cell lines. Breast Cancer Res Treat. 2009 Nov;118(2):293-305. doi: 10.1007/s10549-008-0220-1. Epub 2008 Nov 4.

Laste G, Vidor L, de Macedo IC, Rozisky JR, Medeiros L, de Souza A, Meurer L, de Souza IC, Torres IL, Caumo W. Melatonin treatment entrains the rest-activity circadian rhythm in rats with chronic inflammation. Chronobiol Int. 2013 Nov;30(9):1077-88. doi: 10.3109/07420528.2013.800088. Epub 2013 Jul 23.

Lawn, S. et al. Neurotrophin Signaling via TrkB and TrkC Receptors Promotes the Growth of Brain Tumor-initiating Cells. J. Biol. Chem. 290, 3814–3824 (2015).

Lee, M. Cho, C. Miaskowski, M. DoddImpaired sleep and rhythms in persons with cancer. Sleep Med Rev, 8 (2004), pp. 199-212

Leon-Blanco MM1, Guerrero JM, Reiter RJ, Calvo JR, Pozo D. Melatonin inhibits telomerase activity in the MCF-7 tumor cell line both in vivo and in vitro. J Pineal Res. 2003 Oct;35(3):204-11.

Lerner AB, Case JD, Heinzelman RU. Structure of melatonin. J Am Chem Soc 1959; 81:6084-6085.

Lerner AB, Case Jd, Takahashi Y. Isolation of melatonin and 5-methoxyindole-3-acetic acid from bovine pineal glands. J Biol Chem. 1960 Jul;235:1992-7.

Lévi F, Okyar A, Dulong S, et al. (2010). Circadian timing in cancer treatments. Annu Rev Pharmacol Toxicol. 50:377–421.

Lévi F, Dugué PA, Innominato P, et al. (2014). Wrist actimetry circadian rhythm as a robust predictor of colorectal cancer patients survival. Chronobiol Int. 31:891–900.

Liet C, Amenouche F, Freret T, Boulouard M, Mauvieux B, Lelong-Boulouard V, Bocca ML. Effects of acute administration of melatonin on attentional, executive, and working memory processes in rats. Fundam Clin Pharmacol. 2015 Oct;29(5):472-7. doi: 10.1111/fcp.12134.

Lissoni P, Barni S, Mandalà M, Ardizzoia A, Paolorossi F, Vaghi M, Longarini R, Malugani F, Tancini G. Decreased toxicity and increased efficacy of cancer chemotherapy using the pineal hormone melatonin in metastatic solid tumour patients with poor clinical status. Eur J Cancer. 1999 Nov;35(12):1688-92.

Liu L, Rissling M, Neikrug A, Fiorentino L, Natarajan L, Faierman M, Sadler GR, Dimsdale JE, Mills PJ, Parker BA, Ancoli-Israel S. Fatigue and Circadian Activity Rhythms in Breast Cancer Patients Before and After Chemotherapy: A Controlled Study. Fatigue. 2013;1(1-2):12-26.

Liu, Y.-Z., Wang, Y.-X. & Jiang, C.-L. Inflammation: The Common Pathway of Stress-Related Diseases. Front. Hum. Neurosci. 11, 1–11 (2017).Longman AJ, Braden CJ, Mishel MH. Side-effects burden, psychological adjustment, and life quality in women with breast cancer: pattern of association over time. Oncol Nurs Forum. 1999 Jun;26(5):909-15.

Liu, Y.-Z., Wang, Y.-X. & Jiang, C.-L. Inflammation: The Common Pathway of Stress-Related Diseases. Front. Hum. Neurosci. 11, 1–11 (2017).

Lovenberg W, Weissbach H, Udenfriend S. Aromatic L-amino acid decarboxylase. J Biol Chem. 1962;237:89–93.

Lovenberg W, Jequier E, Sjoerdsma A. Tryptophan hydroxylation: measurement in pineal gland, brainstem, and carcinoid tumor. Science. 1967;155:217–219.

Lu NZ, Wardell SE, Burnstein KL, Defranco D, Fuller PJ, Giguere V, Hochberg RB, McKay L, Renoir JM, Weigel NL, Wilson EM, McDonnell DP, Cidlowski JA. International Union of Pharmacology. LXV. The pharmacology and classification of the nuclear receptor superfamily: glucocorticoid, mineralocorticoid, progesterone, and androgen receptors. Pharmacol Rev. 2006 Dec;58(4):782-97. Review.

Macaluso M1, Cinti C, Russo G, Russo A, Giordano A. pRb2/p130-E2F4/5-HDAC1-SUV39H1-p300 and pRb2/p130-E2F4/5-HDAC1-SUV39H1-DNMT1 multimolecular complexes mediate the transcription of estrogen receptor-alpha in breast cancer. Oncogene. 2003 Jun 5;22(23):3511-7.

Macleod MR, O'Collins T, Horky LL, Howells DW, Donnan GA. Systematic review and meta-analysis of the efficacy of melatonin in experimental stroke. J Pineal Res. 2005;38(1):35–41.

Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. 2004; Neuron 44:5–21

Mamounas EP. Impact of neoadjuvant chemotherapy on locoregional surgical treatment of breast cancer. Ann Surg Oncol. 2015 May; 22(5):1425-33.

Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. The nuclear receptor superfamily: the second decade. Cell. 1995 Dec 15;83(6):835-9. Review

Mao L, Yuan L, Slakey LM, Jones FE, Burow ME, Hill SM. Inhibition of breast cancer cell invasion by melatonin is mediated through regulation of the p38 mitogen-activated protein kinase signaling pathway. Breast Cancer Res. 2010;12(6):R107. doi:10.1186/bcr2794

Marchi AA, Gurgel MSC (2010) Adesão ao rastreamento mamográfico oportunístico em serviços de saúde públicos e privados. Rev Brasil de Ginecol e Obstetr 32:191–197

Martin MB, Angeloni SV, Garcia-Morales P, Sholler PF, Castro-Galache MD, Ferragut JA, Saceda M. Regulation of estrogen receptor-alpha expression in MCF-7 cells by taxol. J Endocrinol. 2004 Mar;180(3):487-96.

Martínez-Campa C, Alonso-González C, Mediavilla MD, Cos S, González A, Ramos S,

Sánchez-Barceló EJ. Melatonin inhibits both ER alpha activation and breast cancer cell proliferation induced by a metalloestrogen, cadmium. J Pineal Res. 2006 May;40(4):291-6.

Martínez-Campa C1, Menéndez-Menéndez J1, Alonso-González C1, González A1, Álvarez-García V2, Cos S1. What is known about melatonin, chemotherapy and altered gene expression in breast cancer. Oncol Lett. 2017 Apr;13(4):2003-2014. doi: 10.3892/ol.2017.5712. Epub 2017 Feb 10.

Masana MI1, Doolen S, Ersahin C, Al-Ghoul WM, Duckles SP, Dubocovich ML, Krause DN. MT(2) melatonin receptors are present and functional in rat caudal artery. J Pharmacol Exp Ther. 2002 Sep;302(3):1295-302.

Masood S. Neoadjuvant chemotherapy in breast cancers. Womens Health (Lond). 2016 Sep; 12(5):480-491.

Mausbach BT, Schwab RB, Irwin SA. Depression as a predictor of adherence to adjuvant endocrine therapy (AET) in women with breast cancer: a systematic review and meta-analysis. Breast Cancer Res Treat. 2015 Jul;152(2):239-46. doi: 10.1007/s10549-015-3471-7.

Maughan KL, Lutterbie MA, Ham PS. Treatment of breast cancer. Am Fam Physician. 2010 Jun 1; 81(11):1339-46.

McDonald BC, Conroy SK, Ahles TA, West JD, Saykin AJ. Alterations in brain activation during working memory processing associated with breast cancer and treatment: a prospective functional magnetic resonance imaging study. J Clin Oncol. 2012; 30(20):2500–2508.

McIsaac WM, Page IH. The metabolism of serotonin (5-hydroxytryptamine) J Biol Chem. 1959;234:858–864

Mendes BM, Trindade BM, Fonseca TC, de Campos TPR. Assesment of radiation-induced secondary cancer risk in the brazilian population from left-sided breast-3D-CRT using MCNPX. Br J Radiol. 2017 Sep 20:20170187. doi: 10.1259/bjr.20170187.

Micale, V., Arezzi, A., Rampello, L. & Drago, F. Melatonin affects the immobility time of rats in the forced swim test: The role of serotonin neurotransmission. *Eur. Neuropsychopharmacol.* 16, 538–545 (2006).

Mills E1, Wu P, Seely D, Guyatt G. Melatonin in the treatment of cancer: a systematic review of randomized controlled trials and meta-analysis. J Pineal Res. 2005 Nov;39(4):360-6.

Ministério da Saúde (BR). Instituto Nacional do Câncer (INCA). Estimativas da incidência e mortalidade por câncer no Brasil, 2016. [online]. [acesso 2017 dez 12]. Disponível em: http://www.inca.org.br.

Mantovani, M., Pértile, R., Calixto, J. B., Santos, A. R. S. & Rodrigues, A. L. S. Melatonin exerts an antidepressant-like effect in the tail suspension test in mice: Evidence for involvement of N-methyl-D-aspartate receptors and the L-arginine-nitric oxide pathway. Neurosci. Lett. 343, 1–4 (2003).

Molis TM1, Spriggs LL, Hill SM. Modulation of estrogen receptor mRNA expression by melatonin in MCF-7 human breast cancer cells. Mol Endocrinol. 1994 Dec;8(12):1681-90.

Molis TM1, Spriggs LL, Jupiter Y, Hill SM. Melatonin modulation of estrogen-regulated proteins, growth factors, and proto-oncogenes in human breast cancer. J Pineal Res. 1995 Mar;18(2):93-103.

NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Breast Cancer V.2.2017. Disponível em: http://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Acessado em julho, 2017.

Nicolini, Ferrari, Duffy. Prognostic and predictive biomarkers in breast cancer: Past, present and future. Semin Cancer Biol. 2017 Sep 4. pii: S1044-579X (17)30052-4. doi: 10.1016/j.semcancer.2017.08.010.

Najafi M, Shirazi A, Motevaseli E, Geraily G, Norouzi F, Heidari M, Rezapoor S. The melatonin immunomodulatory actions in radiotherapy. Biophys Rev. 2017 Apr;9(2):139-148. doi: 10.1007/s12551-017-0256-8. Epub 2017 Mar 27. Review.

Palesh OG, Roscoe JA, Mustian KM, et al. Prevalence, demographics, and psychological associations of sleep disruption in patients with cancer: University of Rochester Cancer Center-Community Clinical Oncology Program. J Clin Oncol 2010; 28:292–298.

Palesh O, Aldridge-Gerry A, Ulusakarya A, Ortiz-Tudela E, Capuron L, Innominato PF. Sleep disruption in breast cancer patients and survivors. J Natl Compr Canc Netw. 2013 Dec 1;11(12):1523-30.

Pandi-Perumal SR, Trakht I, Srinivasan V, Spence DW, Maestroni GJ, Zisapel N, Cardinali DP. Physiological effects of melatonin: role of melatonin receptors and signal transduction pathways. Prog Neurobiol. 2008;85:335–353.

Parganiha A, Taj S, Chandel P, Sultan A, Choudhary V. Effect of hospitalization on rest-activity rhythm and quality of life of cancer patients. Indian J Exp Biol. 2014 May;52(5):549-58.

Partridge AH, Bunnell CA, Winer EP. Quality of life issues among women undergoing high-dose chemotherapy for breast cancer. Breast Dis. 2001; 14:41-50.

Patani N1, Jiang WG, Mokbel K. Brain-derived neurotrophic factor expression predicts adverse pathological & clinical outcomes in human breast cancer. Cancer Cell Int. 2011 Jul 18;11(1):23. doi: 10.1186/1475-2867-11-23.

Pevet P, Challet E, 2011. a. Melatonin: both master clock output and internal time-giver in the circadian clocks network. J. Physiol. Paris 105 (December), 170–182.

Pezet S, McMahon SB.Neurotrophins: mediators and modulators of pain. Annu Rev Neurosci. 2006;29:507-38. Review.

Price DD, Staud R. Neurobiology of fibromyalgia syndrome. J Rheumatol Suppl. 2005 Aug; 75:22-8. Review.

Ram PT1, Dai J, Yuan L, Dong C, Kiefer TL, Lai L, Hill SM. Involvement of the mt1 melatonin receptor in human breast cancer. Cancer Lett. 2002 May 28;179(2):141-50.

Rand, J.L. Otte, D. Flockhart, D. Hayes, A.M. Storniolo, V. Stearns, et al.Modeling hot flushes and quality of life in breast cancer survivors. Climacteric, 14 (1) (2011), pp. 171-180.

Rato AG1, Pedrero JG, Martinez MA, del Rio B, Lazo PS, Ramos S. Melatonin blocks the activation of estrogen receptor for DNA binding. FASEB J. 1999 May;13(8):857-68.

Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocr Rev. 1991c;12:151–180.

Reiter RJ. The melatonin rhythm: both a clock and a calendar. Experientia. 1993;49:654–664.

Reiter RJ, Tan DX, Qi W, Manchester LC, Karbownik M, Calvo JR. Pharmacology and physiology of melatonin in the reduction of oxidative stress in vivo. Biol Signals Recept. 2000;9:160–171.

Reiter RJ, Tan DX, Fuentes-Broto L. Melatonin: a multitasking molecule. Prog Brain Res. 2010; 181():127-51.

Reiter RJ, Tan DX, Kim SJ, Cruz MHC. Delivery of pineal melatonin to the brain and SCN: role of canaliculi, cerebrospinal fluid, tanycytes and Virchow–Robin perivascular spaces. Brain Struct Funct. 2014;219(6):1873–87.

Reiter RJ, Rosales-Corral SA, Tan DX, Acuna-Castroviejo D, Qin L, Yang SF, Xu K.Melatonin, a Full Service Anti-Cancer Agent: Inhibition of Initiation, Progression and Metastasis. Int J Mol Sci. 2017 Apr 17;18(4). pii: E843. doi: 10.3390/ijms18040843. Review.

Reuter-Lorenz, P. A. & Cimprich, B. Cognitive function and breast cancer: Promise and potential insights from functional brain imaging. Breast Cancer Res. Treat. 137, 33–43 (2013).

Ren K, Dubner R. Descending modulation in persistent pain: an update. 2002. Pain 100:1–6

Ren K, Dubner R. Pain facilitation and activity-dependent plasticity in pain modulatory circuitry: role of BDNF-TrkB signaling and NMDA receptors. Mol Neurobiol. 2007; 35:224–235.

Rimmele U, Spillmann M, Bärtschi C, Wolf OT, Weber CS, Ehlert U, Wirtz PH. Melatonin improves memory acquisition under stress independent of stress hormone release. Psychopharmacology (Berl). 2009 Mar;202(4):663-72. doi: 10.1007/s00213-008-1344-z. Epub 2008 Oct 14.

Ripamonti, D. Santini, E. Maranzano, M.Berti, F. Roila Management of cancer pain: ESMO clinical practice guidelines. Ann. Oncol., 23 (2012), pp. 139-154

Rocha-Brischiliari SC, de Oliveira RR, Andrade L, et al. The Rise in Mortality from Breast Cancer in Young Women: Trend Analysis in Brazil. Ahmad A, ed. PLoS ONE. 2017;12(1): e0168950. doi: 10.1371/journal.pone.0168950.

Rodella LF, Favero G, Foglio E, Rossini C, Castrezzati S, Lonati C, Rezzani R.Vascular endothelial cells and dysfunctions: role of melatonin. Front Biosci (Elite Ed). 2013 Jan 1;5:119-29.

Roehrs T, Roth T. Sleep and pain: Interaction of two vital functions. Semin Neurol. 2005;25(1):106–16.

Roenneberg T, Merrow M. Entrainment of the human circadian clock. Cold Spring Harb Symp Quant Biol. 2007;72:293-9. doi: 10.1101/sqb.2007.72.043. Review.

Roesler R, de Farias CB, Abujamra AL, Brunetto AL, Schwartsmann G. BDNF/TrkB signaling as an anti-tumor target. Expert Rev Anticancer Ther. 2011 Oct;11(10):1473-5. doi: 10.1586/era.11.150

Rögelsperger O1, Wlcek K, Ekmekcioglu C, Humpeler S, Svoboda M, Königsberg R, Klimpfinger M, Jäger W, Thalhammer T. Melatonin receptors, melatonin metabolizing enzymes and cyclin D1 in human breast cancer. J Recept Signal Transduct Res. 2011 Apr;31(2):180-7. doi: 10.3109/10799893.2011.557734.

Roscoe JA, Morrow GR, Hickok JT, et al. (2002). Temporal interrelationships among fatigue, circadian rhythm and depression in breast cancer patients undergoing chemotherapy treatment. Support Care Cancer. 10:329–36.

Ruksee N, Tongjaroenbuangam W, Mahanam T, Govitrapong P. Melatonin pretreatment prevented the effect of dexamethasone negative alterations on behavior and hippocampal neurogenesis in the mouse brain. J Steroid Biochem Mol Biol [Internet]. 2014;143:72–80

Samuelsson LB, Bovbjerg DH, Roecklein KA, Hall MH. Sleep and circadian disruption and incident breast cancer risk: An evidence-based and theoretical review. Neurosci Biobehav Rev. 2017;84:35–48. doi:10.1016/j.neubiorev.2017.10.011

Sanchez-Barcelo, E. J., Mediavilla, M. D., Alonso-Gonzalez, C. & Reiter, R. J. Melatonin uses in oncology: breast cancer prevention and reduction of the side effects of chemotherapy and radiation. Expert Opin. Investig. Drugs 21, 819–831 (2012).

Savard J, Simard S, Blanchet J, Ivers H, Morin CM. Prevalence, clinical characteristics, and risk factors for insomnia in the context of breast cancer. Sleep. 2001 Aug 1; 24(5):583-90.

Savard, J. Savard, C. Quesnel, H. IversThe influence of breast cancer treatment on the occurrence of hot flashes. J Pain Symptom Manage, 37 (4) (2009), pp. 687-697.

Schallreuter KU, Wood JM, Pittelkow MR, Gutlich M, Lemke KR, Rodl W, Swanson NN, Hitzemann K, Ziegler I. Regulation of melanin biosynthesis in the human epidermis by tetrahydrobiopterin. Science. 1994;263:1444–1446.

Schernhammer ES1, Hankinson SE. Urinary melatonin levels and breast cancer risk. J Natl Cancer Inst. 2005 Jul 20;97(14):1084-7.

Schneider SM, Offterdinger M, Huber H, Grunt TW. Involvement of nuclear

steroid/thyroid/retinoid receptors and of protein kinases in the regulation of growth and of c-erbB and retinoic acid receptor expression in MCF-7 breast cancer cells. Breast Cancer Res Treat. 1999 Nov;58(2):171-81.

Schwertner A, Conceição Dos Santos CC, Costa GD, Deitos A, de Souza A, de Souza IC, Torres IL, da Cunha Filho JS, Caumo W. Efficacy of melatonin in the treatment of endometriosis: a phase II, randomized, double-blind, placebo-controlled trial. Pain. 2013 Jun;154(6):874-81.

Seely D, Wu P, Fritz H, Kennedy DA, Tsui T, Seely AJ, Mills E. Melatonin as adjuvant cancer care with and without chemotherapy: a systematic review and meta-analysis of randomized trials. Integr Cancer Ther. 2012 Dec; 11(4):293-303.

Sharma R, McMillan CR, Tenn CC, Niles LP. Physiological neuroprotection by melatonin in a 6-hydroxydopamine model of Parkinson's disease. Brain Res. 2006;1068(1):230–6.

Smith M, Edwards R, McCann U, Haythornthwaite J. The Effects of Sleep Deprivation on Pain Inhibition and Spontaneous Pain in Women. Sleep Deprivation Pain Woman [Internet]. 2007;30(4):494–505.

Stefani LC, Muller S, Torres IL, Razzolini B, Rozisky JR, Fregni F, Markus R, Caumo W. A Phase II, Randomized, Double-Blind, Placebo Controlled, Dose-Response Trial of the Melatonin Effect on the Pain Threshold of Healthy Subjects. PLoS One. 2013 Oct 2;8(10):e74107. doi: 10.1371/journal.pone.0074107.

Selamat, Maryam Hafsah; Siew Yim Loh, Lynette Mackenzie, Janette Vardy. Chemobrain Experienced by Breast Cancer Survivors: A Meta-Ethnography Study Investigating Research and Care Implications. PLoS One. 2014; 9(9): e108002. Published online 2014 Sep 26. doi: 10.1371/journal.pone.0108002

Serikov VS, Lyashev YD. Effects of Melatonin on Stress-Induced Changes in the Liver of Rats with Different Resistance to Stress. Bull Exp Biol Med. 2015;159(3):314–7.

Shah PN, Mhatre MC, Kothari LS, 1984. Effect of melatonin on mammary carcinogenesis in intact and pinealectomized rats in varying photoperiods. Cancer Res. 44 (August), 3403–3407

Shapiro CL, Recht A. Side effects of adjuvant treatment of breast cancer. N Engl J Med. 2001 Jun 28;344(26):1997-2008.

Slominski A, Tobin DJ, Zmijewski MA, Wortsman J, Paus R. Melatonin in the skin: synthesis, metabolism and functions. Trends Endocrinol Metab. 2008;19:17–24

Slominski RM1, Reiter RJ, Schlabritz-Loutsevitch N, Ostrom RS, Slominski AT. Melatonin membrane receptors in peripheral tissues: distribution and functions. Mol Cell Endocrinol. 2012 Apr 4;351(2):152-66. doi: 10.1016/j.mce.2012.01.004. Epub 2012 Jan 8.

Slominski AT, Kleszczyński K, Semak I, et al. Local melatoninergic system as the protector of skin integrity. Int J Mol Sci. 2014;15(10):17705–17732. Published 2014 Sep 30. doi:10.3390/ijms151017705

Smirnov AN1. Nuclear melatonin receptors. Biochemistry (Mosc). 2001 Jan;66(1):19-26.

Srinivasan V, Pandi-Perumal SR, Trakht I, Spence DW, Hardeland R, Poeggeler B, Cardinali DP. Pathophysiology of depression: role of sleep and the melatonergic system. Psychiatry Res. 2009 Feb 28; 165(3):201-14.

Stacchiotti A, Favero G, Giugno L, Lavazza A, Reiter RJ, Rodella LF, Rezzani R.Mitochondrial and metabolic dysfunction in renal convoluted tubules of obese mice: protective role of melatonin. PLoS One. 2014 Oct 27;9(10):e111141. doi: 10.1371/journal.pone.0111141. eCollection 2014.

Stehle JH, Saade A, Rawashdeh O, Ackermann K, Jilg A, Sebesteny T, Maronde E. A survey of molecular details in the human pineal gland in the light of phylogeny, structure, function and chronobiological diseases. J Pineal Res. 2011;51:17–43.

Suter R, Marcum JA. The molecular genetics of breast cancer and targeted therapy. Biologics. 2007 Sep; 1(3):241-58.

Szyf M, Pakneshan P, Rabbani SA. DNA methylation and breast cancer. Biochem Pharmacol. 2004 Sep 15;68(6):1187-97. Review.

Tajbakhsh, A. et al. Therapeutic Potentials of BDNF/TrkB in Breast Cancer; Current Status and Perspectives. Journal of Cellular Biochemistry 118, (2017).

Tan DX, Manchester LC, Burkhardt S, Sainz RM, Mayo JC, Kohen R, Shohami E, Huo YS, Hardeland R, Reiter RJ. N1-acetyl-N2-formyl-5-methoxykynuramine, a biogenic amine and melatonin metabolite, functions as a potent antioxidant. FASEB J. 2001;15:2294–2296.

Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J Pineal Res. 2007;42:28–42.

Tan DX, Manchester LC, Fuentes-Broto L, Paredes SD, Reiter RJ. Significance and application of melatonin in the regulation of brown adipose tissue metabolism: relation to human obesity. Obes Rev. 2011;12:167–188.

Tamarkin L, Danforth DN, Lichter A, DeMoss E, Cohen M, Chabner B & Lippman M 1982 Decreased nocturnal plasma melatonin peak in patients with estrogen receptor positive breast cancer. Science 216 1003–1005.

Tengattini S1, Reiter RJ, Tan DX, Terron MP, Rodella LF, Rezzani R. Cardiovascular diseases: protective effects of melatonin. J Pineal Res. 2008 Jan;44(1):16-25

Toffol, E. N. Kalleinen, J. Haukka, O. Vakkuri, T. Partonen, P. Polo-KantolaMelatonin in peri- and postmenopausal women: associations with sleep, mood, anxiety and climacteric symptoms. Menopause (2013), 10.1097/GME.0b013e3182a6c8f3.

Toffol E, Kalleinen N, Haukka J, Vakkuri O, Partonen T, Polo-Kantola P. The effect of hormone therapy on serum melatonin concentrations in premenopausal and postmenopausal

women: a randomized, double-blind, placebo-controlled study. Maturitas. 2014 Apr;77(4):361-9. doi: 10.1016/j.maturitas.2014.01.015.

Tonsfeldt KJ, Chappell PE, 2012. Clocks on top: the role of the circadian clock in the hypothalamic and pituitary regulation of endocrine physiology. Mol. Cell. Endocrinol. 349 (February), 3–12.

Torre LA1, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015 Mar;65(2):87-108. doi: 10.3322/caac.21262. Epub 2015 Feb 4.

Trošt Bobić T. The Impact of Sleep Deprivation on the Brain. Acta Clin Croat [Internet]. 2016;469–73.

Ulmann L1, Hatcher JP, Hughes JP, Chaumont S, Green PJ, Conquet F, Buell GN, Reeve AJ, Chessell IP, Rassendren F. Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. J Neurosci. 2008 Oct 29;28(44):11263-8. doi: 10.1523/JNEUROSCI.2308-08.2008.

Vanhecke E1, Adriaenssens E, Verbeke S, Meignan S, Germain E, Berteaux N, Nurcombe V, Le Bourhis X, Hondermarck H. Brain-derived neurotrophic factor and neurotrophin-4/5 are expressed in breast cancer and can be targeted to inhibit tumor cell survival. Clin Cancer Res. 2011 Apr 1;17(7):1741-52. doi: 10.1158/1078-0432.CCR-10-1890. Epub 2011 Feb 24.

Vanegas H, Schaible HG (2004) Descending control of persistent pain: inhibitory or facilitatory? Brain Res Rev 46:295–309.

Vena, K. Parker, M. Cunningham, J. Clark, S. McMillanSleep-wake disturbances in people with cancer part I: an overview of sleep, sleep regulation, and effects of disease and treatment. Oncol Nurs Forum, 31 (4) (2004), pp. 735-746

Verkasalo PK, Pukkala E, Stevens RG, Ojamo M & Rudanko SL. Inverse association between breast cancer incidence and degree of visual impairment in Finland. British Journal of Cancer 80 1459–1460, 1999.

Villapol S, Fau S, Renolleau S, Biran V, Charriaut-Marlangue C, Baud O. Melatonin promotes myelination by decreasing white matter inflammation after neonatal stroke. Pediatr Res. 2011;69(1):51–5.

Xing L, He Q, Wang YY, Li HY, Ren GS. Advances in the surgical treatment of breast cancer. Chin Clin Oncol. 2016 Jun;5(3):34. doi: 10.21037/cco.2016.05.08.

Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. Biometrics. 1988;

Zarychta E, Rhone P, Bielawski K, Michalska M, Rość D, Ruszkowska-Ciastek B. Anti-angiogenic efficacy in invasive breast carcinoma patients depends on clinicopathological determinants. Adv Med Sci. 2019 Feb 25;64(2):216-223. doi: 10.1016/j.advms.2019.02.001

Zhang C, Li X1, Gao D, Ruan H, Lin Z, Li X, Liu G, Ma Z, Li X. The prognostic value of over-

expressed TrkB in solid tumors: a systematic review and meta-analysis. Oncotarget. 2017 Jul 25;8(59):99394-99401. doi: 10.18632/oncotarget.19561. eCollection 2017 Nov 21.

Zhang, S., Guo, D., Luo, W., Zhang, Q., Zhang, Y., Li, C., Lu, Y., Cui, Z., and Qiu, X. (2010). TrkB is highly expressed in NSCLC and mediates BDNF-induced the activation of Pyk2 signaling and the invasion of A549 cells. BMC Cancer 10, 43.

Zhou D, Zhang Z, Liu L, Li C, Li M, Yu H, et al. The antidepressant-like effects of biperiden may involve BDNF/TrkB signaling-mediated BICC1 expression in the hippocampus and prefrontal cortex of mice. Pharmacol Biochem Behav [Internet]. 2017;157:47–57.

Walker MP. Cognitive consequences of sleep and sleep loss. Sleep Med. 2008 Sep;9 Suppl 1:S29-34. doi: 10.1016/S1389-9457(08)70014-5. Review.

Wang Y, Jin B, Ai F, Duan C, Lu Y, Dong T, Fu Q. The efficacy and safety of melatonin in concurrent chemotherapy or radiotherapy for solid tumors: a meta-analysis of randomized controlled trials. Cancer ChemotherPharmacol, 2012, 69:1213-1220

Wang L, Guyatt GH, Kennedy SA, et al. Predictors of persistent pain after breast cancer surgery: a systematic review and meta-analysis of observational studies. CMAJ. 2016;188(14):E352-E361.

Wefel, R. Lenzi, R. Theriault, A.U. Buzdar,S. Cruickshank, and C. A. Meyers, "Chemobrain" in breast carcinoma?: a prologue,"Cancer,vol.101,no.3,pp.466–475,2004.

Weissbach A. A novel system for the incorporation of amino acids by extracts of E. coli B. Biochim. Biophys Acta. 1960;41:498–509.

Whitehead KJ, Rose S, Jenner P. Halothane anesthesia affects NMDA-stimulated cholinergic and GABAergic modulation of striatal dopamine efflux and metabolism in the rat in vivo. Neurochem Res. 2004 Apr;29(4):835-42.

WHO. The breast cancer conundrum. Bulletin of the World Health Organization, 2013; 91:626–7. http://dx.doi.org/10.2471/BLT.13.020913 pmid:24101777

Yeh CM, Su SC, Lin CW, Yang WE, Chien MH, Reiter RJ, Yang SF. Melatonin as a potential inhibitory agent in head and neck cancer. Oncotarget. 2017 Aug 9;8(52):90545-90556. doi: 10.18632/oncotarget.20079. eCollection 2017 Oct 27.

Yu H, Chen ZY. The role of BDNF in depression on the basis of its location in the neural circuitry. Acta Pharmacol Sin [Internet]. 2011;32(1):3–11. Available from: http://dx.doi.org/10.1038/aps.2010.184

Yu H, Wang DD, Wang Y, Liu T, Lee FS, Chen ZY. Variant brain-derived neurotrophic factor Val66Met polymorphism alters vulnerability to stress and response to antidepressants. J Neurosci. 2012 Mar 21;32(12):4092-101. doi: 10.1523/JNEUROSCI.5048-11.2012.

ANEXO A – REGIMES DE TRATAMENTO ADJUVANTE E NEOADJUVANTE PARA CÂNCER DE MAMA

Quimioterapia Neoadjuvante / Adjuvante Baseada em Evidência [1]	
Regimes preferíveis para doença HER2-negativo[1, f]	
Regime	**Dosagem**
Dose-densa de doxorubicina + ciclofosfamida (AC) seguida de paclitaxel (Categoria 1) [2]	Dia 1: Doxorubicina 60mg/m^2 IV Dia 1: Ciclofosfamida 600mg/m^2 IV 4 sessões (a cada 14 dias) seguido de: Dia 1: Paclitaxel 175mg/m^2 IV
Dose-densa de AC seguida de paclitaxel semanal (Categoria 1) [2]	Dia 1: Doxorubicina 60mg/m^2 IV Dia 1: Ciclofosfamida 600mg/m^2 IV 4 sessões (a cada 14 dias) seguido de: Dia 1: Paclitaxel 80 mg/m^2 IV semanal por12 semanas
Docetaxel + ciclofosfamida (TC) (Categoria 1) [3]	Dia 1: Docetaxel 75 mg/m^2 IV Dia 1: Ciclofosfamida 600mg/m^2 IV 4 sessões (a cada 21 dias)
Outros regimes para doença HER2-negativo[1, f]	
Dose-densa AC (Categoria 1) [2]	Dia 1: Doxorubicina 60mg / m^2 IV Dia 1: Ciclofosfamida 600mg / m^2 IV 4 sessões (a cada 14 dias)
AC (Categoria 2B) [4]	Dia 1: Doxorubicina 60mg / m^2 IV Dia 1: Ciclofosfamida 600mg / m^2 IV 4 sessões (a cada 21 dias)
Docetaxel + doxorubicina + ciclofosfamida (TAC) (Categoria 1) [5]	Dia 1: Docetaxel 75mg / m^2 IV Dia 1: Doxorubicina 50mg / m^2 IV Dia 1: Ciclofosfamida 500mg / m^2 IV 6 sessões (a cada 21 dias)
Ciclofosfamida + metotrexato + 5-fluorouracil (CMF) (Categoria 1) [6]	Dias 1 à 14: Ciclofosfamida 100mg / m^2 VO Dia 1 e 8: Metotrexato 40mg / m^2 IV Dia 1 e 8: 5-fluorouracil 600mg / m^2 IV. 6 sessões (a cada 28 dias)
AC seguido de docetaxel (Categoria 1) [7]	Dia 1: Doxorubicina 60 mg/m^2 IV Dia 1: Ciclofosfamida 600mg / m^2 IV.

	4 sessões (a cada 21 dias) seguido de: Dia 1: Docetaxel 100 mg/m^2 IV 4 sessões (a cada 21 dias)
AC seguido paclitaxel semanal (Categoria 1) [8]	Dia 1: Doxorubicina 60mg / m^2 IV Dia 1: Ciclofosfamida 600mg / m^2 IV 4 sessões (a cada 21 dias) seguido de Dia 1: Paclitaxel 80 mg/m^2 IV semanal por 12 semanas
Epirubicina + Ciclofosfamida (EC) (Categoria 1) [9]	Dia 1: Epirubicina 100mg / m^2 IV Dia 1: Ciclofosfamida 830mg / m^2 IV 8 sessões (a cada 21 dias)
Regimes preferíveis para doença HER2-positivo [1,f,g,h,i]	
AC seguido de paclitaxel + trastuzumab[10,j,k]	Dia 1: Doxorubicina 60mg / m^2 IV Dia 1: Ciclofosfamida 600mg / m^2 IV 4 sessões (a cada 21 dias) seguido: Paclitaxel 80mg / m^2 IV semanal por 12 semanas Trastuzumabe 4mg/kg IV c/ 1ª dose paclitaxel, seguido Trastuzumab 2mg/kg IV semanal por 1 ano
AC seguido de paclitaxel + trastuzumabe +pertuzumabe[10,j,k]	Dia 1: Doxorubicina 60mg / m^2 IV IV Day 1: Ciclofosfamida 600 mg / m^2 IV 4 sessões (a cada 21 dias) seguido de: Dia 1: Pertuzumabe 840mg IV seguido de 420mg IV Dia 1: Trastuzumab 8mg/kg IV seguido de 6mg/kg IV Dias 1, 8, e 15: Paclitaxel 80mg/m2 IV 4 sessões (a cada 21 dias) Dia 1: Trastuzumabe 6mg/kg IV a cada 21 dias / 1 ano
Dose-densa AC seguida de paclitaxel + trastuzumabe[11,j,k]	Dia 1: Doxorubicina 60 mg/m^2 IV Dia 1: Ciclofosfamida 600mg/m^2 IV. 4 sessões (a cada 14 dias) seguido: Dia 1: Paclitaxel 175 mg/m^2 IV 4 sessões (a cada 14 dias), mais: Trastuzumabe 4mg/kg IV com a primeira dose de paclitaxel, seguido de: Trastuzumabe 2mg/kg IV semanal até completar 1 ano
Docetaxel + Carboplatina +	Dia 1: Docetaxel 75 mg/m^2 IV

Trastuzumabe (TCH)[12,k]	Dia 1: Carboplatina AUC 6mg • min/mL IV 6 sessões (a cada 21 dias), com: Trastuzumabe 4mg/kg IV na primeira semana, seguido de: 2mg/kg IV por 17 semanas, seguido de: Trastuzumabe 6mg/kg IV a cada 21 dias até completar 1 ano com trastuzumabe, OU Trastuzumabe 8mg/kg IV primeira semana, seguido: Trastuzumabe 6mg/kg IV a cada 21 dias / 1 ano
TCH + pertuzumabe[13,k]	Dia 1: Trastuzumabe 8mg/kg IV seguido de 6mg/kg IV Dia 1: Pertuzumabe 840mg IV seguido de 420mg IV Dia 1: Docetaxel 75 mg/m^2 IV Dia 1: Carboplatina AUC 6mg • min/mL IV 6 sessões (a cada 21 dias), seguido de: Trastuzumab 6mg/kg IV a cada 21 dias / 1 ano
Outros regimes para doença HER2-positivo [1,f,g,h,i]	
AC seguido de docetaxel + trastuzumabe[12,j,k]	Dia 1: Doxorubicina 60 mg/m^2 IV Dia 1: Ciclofosfamida 600mg/m2 IV 4 sessões (a cada 21 dias), seguido de: Dia 1: Docetaxel 100 mg/m^2 IV 4 sessões (a cada 21 dias), com: Trastuzumabe 4mg/kg IV na primeira semana, seguido de 2mg/kg IV semanal por 11 semanas, seguido de: Trastuzumab 6mg/kg IV a cada 21 dias / 1 ano
AC seguido de docetaxel + trastuzumabe + pertuzumabe[13,j,k]	Dia 1: Doxorubicina 60 mg/m^2 IV Dia 1: Ciclofosfamida 600mg/m2 IV 4 sessões (a cada 21 dias), seguido de: Dia 1: Pertuzumabe 840mg IV seguido de 420mg IV Dia 1: Trastuzumab 8mg/kg IV seguido de 6mg/kg IV Dia 1: Docetaxel 75–100mg/m^2 IV 4 sessões (a cada 21 dias), seguido de: Trastuzumabe 6mg/kg IV a cada cada 21 dias / 1 ano
Docetaxel + ciclofosfamida + trastuzumabe[14,k]	Dia 1: Docetaxel 75 mg/m^2 IV Dia 1: Ciclofosfamida 600mg/m^2 IV 4 sessões (a cada 21 dias), mais:

	Trastuzumab 4mg/kg IV na primeira semana, seguido: Trastuzumab 2mg/kg IV semanalmente/11 semanas, seguido de: Trastuzumab 6mg/kg IV a cada 21 dias até completar 1 ano de terapia OU Trastuzumab 8mg/kg IV na primeira semana, seguido: Trastuzumab 6mg/kg IV a cada 21 dias / 1 ano
Fluorouracil + Epirubicina + Ciclofosfamida (FEC) seguido de pertuzumabe + trastuzumabe + docetaxel[13,j,k]	Dia 1: Fluorouracil 500 mg/m^2 IV Dia 1: Epirubicina 100 mg/m^2 IV Dia 1: Ciclofosfamida 600mg/m2 IV 3 sessões (a cada 21 dias) seguido de: Dia 1: Pertuzumabe 840mg IV seguido de 420mg IV Dia 1: Trastuzumab 8mg/kg IV seguido de 6mg/kg IV Dia 1: Docetaxel 75–100mg/m2 IV 3 sessões (a cada 21 dias), seguido de: Dia 1: Trastuzumabe 6mg/kg IV a cada 21 dias / 1 ano
FEC seguido de pertuzumab + trastuzumab + paclitaxel[13,j,k]	Dia 1: Fluorouracil 500 mg/m^2 IV Dia 1: Epirubicin 100 mg/m^2 IV Dia 1: Ciclofosfamida 600mg/m^2 IV 3 sessões (a cada 21 dias), seguido de: Dia 1: Pertuzumab 840mg IV seguido de 420mg IV Dia 1: Trastuzumab 8mg/kg IV seguido de 6mg/kg IV Dias 1, 8, e 15: Paclitaxel 80 mg/m^2 IV 3 sessões (a cada 21 dias), seguido de: Dia 1: Trastuzumab 6mg/kg IV a cada 21 dias / 1 ano
Paclitaxel + trastuzumabe [15,k,l]	Dia 1: Paclitaxel 80 mg/m^2 IV semanal/12semanas, + Trastuzumab 4mg/kg IV c/ 1ªdose paclitaxel, seguido: Trastuzumab 2mg/kg IV semanalmente por 1 ano
Pertuzumab + trastuzumab + docetaxel seguido de FEC[16,j,k]	Dia 1: Pertuzumab 840mg IV seguido de 420mg IV Dia 1: Trastuzumab 8mg/kg IV seguido de 6mg/kg IV Dia 1: Docetaxel 75–100mg/m^2 IV 4 sessões (a cada 21 dias), seguido de: Dia 1: Fluorouracil 600 mg/m^2 IV Dia 1: Epirubicina 90 mg/m^2 IV

	Dia 1: Ciclofosfamida 600mg/m^2 IV Dia 1: Trastuzumab 6mg/kg IV 3 sessões (a cada 21 dias), seguido de: Dia 1: Trastuzumab 6mg/kg IV a cada 21 dias / 1 ano
Pertuzumabe + trastuzumabe + paclitaxel seguido de FEC[16,j,k]	Dia 1: Pertuzumab 840mg IV seguido de 420mg IV Dia 1: Trastuzumab 8mg/kg IV seguido de 6mg/kg IV Dias 1, 8, e 15: Paclitaxel 80 mg/m^2 IV 4 sessões (a cada 21 dias), seguido de: Dias 1: Fluorouracil 600 mg/m^2 IV Dia 1: Epirubicina 90 mg/m^2 IV Dia 1: Ciclofosfamida 600mg/m^2 IV Dia 1: Trastuzumab 6mg/kg IV 3 sessões (a cada 21 dias), seguido de: Dia 1: Trastuzumab 6mg/kg IV a cada 21 dias / 1 ano

ANEXO B – CARTA DE ACEITE DO COMITE DE ÉTICA

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

COMISSÃO CIENTÍFICA

A Comissão Científica do Hospital de Clínicas de Porto Alegre analisou o projeto:

Projeto: 140701
Data da Versão do Projeto: 19/12/2014

Pesquisadores:
WOLNEI CAUMO
LUCIANA DA CONCEIÇÃO ANTUNES
ANA CLÁUDIA DE SOUZA
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Título: MELATONINA COMO SINCRONIZADORA DO RITMO SONO-VIGÍLIA, NEUROMODULADORA E MIELOPROTETORA NA QUIMIOTERAPIA POR CÂNCER DE MAMA: ENSAIO CLÍNICO, RANDOMIZADO, DUPLO-CEGO, EM PARALELO, CONTROLADO COM PLACEBO

Este projeto foi APROVADO em seus aspectos éticos, metodológicos, logísticos e financeiros para ser realizado no Hospital de Clínicas de Porto Alegre.
Esta aprovação está baseada nos pareceres dos respectivos Comitês de Ética e do Serviço de Gestão em Pesquisa.

- Os pesquisadores vinculados ao projeto não participaram de qualquer etapa do processo de avalição de seus projetos.

- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao Grupo de Pesquisa e Pós-Graduação (GPPG)

Porto Alegre, 15 de maio de 2015.

Prof. José Roberto Goldim
Coordenador CEP/HCPA