Universidade Federal do Rio Grande do Sul
Programa de Pós-Graduação em Biologia Celular e Molecular

Efeitos crônicos não-térmicos das ondas eletromagnéticas não-ionizantes sobre o fígado e o córtex cerebral de ratos com diferentes idades

Orlando Vieira Furtado Filho

Orientadora: Jenifer Saffi
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Porto Alegre-RS, dezembro de 2012.

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Tese apresentada ao Programa de Pós-Graduação em Biologia Celular e Molecular da Universidade Federal do Rio Grande do Sul para obtenção do título de Doutor em Ciências Biológicas.

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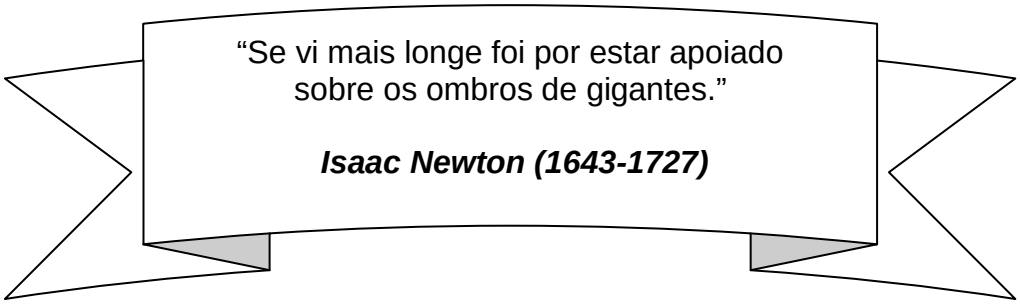
Este trabalho foi realizado nas instalações do **Laboratório de Radiobiologia Molecular** do Centro de Biotecnologia desta Universidade.


“Se vi mais longe foi por estar apoiado
sobre os ombros de gigantes.”
Isaac Newton (1643-1727)

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LISTA DE ABREVIATURAS

AA – ácido aracdônico

AG – ácido graxo

AGE – produtos finais de glicação avançada

AGI / UFA – ácidos graxos insaturados

AH_2 – ácido ascórbico

ALA – ácido alfa-linoleico

ALP – fosfatase alcalina

ALT – alanina aminotransferase

ANATEL – Agência Nacional de Telecomunicações

AP – sítio apurínico e apirimidínico

ASP – aspartato aminotransferase

$A^{-\bullet}$ – radical ascorbil

BER – reparo por excisão de base

BHT – butil hidróxido tolueno

Cat – catalase

CCD / RCC – córtex cerebral direito

CCE / LCC – córtex cerebral esquerdo

CEMs – campos eletromagnéticos

Co – cobalto

COX-2 – cicloxigenase 2

Cr – cromo

CTE – cadeia de transporte de elétrons

Cu – cobre

CuZnSod – cobre zinco superóxido dismutase

D5D – delta-5 dessaturase

DHA – ácido docosahexanóico

DI – índice de dano

DMSO – dimetil sulfóxido

DNFH – 2,4 - dinitrofenil hidrazina

E – campo eletromagnético

EA – ácido ecoisatrienóico

EC-Sod – superóxido dismutase extracelular

ED – sistema de alongamento e dessaturação

EDTA – ácido etileno diamino tetra-cético

EGF – fator de crescimento epidermal

EMR – radiação eletromagnética

EO / OS – estresse oxidativo

EPA – ácido ecoisapentanóico

ERK – cinase regulada por sinal extracelular

ERN – espécie reativa de nitrogênio

ERO / ROS – espécies reativas de oxigênio

Fe – ferro

FEN 1 – flap endonuclease 1

Fe-S – proteína ferro-enxofre

FeSod – ferro superóxido dismutase

FID – detector de ionização de chama

FOX – oxidação ferrosa em alaranjado de xilenol

G6PDH – gliceraldeído 6-fosfato desidrogenase

GC – cromatografia gasosa

GI-GPx – glutationa peroxidase gastrointestinal

GPx – glutationa peroxidase

GR – glutationa redutase

GSH – glutationa reduzida

GSSG – glutationa oxidada

GST – glutationa S-transferase

H_2O_2 – peróxido de hidrogênio

Hb-EGF – fator de crescimento epidermal ligante à heparina

HBSS – solução salina equilibrada de Hank

HCl – ácido clorídrico

HNE – 4-hidroxi-2-nonenal

ICNIRP – Comissão Internacional de Proteção Contra Radiação Ionizante

LA – ácido linoleico

LDL – lipoproteína de baixa densidade

LMP – baixo ponto de fusão

LOOH – hidroperóxido lipídico

$LOO^{\bullet}$ – radical peroxil

LPO – lipoperoxidação

LPS – lipopolissacarídeo

MAPK – proteína cinase ativada por mitógeno

MDA – malonaldeído

MMP – metaloproteínase matricial

MnSod – manganês superóxido dismutase

NaCl – cloreto de sódio

NADH – nicotinamida adenina dinucleotídeo reduzida

NADPH – nicotinamida adenina dinucleotídeo fosfato reduzida

NAG – N-acetil-β-D-glicosaminidase

NaOH – hidróxido de sódio

NER – Reparo por excisão de nucleotídeos

Ni – níquel

$NO^{\bullet}$ – óxido nítrico

8-OHdG – 7,8-hidróxi-8-oxo-2 desoxiguanina

$O_2^{-\bullet}$ – radical superóxido

OA – ácido oleico

OEM / EMW – ondas eletromagnéticas

OH^{-} – aniôn hidroxila

$OH^{\bullet}$ – radical hidroxila

$ONOO^{-}$ – peroxinitrito

PBS-Tween – solução salina de tampão fosfato com tween

PC / CP – proteínas carboniladas

PFGE – eletroforese em gel de campo pulsado

PHGPx – fosfolipídio hidroperóxido glutationa peroxidase

PLGPx – glutationa peroxidase plasmática

PUFA – ácido graxo poliinsaturado

PVDF – fluoreto de polivinilideno

Q – coenzima Q

RC / CR – ratos controle

RE / ER – ratos expostos

RetOH - retinol

RNI – radiação não-ionizante

RNS – espécies reativas de nitrogênio

SAR – taxa de absorção específica

SDS – dodecil sulfato de sódio

PAGE – eletroforese em gel de poliacrilamida

SNC – sistema nervoso central

Sod – superóxido dismutase

Sod-1 – superóxido dismutase 1

TBA – ácido triobarbitúrico

TBARS – substâncias reativas com ácido tiobarbitúrico

TERL – teoria do envelhecimento por radicais livres

UHF – ultra-alta freqüência

UHF-EMR – radiações eletromagnéticas de ultra-alta freqüência

V – vanádio

WB – Western blotting

XO – xantina oxidase

Zn – Zinco

α,β,γ,δ–TOH – tocoferóis e tocotrienóis

α,β,γ,δ–TO$^{\bullet}$ – radical tocoferil

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Parte I

RESUMO

O desenvolvimento tecnológico aumentou a exposição dos organismos às ondas eletromagnéticas (OEM). Dependendo das condições de exposição, este agente físico pode causar mudanças comportamentais, fisiológicas, celulares e moleculares. Neste nível de organização biológico, a literatura científica vem relacionando as OEM com o metabolismo das espécies reativas de oxigênio (ERO). Estas podem causar danos oxidativos a ácidos nucleicos, lipídios e proteínas. Entretanto, para se defender destas lesões, os sistemas biológicos apresentam defesas antioxidantes. O desequilíbrio entre oxidantes e antioxidantes ocasiona estresse oxidativo (EO), que pode ser observado em várias patologias neurodegenerativas e cardiovasculares, bem como nos processos de isquemia-reperfusão e envelhecimento. Órgãos com altas taxas metabólicas e contendo muitos substratos oxidáveis, tais como o cérebro e fígado, são mais susceptíveis aos danos oxidativos. Sendo assim, o objetivo deste estudo foi verificar o efeito das OEM de UHF (ultra-alta-frequência) sobre os danos ao DNA, aos lipídios e às proteínas bem como sobre a expressão da catalase e o metabolismo de ácidos graxos insaturados (AGI) no fígado e córtex cerebral de ratos com diferentes idades (0, 6, 15 e 30 dias). ***Rattus norvergicus*** foram divididos em 2 grupos com 6 animais cada: ratos controles (RC) e ratos expostos (RE) às OEM com uma freqüência de 950 MHz, onda contínua, 1 W de potência, antena de polarização vertical, ½ hora por dia, durante 51 dias (21 da gestação + 30 de nascido). A taxa de absorção específica dos RE variou de 1,3 a 1,0 W/kg. Depois do período de exposição, os animais foram dissecados, o material foi congelado em nitrogênio líquido e armazenado no ultra-congelador. Os danos ao DNA foram verificados pelo ensaio cometa alcalino; os danos oxidativos a proteínas, por PC (proteínas carboniladas); os danos oxidativos a lipídios, por TBARS (substâncias reativas com ácido tiobarbitúrico); a expressão da catalase foi verificada por immunoblotting; e a quantificação e a qualificação de ácidos graxos, por cromatografia gasosa. Nos resultados do fígado, os ratos 0 dia apresentaram menores níveis de TBARS e concentrações de AGI após exposição. Não houve diferença significativa de proteínas carboniladas em nenhuma das idades. Os danos ao DNA de RE de 15 e 30 dias foram significativamente diferentes. Os ratos com 0 dia expostos mostraram menor expressão de catalase. Nos resultados de córtex cerebral de 0 dia, não houve diferenças de TBARS e nem de PC no CCD (córtex cerebral direito) nem no CCE (córtex cerebral esquerdo). Os animais com 6 dias também não mostraram diferenças significativas de PC no CCE mas o CCD dos RE apresentaram maiores níveis de PC o que não foi observado em cometa. Os RE com 6 dias apresentaram menor concentração de glicose sangue total. Nossos resultados do fígado indicam que não há EO e nem genotoxicidade nos ratos com 0, 6, 15 dias de idade, mas há alteração na concentração de ácidos graxos polinsaturados de neonatos. Nos ratos com 30 dias, não há EO porém as OEM são genotóxicas. Os resultados do córtex cerebral de 0 e 6 dias indicam que não há lateralidade oxidativa e nem EO nos córtex. Entretanto, os maiores níveis de PC no CCD podem ser resultado de produtos finais de glicação avançada neste órgão. São necessários mais estudos para se entender os mecanismos das alterações em fígado de 0 e 30 dias bem como em CCD de animais com 6 dias de idade.

Palavras-chave: radiação eletromagnética, estresse oxidative, envelhecimento, AGI, rato.

ABSTRACT

Technological development has increased the exposure of organisms to electromagnetic waves (EMW). Depending on the exposure conditions, this physical agent can cause behavioral changes, physiological, cellular and molecular. At this level of organization of animals, the scientific literature relating the OEM comes with the metabolism of reactive oxygen species (ROS). These can cause oxidative damage to nucleic acids, lipids and proteins. However, to protect these lesions, biological systems exhibit antioxidant defenses. The imbalance between oxidants and antioxidants cause oxidative stress (OS), which can be observed in several neurodegenerative disorders and cardiovascular disorders and in cases of ischemia-reperfusion injury, and aging. Organs with high metabolic rates and containing many oxidizable substrates such as the brain and liver, are more susceptible to oxidative damage. Therefore, the objective of this study was to investigate the effect of OEM UHF on damage to DNA, lipids and proteins as well as on catalase expression and metabolism of unsaturated fatty acids (UFA) in the liver and cerebral cortex of rats with different ages (0, 6, 15 and 30 days). ***Rattus norvegicus*** were divided into 2 groups of 6 animals each: control rats (CR) and exposed rats (ER) to the EMW with a frequency of 950 MHz, continuous wave, 1 W of power, vertical polarization antenna, ½ hour per day, for 51 days (21 days of gestation and 30 days of life outside the womb) . The specific absorption rate of ER ranged from 1.3 to 1.0 W / Kg. After the exposure period, the animals were dissected, material was frozen in liquid nitrogen and stored in ultra-freezer. The DNA damage were verified by alkaline comet assay, oxidative damage to proteins, for CP (protein carbonyls); oxidative damage to lipids by TBARS (thiobarbituric acid reactive substances), catalase expression was detected by immunoblotting, and quantification and qualification of fatty acids by gas chromatography. The results of the liver, 0 day rats had lower levels of TBARS concentrations and UFA after exposure. There was no difference in CP for any age. Damage to the DNA of ER with 15 and 30 days were different. Neonates (0 day) exposed showed lower expression of catalase. The results of the cerebral cortex of 0 day, there were no differences in TBARS and CP nor the RCC (right cerebral cortex) or the LCC (left cerebral cortex). The animals with 6 days also showed no differences in CP of LCC but the RCC of RE showed higher levels of CP which was not observed in comet. The ER with 6 days had lower total blood glucose concentration. Our results indicate that the liver no OS nor genotoxicity in rats with 0, 6, 15 days old but changed the concentration of polyunsaturated fatty acids in rats 0 day. In animals with 30 days no OS but the EMW are genotoxic. The results of the cerebral cortex of 0 day and 6 days indicated no oxidative lateral and OS in the cortex. However, the highest levels of the CP in RCC may be the result of advanced glycation end products in this organ. Further studies are needed to understand the mechanisms of changes in liver of 0 day and 30days as well as in animal RCC with 6 days old.

Keywords: electromagnetic radiation, oxidative stress, aging, UFA, rat..

1 Introdução Geral

No nosso dia-a-dia, somos constantemente expostos a muitos tipos de ondas eletromagnéticas. As radiações dessas ondas são produzidas naturalmente pelo Sol mas, atualmente, com o desenvolvimento tecnológico, ficamos em contato com as ondas eletromagnéticas produzidas artificialmente por estações de rádio e TV, redes de alta tensão, sistemas de comunicação à base de microondas, telefones sem fio, estações rádio-base e telefones celulares. Estes são motivo preocupação dos pesquisadores, pois realizam emissão de ondas eletromagnéticas de ultra-alta freqüência.

Há vários estudos na literatura relacionando as radiações eletromagnéticas de ultra-alta freqüência (UHF-EMR) com alterações bioquímicas e comportamentais. Estas mudanças podem ser de deficit de aprendizagem, de modificações elétricas cerebrais, de danos e síntese de DNA, de transcrição de RNA, de aberrações cromossomais, de formação de micronúcleo, de permeabilidade da barreira hematocefálica e de fluxo de íons através das membranas (SALLES *et al.,* 2003; NAZIROĞLU *et al.,* 2012). Em 2006, Hardell e colaboradores demonstraram que o uso prolongado de telefones celulares e sem fio está relacionado com o aparecimento de tumores cerebrais, principalmente meningioma e neuroma acústico. Outro tipo de câncer relacionado à radiação eletromagnética (EMR) é a leucemia (WERTHEIMER & LEEPER, 1979; VILLENEUVE *et al*., 2000a; LINET *et al*., 2003; TYNES & HALDORSEN, 2003; POOLE *et al.,* 2006; MEZEI & KHEIFETS, 2006), principalmente a infantil.

1.1 Radiação Eletromagnética

A existência das ondas eletromagnéticas foi prevista no século XIX por James Maxwell. Este cientista escocês resumiu as leis da eletricidade e do magnetismo numa fórmula matemática. Ainda no século XIX, Henrique Hertz produziu, pela primeira vez, as ondas eletromagnéticas em laboratório. Das descobertas destes pesquisadores até os dias de hoje, a exposição à EMR vem aumentando devido ao desenvolvimento tecnológico. Neste, o ramo que mais

cresce no Brasil é o da telefonia celular. Em 2011, segundo a ANATEL (Agência Nacional de Telecomunicações), o nosso país ultrapassou 242 milhões de telefones celulares.

Esses aparelhos utilizam radiação eletromagnética não-ionizante (RNI) que possui energia inferior a 10 elétron-volts e um comprimento de onda eletromagnética superior a 200 nanômetros. Esta energia é incapaz de produzir emissão de elétrons (EBLEM, 2006; JÚNIOR & MARTIN, 2006). A telefonia celular, normalmente, usa a faixa de freqüência de 800 a 2.200MHz (figura 1). Este intervalo é classificado como UHF (ultra-alta-freqüência) que vai de 300 MHz a 3 GHz (VERSACHAVE & MAES 1998; FIGURA & TEIXEIRA, 2007).

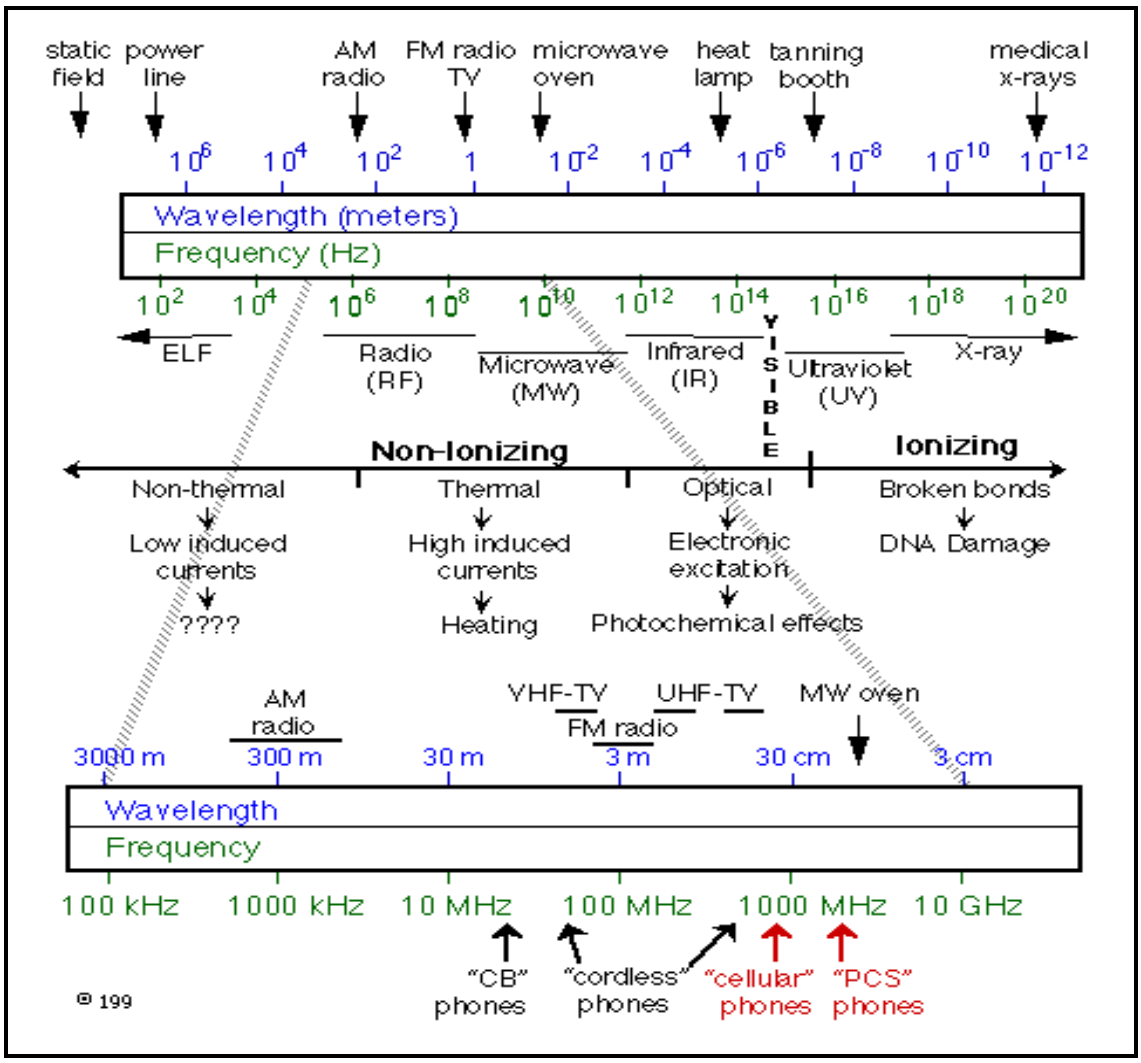

Figura 1. Espectro eletromagnético (MOULDER, 1999)

O calor também é produzido pelas RNI. Para eliminar a possibilidade dos efeitos térmicos sobre o organismo, utiliza-se a taxa de absorção específica (SAR). Esta grandeza quantifica os limites básicos de exposição às ondas eletromagnéticas. Nesta medida, mostra-se a potência absorvida pela massa e é dada em Watt por quilograma. Não há dano térmico quando SAR é igual ou inferior a 4 W/kg visto que a temperatura do tecido não ultrapassa um grau centígrado. Quando a SAR ultrapassa 4 W/kg, pode haver uma incapacidade termo-regulatória do corpo, resultando em níveis danosos (BERNHARDT, 1992; ICNIRP, 2009). A SAR permitida pelas normas internacionais de proteção contra as RNI (ICNIRP, 2009), para os campos eletromagnéticos (CEMs) na faixa da radiofrequência, é menor que 2 mW/g. Este valor considera somente os efeitos térmicos, representando metade do valor para provocar elevação de temperatura.

1.2 Radicais Livres em Sistemas Biológicos

A ligação entre radicais livres e dano biológico vem do início da radiologia, quando ficou estabelecido que a exposição de células à radiação de alta energia leva à formação de radicais livres e, ao mesmo tempo, à mutação e morte celular. A partir da caracterização da enzima antioxidante superóxido dismutase, por MCCORD & FRIDOVICH (1969), uma série de estudos mostraram que os radicais livres são formados durante o metabolismo normal (AUGUSTO, 2006) e durante o metabolismo de xenobióticos (revisado por HALLIWELL & GUTTERIDGE, 1999; STOHS, 2011).

De acordo com a classificação de HALLIWELL & GUTTERIDGE (1999), várias doenças estão ligadas aos radicais livres, tais como aterosclerose, doença de Parkinson e processo de isquemia e reperfusão. Até mesmo o processo de envelhecimento natural tem a participação dos radicais livres. A teoria do envelhecimento por radicais livres sugere que as espécies reativas de oxigênio (ERO), produzidas durante a respiração aeróbica celular, causam danos oxidativos que se acumulam com o tempo, resultando em envelhecimento e morte celular (WOO & SHADEL, 2011). Em 1995, SOHAL e colaboradores relacionaram a concentração do radical ânion superóxido e do peróxido de hidrogênio com o

tempo máximo de vida de moscas e observaram que esta relação é inversamente proporcional. PAMPLONA e colaboradores (1995) observaram que aves têm maior longevidade do que mamíferos, embora possuam taxa metabólica semelhante. Isso é devido à maior geração de ERO na mitocôndria de ratos do que na de pombos e devido, também, às maiores quantidades de ácidos graxos poliinsaturados nas mitocôndrias de ratos. Essas moléculas são um dos principais alvos dos radicais livres (LIMA *et al*., 2001), causando peroxidação lipídica e o acúmulo dos produtos da peroxidação lipídica é um dos fatores que caracterizam o envelhecimento.

1.2.1 Bioquímica das Espécies Reativas de Oxigênio (ERO)

Na terceira etapa da respiração celular, na fosforilação oxidativa mitocondrial, o oxigênio é reduzido completamente por 4 elétrons, na cadeia de transporte de elétrons (CTE) da crista mitocondrial, formando 2 moléculas de água. Em mamíferos, parte dos elétrons que passam na CTE vaza e reduz o oxigênio incompletamente (com um elétron), formando o radical livre superóxido ($O_2^{-\cdot}$). A produção desta ERO dependerá da tensão de O_2 e da sua concentração no tecido (ADDABBLO *et al.,*2009).

Qualquer radical livre é definido como uma espécie química independente, contendo elétron desemparelhado, ou seja, sozinho no seu orbital (CADENAS, 1995; HALLIWELL & GUTTERIDGE, 1999; AUGUSTO, 2006; HALLIWELL, 2007).

Quando as cargas elétricas rotam, um campo magnético é gerado. Este efeito é neutralizado pelo pareamento de elétrons de spins opostos, porém o elétron desemparelhado torna-se paramagnético (um pouco atraído pelo campo magnético), fazendo com que esta se torne altamente reativa. O oxigênio molecular é paramagnético, contendo dois elétrons desemparelhados e com o mesmo spin (FRIDOVICH, 1998; HALLIWELL & GUTTERIDGE, 1999; AUGUSTO, 2006; HALLIWELL, 2007). Sendo assim, o O_2 tende a ocupar estes spins por meio de redução monoeletrônica, levando à formação de $O_2^{-\cdot}$ e O_2^{2-} (superóxido e peróxido, respectivamente – reações 1 e 2).

$$O_2 + e^- \rightarrow \mathbf{O_2^{-\bullet}} \quad (1)$$

$$O_2^{-\bullet} + e^- \rightarrow \mathbf{O_2^{2-}} + 2H^+ \rightarrow \mathbf{H_2O_2} \quad (2)$$

$$H_2O_2 + e^- \rightarrow OH^- + \mathbf{OH^\bullet} \quad (3)$$

$$\mathbf{OH^\bullet} + e^- \rightarrow OH^- + H^+ \rightarrow H_2O \quad (4)$$

Quando o H_2O_2 é cindido, a ligação O-O é rompida heteroliticamente, formando o radical livre hidroxil ($OH^{\cdot}$) e o ânion hidroxila (OH^-) (reação 3). Na redução completa do oxigênio, o $OH^{\cdot}$ é reduzido, formando o ânion OH^- que reagirá com um próton de hidrogênio e formará a água (reação 4).

Nos organismos, metais de transição como ferro e cobre podem doar elétrons ao H_2O_2 através da reação de Fenton (reação 5) e formar o $OH^{\cdot}$. O íon ferroso utilizado nesta reação é reciclado pela redução realizada pelo superóxido (reação 6). A soma das reações 5 e 6 resulta na reação de HABER-WEISS (reação 7) (MCCORD & DAY, 1978; HALLIWELL & GUTTERIDGE,1992).

$$\text{Fe (II)} + \mathbf{H_2O_2} \rightarrow \text{Fe (III)} + OH^- + \mathbf{OH^\bullet} \quad \text{(5) (reação de Fenton)}$$

$$\text{Fe (III)} + \mathbf{O_2^{-\bullet}} \rightarrow \text{Fe (II)} + O_2 \quad \text{(6)}$$

$$\mathbf{O_2^{-\bullet}} + H_2O_2 \rightarrow O_2 + OH^- + \mathbf{OH^\bullet} \quad \text{(7) (reação de Haber-Weiss)}$$

O superóxido é produzido por autoxidação de pequenas moléculas (MACARTHUR *et al*., 2000), pela ativação de macrófagos, pelas atividades de oxidases e pela CTE cloroplástica e mitocondrial (CADENAS, 1995; HALLIWELL & GUTTERIDGE, 1999). Em pH neutro, a sua meia-vida é de segundos, tendo baixa reatividade mas é potencialmente danoso. Ele pode se difundir a distâncias consideráveis, através de canais aniônicos, até encontrar um alvo compatível para reagir e causar danos oxidativos às estruturas celulares diretamente ou pela reação de Haber-Weiss (FRIDOVICH, 1998; ANDERSON *et al.,*1998; HALLIWELL & GUTTERIDGE, 1999) onde forma o radical hidroxil (CUZZOCREA *et al.,* 2001).

As enzimas superóxido dismutase, glicolato oxidase, D-aminoácido oxidase e urato oxidase produzem o H_2O_2. Esta ERO é um agente oxidante fraco, mas mesmo assim é capaz de inativar diretamente algumas enzimas por meio da

oxidação dos seus grupos tióis (HALLIWELL & GUTTERIDGE, 1990, 1999; HALLIWELL, 2009). Em 1995, SOHAL e colaboradores encontraram que a concentração de superóxido e peróxido de hidrogênio nas mitocôndrias é inversamente proporcional ao tempo de vida de alguns animais.

A ação tóxica do H_2O_2 depende de metais de transição (via reação de Fenton) e também do $O_2^{\cdot-}$ (via reação de Haber-Weisss) que levam à formação de $OH^{\cdot}$. Este radical livre é a ERO mais reativa, podendo reagir com todos os tipos de moléculas (principalmente os ácidos graxos poliinsaturados, os PUFA) com elevada velocidade (HALLIWELL & GUTTERIDGE, 1999; HALLIWELL, 2007). A constante de velocidade de reação do radical hidroxil com biomoléculas está na faixa de 10^9 M^{-1} s^{-1} (ISCHIROPOULOS, 1992). O radical $OH^{\cdot}$ tem pequena difusão graças à sua alta reatividade, causando danos oxidativos próximo aos locais onde é gerado (CUZZOCREA *et al.,* 2001).

1.2.1.1 Danos Radicalares

Ao reagirem com biomoléculas, os radicais livres podem danificá-las ou mesmo alterar a sua função original (BARBOSA *et al.,* 2006).

1.2.1.1.1 Peroxidação Lipídica

Quando as espécies reativas de oxigênio agem sobre os ácidos graxos insaturados das membranas celulares causam lipoperoxidação (LPO), formando lipoperoxil, alcoxil e hidroperóxidos lipídicos (VACA *et al.*, 1988; LIMA *et al*., 2001). A LPO altera a sua fluidez e pode levar ao seu rompimento. Neste último evento, podem ocorrer liberação de lipases e proteases das organelas. Além dessas enzimas, também pode acontecer a liberação de íons que podem ativar outras enzimas e vias de transdução de sinal.

A lipoperoxidação é um processo envolvido na fisiopatologia da hemocromatose (HERMES-LIMA *et al.,* 1995; SANTOS, 1998) e na aterogênese (DARLEY-USMAR *et al.,* 1995). O início da LPO ocorre quando a espécie reativa ataca um ácido graxo poliinsaturado (PUFA) da bicamada lipídica, abstraindo um

átomo de hidrogênio. A saída deste H deixa um elétron não-pareado no átomo de C o qual reage com oxigênio molecular, gerando o lipoperoxil ($LOO^{\cdot}$). Após esta reação, novamente, o $LOO^{\cdot}$ pode reagir com outro PUFA, reiniciando uma nova cadeia de oxidação. Ao reagir com esse ácido graxo, o $LOO^{\cdot}$ se liga ao H, formando o hidroperóxido lipídico (LOOH) (VACA *et al.,* 1988; HERMES-LIMA, 2004). O LOOH pode reagir com metais de transição produzindo os radicais livres alcoxil e peroxil (HALLIWELL & GUTTERIDGE, 1999). Estes radicais podem retirar átomos de hidrogênio de outros PUFA e LOOH, formando mais radicais lipídicos e contribuindo para a propagação da peroxidação lipídica. Quando LOOH se decompõe, radicais livres e outros proddutos não-radicalares são gerados. Dentre estes, aldeídos, cetonas e epóxidos. Estas moléculas são de baixa massa molecular e mais estáveis que os radicais livres que iniciaram o processo. Assim, o processo de peroxidação lipídica – após iniciado - torna-se autocatalítico, levando à formação de hidroperóxidos e produtos secundários (Figura 2) (MARBAN *et al.,* 1994; CHEN *et al.*, 1995; HALLIWELL & GUTTERIDGE, 1999; HERMES-LIMA 2004).

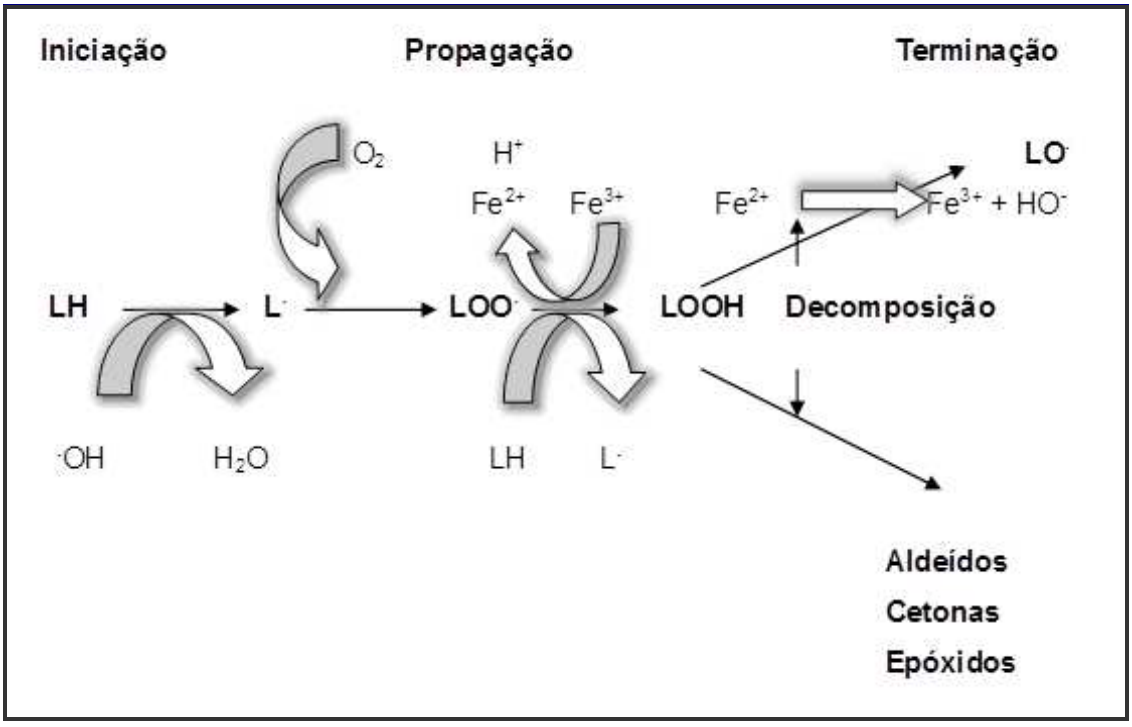

Figura 2. Representação geral das fases da peroxidação lipídica (modificado de VACA *et al.,* 1988). LH, ácido graxo insaturado; $L^{\cdot}$, radical alquil; $LO^{\cdot}$, radical alcoxil; $LOO^{\cdot}$ Radical lipoperoxil; LOOH, hidroperóxido lipídico.

1.2.1.1.2 Danos Oxidativos a Proteínas

As proteínas carboniladas são marcas características da modificação oxidativa provocada pelo radical $OH^{\cdot}$ ao reagir com a porção aminoterminal de peptídeos (STADTMAN, 1992). Diretamente, a oxidação das ERO pode formar grupos carbonil nos resíduos de lisina, arginina e prolina (STADTMAN, 1992; STADTMAN & LEVINE, 2000). Indiretamente, aldeídos, açúcares reduzidos ou seus produtos de oxidação podem também levar à formação de grupos carbonila (KRISTAL & YU, 1992). Além das modificações aminoterminais, as proteínas podem sofrer alterações oxidativas nas sulfidrilas, provocando mudanças conformacionais, alteração na atividade enzimática, clivagem de ligações peptídicas, modificação de carboidratos em glicoproteínas e perda de metais em metaloproteínas (STADTMAN, 1992; DEAN *et al.* 1997; LIMA & ABDALLA, 2001). No citosol, as proteínas oxidadas podem ser degradadas por proteases (STADTMAN, 1992) e/ou por proteassomas (HERSHKO & CIECHANOVER, 1992; BREUSING *et al.,* 2009) que são sistemas proteolíticos dependentes de ubiquitina (JÚNIOR *et al.* 2005).

Os níveis de proteínas carboniladas se apresentam elevados após processos de isquemia-reperfusão e de envelhecimento natural de diversas espécies. Além disso, a concentração do grupo carbonil também se encontra alta nas doenças de Alzheimer (CALABRESE *et al.,* 2003; DI DOMENICO et al., 2011), Parkinson, diabetes e aterosclerose dentre outras (STADTMAN & LEVINE, 2000; HERMES-LIMA, 2004; SOROLLA et al., 2010).

1.2.1.1.3 Danos ao DNA

ERO, ERN e produtos da LPO podem danificar o DNA, provocando quedra da sua fita e/ou modificando oxidativamente as suas bases nitrogenadas, gerando uma série de produtos (8-hidroxiguanina, 5-hidroximetil-glutaril e citosina-glicol) que podem levar a processos mutagênicos e carcinogênicos (HALLIWELL e GUTTERIDGE, 1990; ARUOMA & HALLIWELL, 1995; HERMES-LIMA *et al.* 1998b; COOKE & EVANS, 2005). Reações cruzadas entre proteínas e DNA, bem

como danos a açucares ocorrem após ataques por radicais livres. Os danos múltiplos ao DNA (todas as lesões provocadas em regiões próximas ao DNA) juntamente com a quebra de cadeias duplas não genotóxicas podem ser letais, mutagênicas e carcinogênicas (revisado por SAFFI & HENRIQUES, 2003; SALVADOR & HENRIQUES, 2004; JEGGO & LOBRICH, 2007).

Diretamente, $O_2^{-\cdot}$ e o H_2O_2 não atacam o DNA mas o $OH^{\cdot}$ e o oxigênio singlete causam lesão direta. O hidroxil modifica as bases nitrogenadas, quebrando cadeia de DNA e formando sítio apurínicos e apirimidínicos (AP). O oxigênio singlete pode causar ciclo-adição aos carbonos de ligação dupla do anel imidazol, gerar sítios AP álcali-lábeis e quebra de cadeias ao lado de guaninas. O principal alvo das ERO é a guanina e o aduto mais comum é a 7,8 dihidroxi-8-oxo-2 desoxiguanina (revisado por SAFFI & HENRIQUES, 2003; FOTI et al., 2012).

Os danos oxidativos ao DNA são, preferencialmente, reparados pelo sistema de reparo por excisão de bases (BER) (BOITEUX & GUILLET, 2004; LIU & WILSON, 2012). Este sistema consiste em (1) remover a base danificada por ação de uma DNA-glicosilase; (2) fazer uma incisão no sítio AP por uma AP-endonuclease; (3) processar as extremidades terminais; (4) preencher o sítio AP; e (5) reconstituir a fita danificada (DAVID *et al.,* 2007; LIU & WILSON, 2012) (figura 3).

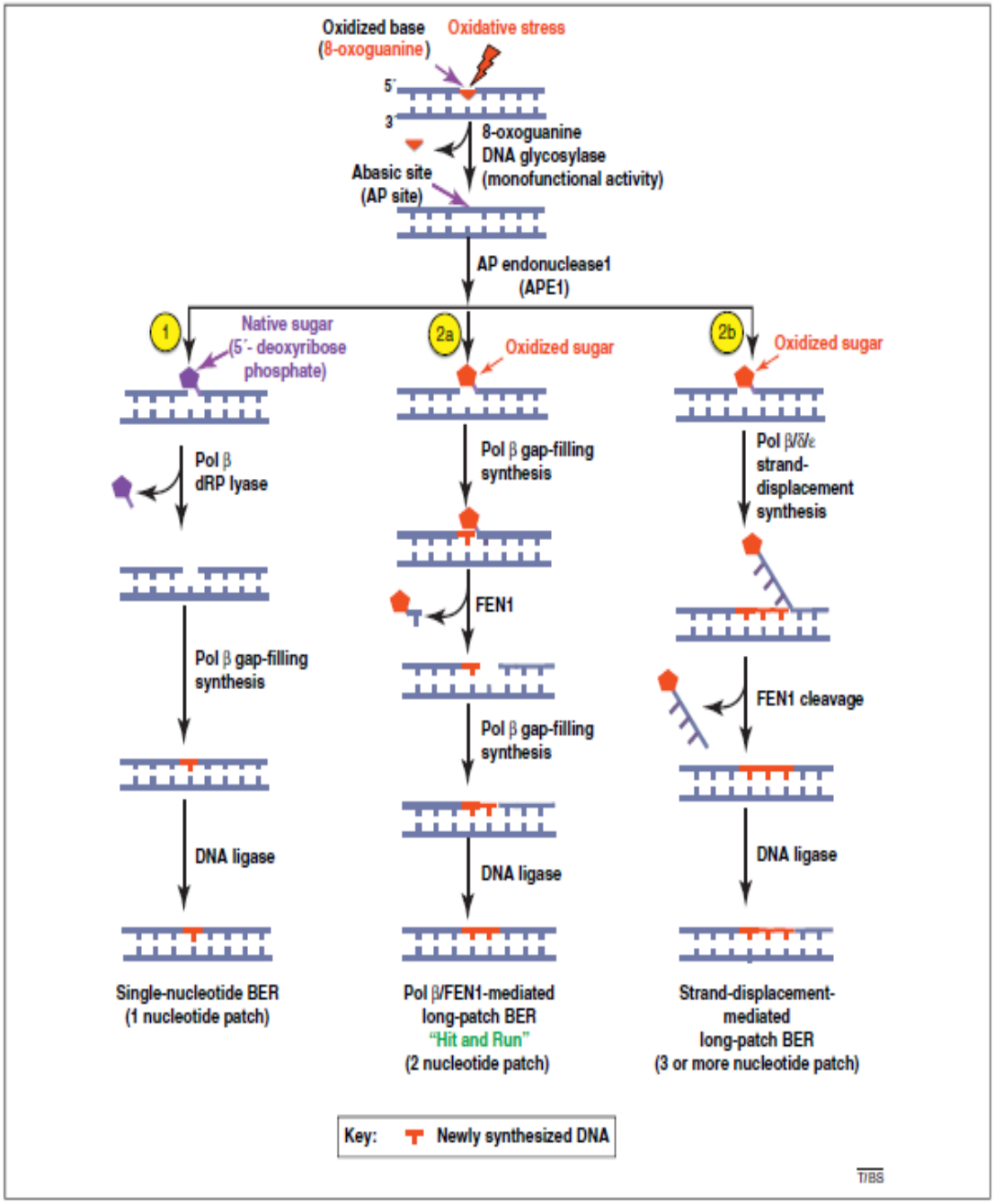

Figura 3. Sistema de reparo BER. BER, reparo por excisão de base; OGG1, DNA glicosilase 8-oxoguanina; AP, sítio apurínico/apirimídico; APE1, AP endonuclease 1; Pol β, polimerase beta; dRP, desoxirribose fosfato; FEN 1, Flap endonuclease 1 (LIU & WILSON, 2012).

As lesões oxidativas ou o próprio reparo das lesões oxidativas levam a formação de quebras duplas, que são reparadas pelo sistema de recombinação homóloga (HR) ou pelo sistema de junção de extremidades não homólogas (NHEJ).

1.3 Antioxidantes

Uma substância que iniba ou retarde a velocidade de oxidação de um substrato, estando em menor concentração que este, é considerado um antioxidante (HALLIWELL & GUTERIDGE, 1999; HALLIWELL, 2008).

Os organismos aeróbicos desenvolveram, com o passar do tempo, defesas antioxidantes que possibilitaram a sobrevivência nas condições ambientais apenas dos mais aptos a se defenderem dos danos provocados pelas espécies reativas (ÇIMEN, 2008). Enzimaticamente, essas defesas incluem principalmente a superóxido dismutase (Sod), que catalisa a dismutação do radical superóxido; a catalase (Cat), que decompõe o peróxido de hidrogênio, formando água e oxigênio; e a glutationa peroxidase (GPx) que decompõe não só o peróxido de hidrogênio como também hidroperóxidos orgânicos, usando glutationa como co-substrato (revisado por HERMES-LIMA *et al.* 1998a, 2001; ÇIMEN, 2008).

Estes organismos também utilizam as defesas antioxidantes não-enzimáticas. Estas são moléculas de baixa massa molecular podendo estar no meio aquoso (vitamina C e glutationa, por exemplo) ou não (vitamina E e carotenóides, por exemplo) (BIANCHI & ANTUNES, 1999; CERQUEIRA *et al.*, 2007). Essas defesas funcionam interrompendo a cadeia de reações oxidativas, não deixando acontecer a propagação de reações radicalares (DI MASCIO *et al.* 1991; CADENAS, 1995).

Outra forma de defesa antioxidante não-enzimática é por meio da prevenção da formação de ERO. Este é o mecanismo realizado pelos quelantes de metais de transição. Estas moléculas ligam-se aos metais e não os deixam livres para realizar a reação de Fenton. Exemplos de quelantes proteicos são a ceruloplasmina (quelante de cobre) e a transferrina (quelante de ferro) (AHMAD, 1995; ÇIMEN, 2008).

Há algum tempo, alguns pesquisadores têm verificado que metais, como o zinco e o magnésio, também exercem função antioxidante, estabilizando membranas e/ou proteínas e minimizando a susceptibilidade ao ataque de ERO (POWELL, 2000).

1.4 Estresse Oxidativo

O desequilíbrio entre a produção de oxidantes e a concentração de defesas antioxidantes, levando a danos celulares, é chamado de estresse oxidativo. Este pode ocorrer devido à elevada produção de ERO, pela diminuição das defesas ou por ambos os processos simultaneamente (SIES, 1993).

Os agentes oxidantes são formados no processo normal do metabolismo e em várias condições patológicas. Eles podem ser produzidos em taxas muito elevadas, levando ao estresse oxidativo e, possivelmente, à morte celular. O estresse oxidativo está relacionado a doenças como o câncer, hemocromatose, artrite reumatóide, diabetes, porfirias hepáticas, doenças neurodegenerativas, patologias cardiovasculares e com o processo natural de envelhecimento (BRAUGHLER & HALL, 1989; KLEINVELD *et al.,* 1989; STADTMAN, 1992; KAUL *et al*., 1993; LEVINE, 1993; HERMES-LIMA, 2004).

Há, também, indutores exógenos que geram ERO. Esses podem ser: substâncias de efeito redox (paraquat, diquate e aloxano) (RINCHEVAL *et al.,* 2012); drogas oxidantes (cloreto de carbono e acetaminofeno) (GIRISH & PRADHAN, 2012); tabaco (PAL *et al.,* 2012); luz solar e outras radiações ionizantes (POLJŠAK & DAHMANE, 2012); agentes quimioterápicos (HE *et al.,* 2012); citoquinas pró-inflamatórias (WESSELS et al., 2012); fatores de crescimento (RAY et al., 2012); e infecção por parasitas de vírus e bactérias (PAN et al., 2012).

1.5 Envelhecimento

A preocupação com o envelhecimento não é recente. Em 1798, o Dr Christopher Eilhelm Hufeland publicou o livro “Art of Increasing Human Life Span“, que descreveu métodos para aumentar o tempo máximo de vida. O envelhecimento pode ser definido como uma série de mudanças funcionais e estruturais de efeitos cumulativos, progressivos e deletérios que podem acontecer dependente do tempo ou não e que levam à morte (ARKING, 1998).

O envelhecimento é um processo multifatorial, onde fatores genéticos e ambientais (temperatura, atividade e nutrição, entre outros) podem afetar a longevidade de um organismo. ROBERT ARKING (1998) classifica as teorias do envelhecimento quanto à localização e à natureza dos efeitos das mudanças. Quanto à localização, as mudanças podem ser intracelulares ou de natureza intercelular. Quanto à origem das mudanças, as teorias classificam-se em estocásticas e sistêmicas. Nas estocásticas, acontecem eventos randômicos, que levam ao acúmulo das alterações, como, por exemplo, a mutação gênica. Já na sistêmica, há ocorrência de uma cascata hierárquica de eventos interconectados. Assim como a teoria do envelhecimento de proteínas alteradas, da mutação somática, da dos danos e reparos em DNA, entre outras – a teoria do envelhecimento por radicais livres (TERL) ou teoria dos danos oxidativos é classificada como estocástica. Nesta teoria, a longevidade é inversamente proporcional à extensão dos danos oxidativos e diretamente proporcional às atividades das defesas antioxidantes enzimáticas e não-enzimáticas. A TERL foi proposta por HARMAN, em 1956, onde o envelhecimento resultaria da proteção imperfeita contra os danos teciduais produzidos por radicais livres. Esta teoria serviu de base biológica para explicar a senescência e uma série de doenças.

A maioria das teorias para explicar o processo de envelhecimento, sugere o acúmulo de danos de constituintes celulares relacionados com a idade do indivíduo (ASHOK & ALI 1999), o que leva à redução da capacidade de responder aos desafios da vida, caracterizando, assim, a senescência.

Múltiplos fatores bioquímicos podem interferir na longevidade dos organismos. Dentre eles, pode-se citar a capacidade proteolítica, o acúmulo de lipofuccina, a restrição calórica, a quantidade e/ou atividade de antioxidantes, a taxa metabólica e a concentração e composição de ácidos graxos insaturados (UFA), particularmente os poliinsaturados (BECKMAN & AMES 1998; MUNRO & BLIER, 2012).

1.5.1 Capacidade Proteolítica

O envelhecimento é acompanhado por perda da capacidade proteolítica e pelo acúmulo de formas de enzimas cataliticamente menos ativa e termodinamicamente mais sensíveis. Foi proposto por Stadtman (2002) que o acúmulo de proteínas oxidadas - que normalmente acompanha o envelhecimento - seria responsável pela diminuição da renovação das enzimas. É possível que a perda da homeostase celular possa resultar da oxidação seletiva de proteínas. O acúmulo de proteínas oxidadas no envelhecimento pode ser explicado pela redução da capacidade antioxidante celular e pela redução da atividade de proteases e proteassomas que degradam essas proteínas oxidadas (BULTEAU et al., 2002; BREUSING *et al.,* 2009).

Pode-se dizer que no envelhecimento o sistema antioxidante também envelhece junto com os organismos.

1.5.2 Quantidade de Antioxidantes

Se os radicais livres estão envolvidos no processo de envelhecimento, então a utilização de antioxidantes – teoricamente – aumentaria o tempo de vida dos organismos. Entretanto, esta afirmação não é tão simples assim. A administração de Sod exógena inibe a produção celular de Sod endógena. Há também a dificuldade da entrada de antioxidantes nas células e nos locais de produção de ERO, como, por exemplo, na mitocôndria (BALIN, 1983).

Outra estratégia sugerida para aumentar o tempo de vida dos organismos seria aumentar endogenamente a expressão de genes de enzimas antioxidantes. Nesse sentido, ORR & SOHAL (1994) criaram drosófilas que superexpressavam Sod e Cat. Os autores demonstraram que houve aumento em 1/3 do tempo médio e máximo de vida e uma diminuição nos níveis de proteínas oxidadas nesses insetos.

Tem sido mostrado por alguns autores que a atividade de enzimas antioxidantes diminui com a idade. KASAPOGLU & OZBEN (2001) encontraram que a atividade de glutationa-peroxidase Se-dependente diminui nos eritrócitos de

humanos na faixa de 60-69 anos de idade, mas não a atividade de Sod e Cat. Porém, não há uma relação certa entre os antioxidantes (enzimáticos e não-enzimáticos) e o envelhecimento (BARJA, 2002; RAAMSDONK et al., 2010).

1.5.3 Concentração de PUFA

Há um mais elevado tempo máximo de vida das aves em relação à maioria dos mamíferos, de tamanho corpóreo e taxa metabólica semelhantes. Por exemplo, enquanto um rato vive até 4 anos, um pombo vive até 35. PAMPLONA e colaboradores (1995) propuseram que a menor longevidade de ratos – comparando com pombos – é devido a maiores quantidades de PUFA (ácidos graxos poliinsaturados) nas mitocôndrias do fígado de ratos.

Numa comparação entre oito espécies de mamíferos (***Mus musculus, Rattus norvegicus, Cavia porcellus, Oryctolagus caniculus, Ovis aries, Sus scrofa, Bos taurus*** e ***Equus caballus***), variando o tempo máximo de vida de 3,5 (***Mus musculus*)** a 46 anos (***Equus caballus***), foi observado que o conteúdo de duplas ligações totais – dos ácidos graxos dos fosfolipídeos do coração – é maior em pequenos mamíferos de vida mais curta (25± 0,96% dos ácidos graxos são de ácido docosahexanóico, DHA, em coração de camundongos) do que nos animais maiores de vidas mais longas (0,18 ± 0,02% de DHA em coração de cavalos). Neste novo trabalho, PAMPLONA *et al*. (1999) mostraram que há uma redistribuição dos ácidos graxos totais nos fosfolipídeos do coração, em especial do DHA (seis insaturações) para o alfa-linolênico (três insaturações). Isso deve ter acontecido devido à menor atividade de delta-6 dessaturase nos mamíferos maiores de vidas mais longas. A formação de AG poliinsaturados pode ser por degradação parcial de outros PUFA (SPRECHER, 2000) ou pela ação de um sistema de dessaturação e alongamento (DE) de AG saturados. O sistema de dessaturação de AG saturados e insaturados em poliinsaturados é dependente da atividade da delta-6-dessaturase. O sistema ED acontece no retículo endoplasmático, também chamado de sistema microssomal. Neste sistema, os AG principalmente da série ômega-3 são alongados e dessaturados formando os ácidos EPA e DHA, importantíssimos para o SNC. O DHA encontra-se em altas

concentrações no córtex cerebral é necessário para o desenvolvimento cerebral (MAYES, 1994).

1.6 Relação entre a EMR e as ROS

Há algum tempo, as EMR vêm sendo relacionadas com alteração do fluxo de íons, da permeabilidade da barreira hemato-cefálica e de células do sistema imunológico. Estas mudanças, assim como o desenvolvimento de alguns tipos de câncer, são ligadas aos efeitos não-térmicos desses campos.

Não se sabe exatamente como os EMR interagem com os sistemas biológicos e provocam as alterações supracitadas. Uma hipótese que vem sendo testada nos dias de hoje é a de que as ERO estejam envolvidas nessas mudanças. BEDIZ *et al.,* (2006) demonstraram proteção do cérebro de ratos contra a peroxidação lipídica, após o uso de zinco. Este elemento químico está relacionado à ativação do sistema antioxidante (POWELL, 2000; OZDEMIR & INANC, 2005).

O hormônio melatonina é um reconhecido “scavenger” de radicais livres. Além disso, ela estimula a atividade/expressão da GPx e da Sod (TAN et al., 1993). OKTEM et al*.,* (2005) mostraram que esse hormônio protegeu o rim de ratos expostos à radiação eletromagnética de 900 MHz emitidas por telefone celular, reduzindo os níveis de malonaldialdeído (MDA) que antes estava aumentado após exposição às radiações eletromagnéticas.

Além dos danos em lipídios induzidos pelos EMR, há casos de danos em DNA como resultado do aumento dos radicais livres de oxigênio (PHILLIPS *et al.,* 1998; LAI & SINGH, 2004; YOKUS *et al.,* 2005).

Em 2006, FERREIRA e colaboradores encontraram aumento na freqüência de micronúcleo em neoblastos da planária ***Giardia tigrina*** após exposição ao UHF-EMR no período de 1 – 6 dias (8h/d). Neste trabalho, a Sod teve atividade aumentada após 6 dias, enquanto a Cat teve sua atividade diminuída. O mesmo grupo de pesquisadores encontrou aumento significativo de micronúcleos em eritrócitos de filhotes de ratas grávidas (***Ratus norvegicus***)

expostas ao EMR, mostrando que o UHF-EMR foi capaz de induzir resposta genotóxica no tecido hematopoiético durante o processo de embriogênese.

2 Justificativa

O aumento constante das atividades diárias, a necessidade de se comunicar em diversos locais diferentes de casa, além da busca por uma comodidade, faz crescer cada vez mais o uso dos telefones celulares e, consequentemente, de estações rádio-base no mundo. Estes aparelhos emitem ondas eletromagnéticas de ultra-alta freqüência que podem variar de 800 a 2.200 MHz. Há uma série de indícios relacionando essas radiações eletromagnéticas com problemas de saúde. Leucemia infantil (WERTHEIMER & LEEPER, 1979), diminuição de memória em tarefas comportamentais (SEAMAN *et al.,* 1998; LAI, 1994; 1996), alteração no processo do envelhecimento (IVANCSITS *et al.,* 2003) e tumores cerebrais (HARDELL *et al.*, 2006) são efeitos biológicos relacionados com EMR que justificam este trabalho. Tendo em vista os resultados controversos da literatura, ainda se faz necessário ampliar o conhecimento sobre as ações biológicas dessas radiações com relação à estabilidade genômica e o estado redox celular.

Neste trabalho, foram utilizados ratos da espécie ***Ratus norvegicus*** com 0, 6, 15 e 30 dias de idade. As alterações fisiológicas e bioquímicas que ocorrem nestes animais com o seu desenvolvimento são importantes para se verificar os efeitos da UHF-EMR sobre o fígado e o córtex cerebral desses animais.

3 Objetivos

3.1 Geral

Investigar a ação crônica da radiação eletromagnética de ultra-alta-frequência de 950 MHz de freqüência (UHF-EMR), ondas contínuas, 35 v/m de campo elétrico, 1 w de potência e polarização vertical sobre os mecanismos moleculares do córtex cerebral e do fígado de ratos com diferentes idades.

3.2 Específicos

Capítulo 1 - Avaliar os efeitos da radiação eletromagnética sobre o metabolismo das espécies reativas de oxigênio e suas consequências nos animais expostos;

Capítulo 2 - Avaliar o efeito da radiação eletromagnética sobre os danos oxidativos a lipídios, proteínas e DNA, bem como, verificar a concentração de ácidos graxos insaturados, a expressão da enzima catalase no fígado de ratos com 0, 6, 15 e 30 dias de idade irradiados da concepção até o nascimento;

Capítulo 3 - Avaliar o efeito da radiação eletromagnética sobre o metabolismo de ERO no córtex cerebral esquerdo e no córtex cerebral direito, bem como, a concentração de glicose no sangue total de ratos recém-nascidos irradiados da concepção até os 6 dias de idade.

Parte II

Capítulo 1

Bioelectromagnetics – BEM-12-0217
(Submetido)

Ultra-High-Frequency Electromagnetic Radiation and Reactive Oxygen Species in Mammals

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Abstract

The increasing emission of electromagnetic waves by various devices has increased concerns of possible health effects. Exposure to ultra-high-frequency (UHF) radiation is increasing due to the development of wireless communication technologies. This type of radiation has been linked to changes in organisms through the generation of ROS (Reactive Oxygen Species). The aim of this review was to discuss the effects of ultra-high-frequency electromagnetic radiation (UHF-EMR) on the metabolism of ROS and its consequences for mammals. We discuss the relationship of UHF electromagnetic waves with oxidants and antioxidants in mammals. After reviewing several studies, we conclude that UHF-EMR can cause changes in biomarkers of oxidative damage and can cause decreases in antioxidant defenses, which may result from these biomarkers' fight against the oxidative damage to macromolecules induced by ROS.

Keywords: electromagnetic radiation; ROS; oxidative damage; antioxidant; oxidative stress.

1 Introduction

Technological advancement has increased the exposure of organisms to electromagnetic waves. Electrical networks, broadcast radio and television stations, aircraft radar, microwave ovens, computer monitors and lights are some

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examples of objects that produce electromagnetic waves. Recently, the world has seen an increase in the number of mobile phones and base stations. These devices produce ultra-high-frequency electromagnetic radiation (UHF-EMR). Currently, this type of EMR has been linked to changes in biological systems. Physiological, molecular and behavioral changes have been observed in various organisms. In this review, we analyze the data reported in the literature on the effect of UHF-EMR on the metabolism of reactive oxygen species (ROS) and its consequences for mammals.

2 Electromagnetic Radiation

In the nineteenth century, James Maxwell expressed the existence of electromagnetic waves by expressing mathematically the laws of electricity and magnetism in a formula. Later that century, Heinrich Hertz produced electromagnetic waves for the first time in his laboratory. Since these two researchers' discoveries, the use of electromagnetic radiation has increased due to the development of new technologies, including wireless and mobile telephony.

In addition to the technologies mentioned above, devices such as radio, TV, the electric transmission network, visible lights and microwaves emit non-ionizing radiation. These emissions have energies lower than 10 eV and wavelengths greater than 200 nm. They have insufficient energy to produce electron emission [Eblem, 2006; Júnior and Martin, 2006]. The frequency range from 800 to 2200 MHz is used for mobile telephony. These frequencies are in the range classified as UHF (from 300 MHz to 3 GHz) [Verschaeve and Maes, 1998; Figura and Teixeira, 2007].

The scientific literature has shown that, given certain conditions, UHF-EMR induces ROS production in several biological systems [Moustafa et al., 2001; Zmyslony et al., 2004; Simko et al., 2006; Yurekli et al., 2006; Arthur, 2007; Valko et al., 2007; Phillips et al., 2009].

3 Reactive Oxygen Species (ROS)

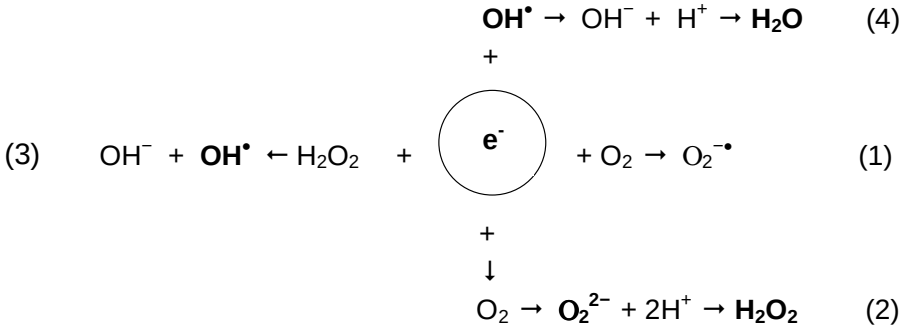
A chemical species capable of independent existence that contains one or more unpaired electrons is defined as free radical. An unpaired electron is one that occupies an atomic or molecular orbital alone [Cadenas, 1995; Halliwell and Gutteridge, 1999]. The superoxide and hydroxyl radicals are examples of oxygen-based free radicals.

ROS are composed of free radicals and potential ROS-forming molecules. Hydrogen peroxide and singlet oxygen are examples of ROS.

3.1 Biochemistry of ROS

During oxidative energy production in mitochondria, dioxygen is reduced by four electrons from cytochrome oxidase, forming two molecules of water. However, in mammals, a leak of electrons from the respiratory chain can result in oxygen being reduced by one electron, producing superoxide ($O_2^{-\bullet}$) [Lenaz, 2012]. The generation of ROS by mitochondria depends on the tension of O_2 and its concentration in the tissue.

The rotation of electrical charges generates a magnetic field. The pairing of electrons of opposite spins counteracts this effect, but an unpaired electron causes a molecule to be paramagnetic (slightly attracted by a magnetic field) and possibly highly reactive. Oxygen gas is paramagnetic, containing two unpaired electrons with the same spin [Halliwell and Gutteridge, 1999; Fridovich, 1998]. Thus, the O_2 electrons tend to occupy these spins through monoelectronic reduction reactions leading to the formation of species $O_2^{-\bullet}$ and O_2^{2-} (superoxide and peroxide, respectively, i.e., reactions 1 and 2).



The monoelectronic reduction of H_2O_2 occurs by a heterolytic fission of the O-O bond leading to the formation of a hydroxyl radical ($OH^{\bullet}$) and a hydroxyl anion (OH^-) (reaction 3). When the hydroxyl radical is reduced, it forms the hydroxide anion, which can react with a proton to form water (reaction 4). In vivo, the electron donors for H_2O_2 are usually transition metals such as iron and copper through the Fenton reaction (reaction 5). Fe (III) ions produced by the reaction can be recycled by reaction with reducing agents such as $O_2^{-\bullet}$ to generate Fe (II) (reaction 6). The sum of reactions 5 and 6 results in the Haber-Weiss reaction (reaction 7) [Halliwell and Gutteridge, 1999; Mccord and Day, 1978].

$$Fe\ (II) + \mathbf{H_2O_2} \rightarrow Fe\ (III) + OH^- + \mathbf{OH^{\bullet}} \qquad (5)\ \text{(reação de Fenton)}$$

$$Fe\ (III) + \mathbf{O_2^{-\bullet}} \rightarrow Fe\ (II) + O_2 \qquad (6)$$

$$\mathbf{O_2^{-\bullet}} + H_2O_2 \rightarrow O_2 + OH^- + \mathbf{OH^{\bullet}} \qquad (7)\ \text{(reação de Haber-Weiss)}$$

The superoxide radical is generated in all cellular compartments. It can be produced by the autoxidation of small molecules such as catecholamines and hydroquinones, activation of macrophages and neutrophils (the action of NADPH oxidase), the activity of various oxidases such as xanthine oxidase, cytochrome P450 and the electron transport chain of mitochondria and chloroplasts through Fe-S protein and coenzyme Q [Cadenas, 1995; Halliwell and Gutteridge, 1999].

The superoxide radical, at neutral pH, has a half-life of seconds. It has low reactivity and is unable to cross membranes. However, it is potentially harmful, as it is able to diffuse considerable distances to find a target. Diffusion occurs because superoxide can pass through ion channels. This radical can cause significant oxidative damage in subcellular structures through the formation of hydroxyl radicals via the Haber-Weiss reaction. Some enzymes such as aconitase can be inactivated directly by $O_2^{-\bullet}$, and the low activity of this enzyme is considered an indicator of oxidative stress in vivo [Halliwell and Gutteridge, 1999; Fridovich, 1998; Anderson et al., 1998].

Hydrogen peroxide is produced mainly by the dismutation of superoxide, but there are enzymes that produce it without the intermediary of $O_2^{-\bullet}$ (e.g.,

glycolate oxidase, D-amino acid oxidase, urate oxidase). Although H_2O_2 is a weak oxidizing agent, it is capable of inactivating some enzymes directly, usually by oxidation of the thiol groups (such as glyceraldehyde-3-phosphate dehydrogenase) [Halliwell and Gutteridge, 1999]. Sohal and Weindruch [1996] found that the higher the concentration of superoxide and hydrogen peroxide in the mitochondria, the lower the lifespan of some animals.

The toxicity of superoxide and hydrogen peroxide is dependent on the presence of iron or copper, which leads to the formation of hydroxyl radicals via the Haber-Weiss reaction (reaction 7). Thus, the nature and location of the hydroxyl radical damage depends on the presence of transition metals. The hydroxyl radical is one of the most reactive chemical species known; it can rapidly react with all types of cellular molecules (sugars, amino acids, proteins, nucleic acids and lipids, especially those containing polyunsaturated fatty acids) [Halliwell and Gutteridge, 1999]. The rate constants for the reaction of hydroxyl radical with biomolecules are in the range of 10^9 M^{-1} s^{-1} [Ischiropoulos, 1992]. Due to its high reactivity, the hydroxyl radical has a small diffusion radius inside the cell, causing damage near the sites where it is generated.

4 Production of ROS by EMR

ROS are generated naturally by cellular metabolism or induced by exogenous agents, such as parasites, pollutants and radiation. Electromagnetic radiation can increase ROS by the action of NADH oxidase, via the Fenton reaction or by altering the expression or activity of antioxidant enzymes.

4.1 Via NADH oxidase

NADH oxidase is a plasma membrane protein responsive to exogenous agents. This oxidase is present in animals and plants [Castillo-Olivares, 2000; Ushio-Fukai, 2006]. Its enzymatic activity is stimulated by hormones and growth factors in rat liver [Bruno et al., 1992] and by LPS (lipopolysaccharide) in mouse cardiomyocytes [Peng, 2005]. In the latter work, the increased activity of NADH

oxidase induced higher expression of COX-2 (cyclooxygenase 2). Once stimulated, the enzyme produces the free radical $O_2^{-\bullet}$. Some studies have shown that non-ionizing electromagnetic waves at UHF frequencies induce the formation of ROS through the activation of plasma membrane NADH oxidase [Friedman et al., 2007; Desai et al., 2009]. The ROS then activate extracellular matrix metalloproteinases that form Hb-EGF (heparin-binding epidermal growth factor) after cleavage of pro-Hb-EGF from the plasma membrane. This epidermal growth factor (EGF) stimulates the EGF receptor that triggers the activation of MAPK (mitogen-activated protein kinase), especially ERK (extracellular-signal-regulated kinase) [Friedman et al., 2007] (figure 1).

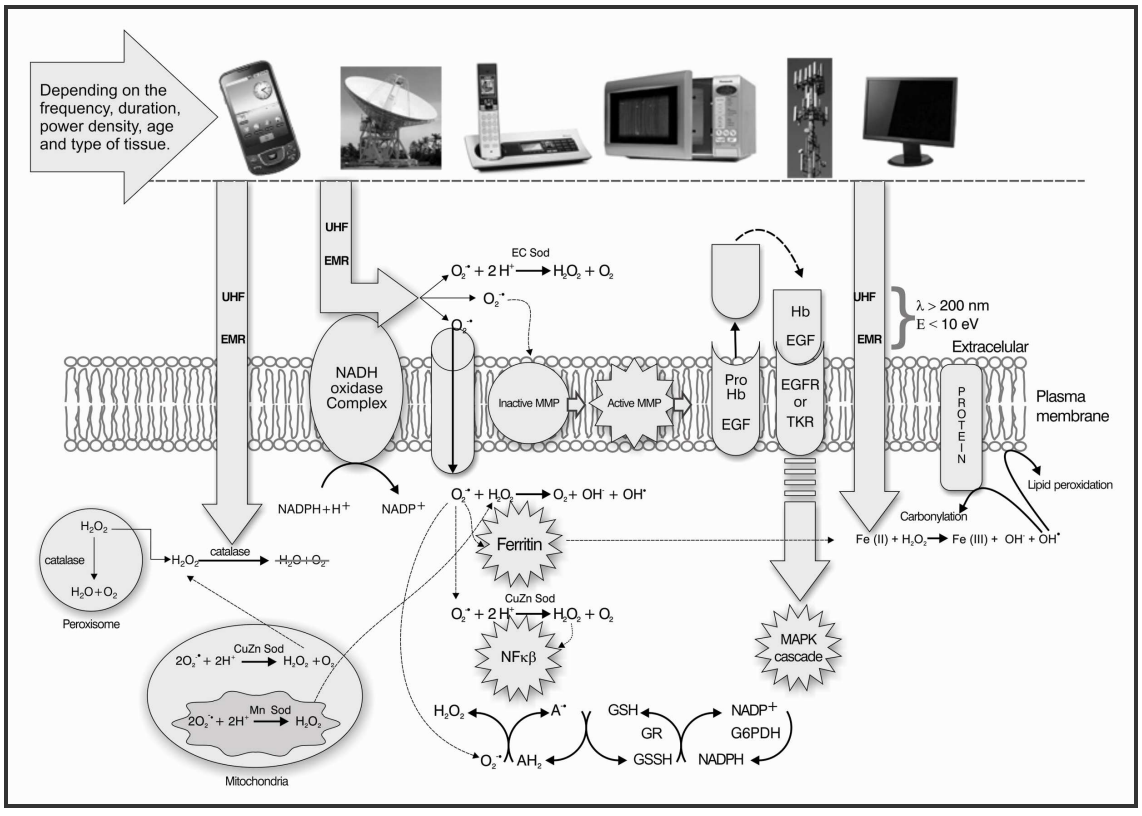


Figure 1. Mechanisms by which UHF-EMR generates ROS in the plasma membrane and cytoplasm. This figure also shows the consequences for cell signaling and antioxidant defense systems in addition to oxidative damage. UHF-EMR: ultra-high-frequency electromagnetic radiation, EC Sod, extracellular superoxide dismutase, CuZn Sod: copper-zinc superoxide dismutase, Mn Sod: manganese superoxide dismutase; MMP: extracellular matrix metalloproteinases, EGF: epidermal growth factor; Hb-EGF: heparin-binding epidermal growth factor, MAPK: mitogen-activated protein kinase, NF-ƙB: nuclear factor kappa-light-chain-enhancer of activated B cells, GR: glutathione reductase, G6PDH: glucose-6-phosphate dehydrogenase, GSH, reduced glutathione, GSSG, oxidized glutathione, AH_2: ascorbic acid, $A^{-\bullet}$: ascorbyl radical; $O_2^{-\bullet}$: superoxide radical, H_2O_2: hydrogen peroxide; $OH^{\bullet}$: hydroxyl radical, OH^-: hydroxyl anion.

4.2 Via the Fenton reaction

In 1876, Fenton found that the reaction of ferrous iron with hydrogen peroxide oxidizes organic compounds. Today, the Fenton reaction is used to destroy pollutants and to improve the biodegradability of organic molecules [Mackul'Ak et al., 2011]. This reaction is also related to a number of neurodegenerative [Mackul'Ak et al., 2011; Halliwell, 2006; Altamura and Muckenthaler, 2009; Uttara et al., 2009] and cardiovascular [Madamanchi et al., 2005] diseases. As described in section 3.3 of this review, the products of this reaction are ferric iron, hydroxyl anion and hydroxyl free radical [Halliwell and Gutteridge, 1999]. This is extremely reactive with a rate constant of nonspecific reaction of 10^9 M^{-1} S^{-1} [Augusto, 2006]. Some studies show that the interaction of electromagnetic fields with the Fe^{+2} ion in the Fenton reaction [Phillips et al., 2009], leads to the formation of hydroxyl radical. Céspedes and Ueno [2009] discovered the functional alteration of the protein ferritin, in organisms with large amounts of iron, after exposure to magnetic field. Depending on the molecular environment, the release of iron by ferritin may lead to the formation of ROS (figure 1).

4.3 Via alteration of antioxidant enzymes

A decrease in antioxidants is one way to cause oxidative stress [Sies, 1993]. An increase in ROS by UHF-EMR via NADH oxidase, can lead to the inactivation of antioxidant enzymes. The decrease in enzyme activity may be caused by the oxidation of sulfhydryls or by the loss of metals in metalloproteins, leading to structural changes in these proteins [Dean et al., 1997].

The interaction of electromagnetic waves with metals present in enzymes can lead to structural changes of these molecules, altering the activity of some antioxidant enzymes [Kesari and Behari, 2008]. The presence of iron in catalase and copper in superoxide dismutase could make these proteins more susceptible to structural changes induced by UHF-EMR, leading to changes in enzyme activity (figure 2).

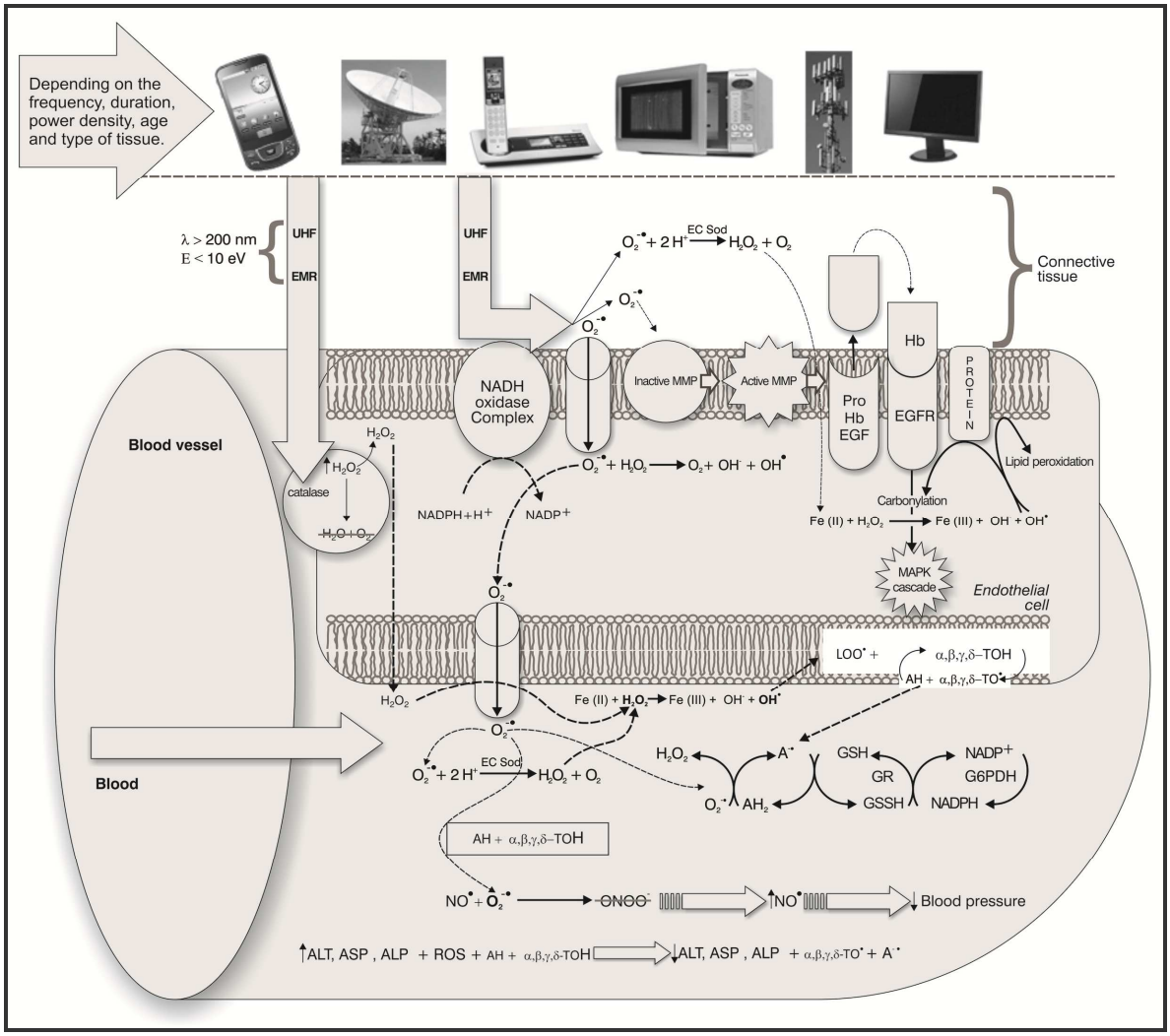


Figure 2. Mechanisms proposed to explain the effects of UHF-EMR on the metabolism of ROS in epithelial cells and inside blood vessels. This figure also shows the consequences for antioxidant defense systems in addition to oxidative damage in epithelial cells and blood vessels. UHF-EMR: ultra-high-frequency electromagnetic radiation, EC Sod: Extracellular superoxide dismutase; CuZn Sod: Copper-Zinc Superoxide dismutase, Mn Sod: Manganese superoxide dismutase, MMP: extracellular matrix metalloproteinases, EGF: epidermal growth factor, Hb-EGF: heparin-binding epidermal growth factor, MAPK: mitogen-activated protein kinase, GR: glutathione reductase, G6PDH: glucose-6-phosphate dehydrogenase, GSH: reduced glutathione; GSSG: oxidized glutathione, AH_2: ascorbic acid, $A^{-\bullet}$: ascorbyl radical, $O_2^{-\bullet}$: superoxide radical, H_2O_2: hydrogen peroxide, $OH^{\bullet}$: hydroxyl radical, OH^-: hydroxyl anion, α,β,γ,δ–TOH: vitamin E, α,β,γ,δ–$TO^{\bullet}$: tocopheryl radical, $LOO^{\bullet}$: lipoperoxyl radical, $NO^{\bullet}$: nitric oxide, $ONOO^-$: peroxynitrite, ALT: alanine aminotransferase, ASP: aspartate aminotransferase, ASP: alkaline phosphatase.

5 Oxidative Damages to Biomolecules

5.1 Lipids

The action of ROS on biological membranes promotes chain reactions that can lead to peroxidation of membrane phospholipids containing unsaturated fatty acids, generating peroxyl radicals, alkoxyl and lipid hydroperoxides. The peroxidation of membrane lipids alters membrane fluidity and can lead to rupture. In the case of membrane rupture, organelles could release proteases, lipases and ions. Calcium, for example, can activate a series of Ca^{2+}-dependent enzymes and several signal transduction pathways. Lipid hydroperoxides can be broken down into low molecular weight molecules (such as alkanes, ketones and aldehydes), which are also toxic to the cell [Marban et al., 1994; Chen et al., 1995; Halliwell and Gutteridge, 1999; Hermes-Lima et al., 2004]. Peroxidation of liver membranes of mitochondria-mediated iron ions is an important event in the pathophysiology of hemochromatosis [Hermes-Lima et al., 1995; Santos, 1998]. The peroxidation of low-density lipoprotein (LDL) is an important phenomenon in the process of atherogenesis [Darley-Usmar et al., 1995; Nageswara et al., 2005].

Lipid peroxidation is initiated by the attack on the lipid bilayer by any species sufficiently reactive to remove a hydrogen atom from a polyunsaturated fatty acid (PUFA). The removal of hydrogen generates an unpaired electron on a carbon atom, which reacts with molecular oxygen, forming peroxyl radical ($LOO^{\bullet}$). This reaction is capable of reacting with another PUFA, initiating a new chain of oxidation. The peroxyl radical combines with the abstracted hydrogen atom, forming a lipid hydroperoxide (LOOH) [Vaca et al., 1988; Hermes-Lima et al., 2004]. This hydroperoxide is unstable in the presence of transition metals, for example, iron and copper, which react with it to form alkoxyl and peroxyl radicals [Halliwell and Gutteridge, 1999]. The alkoxyl radical can remove hydrogen atoms from other PUFA and lipid hydroperoxides to form lipid and peroxyl radicals, thus contributing to the propagation of lipid peroxidation. The decomposition of LOOH generates not only radicals that propagate lipid peroxidation but also radical products such as aldehydes and ketones among others.

Aldehydes are a biomarker of oxidative damage to lipids. These end products of lipid peroxidation are used as markers of the action of some exogenous agents. Electromagnetic radiation can induce an increase in aldehydes. Of these, the one most often measured is MDA (malondialdehyde). Most studies published in the literature show an increase in tissue MDA irrespective of the tissue analyzed [Moustafa et al., 2001; Ozguner et al., 2005; Oktem et al., 2005; Meral et al., 2007; Ozgur et al., 2007; Sokolovic et al., 2008; Moussa, 2009; Dindic et al., 2010; Hassan et al., 2010] (figures 1 and 2).

Another biomarker of oxidative damage to lipids is lipoperoxide. There are few studies quantifying lipoperoxide in relation to EMR. In one, Tomruk et al. [2010] found increased levels of FOX (Ferrous Oxidation in Xylenol Orange) in the liver of rabbits after exposure to UHF-EMR.

The increase in lipoperoxides and aldehydes resulting from exposure to EMR is explained by the fact that radiation generates ROS. These reactive species attack PUFAs, leading to the formation of intermediate products, and finally, lipoperoxidation.

5.2 Proteins

The introduction of carbonyl groups into proteins is a hallmark of oxidative modification and has been linked to oxidative damage via hydroxyl radical reaction with portions of the amino-terminal peptide [Stadman, 1992]. Oxidation – mediated by ROS – of some amino acid residues such as lysine, arginine and proline leads to the formation of carbonyl groups [Stadman, 1992; Kristal and Yu, 1992]. These groups can also be formed as the result of secondary reactions of some amino acid side chains with lipid oxidation products, or even reduced sugars or their oxidation products [Kristal and Yu, 1992]. Proteins can undergo other oxidative changes, for example, the oxidation of sulfhydryl groups, leading to conformational changes and the alteration of enzyme activity, cleavage of peptide bonds, modification of carbohydrates in glycoproteins and the loss of metals in metalloproteins [Stadman, 1992; Dean et al., 1997]. Oxidatively damaged proteins are degraded by cytosolic proteases [Stadman, 1992]. Proteins containing

oxidized amino acids are recognized and degraded by ubiquitin-dependent proteolytic systems. The protein complexes that degrade these oxidized proteins are called proteasomes and are present in many copies in the cytosol [Hershko and Ciechanover, 1992] and cell nucleus [Peters et al., 1994].

The oxidation of proteins is associated with oxidative stress situations, such as ischemia-reperfusion, hyperoxia, smoking, estrogen administration, artificial ventilation, forced exercise and in cultured cells exposed to H_2O_2 or xanthine oxidase. High levels of carbonyl groups are associated with various diseases such as Alzheimer's, Parkinson's, diabetes, rheumatoid arthritis and muscular dystrophy, and can induce kidney tumors [Hermes-Lima, 2004]. In addition, protein carbonyl levels are directly associated with the natural aging of various species [Stadman and Levine, 2000; Hermes-Lima et al., 2004].

There are few studies with measurements of protein carbonyls as a function of exposure to ultra-high-frequency electromagnetic waves. The work of Sokolovic et al. [2008] showed increased levels of carbonylated proteins after exposure to UHF-EMR. Arendash et al. [2010] found that even with a decrease in MnSOD activity, protein carbonyl content was not changed in the hippocampus of rats.

The increase in protein carbonylation may be due to the direct attack of ROS on the amino acids of the polypeptide. Indirectly, the protein may be carbonylated by reaction with aldehydes generated by lipid peroxidation (figures 1 and 2).

5.3 Nucleic acids

DNA is also damaged by ROS, lipid peroxidation products and reactive nitrogen species (RNS). These molecules can cause the DNA strand breaks and oxidative modifications to bases, creating a series of products (8-hydroxyguanine, glycol 5-hydroxymethyl-glutaryl and cytosine) that can lead to mutagenic and carcinogenic processes [Halliwell and Gutteridge, 1999; Hermes-Lima et al., 1998b; Saffi and Henriques, 2003]. Free radical attack may also lead to cross-reactions between DNA and proteins. Damage to the sugar moiety is also

frequently observed. These multiple types of DNA damage (i.e., all the injuries in regions close to the DNA) together with the double-strand break can be genotoxic, mutagenic and carcinogenic [reviewed by Saffi and Henriques, 2003].

The ROS superoxide and hydrogen peroxide do not attack the DNA directly, where as hydroxyl radicals and singlet oxygen can cause direct injury. The first reacts with the four nitrogenous bases, generating modified bases, breaking DNA chains and forming apurinic and apyrimidinic (AP) sites. Singlet oxygen can cause cyclo-addition to the carbon double bond of the imidazole ring, generating alkali-labile AP sites and breaks in the chain next to guanines. The preferred target of ROS is guanine and most common product is 7.8-hydroxy-8-oxo-2 deoxyguanine (8-OHdG) [reviewed by Saffi and Henriques, 2003].

Damage to DNA can be measured by the comet assay, by monitoring levels of 8-OHdG and by PFGE (Pulsed-Field Gel Electrophoresis). The results of the experiments using continuous UHF-EMR showed no differences in DNA fragmentation in rat brains, rat glial cell cultures and in rabbit livers [Belayaev et al., 2006; Campisi et al., 2010; Di Mascio et al., 1991; Tomruk et al., 2010].

6 Antioxidant Defenses

Any substance which, when present in low concentrations compared with the oxidant, slows or inhibits the rate of oxidation of a substrate is considered an antioxidant [Halliwell and Gutteridge, 1999].

Aerobic organisms evolved enzymatic antioxidant defenses and related non-enzymatic adaptations to environmental conditions, due to survival by those most able to defend themselves from damage caused by ROS.

6.1 Non-enzymatic

The non-enzymatic defenses are mainly composed of low molecular weight molecules. They include vitamins C and E, beta-carotene, glutathione, bilirubin, lactate and uric acid. Most non-enzymatic antioxidants are found in water (plasma, cytoplasm, mitochondrial matrix, etc.); vitamin E and carotenoids are

found in the membranes. The mechanism of action of these defenses is to interrupt the chain of oxidative reactions, stopping the spread of radical reactions [Cadenas, 1995; Di Mascio et al., 1991].

6.1.1 Vitamin A

Vitamin A (retinol) is formed from carotenoids. In this process, the reaction of the carotenoid with O_2 is catalyzed by dioxygenase to produce two molecules of retinol [Halliwell and Guteridge, 1999]. The antioxidant activity of vitamin A (RetOH) is due to polyene units that act on singlet oxygen and peroxyl free radical ($ROO^{\bullet}$) (reaction 8). In addition to these ROS, retinol also acts on tiils radicals. Depending on the reactive species, retinol can act as a "quencher" (singlet oxygen) or "scavenger" (peroxyl radical). In addition to the antioxidant activities mentioned, Barber et al. [2000] found that rats deficient in vitamin A had a decreased ratio of GSH / GSSG in rat liver mitochondria. These researchers also observed an increase in the level of MDA and 8-oxodG. These results were reversed after re-feeding with vitamin A.

Reaction

$$\mathbf{RetOH} + \mathbf{ROO^{\bullet}} \Longrightarrow \mathbf{RetO^{\bullet}} + \mathbf{ROOH} \quad (8)$$

The discovery that electromagnetic radiation can generate oxidative stress (OS) [Elhag et al., 2007] has led to experimental designs that include non-enzymatic antioxidants, dietary or not, along with radiation. Retinol levels were lower (relative to the control group) due to oxidative damage induced by UHF-EMR in the heart and plasma of rats [Elhag et al., 2007; Türker et al., 2011]. However, the same result was not found in the plasma and brain of guinea pigs [Meral et al., 2007].

Vitamin A is consumed to reduce oxidative damage by acting as a quencher or scavenger of ROS generated by electromagnetic radiation.

6.1.2 Vitamin C

Vitamin C (ascorbic acid or AH_2) is formed from glucose in many organisms. Monkeys do not produce this vitamin. This is because the enzyme responsible for the synthesis of ascorbic acid (L-gulono-1, 4-lactone oxidase) is not transcribed [Cerqueira et al., 2007]. Therefore, these organisms need to acquire this acid in their diet because it has many biological functions. Some of these are the post-translational modification of collagen, the production of carnitine, the transformation of dopamine into norepinephrine, and the metabolism of tyrosine. In addition to its function as a cofactor, vitamin C participates in the transport of iron, and, depending on its concentration, it is an important antioxidant.

AH_2 is water-soluble and is a strong reducing agent [Cerqueira et al., 2007]. These characteristics make it an excellent antioxidant in aqueous intra- and extracellular milieus. It acts directly as an antioxidant when it reduces ROS, e.g., superoxide, hydroxyl radical and nitrogen dioxide. Vitamin C acts indirectly as an antioxidant in alpha-tocopherol recycling. This recycling protects membranes against lipid peroxidation. After recycling of alpha-tocopherol (vitamin E) from the tocopheryl radical, vitamin C becomes the ascorbyl radical ($A^{-\bullet}$) (reaction 9). This radical has low reactivity and can be transformed back into ascorbic acid by the action of NADH-dependent reductases or by the action of GSH.

Reaction

$$\mathbf{AH_2 + O_2^{-\bullet} \Longrightarrow A^{-\bullet} + H_2O_2} \qquad (9)$$

The levels of vitamin C have been monitored in experiments with electromagnetic radiation. Türker et al. [2011] found lower levels of vitamin C in the heart tissue of rats exposed to UHF-EMR of 2.45 GHz. Elhag et al. [2007] also observed decreased levels of vitamin C in the plasma of rats exposed to 900 MHz UHF-EMR. The decrease in content of vitamin C comes from its use as a reducing agent of ROS, especially superoxide generated by EMR (figure 1 and 2).

6.1.3 Vitamin E

The tocopherols and tocotrienols together constitute vitamin E (α,β,γ,δ–TOH). These two groups of molecules are formed by a chromanol nucleus and an aliphatic chain. This formation facilitates the insertion of the vitamin in the phospholipid layer, while the core chromanol ring is responsible for the lipid radical ($LOO^{\bullet}$), interrupting the lipid peroxidation chain and forming the tocopheryl radical ($\alpha,\beta,\gamma,\delta–TO^{\bullet}$) (reaction 10). This layer is recycled to the alpha-tocopherol by ascorbic acid (AH_2) (reaction 11).

Reactions

$$\alpha,\beta,\gamma,\delta–TOH + LOO^{\bullet} \Longrightarrow \alpha,\beta,\gamma,\delta–TO^{\bullet} + LOOH \quad (10)$$

$$\alpha,\beta,\gamma,\delta–TO^{\bullet} + AH_2 \Longrightarrow \alpha,\beta,\gamma,\delta–TOH + A^{-\bullet} \quad (11)$$

Türker et al. [2011] observed a decrease in the levels of this vitamin in rats exposed to 2.45 GHz UHF-EMR. The activity of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (ASP) and alkaline phosphatase (ALP) indicate the functional state of the liver. The activity of these enzymes increases when there is illness or injury by toxic agents to the liver [Pashovkina et al., 2001]. Exposure to UHF electromagnetic waves increases the activity of ALT, ASP and ALP in the blood of rats [Moussa, 2009; Aziz et al. 2010]. When rats were exposed to UHF-EMR and received vitamins C and E, the enzyme activity decreased [Aziz et al. 2010]. This can be explained due to the antioxidant action of those compounds that decreased the liver damage caused by ROS (figure 2). The reduction of damage led to a decrease of measured enzyme activities.

Another action of vitamin E and C is related to the cardiovascular system. The use of these vitamins caused decreases in the blood pressure of rats exposed to EMR [Awed, 2011]. The antioxidant capacity of these vitamins decreased the ROS formed by the UHF-EMR. This led to the increased availability of nitric oxide, which lowered the blood pressure of the rats (figure 2).

6.1.4 GSH

Reduced glutathione (GSH) (γ-glutamyl-cysteinyl-glycine) is present in most cells and it is the most abundant thiol in the intracellular environment [Meister and Anderson, 1983]. This tripeptide is free in animal tissues, its concentration ranges from 0.5 to 10 x 10^{-3} M. Its reductive capacity is determined by the sulfhydryl (-SH) group, of the amino acid cysteine.

Glutathione is an important non-enzymatic antioxidant defense and participates in the regeneration of ascorbate and alpha-tocopherol (reaction 13) and is a co-substrate of GPx enzyme reaction (18, 19) and glutathione S-transferase. GSH can be considered one of the most important agents of the antioxidant defense system of cells, protecting them against injury that results from exposure to agents such as iron ions [Galeano and Puntarulo, 1995], hydroxyl radicals (reaction 14), and alkoxyl and peroxyl ($ROO^{\bullet}$) radicals (reaction 15), as well as superoxide, hyperbaric oxygen, radiation and ultraviolet light [Deneke and Fanburg, 1989]. In addition, GSH decreases the susceptibility to renal injury caused by ischemia and reperfusion [Shan et al, 1990], and it acts as a carrier and reservoir of cysteine and participates in the detoxification of xenobiotics and lipid peroxidation products [Ferreira and Matsubara, 1997]. An increase in the generation of ROS in the cell leads to an increased ratio of GSSG / GSH (glutathione disulfide / reduced glutathione), and this ratio used as an important indicator of oxidative stress (figures 1 and 2).

Reactions

$$\alpha,\beta,\gamma,\delta\text{–}TO^{\bullet} + AH_2 \Longrightarrow \alpha,\beta,\gamma,\delta\text{–}TOH + A^{-\bullet} \qquad (12)$$

$$A^{-\bullet} + 2\,GSH \Longrightarrow AH_2 + GSSG \qquad (13)$$

$$GSH + OH^{\bullet} \Longrightarrow GS^{\bullet} + H_2O \qquad (14)$$

$$GSH + RO_2^{\bullet} \Longrightarrow GS^{\bullet} + ROOH \qquad (15)$$

$$GS^{\bullet} + GS^{\bullet} \Longrightarrow GSSG \qquad (16)$$

GSH was measured in the brain, blood [Meral et al., 2007], liver, kidney [Moussa, 2009], hippocampus [Arendash et al., 2010] and reproductive tissue [Mailankof et al., 2009] of mammals after exposure to UHF-EMR. There was a decrease in these levels in all the organs. Although most studies show a reduction in the concentration of GSH, Praputpittaya et al. [2008] found no differences in the brains of rats exposed to 900 MHz UHF-EMR for 30 minutes a day for one week.

The decrease in GSH levels comes from its direct use in hydroxyl radical and singlet oxygen scavenging. In addition, the role of glutathione as co-substrate in the reactions of GPx and GST (glutathione S-transferase) causes a reduction in its concentration. The production of ROS and RNS induced by electromagnetic fields leads to the formation of organic peroxides, which are metabolized by some types of GST and GPx (figure 2).

6.1.5 Melatonin

Melatonin is produced largely by the pineal gland from serotonin. This happens mostly at night. The best-known function of this molecule is circadian rhythm control. Today, it is known that the hormone melatonin is a "scavenger" of free radicals. In addition, it stimulates the activity/expression of GPx and SOD [Tan et al., 1993]. Melatonin's potent antioxidant capacity comes from its ability to cross biological membranes. Treatment with melatonin prevented an increase in lipid peroxidation and in the activity of xanthine oxidase (XO) in rat brain 40 days after exposure to EMR but did not prevent a decrease of catalase activity and an increased protein carbonyl content [Sokolovic et al., 2008]. In other studies, the administration of melatonin to rats reduces an UHF-EMR-induced increase of lipid peroxidation in the hippocampus [Köylu et al., 2006] and kidney [Oktem et al., 2005]. In that organ, there was also an increase in the concentration of NAG (N-acetyl-β-D-glucosaminidase), a marker of damage to renal tubules. NAG levels after exposure to EMR decreased after administration of melatonin.

The hormone melatonin induces an increase in gene expression and in the activity of Sod and GPx that can then consume greater amounts of $O_2^{-\bullet}$ and

H_2O_2, respectively, generated by UHF-EMR. The reduction of these ROS leads to reduced lipid peroxidation, protein carbonylation and kidney damage.

6.1.6 Zinc

Some researchers have found that certain metals have pro-antioxidant function. An example is zinc. This transition metal is the thirtieth element of the periodic table and sits in the fifth period of that table. Unlike most of this period of transition metals, Zn has antioxidant activity rather than oxidant activity as observed in Fe, Cu, V, Ni, Co and Cr [Valko et al., 2005]. Zinc stabilizes biological membranes and / or proteins, minimizing their susceptibility to attack by ROS [Bettger and O'Dell 1981; Powell, 2000]. This metal does not participate in redox reactions. Its antioxidant action is due to its binding to sulfhydryl groups of proteins, which protects them from ROS attack [Gibbs et al., 1985].

The antioxidant properties of zinc can be acute (described in the preceding paragraph) or chronic. This happens when an organism is treated with Zn for a long period, leading to increased synthesis of metallothioneins. These are metal-binding proteins, possessing large numbers of cysteines that bind to Zn, storing it [Powell, 2000]. Another situation in which the Zn has antioxidant activity occurs when it moves the Fe-binding site, inhibiting reactions that lead to the formation of ROS. In addition to the above mentioned antioxidant mechanisms, Zn stabilizes the structure of CuZnSod, while Cu participates in the catalysis of reactions through oxidation and reduction where the superoxide is consumed [Ahmad, 1995].

There are few studies relating non-ionizing electromagnetic radiation and quantification of metals in vivo. In one, Kiliçalp et al. [2009] investigated the action of 900 MHz UHF-EMR on the liver of ***Cavia porcellus***. The Zn content did not change in the liver but decreased in the testicles. The Zn levels did not change in the liver due to its high quantity and activity of enzymatic and non-enzymatic antioxidants (other than zinc), which might have decreased the antioxidant levels instead of Zn. As the testis does not contain such a wealth of antioxidants, the Zn

is used to defend against oxidative damage that can be caused by the generation of ROS induced by UHF-EMR.

6.2 Enzymatic

These defenses include mainly superoxide dismutase (Sod), catalase and glutathione peroxidase (GPx) [Hermes-Lima et al., 1998b; 2001].

6.2.1 Sod

This enzyme catalyzes the dismutation of superoxide to hydrogen peroxide and molecular oxygen (reaction 17). It is widely distributed in aerobic organisms. Although there are four types of superoxide dismutase – FeSod, MnSod, CuZnSod and EC-Sod – only the last three are present in mammals. Regardless of type, all these superoxide dismutases have the same catalytic mechanism. MnSod is present in the mitochondrial matrix, CuZn Sod occurs in the cytosol, nucleus, lysosome and mitochondrial intermembrane space, and EC-Sod is found in the extracellular medium (plasma, lymph and cerebrospinal fluid) (figures 1 and 2).

Reaction

$$O_2^{-\bullet} + O_2^{-\bullet} + 2H^+ \xrightarrow{\text{FeSod, MnSod; CuZnSod, E C-Sod}} H_2O_2 + O_2 \qquad (17)$$

In most studies where animals were exposed to UHF-EMR, a decrease in SOD activity was observed. These results occurred independently in various organs of experimental models [Moustafa et al., 2001; Ozguner et al., 2005; Oktem et al., 2005; Ozgur et al., 2007; Elhag et al., 2007; Kesari and Behari, 2009; Arendash et al., 2010; Hassan et al., 2010].

6.2.2 Catalase

Catalase decomposes hydrogen peroxide, forming water and oxygen (reaction 18) 4. This enzyme is present in most aerobic organisms [Halliwell and Gutteridge, 1999]. Although catalase exists in other organelles, it is present mainly in peroxisomes where there is a greater concentration of hydrogen peroxide (figures 1 and 2).

Reaction

$$H_2O_2 + H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \qquad (18)$$

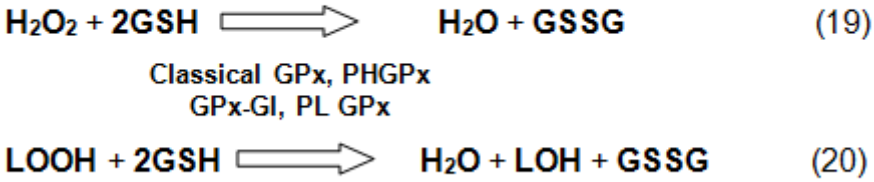
Four heme groups constitute the majority of catalases. These groups are linked to the enzyme active site. This site is stabilized by NADPH that is connected to each subunit of catalase [Vainshtein et al., 1981].

Catalase activity decreased in most studies where organisms were exposed to UHF-EMR. Except for a few experiments [Kesari and Behari, 2009; Ferreira et al., 2006], exposure for less than 10 days in rats or guinea pig – whether measured in the brain [Meral et al., 2007; Sokolovic et al., 2008], heart [Ozguner et al., 2005], kidney [Oktem et al., 2005] or blood [Hassan et al., 2010] – has always shown decreased activity for this enzyme.

6.2.3 GPx

This enzyme breaks down not only hydrogen peroxide but also organic hydroperoxides, using glutathione as a reductant (reaction 19 and 20). There are four types of GPx: the classical Se-dependent GPx (located in the cytosol and mitochondrial matrix) depends on phospholipase A_2 to act on membrane hydroperoxides; PHGPx (located in the cytosol) uses phospholipid hydroperoxides as substrates without the need for phospholipase; GI-GPx (in the cytosol of cells of the gastrointestinal tract); and PLGPx (plasma). Even with different sizes in the active site of these enzymes, there is always an atom Se replacing the cysteine S.

Reactions



Although there are some studies that show no significant change in GPx activity in animals exposed to UHF-EMR [Irmak et al., 2002; Ferreira et al., 2006; Ozgur et al., 2007], other studies showed that the enzyme activity decreases significantly in liver, brain, sperm [Kesari and Behari, 2009], blood [Moustafa et al., 2001; Hassan et al., 2010], heart [Ozguner et al., 2005] and kidney [Oktem et al., 2005] after continuous exposure to radiation mainly at a frequency of 900 MHz.

The low total GPx activity can be explained by a decreased concentration of reduced glutathione. The concentration of this co-substrate decreases after exposure to UHF-EMR.

7 Oxidative stress

The imbalance between the production of oxidants and the concentration of antioxidant defenses leading to cell damage is called oxidative stress (OS). This can occur due to a high production of ROS by a reduction of antioxidant defenses or by both processes simultaneously [Sies, 1993].

Oxidizing agents are formed in the normal process of metabolism and in various pathological conditions. They can be produced at very high rates, leading to oxidative stress and possibly to cell death and / or apoptosis. Oxidative stress is related to diseases such as cancer, hemochromatosis, rheumatoid arthritis, diabetes, hepatic porphyria, neurodegenerative diseases, cardiovascular diseases and aging [Braughler and Hall, 1989; Kleinveld et al., 1989; Stadman, 1992; Kaul et al., 1993; Levine, 1993; Hermes-Lima, 2004; Reuter et al., 2010].

Recently, the OS has been observed in mammalian organs when they are exposed to UHF-EMR [Kesari and Behari, 2008; Reuter et al., 2010]. As this

radiation can induce the generation of ROS, it is likely that these animals become more susceptible to the initiation and / or worsening of various pathological conditions.

8 Conclusion

The main question addressed in this work was: Does UHF-EMR alter the metabolism of ROS in mammals? To answer this question we carried out an extensive literature review relating UHF radiation to oxidative damage and antioxidants. The majority of the studies reviewed show that UHF-EMR can cause changes in biomarkers of oxidative damage. Of these, the products of lipid peroxidation show the greatest increase. Commonly, if reduced antioxidant defenses are observed, it is because they have been depleted (mainly GSH) by fighting against oxidative damage to macromolecules induced by ROS. Increased concentrations of aldehydes can lead to the aggravation of cancers, Alzheimer's disease, atherosclerosis and diabetes, as MDA is a biomarker of these diseases. Therefore, people with any of these diseases should avoid exposure to UHF-EMR.

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Capítulo 2

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Effect of 950 MHz UHF-EMR on biomarkers of oxidative damage, UFA metabolism and antioxidants in the liver of rats of different ages

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Abstract

Purpose: To assess the effect of 950 MHz UHF EMR (ultra-high-frequency electromagnetic radiation) on biomarkers of oxidative damage, as well as to verify the concentration of unsaturated fatty acids (UFA) and the expression of the catalase in the livers of rats of different ages.
Materials and methods: Twelve rats were equally divided into two groups as controls (CR) and exposed (ER), for each age (0, 6, 15 and 30 days). Radiation exposure lasted half an hour per day for up to 51 days (21 days of gestation and 6, 15 or 30 days of life outside the womb). The SAR (specific absorption rate) ranged from 1.3 - 1.0 W/kg. The damage to lipids, proteins and DNA was verified by TBARS (thiobarbituric acid reactive substances), protein carbonyls and comets, respectively. The UFA were determined by gas chromatography with a flame ionization detector. The expression of catalase was by Western blotting.
Results: The 0 day had low levels of TBARS and concentrations of UFA after exposure. There was no age difference in the accumulation of protein carbonyls for any age. The DNA damage of ER 15 or 30 days was different when compared to the control group.The exposed 0 day exhibited lower expression of catalase.
Conclusions: 950 MHz UHF EMR does not cause Oxidative Stress (OS), and it is not genotoxic to the livers of 0 day or those of 6 and 15 day old rats, but it changes the concentrations of PUFA in 0 day. For rats of 30 days, no OS, but it is genotoxic to the livers of ER to total body irradiation.

Keywords: EMR; oxidative damage; antioxidant; aging; PUFA.

1 Introduction

Every day, technological development results in increased numbers of devices that emit electromagnetic waves. These devices can be seen in airports, homes, schools, hospitals and industries. Appliances such as radar, microwave ovens, computer monitors, MRI machines, radio base stations and cell phones are all examples of sources of ultra-high frequency electromagnetic radiation (UHF-EMR).

UHF-EMR occupies a frequency band that ranges from 300 MHz to 3 GHz (Versachaeve and Maes 1998; Figura and Teixeira 2007). Some studies correlate this type of electromagnetic radiation with behavioral (Salford et al. 2003), physiological (D'andrea et al. 2003), structural (Fejes et al. 2005) and molecular (Paulraj and Behari 1997) changes. These changes seem to be due to the generation of reactive oxygen species (ROS) (Moustafa et al. 2001; Zmyslony et al. 2004; Oktem et al. 2005; Simko et al. 2006; Yurekli et al. 2006; Arthur 2007; Valko et al. 2007; Phillips et al. 2009; Ozgur et al. 2010; Nazıroğlu et al. 2012; Avci et al. 2012).

ROS are composed of free radicals and the molecules that can generate them. A free radical is any chemical species that has an unpaired electron in an orbital (Halliwell and Gutteridge 1999). These molecules cause oxidative damage to lipids, proteins and nucleic acids. Increased damage from these molecules occurs in various diseases and biological processes. ROS contribute to neurodegenerative and cardiovascular diseases, as well as to ischemia-reperfusion injuries (Reuter et al. 2010).

In addition to diseases, ROS contribute to the natural aging process. This multifactorial process is a series of functional and structural changes of cumulative, progressive and deleterious effects that may or may not occur as a function of time and that lead to death (Arking 1998). The most accepted explanation for aging is based on the theory that free radicals cause aging. This explanation was proposed in 1956 by Harman. In this theory, longevity is inversely proportional to the extent of oxidative damage and directly proportional to the activities of the antioxidant defenses.

Given the above, the objective of this study was (1) to assess the effect of UHF EMR on oxidative damage to lipids, proteins and DNA, (2) to verify the concentration of unsaturated fatty acids and (3) to determine the expression of the enzyme catalase in the livers of rats of different ages.

2 Experimental procedures

2.1 Animals

The rats were obtained from the Center for Reproduction and Animal Experimentation Laboratory of the Federal University of Rio Grande do Sul. Pregnant rat (***Rattus norvegicus***) was placed in a box from the first day of pregnancy in the company of other pregnant rats to avoid the stress of isolation. The first day of pregnancy was considered to be the day on which spermatozoids were observed in the vagina. This condition was observed by optical microscopy (Zeiss Axiophot, magnification, 100x). A total of twelve pregnant rats were used, six rats in the control group and six in the group irradiated with UHF-EMR. The control group was subjected to the same conditions as the irradiated group but without the system that generated electromagnetic waves. The pregnant rats were irradiated for half an hour per day from the day of sperm detection until the birth of pups. After the births, 6 pups from the exposed rats (ER) and 6 pups from the control rats (CR) were separated and sacrificed for each time period (0 day, 6 days, 15 days and 30 days of age).

Before exposure, the pregnant rats were kept in individual standard boxes (40.5 x 33.3 x 17.5 cm) under a cycle of 12 hours light/12 hours darkness, at a temperature of 23 ± 0.5℃, with water and a balanced diet *ad libitum*. During exposure to UHF-EMR, the animals were not allowed to drink water and were not fed. The rats were exposed for 30 minutes a day, between 12:00 pm and 3:00 pm, for 30 days. The control rats (not exposed to UHF EMR) were removed from the boxes and placed inside a Faraday box without an antenna at the same time.

All experiments were performed according to the guide for care and use of laboratory animals of the National Institute of Health (NIH publication No 85-23,

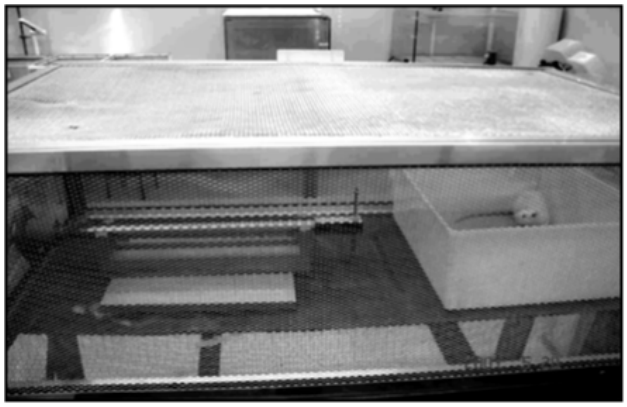
revised 1996). This research was filed with and approved by the Ethics Committee of the University under the number 2006-25A.

2.2 Collection and storage of material

After up to 51 days of exposure (21 days of gestation and 6, 15 or 30 days of life outside the womb), the rats were decapitated with a guillotine. The decapitation occurred in the late afternoon to avoid the antioxidant action of melatonin in rats at 15 and 30 days old (Oktem et al. 2005). The right lobe of the liver was removed, immersed in liquid nitrogen and stored at – 80 °C in an ultra-freezer.

2.3 Exposure System for UHF EMR

The animals underwent daily irradiation for 30 minutes from the first day of gestation up to 30 days of life outside the womb. The boxes of the irradiated groups were placed 11 cm away from the antenna. The antenna and the boxes were kept in an aluminum Faraday cage for the entire duration of the experiment. The electric field (**E**) outside the cage measured to be 0.3 V / m. The cage with the control group was 92 cm away from the cage containing the irradiated group. The cages were placed on a wooden tray. The **E** in the control cage measured 0.15 V / m. The **E** generated by the exposure system was 35 V/m. A generator of electromagnetic waves, composed of an Aural Broadcast STL generator, model PCL-303 (Moseley PCL-303), and a Precision Half-Wave dipole antenna, were used to produce UHF EMR at a power of 1 W and a frequency of 950 MHz. The antenna was configured for vertical polarization (Picture 1). An EMR-30 Radiation Meter (manufactured by Wandel & Goltermann) was used to measure the intensity of the **E** inside and outside of the boxes.



Picture 1. Exposure System

The SAR (specific absorption rate) of the tissue was calculated. SAR is a measure of the rate at which energy is absorbed from an electromagnetic radiation into biological tissue. The value was obtained using the formula below, according to ICNIRP (ICNIRP 2009).

$$\mathbf{SAR = (\sigma/\rho \times (E)^2\ [W / kg],}$$

where σ is the electrical conductivity of the sample, E is the average value of electric field and ρ is the density of the sample (liver). In this study, the SAR of the exposed group was 1.3 W / kg for 0 day and 6 day old rats, 0.9 W / kg for 15 day old rats and 1.0 W / kg for 30 day old rats. The pregnant rats were exposed to a SAR ranging from 0.6 W / kg on the first day to 0.4 W / kg on the twenty-first day of gestation. These values were found using a conductivity of 1.1 S/m for 0 day and 6 day old rats, 0.76 S/m for 15 day old rats and 0.86 S/m for 30 day old rats and a density of 1040 kg/m^3 for tissue. These values were calculated from the parameters measured by Peyman et al (2001). The specific absorption rates of the control groups were 2.37 x 10^{-5} W / kg for 0 day and 6 day old rats, 1.64 x 10^{-5} W / kg for 15 day old rats and 1.86 x 10^{-5} W / kg for 30 day old rats.

2.4 Lipid peroxidation assay

In this technique, lipid peroxidation is assayed by the reaction of thiobarbituric acid (TBA) with aldehyde compounds, primarily malondialdehyde (MDA), present in the tissue. The reaction results in the formation of TBARS, which can be

quantified by absorbance at 532 nm (Burge and Aust 1978; Hermes-Lima and Storey 1996; Ramos 1999).

Initially, the tissues were homogenized in a glass-glass homogenizer in 0.2% phosphoric acid; then, the same amount of phosphoric acid was added at a concentration of 2%. The final dilution was 1:40 (w:v). The two-stage homogenization reduced the formation of bubbles.

The material was kept on ice. After homogenization, 400 μL of the homogenate was transferred to tubes containing 200 μL of 7% phosphoric acid and 400 μL of 1% TBA (in 50 mM NaOH and 0.1 mM BHT). A mixture of 400 μL of the sample, 200 μL of phosphoric acid and 400 μL of 3 mM HCl was used. The pH was adjusted to 1.6. After the pH was verified, the samples were incubated at 100 ºC for 15 minutes. The tubes were left to cool at room temperature for 10 minutes. After cooling, 1.5 ml of butanol was added to the tubes, and the mixture was vortexed for 40 seconds and centrifuged for five minutes at 1000×g to separate the organic phase. This phase (pink, top) was removed, and the absorbance was read at 532 and 600 nm. The extinction coefficient used was 156 mM^{-1} cm^{-1}. The results were expressed as nmol TBARS/g wet tissue.

2.5 Quantification of Fatty Acid

The quality and quantity of the fatty acids were assessed by gas chromatography with a flame ionization detector (GC/FID), using external standardization. The lipids were extracted from the liver samples (10.2 to 11.6 mg) using chloroform and methanol in the ratio 2:1 (2 ml of chloroform and 1 ml of methanol) and kept on ice. Afterwards, the tube homogenizer (glass-glass) was washed with 1 mL of Milli-Q water. The homogenate was then added to this volume in a conical tube. The entire volume was vortexed for 50 seconds at high speed and centrifuged at 3000xg for 15 min at 4 ℃. After centrifugation, the organic phase (bottom) was transferred to another tube containing 2 ml of 3 mM HCl, vortexed and centrifuged under the same conditions. The organic phase (bottom) was removed and placed in a tube with 2 mL 3 M NaCl (saturating conditions). The solution was vortexed for 50 seconds at high speed and

centrifuged at 3000xg for 15 min at 4° C. The organ ic phase was then transferred to a test tube. Subsequently, the samples were dried with a flow of nitrogen gas and derivatized in methanolic NaOH (2%) using BF_3 and heptane. For the evaluation of the profile, we used external standards of single and mixed fatty acids: oleic acid, linoleic acid, linolenic acid, cis-8,11,14-eicosatrienoic acid, arachidonic acid, cis-5,8,11,14,17-eicosapentaenoic acid and cis-4,7,10,13,16,19-docosahexaenoic acid. The fatty acids were esterified by the method of Metcalfe et al. (1966, modified) to form methyl esters. The fatty acid analysis was performed on a gas chromatograph (GC) equipped with a polar column DB-Waxetr (30 m-0.25 µm, 0.250 mm) with a detector temperature of 275º C and an inlet temperature of 220° C. The initial column temperatu re was 150º C, which was maintained for one minute, followed by a heating ramp of 15° C / min up to 200° C, which was maintained for 2 minutes, followed by another ramp of 2° C / min up to 250° C, which was maintained for 5 minutes. The det ector was of the FID (Flame Ionization Detector) type.

2.6 Protein oxidation assay

The oxidized proteins were determined by measuring the levels of carbonyled proteins (adapted from Lenz et al. 1989). The protein carbonyl group reacts with 2,4-dinitrophenyl-hydrazine (DNFH), forming a product quantified by absorbance at 370 nm (Furtado-Filho et al. 2007).

Samples of the livers were homogenized in 5% sulphosalicylic acid, a dilution of 1:40 (w:v), and centrifuged for 5 minutes at 13,000xg. The supernatant was discarded, and the precipitate was added to 500 µL of 10 mM 2,4-dinitrophenyl-hydrazine (DNFH) prepared in 2 M HCl. The samples were kept at room temperature for one hour and vortexed every 15 minutes (4 times) to allow the reaction to occur between the DNFH and carbonyl groups. After this process, 500 µL of 20% trichloroacetic acid was added, and the reaction was centrifuged at 13,000xg for 3 minutes. The supernatant was discarded. Excess DNFH was removed by washing the precipitate with 1 ml of ethanol:ethyl acetate at a ratio of 1:1. The precipitate was vortexed for 40 seconds and centrifuged at 13,000xg for 3

minutes. This procedure (washing, spinning and disposal) was performed three times. The proteins were resuspended in 6 M guanidine chloride and incubated for 15 minutes. The carbonyl groups were quantified spectrophotometrically at 370 nm. The extinction coefficient used was 22 $mM^{-1}cm^{-1}$.

2.7 DNA damage assay

Damage to DNA was evaluated by the alkaline comet assay, according to Collins (2004). This assay was performed with frozen liver tissue (Recio et al. 2012). The tissues were fragmented in liquid nitrogen and weighed (0.010 to 0.012 g). The samples were thawed in an ice and water cooler for 15 minutes. A 1000 μL quantity of mincing solution (HBSS with 20 mM EDTA, 10% DMSO, pH 7.5) was added to 24-well plates on an ice shelf. The tissue was added to the mincing solution and left to soak for 15 minutes to remove excess blood. The spent mincing solution (1000 μL) was then discarded, and 500 μL of fresh solution was added. The tissue was suspended with the aid of two 200 μL tips. Soon after, under yellow light, 20 μL aliquots of cell suspension were collected and dissolved in 90 μL of 0.75% LMP agarose. The cell suspensions and agarose were immediately pipetted onto glass slides pre-coated with a layer of normal 1% agarose. The preparations were covered with a coverslip and left to dry in a refrigerator for 15 minutes. The coverslips were then removed, and the slides were placed in a vertical bucket (previously covered with foil) and incubated in cold lysis solution (2.5 M NaCl, 10 mM Tris, 100 mM EDTA, 1% Triton X-100, 10% DMSO, pH 10.0). The slides were protected from light and incubated in a refrigerator for 1 hour to remove the nuclear membranes and cytoplasmic contents, leaving the DNA as nucleoids. After the lysis procedure, the slides were placed in a horizontal electrophoresis tank. This tank was placed inside a plastic tray filled with ice. Electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH 13.0) was added to cover the slides, allowing the DNA to relax and the alkali labile sites to be cleaved. Electrophoresis was performed with a source set at a voltage of 25 V and an electric current of 300 mA for 20 minutes (the current was controlled by the volume of buffer). All previous steps were performed under yellow light or in darkness to

prevent further damage to the DNA. The slides were then placed vertically inside the bucket and covered with the neutralization buffer (0.4 M Tris, pH 7.5) for 5 minutes. This procedure was repeated three times. Subsequently, the slides were kept in distilled water for 5 minutes. The water was discarded, and the procedure was repeated twice. The slides were dried overnight and hydrated for 5 minutes in distilled water. Following that, the slides were stained with silver nitrate solution according to the protocol described by Nadin et al. (2001). After staining, the slides were dried at room temperature overnight. Finally, samples were analyzed using an optical microscope. One hundred cells on each of two duplicate slides were selected. The cells were visually scored into five classes according to the length of the tail: class 0, undamaged, no tail; class 1, with a tail shorter than the diameter of the head (nucleus); class 2, with a tail length of 1-2x the diameter of the head; class 3, with a tail with more than 2 times the diameter of the head; and class 4, with a tail with more than three times the diameter of the head or with no heads. The damage index (DI) for each group ranged from zero (100x0, 100 cells completely undamaged) to 400 (100x4, 100 cells observed with the maximum damage).

2.8 Immunoblotting of catalase

The expression of catalase was verified by Western blotting. Rat liver was rapidly homogenized in a solution containing 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 10% glycerol and Complete^{TM} protease inhibitor cocktail (Roche). Laemmli buffer (60 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 14.4 mM β-mercaptoethanol and 0.1 % BFB) was added to the samples. Normalization was performed using the Qubit System (Invitrogen). Proteins were separated by SDS-PAGE and transferred (electroblotted) to PVDF membranes. Proteins were detected by Coomassie Blue R staining. After staining, the membranes were bleached with 10% acetic acid and 50% methanol and blocked with 5% albumin for 75 minutes with slight agitation. Subsequently, the membranes were washed with distilled water and incubated for 10 minutes in 0.1% PBS-Tween 20 with slight agitation at room temperature. The PVDF

membrane was incubated overnight with primary antibodies (goat polyclonal antibodies) to catalase and vinculin. The membranes were subsequently washed with distilled water and placed in PBS-tween 20 with mild agitation for 5 minutes. The membranes were then incubated for two hours with horseradish peroxidase-linked anti-IgG secondary antibody. The membranes were placed in PBS-tween for five minutes and rinsed once again with distilled water. All antibodies were diluted 1:1000 in 0.1% PBS-tween. The immunoreactivity was detected by enhanced chemiluminescence using ECL solution at a ratio of 1:1 (v:v) luminol and hydrogen peroxide. The internal control used was vinculin. All antibodies were purchased from Santa Cruz Biotechnology.

2.9 Statistical analysis

All data are reported as the mean ± SEM. For comparisons between two different groups, we used Student's t-test (two-tailed). To compare more than two different groups, the test used was post-ANOVA SNK (Student-Newman-Keuls). Values of $p < 0.05$ were considered significant for both the t-test and the post-ANOVA test.

3 Results

3.1 Lipid peroxidation

Lipid peroxidation, determined by TBARS, was performed with the livers of control rats (CR) and exposed rats (ER). The TBARS levels differed between the ER and CR groups in rat 0 day. The exposed group (30.0 ± 1.08 nmol TBARS/g wet wt) exhibited lower levels of TBARS compared to the control group (43.00 ± 5.0 nmol TBARS/g wet wt). In the groups of 6, 15 and 30 days of age, no differences were detected between the CR and the ER (figure 1).

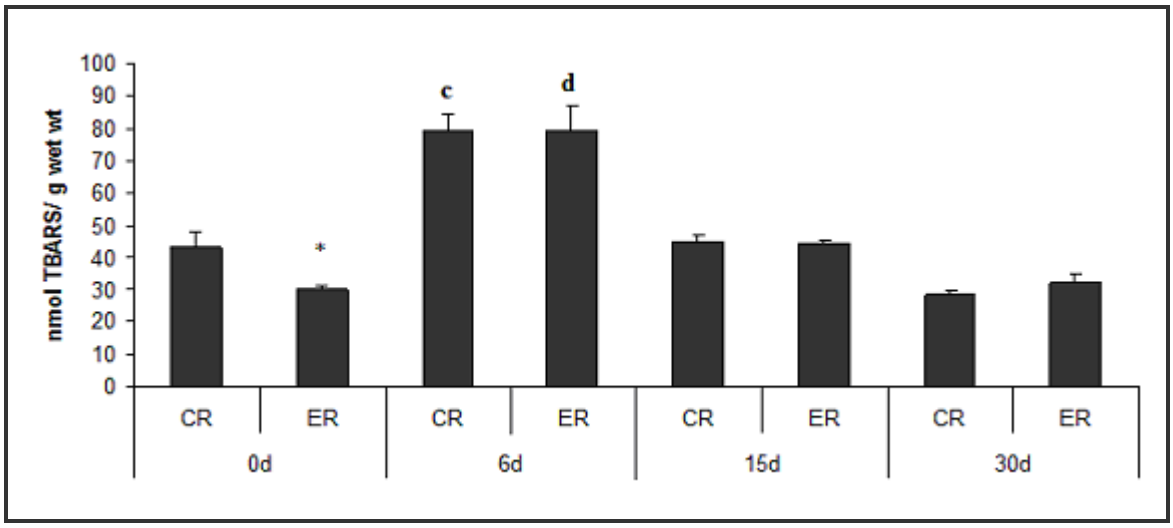


Figure 1. Determination of lipid peroxidation of the liver in rats with 0, 6, 15 and 30 days of age exposed (ER) and not exposed (CR) to UHF- EMR. The results are presented as mean ± SEM, n= 6. *: Significantly different from the CR value, p<0.05 (two tailed t-test). [c, d]: Significantly different when compared to all CR and ER, respectively, P<0.005 (SNK test post-ANOVA)

3.2 Quantification of Fatty Acid

Verification of the profiles and concentrations of fatty acids was performed by GC / FID with external standardization. There were changes in the concentrations of the ω–3, ω–6 and ω–9 fatty acids of the ER group compared to the CR group. Only the concentrations of linoleic acid and oleic acid did not change after exposure to UHF EMR. The concentrations of all other fatty acids decreased after the animals were irradiated. The omega-3 series was represented by the fatty acids linolenic acid (CR, 0.405865 ± 0.033137 μg fatty acid / mg wet wt and ER, 0.349447 ± 0.010999 μg fatty acid / mg wet wt), eicosapentaenoic acid (CR, 0.449045 ± 0.03266 μg fatty acid / mg wet wt and ER, 0.300933 ± 0.011412 μg fatty acid / mg wet wt) and docosahexaenoic acid (CR, 1.22296 ± 0.155778 μg fatty acid / mg wet wt and ER, 0.222408 ± 0.0.103971 μg fatty acid / mg wet wt). The omega 6 series was represented by the fatty acids linoleic acid (CR, 7.25326 ± 0.71032 μg fatty acid / mg wet wt and ER, 3.96454 ± 0.279468 μg fatty acid / mg wet wt) and arachidonic acid (CR, 4.75899 ± 0.577148 μg fatty acid / mg wet wt and ER, 1.81219 ± 0.222063 μg fatty acid / mg wet wt). The omega 9 series was represented by oleic acid (CR, 12.5435 ± 1.14056 μg fatty acid / mg wet wt and ER, 11.6191 ± 0.54939 μg fatty acid / mg wet wt) and eicosatrienoic acid (CR,

0.429848 ± 0.0382584 μg fatty acid / mg wet wt and ER, 0.296353 ± 0.0174513 μg fatty acid / mg wet wt) (Figure 2). Among all of the fatty acids that decreased in concentration, the docosahexaenoic acid exhibited the greatest reduction (5.5 times).

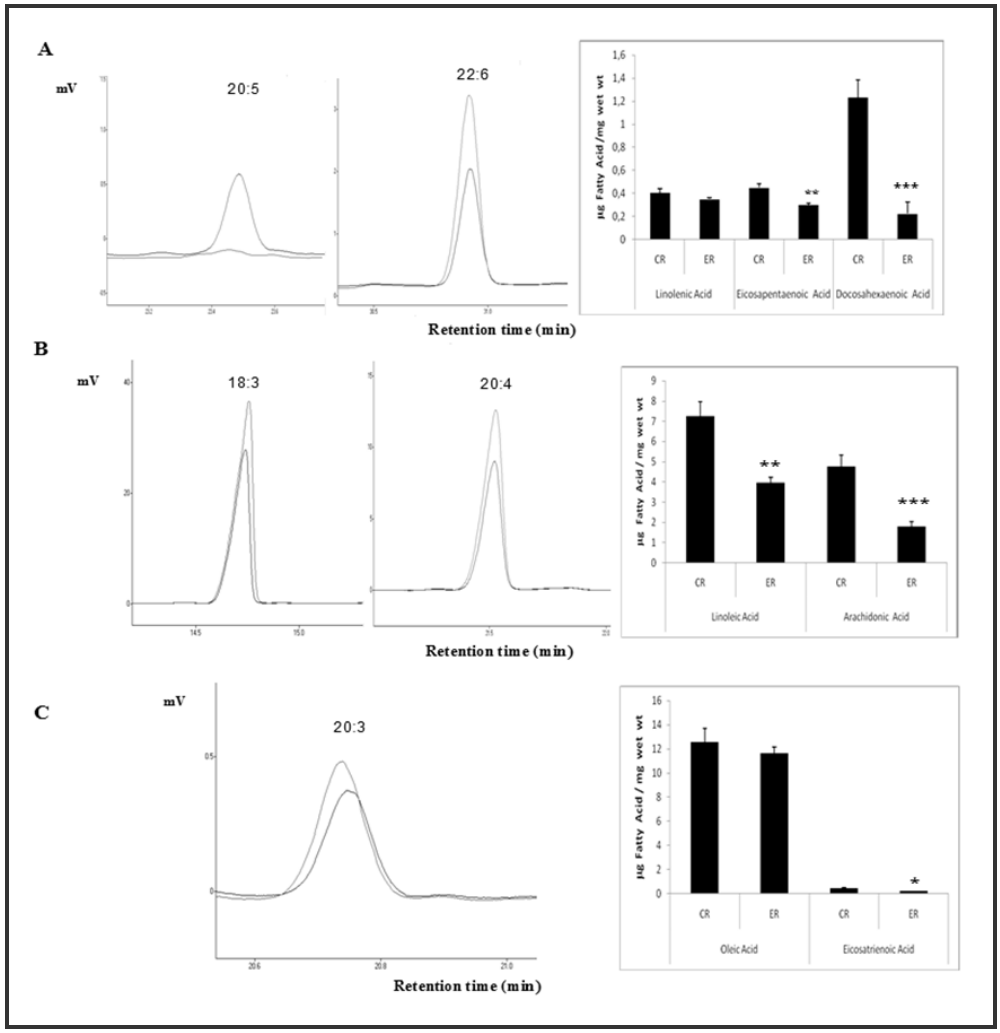


Figure 2. Determination of the concentration of fatty acids by gas chromatography of livers of rats exposed 0 day (ER) and unexposed (CR) to UHF EMR. The results are presented as mean ± SEM, n =6. *=p<0.01, **=p<0.005, ***=p<0.001 significant difference from the CR values (two tailed t-test). **A**, fatty acids ω–3 series; **B**, fatty acids ω–6 series; **C**, fatty acids ω–9 series.

3.3 Protein oxidation

The oxidation of proteins was measured by the levels of protein carbonyls formed. The levels of the ER groups were not different from those of the CR groups for any age (figure 3).

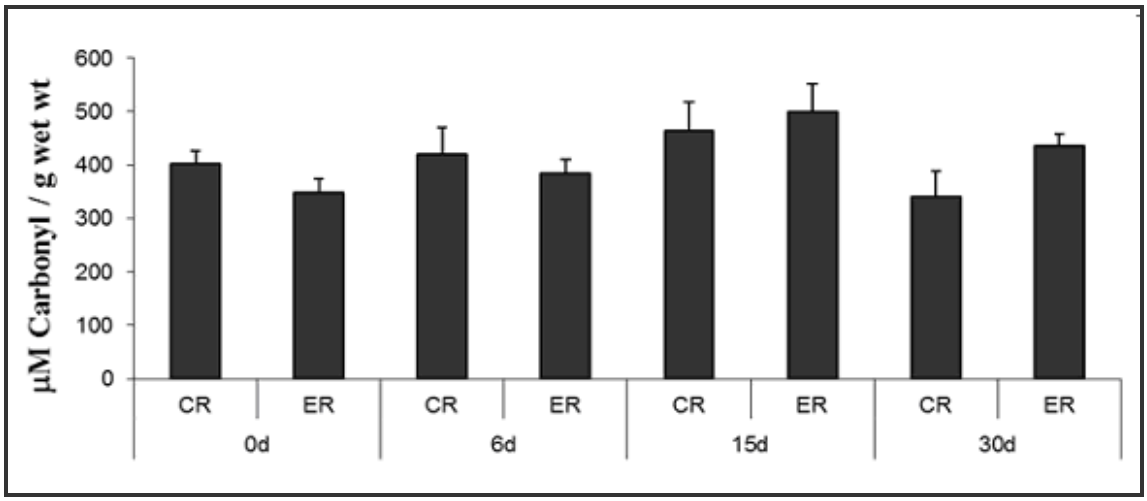


Figure 3. Determination of protein carbonyl in rats livers with 0, 6, 15 and 30 days of age exposed and not exposed to UHF-EMR. The results are presented as mean ± SEM, n = 6. No different from the CR value (two tailed t-test).

3.4 DNA damage

Damage to single-stranded and double stranded DNA was determined using the alkaline comet assay. The DI of neonatal rats and 6 day old rats exposed to UHF EMR was not significantly different from the DI of CR rats (figure 4). In contrast, the DI of ER animals 15 or 30 days old was different than that of the CR group. The 15 day old animals in the ER group had a lower DI (177.6 ±10.52) than those in the CR group (238.2 ±7.77). Rats that were 30 days old in the ER group exhibited a DI of 233.5 ± 22.796, which was significantly higher than that of the CR group (163.667 ± 14. 249) (figure 4).

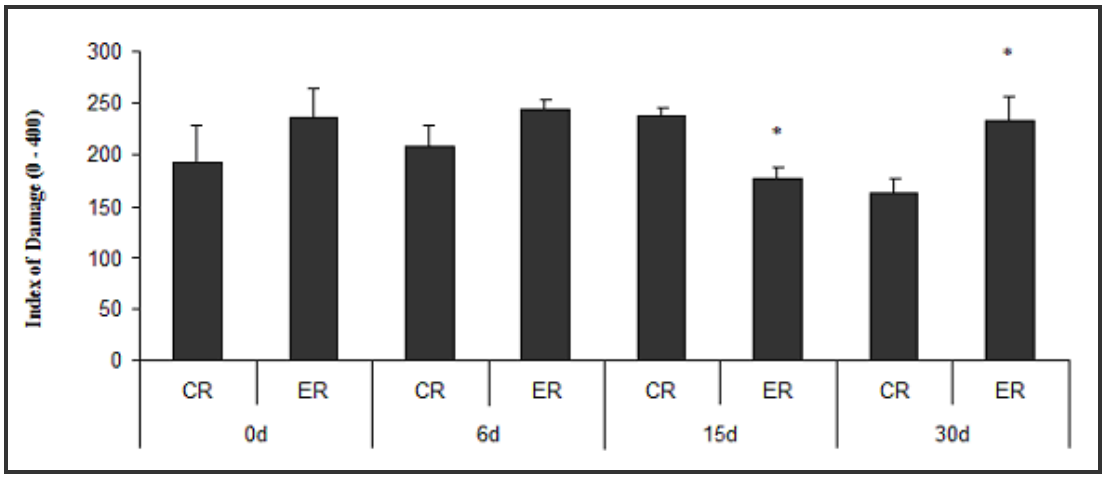


Figure 4. Determination of the damage index (DI) of livers of rats with 0, 6, 15 and 30 days of age exposed (ER) and not exposed (CR) to UHF EMR. The results are presented as mean ± SEM, n =6. * = p<0.05, T-test (two tailed).

3.5 Immunoblotting of catalase

Catalase decomposes hydrogen peroxide, preventing it from causing oxidative damage to other molecules. The UHF EMR did not alter the expression of the antioxidant enzyme catalase, in rat liver with 6, 15 or 30 days old when the CR and ER groups were compared. The exposed 0 day exhibited lower expression levels of this enzyme (figure 5).

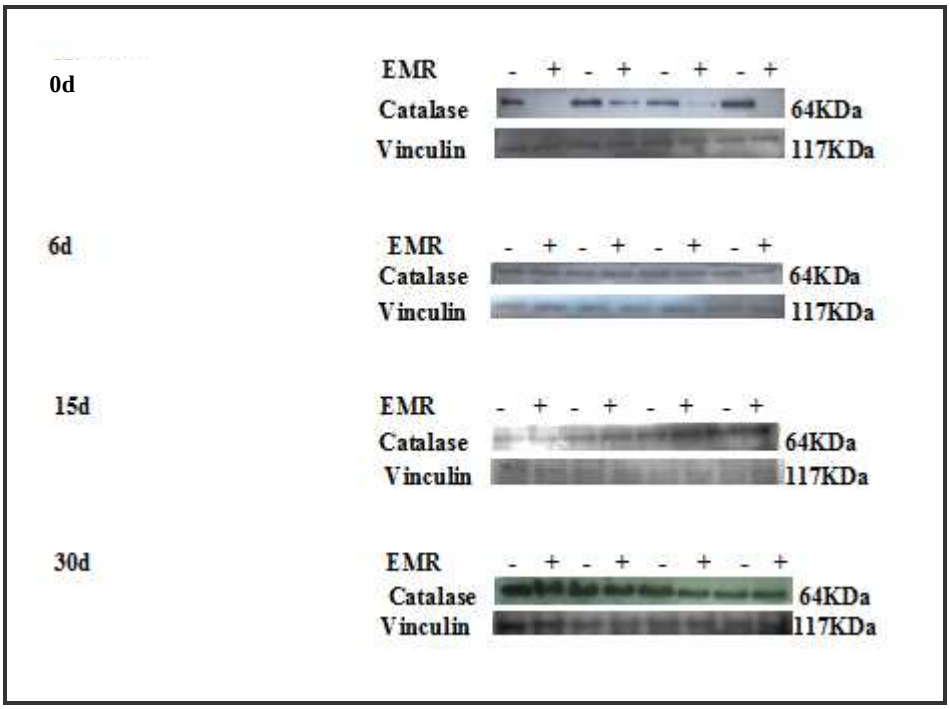


Figure 5. Immunoblotting of liver catalase in rats with 0, 6, 15 and 30 days of age exposed (ER) and not exposed (CR) to UHF EMR.

4 Discussion

This work evaluated the effects of electromagnetic radiation of ultra-high frequency on biomarkers of damage to lipids, proteins and DNA, as well as the concentrations of unsaturated fatty acids. In addition, this study analyzed the expression of catalase. These analyses were performed on the liver tissues of rats, 0, 6, 15 or 30 days old that were exposed to UHF EMR from the earliest days of gestation to their birth. Pups continued to be irradiated for 30 min/day until they reached adulthood (30 days).

4.1 Lipoperoxidation

Surprisingly, in 0 day, the TBARS levels of the ER group were lower those of the CR group. This result was not expected because the 0 day is structurally less protected. They have no fur or strong bone structure. In addition, the amount of water in these rodents is large compared to the amount of water in older animals. The increase in water content is a major factor in the absorption of electromagnetic waves in the tissues (Elbem 2006).

Based on the results of decreased TBARS and knowing that unsaturated fatty acids (UFA) are targets for ROS, we analyzed the profiles and the concentrations of UFA. As expected, the concentrations of most of the polyunsaturated fatty acids (PUFA) decreased, leading to lower levels of aldehydes in the exposed 0 day. Among the six PUFA tested, only the linolenic acid concentration did not decrease in the ER group.

The decrease in the concentrations of eicosatrienoic acid, arachidonic acid and eicosapentaenoic acid can be explained by the lower activity of delta-5 desaturase. This enzyme catalyzes reactions that lead to the formation of the aforementioned fatty acids. Its lower activity can be explained by the interaction of the UHF-EMR with the non-heme iron bonded to histidine residues present in the catalytic center of this enzyme (Nakamura et al. 2004). The reduction of the concentration of eicosapentaenoic acid in the exposed 0 day led to lower concentrations of docosahexaenoic acid because the first is the precursor of the second (Mayes et al. 1994).

There was a balance between the oxidant and antioxidant activities and between the UFA concentrations of the ER and CR groups of animals at 6, 15 or 30 days of age. The UFA concentrations in the livers of 30 day old rats of the ER group did not change compared to those of the CR group (data not shown).

It was expected that oxidative damage to lipids would increase with increasing age (Sohal, 2002). This increase was only observed when the 0 day were compared with 6 day old rats ($p < 0.005$, test SNK). However, rats 15 or 30 days old exhibited no increase but rather a decrease of TBARS. The large increase in TBARS in 6 day old rats compared to the other groups is most likely

due to a reduction in the expression or activity of aldehyde dehydrogenase. This enzyme oxidizes aldehydes to carboxylic acids, which, in turn, leave the liver and are subsequently metabolized in the muscles (Crabb et al. 2004). The decrease in expression or activity of this enzyme, and not the increase of ROS, may have led to the increase in TBARS.

4.2 Protein carbonylation

Despite a tendency toward decreases in oxidative damage to proteins of the exposed 0 day, the results showed no significant differences in the concentration of carbonylated proteins. Ferreira et al. (2006) also observed no changes in the levels of carbonylated proteins in young rats exposed to UHF EMR throughout gestation. Like 0 day, rats that were 6, 15 or 30 days old exhibited no differences between the ER groups and the CR groups. Similar to the TBARS results, protein carbonyl levels were not significantly different between the CR and ER groups in 6, 15 or 30 day old rats. The formation of aldehydes such as MDA and HNE can induce the formation of oxidized proteins (Refsgaard et al. 2000). Thus, the equality of the protein carbonyl levels in the CR and ER groups can be explained by the TBARS results.

There was no age difference (SNK test) in the accumulation of protein carbonyls in 0, 6, 15 or 30 day old rats. This lack of difference was most likely observed because the activities of the proteolytic (Stadtman 1992) and proteasomic systems (Hershko and Ciechanover 1992) against oxidized proteins did not change with age.

4.3 DNA damage

Like the protein carbonyls, DNA damage did not differ in 0 day. A decrease in DNA damage was expected because the levels of TBARS decreased in the ER group. This expectation was based on the fact that the aldehydes, as measured by the TBARS assay, decreased in the ER group. These end products of lipid peroxidation induce DNA damage (Onuki et al. 2002). Because the amount

of aldehydes decreased, it was expected that the damage to DNA would also diminish. Animals that were 6 days of age also exhibited no difference in the DI.

The DI values for rats 15 and 30 days old are very peculiar. The 15 day old rats exhibited less DNA damage in the ER group than in the CR group, whereas the 30 day old rats exhibited a higher DI for the ER group than for the CR group. Güler et al (2012) also found a higher sensitivity to DNA rabbits exposed to radiation than non-exposed. The rats that were 30 days old were more sensitive to UHF EMR than younger animals. This sensitivity may be due to the decline in BER activity in aging animals (Swain and Rao 2012), coupled with greater direct contact of DNA with electromagnetic waves.

There was no difference (SNK test) in DI with the small age difference between the animals (0, 6, 15 or 30 days). This lack of difference was most likely due to the efficient action of the repair systems for DNA damage (primarily NER and BER), which did not decline with age.

4.4 Immunoblotting of catalase

The ER 0 day exhibited less expression of catalase than the CR 0 day. This reduction in catalase can be explained by the reduction in the concentrations of PUFA observed by GC / FID. The β-oxidation of fatty acids forms hydrogen peroxide (Browning and Horton 2004). A lower concentration of lipids leads to less β-oxidation, forming less H_2O_2. The low concentration of hydrogen peroxide induced a small amount of catalase in the group of 0 day exposed to UHF EMR. This phenomenon was not observed in animals that were 6, 15 or 30 days old.

Conclusion

Based on these results and on the findings of other studies, it is possible to conclude that UHF EMR does not cause oxidative stress, and it is not genotoxic to the livers of 0 day or those of 6 and 15 day old rats, but it changes the concentrations of PUFA in 0 day. For rats of 30 days of age, our experimental conditions do not cause oxidative stress, but they are genotoxic to the livers of rats exposed to total body irradiation.

The age differences among the groups of rats that were 0, 6, 15 or 30 days old were not adequate for the older rats to have higher levels of their biomarkers.

Further investigations should be performed for a better understanding of the antioxidant defense system, the decreases in the concentrations of PUFA in neonatal rats exposed to UHF EMR and the mechanism leading to increased DNA damage in 30 day old rats.

Declaration of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Capítulo 3

A ser submetido

Effects of chronic exposure to 950 MHz ultra-high frequency electromagnetic radiation on ROS metabolism in the right and left cerebral cortex of rats

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Abstract

The aim of this work was to investigate the damage caused by 950 MHz ultra-high-frequency electromagnetic radiation (UHF-EMR) to DNA, proteins and lipids in the cerebral cortex of 0-day and 6-day-old rats. The left cerebral cortex (LCC) and right cerebral cortex (RCC) were examined in exposed rat (ER) groups and control rat (CR) groups after exposure to 950 MHz UHF-EMR. Radiation exposure was for 30 minutes per day for 27 days (21 days of gestation and 6 days postnatally). The specific absorption rate (SAR) was 1.3 W/kg. Three methods were used to assess the damage to lipids, proteins and DNA: the thiobarbituric acid reactive substance (TBARS) method, the presence of carbonylated proteins (CP) and the alkaline comet assay. The concentration of glucose in the peripheral blood of the rats was measured by the Accu-Chek Active Kit (Roche) due to increased CP in RCC. In 0 day, no modification of the biomarkers tested was detected. On the other hand, there was an increase in the levels of CP in the RCC of the 6-day-old ER group. Interestingly, the concentration of blood glucose was decreased in the 6-day-old ER group. This decrease in blood glucose may be the result of an increase in the uptake of glucose by cells in the ER group. This may lead to an increase in protein carbonylation in the RCC of the ER group.Our data suggests that oxidative stress is not significantly induced by 950 MHz UHF-EMR in 0-day and 6-day-old rats.

Keywords: brain; oxidative damage; AGE; ultra-high-frequency electromagnetic radiation.

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Introduction

Advances in technological development have led to an increase in devices that emit electromagnetic waves. Devices that emit ultra-high-frequency electromagnetic radiation (UHF-EMR) are present everywhere, and these devices are seen in airports, homes, schools, hospitals and industry. Examples of these devices are radar detectors, microwave ovens, computer monitors, MRI machines, radio base stations and cellular telephones.

The UHF-EMR is contained in a frequency band that ranges from 300 MHz to 3 GHz (Versachaeve and Maes, 1998; Figura and Teixeira, 2007). Several studies correlate this type of electromagnetic wave with behavioral (Salford et al. 2003), physiological (D'andrea et al. 2003), cellular (Fejes et al. 2005) and molecular (Paulraj and Behari 1997; Friedman et al. 2007) changes that have been associated with the generation of reactive oxygen species (ROS) **(**Moustafa et al., 2001; Zmyslony et al., 2004; Simko et al., 2006; Yurekli et al., 2006; Arthur, 2007; Valko et al., 2007; Phillips et al., 2009; Ozgur et al. 2010; Nazıroğlu et al. 2012; Avci et al. 2012).

ROS are known to cause oxidative damage to lipids, proteins and nucleic acids. Increased damage of these molecules is associated with diseases and physiological processes such as neurodegenerative and cardiovascular diseases, as well as ischemia-reperfusion injury and aging (Reuter et al., 2010).

The nervous system possesses cells with high lipid content. The cerebral cortex has a high concentration of polyunsaturated fatty acids, particularly eicosapentaenoic (20:5) and docosahexaenoic (22:6) fatty acids (Mayes, 1994; Sinclair et al., 2007). The cerebral cortex is susceptible to oxidative damage due to (1) a high rate of oxidative metabolic activity, (2) a large membrane surface compared to the cytoplasmic volume, (3) an increased axonal morphology that is prone to peripheral injury, (4) low regeneration of neuronal cells (Evans 1993), and (5) low levels of antioxidant enzymes, and/or non-enzymatic antioxidants, in the brain (Hermes-Lima, 2004; Sheweita and Sheikh, 2011).

In addition to the previously mentioned features of the mammalian brain, there is an anatomical, physiological and neurochemical asymmetry between the right and left hemispheres of the brain (Nalivaeva et al. 1995). These features are

dependent on sex, age and the hormonal state of the organism (Denenberg, 1981; Chuyan, 2004; Xu et al. 2008).

The objective of this study was to investigate the effects of UHF-EMR on ROS metabolism in the left and right cerebral cortex of 0 day and 6-day-old rats that were prenatally exposed to electromagnetic waves.

2 Experimental procedures

2.1 Animals

The rats were obtained from the Center for Reproduction and Animal Experimentation Laboratory of the Federal University of Rio Grande do Sul. Rats in the early stage of pregnancy were placed in boxes in the company of other pregnant rats to avoid isolation stress. The first day of pregnancy was defined as the day on which spermatozoids were observed by optical microscopy (Zeiss Axiophot, 100x magnification) in the vagina. A total of twelve pregnant rats were used: six rats in the control group and six rats in the group irradiated by UHF-EMR. The control group was exposed to the same conditions as the irradiated group without the generation of electromagnetic waves. Pregnant rats were irradiated for 30 minutes per day from the first day of pregnancy until the birth of the pups.

After birth, twelve 0 day (of both sexes) were separated into two groups consisting of six control rats (CR) and six exposed rats (ER), and these groups were then dissected. The remaining pups continued to be exposed to UHF-EMR until they were 6 days old. Then, six ER and six CR (of both sexes) were separated and sacrificed. The 0-day and 6-day-old rats weighed 6 and 11 grams, respectively. This research protocol was filed with and approved by the University Ethics Committee (#2006-25A).

All experiments were performed according to the guide for the care and use of laboratory animals of the National Institute of Health (NIH publication No 85-23, revised 1996). Before exposure, pregnant rats were kept in individual standard boxes (40.5 x 33.3 x 17.5 cm) under a cycle of 12 h light/12 h dark at a

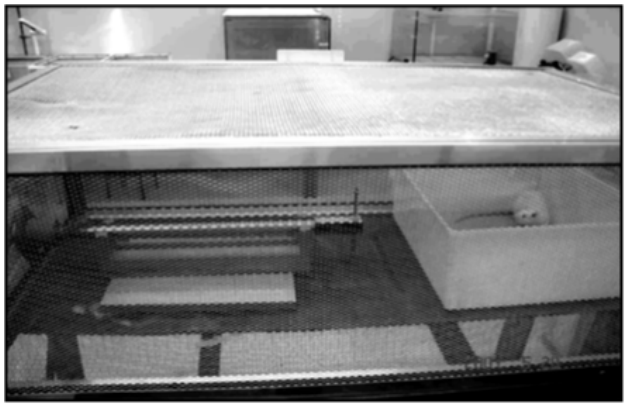
temperature of 23 ± 0.5 ℃, with water and a balanc ed diet provided ad libitum. During exposure to UHF-EMR, the animals were not allowed to drink water and were not fed. The rats were exposed for 30 minutes a day, between 12:00 pm and 3:00 pm, for 27 days. The control group, which was not exposed to EMR, was removed from the boxes and placed inside a Faraday cage (without an antenna) during the exposure time of the ER group.

2.2 Collection and storage of material

After 27 days of exposure (21 days of gestation plus 6 days postnatally), rats were decapitated with a guillotine. Decapitation occurred in the late afternoon. The left cerebral cortex (LCC) and the right cerebral cortex (RCC) were removed, washed with saline, immersed in liquid nitrogen and stored at – 80 ℃ in an ultra-freezer.

2.3 Exposure System of UHF-EMR

The irradiation time occurred daily for 30 minutes per day from the first day of gestation to 6 days postnatally. The boxes of the irradiated groups were placed 11 cm from the antenna. The antenna and boxes were always contained within an aluminum Faraday cage. The electric field (**E**) outside the cage measured 0.3 V / m. The cage with the control group was 92 cm away from the cage containing the irradiated group. The cages were held on a wooden tray. The **E** of the control cage measured 0.15 V / m. The electric field generated by the exposure system was 35 V/m. An electromagnetic wave generator (Aural Broadcast STL generator, Moseley PCL-303) and a Precision Half-Wave dipole antenna were used to produce vertically polarized UHF-EMR with a power of 1 W at a frequency of 950 MHz (Picture 1). An EMR-30 Radiation Meter (manufactured by Wandel & Goltermann) was used to measure the intensity of **E** inside and outside the box.



Picture 1. Exposure System

The SAR (specific absorption rate) of the tissue was calculated. The SAR is a measure of the energy absorbed by tissue exposed to electromagnetic radiation. The SAR value was calculated by the following formula according to ICNIRP (2009):

$$\mathbf{SAR = (\sigma/\rho) \times (E)^2\ [W/kg]},$$

where σ is the electrical conductivity of the sample, **E** is the average value of electric field and ρ is the density of the sample (cerebral cortex). In this study, the SAR of the exposed group was 1.3 W/kg for both age groups. These values were determined by using 1.1 S/m for tissue conductivity and a 1040 kg/m^3 for tissue density. These values were calculated for 0-day and 6-days-old rats as previously described by Peyman et al. (2001). The SAR of the control group was 2.37 x 10^{-5} W/kg.

2.4 Lipid peroxidation assay

Lipid peroxidation was determined by the reaction of thiobarbituric acid (TBA) with the aldehydic compounds present in the tissue, primarily malonaldehyde (MDA). The result of this reaction is the formation of an adduct that has an absorbance of 532 nm (Hermes-Lima & Storey, 1996).

Tissue was homogenized (glass-glass homogenization) in 0.2% phosphoric acid followed by the addition of an equal volume of 2% phosphoric

acid. The final dilution was 1:40 (w:v). This two-stage homogenization decreased the formation of bubbles.

After homogenization, the homogenate was kept on ice, and 400 μL of the homogenate was transferred to individual tubes containing 200 μL of 7% phosphoric acid and 400 μL of 1% TBA (in 50 mM NaOH and 0.1 mM BHT). Initially, a system was used containing 400 μL of homogenate, 200 μL of phosphoric acid and 400 μL of 3 mM HCl. However, in this system the pH had to be continuously adjusted to 1.6. After pH adjustment, the samples were incubated at 100 ºC for 15 minutes. Tubes were then cooled to room temperature over 10 minutes. After reaching room temperature, 1.5 ml of butanol was added to the tubes, which were mixed by vortex for 40 seconds. The tubes were then centrifuged at 1000 x g for 5 minutes to separate the organic phase. The organic phase, and the top pink phase, were removed. Spectrophotometric readings were then performed at 532 and 600 nm. The extinction coefficient used was 156 mM^{-1} cm^{-1}. The results were expressed as nmol TBARS/g wet tissue.

2.5 Protein oxidation assay

Protein oxidation was determined by measuring the levels of carbonylated proteins (adapted from Lenz et al., 1989). Protein carbonyl groups react with 2,4-dinitrophenyl-hydrazine (DNPH), forming an adduct with an absorbance of 370 nm (Furtado-Filho et al., 2007).

Samples of the cerebral cortex were homogenized in 5% sulphosalicylic acid (final dilution 1:40 (w:v)), and these samples were then centrifuged for 5 minutes at 13,000 rpm. The supernatant was discarded, and 500 μL of 10 mM 2,4-dinitrophenyl-hydrazine (DNPH), prepared in 2 M HCl, and was added to the precipitate. The samples were kept at room temperature for 1hour and were mixed by vortex every 15 minutes (a total of 4 times) to allow for the reaction to occur between DNPH and the carbonyl groups. This was followed with the addition of 500 μL of 20% trichloroacetic acid. This mixture was centrifuged at 13,000 rpm for 3 minutes. The supernatant was discarded. Excess DNPH was removed by washing the precipitate with 1 ml of a 1:1 ratio of ethanol:ethyl acetate. This

mixture was then mixed by vortex for 40 seconds and centrifuged. This procedure (washing, spinning and disposal) was performed three times. The proteins were resuspended in 6 M guanidine chloride and incubated for 15 minutes. The carbonyl groups were quantified spectrophotometrically at an absorbance of 370 nm. The extinction coefficient used was 22 $mM^{-1}cm^{-1}$.

2.6 DNA damage assay

DNA damage was evaluated by the alkaline comet assay as previously described (Collins 2004; Recio et al. 2012). Tissue was disrupted with liquid nitrogen and weighed (0.010 to 0.012 g). Samples were then thawed in an ice and water bath for 15 minutes. A 24-well plate was prepared with 1000 µL mincing solution (HBSS with 20 mM EDTA and 10% DMSO, pH 7.5) on an ice shelf. Tissue was added to the mincing solution and left to soak for 15 minutes to remove excess blood. Following this wash, the mincing solution was discarded. The tissue was then perforated with two 200 µL pipette tips, followed by a second wash with 500 µL mincing solution. Under yellow light, a 20 µL aliquot of cell suspension was collected and dissolved in 90 µL of 0.75% LMP agarose. Immediately following dissolution, under yellow light, the cell suspension-agarose mixture was pipetted onto a glass slide, pre-coated with a layer of 1% agarose and covered with a coverslip.

The blade, sample, agarose and coverslip were dried in a refrigerator for 15 minutes. Following drying, the coverslips were removed, and the slides were placed in a vertical bucket (previously covered with foil) and incubated in cold lysis solution (2.5 M NaCl, 10 mM Tris, 100 mM EDTA, 1% Triton X-100, 10% DMSO, pH 10). The slides were incubated for 1 hr in a refrigerator to remove the nuclear membranes and cytoplasmic contents, leaving the DNA as a nucleoid. After the lysis procedure, the slides were placed in a horizontal electrophoresis tank, which was then placed inside a plastic tray filled with ice. Electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH 13.0) was added to cover the wells, which allowed for DNA relaxation. Electrophoresis was performed at 25 V with an electric current of 300 mA for 20 minutes (the current was controlled by the volume of buffer). All of

the previous steps were performed under yellow light, or under dark conditions, to prevent further DNA damage.

Following electrophoresis, the slides were placed vertically inside the bucket and covered with neutralization buffer (0.4 M Tris, pH 7.5) for 5 minutes. This procedure was repeated three times. The slides were then placed in distilled water for 5 minutes. The distilled water was then discarded. This step was repeated twice more. Following the final wash, the slides were dried overnight and re-hydrated the next day for 5 minutes in distilled water. Slides were then stained with a silver nitrate solution as previously described (Nadin et al. 2001). After staining, the slides were dried at room temperature overnight.

Samples were analyzed using an optical microscope. One hundred cells of each slide were selected in duplicate. These cells were visually scored according to the length of the tail in five classes: class 0, undamaged, no tail, class 1, a shorter tail than the diameter of the head (nucleus), class 2, a tail length of 1-2X the diameter of the head, class 3, a tail with more than twice the diameter of the head and class 4, a tail with more than three times the diameter of the head, or comets with no heads. The damage index (DI) for each group studied ranged from zero (100x0, 100 cells completely undamaged) to 400 (100x4, 100 cells observed with maximum damage).

2.7 Glucose measure

The concentration of glucose in the peripheral blood of the rats was measured by the Accu-Chek Active Kit (Roche) according to the manufacturer's instructions.

2.7 Statistical analysis

All data are reported as the mean ± SEM. For comparisons between two different groups, we used a Student's t-test (two-tailed). To compare more than two different groups, we used a post-ANOVA SNK (Student-Newman-Keuls) test.

Values of $p<0.05$ were considered significant for both the t-test and the post-ANOVA test.

3 Results

3.1 Lipid peroxidation

Lipid peroxidation was measured in the right (RCC) and left (LCC) cerebral cortex separately. No difference was found between the exposed rat group and the control rat group at either age, in either cortex (figure 1).

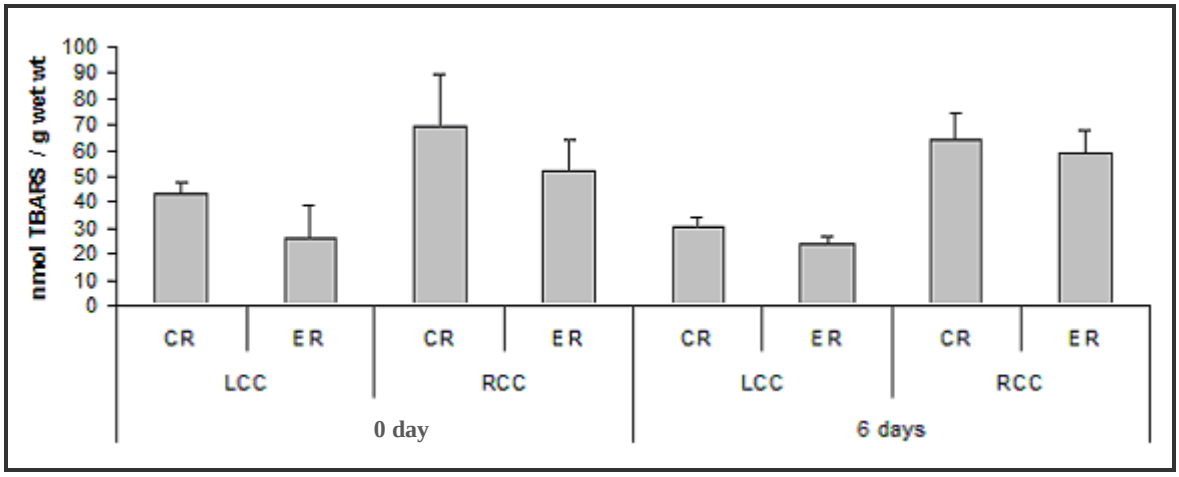


Figure 1. Determination of the levels of lipid peroxidation in left and right cerebral cortexes in 0 day rats and 6 days of age exposed (ER) and not exposed (CR) to UHF- EMW. The results are presented as mean ± SEM, n= 6. No different from the CR value (SNK test post-ANOVA) (SNK test post-ANOVA). LCC, left cerebral cortex, RCC, right cerebral cortex.

3.2 Carbonyl Protein

Similar to the results of TBARS, the carbonylated protein results did not display differences in the LCC and RCC of 0 day. However, the RCC of the rats at 6 days of age displayed a higher content of carbonyl protein when the ER group was compared to the CR group (figure 2).

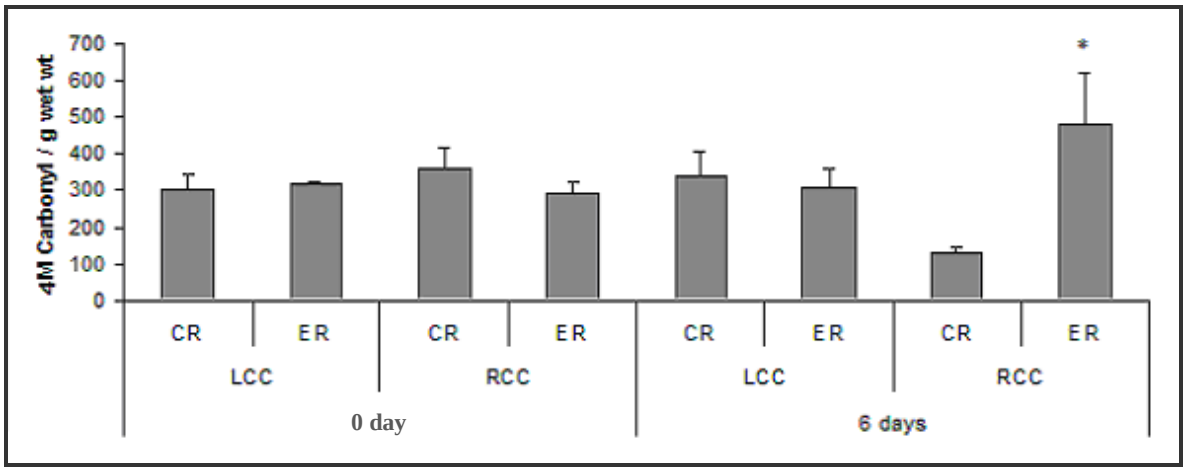


Figure 2. Determination of protein carbonyl concentrations in the left and right cerebral cortex in 0 day rats and 6 days.The results are presented as mean ± SEM, N= 6. No different from the CR value (SNK test post-ANOVA).

3.3 DNA damage

Although there was no statistical difference in DNA damage in the 6-day-old rats, the RCC of treated animals showed a tendency to an increase in DNA damage ($p < 0.1$ in two tails t-test). The LCC showed no change (fig 3).

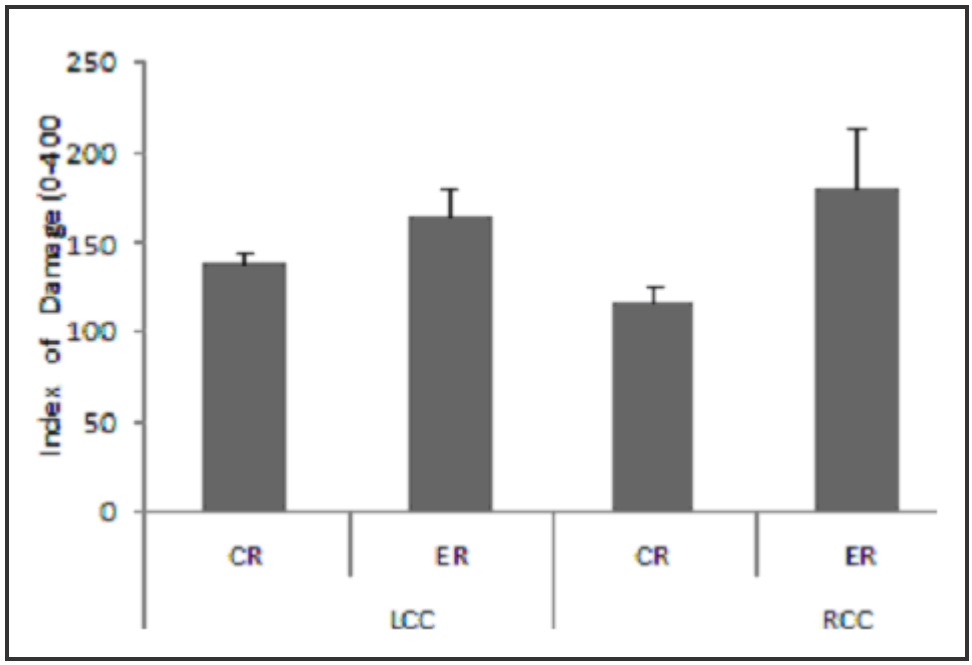


Figure 3. Determination of damage index (DI) by alkaline comet assay in LCC and RCC of ER and CR to UHF EMR in rats with 6 days old. The results are presented as mean ± SEM, n =6. The results are presented as mean ± SEM, N= 6. No different in comparison between groups (SNK test post-ANOVA).

3.4 Glucose Measure

The 6-day-old ER group displayed a 20% reduction of whole blood glucose concentration compared to the CR group (fig. 4).

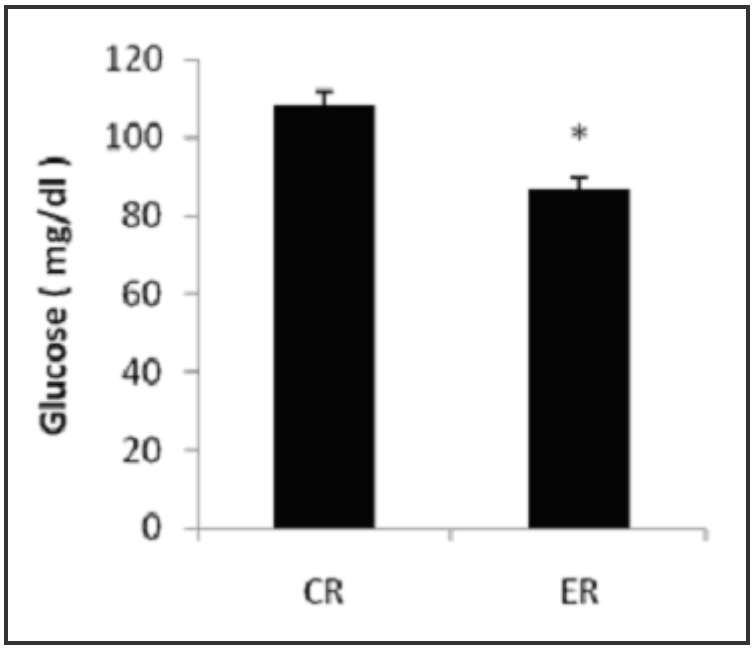


Figure 4. Determination of glucose concentration in whole blood in rats with 6 days old. The results are presented as mean ± SEM, n= 6. *: Significantly different from control group, p <0.05 (two-tailed t test).

4 Discussion

This study evaluated the effects of electromagnetic radiation of ultra-high-frequency on ROS metabolism in the LCC and RCC of rats by determining the lipid peroxidation, protein carbonylation and DI in DNA.

The right and the left cerebral cortexes showed no difference in the level of lipid peroxidation when the ER group was compared to the CR group in 0-day and 6-day-old rats. Therefore, 950 MHz UHF-EMR did not cause an imbalance between the oxidants and antioxidants in the LCC and RCC of 0-day and 6-day-old rats (Ferreira et al., 2006).

The concentration of carbonylated proteins in the LCC and RCC was unchanged in both the ER and CR groups in neonatal rats. The LCC of 6-day-old rats also showed no difference in this comparison. On the other hand, the RCC of

6-day-old rats showed an increase in protein carbonylation in the ER group compared to the CR group. This difference was not observed by the TBARS or comet method; therefore, it is likely that it is not the result of oxidative damage induced by ROS. Oxidative stress is not the only process that results in the formation of carbonylated proteins. For example, hyperglycemia can lead to the formation of advanced glycation end products (AGEs) that can induce protein carbonylation (Barbosa et al., 2008).

The LCC and RCC showed no difference in the rate of DNA damage when comparing the ER group with the CR group. Additionally, Malyapa et al. (1997) found no difference in DNA damage in mammalian cells. These results demonstrate that the UHF-EMR did not alter the damage to single-stranded or double-stranded DNA in those organs. Although there is no statistical difference (two-tailed t-test) between the ER and CR groups, the RCC group exposed rats had a tendency ($p<0.1$ two-tailed t-test) to follow the damage profile of proteins. This tendency of difference in DNA damage between the LCC and RCC can be explained by the carbonylation of the DNA repair proteins.

Blood glucose was measured in 6-day-old rats after the TBARS method determined that the increase in carbonylated proteins and DI in the DNA of the RCC was not the result of ROS-induced damage. Interestingly, the concentration of glucose in whole blood decreased in the ER group compared to the CR group. This decrease in blood glucose may be the result of an increase in the uptake of glucose by cells in the ER group. This may lead to an increase in protein carbonylation in the RCC of the ER group.

The formation of AGE caused by the increased glucose in the cell may have led to the inhibition of the proteasome (Bulteau et al., 2001), and consequently, to the accumulation of carbonylated proteins in the RCC.

There is a known asymmetry between the RCC and LCC that is caused by the neurochemistry of age and hormonal status (Denenberg, 1981; Chuyan, 2004). This asymmetry may alter the effects of UHF-EMR on the RCC and LCC, and may explain the increase in detected protein damage in the RCC and not in the LCC.

Conclusion

The results of this investigation show that continuous wave UHF-EMR (SAR 1.3 W/kg), administered for 30 minutes a day for 27 days (21 days of gestation and 6 days postnatally), is not genotoxic and does not cause oxidative stress in the LCC and RCC of 0-day and 6—day-old rats. However, the RCC displays a significant increase in carbonylated protein concentration that is probably not related to oxidative stress generation. More studies are needed in order to understand the actual mechanism that is implicated in this process.

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Parte III

Discussão

Neste trabalho, verificou-se o efeito da radiação eletromagnética de ultra-alta-freqüência, de onda contínua, sobre o metabolismo de espécies reativas de oxigênio em ratos de diferentes idades. Biomarcadores de danos oxidativos em lipídios, proteínas e DNA, bem como antioxidantes enzimáticos e não-enzimáticos foram pesquisados. Além disso, foi analisada a concentração de ácidos graxos insaturados ω–3, ω–6 e ω–9 no fígado de ratos neonatos e no córtex cerebral direito e esquerdo de ratos com 30 dias de idade.

Na revisão das pesquisas realizadas com radiação eletromagnética e espécies reativas de oxigênio, constatou-se que há aumento de lipoperoxidação, formando, principalmente, MDA após irradiação de UHF-EMR (MOUSTAFA et al., 2001; OZGUNER et al., 2005; OKTEM et al., 2005; MERAL et al., 2007; OZGUR et al., 2007; SOKOLOVIC et al., 2008; MOUSSA, 2009; DINDIC et al., 2010; HASSAN et al., 2010). A elevação dos níveis de aldeídos é explicada pelo fato que, em algumas condições, a EMR gera ROS. Estas atacam PUFA, levando à lipoperoxidação que, no final do processo, forma MDA.

Os resultados de proteínas carboniladas de organismos expostos à UHF-EMR são controversos (SOKOLOVIC et al,. 2008; ARENDASH et al., 2010). O trabalho de SOKOLOVIC et al. (2008) mostrou aumentados níveis de proteínas carboniladas após exposição. ARENDASH et al. (2010), por sua vez, encontraram que, mesmo com um diminuição na atividade da MnSOD, o conteúdo de proteínas carboniladas não se alterou no hipocampo de camundongos.

O aumento de carbonilação pode ser devido ao ataque direto de ROS aos aminoácidos do polipeptídeo. Indeiretamente, as proteínas podem ser carboniladas pela reação com aldeídos resultantes da lipoperoxidação. A diferença de carbonilação entre os grupos pode também ser explicada pela diminuição da quantidade e/ou atividade de proteassomas. Estas estruturas também agem sobre proteínas oxidadas (HERSHKO & CIECHANOVER, 1992; BREUSING et al., 2009) ou glicadas (BULTEAU et al., 2001).

Os experimentos usando ondas eletromagnéticas UHF contínuas não mostraram nenhuma diferença de fragmentação do DNA no cérebro de ratos,

culturas de células gliais de ratos e nem no fígado de coelhos (DI MASCIO et al., 1991; BELYAEV et al. 2006; CAMPISI et al., 2010; TOMRUK et al. 2010). Essas ondas não foram capazes de alterar a geração de sítios AP ou mesmo de mudar o sistema de reparo do DNA, particularmente, o BER.

A literatura mostra que antioxidantes não-enzimáticos como as vitaminas (A, C e E) e GSH diminuem a sua quantidade após exposição à UHF-EMR. Concomitantemente, as atividades de enzimas antioxidantes também diminuem. O fornecimento das vitaminas C e E induz a diminuição das enzimas alanina aminotransferase (ALT), aspartato aminotransferase (ASP) e fostase alcalina (ALP) (AZIZ et al., 2010). A redução de danos oxidativos provocada pela ação antioxidante das vitaminas C e E faz com que as enzimas (ALT, ASP e ALP) – que ficam com altas atividades durante danos por toxicidade – diminuam essa atividade.

A concentração de GSH apresenta-se baixa após exposição à UHF-EMR (MERAL et al., 2007; MOUSSA,, 2009; ARENDASH et al., 2010; MAILANKOF et al., 2009). Além de agir diretamente como antioxidante, este tripeptídeo é co-substrato de GPx e GST onde tem uma ação antioxidante indireta. Estas ações diminuem a relação GSH/GSSG que é aumentada após administração da vitamina A (BARBER et al., 2000).

O zinco é um metal antioxidante quando (1) se liga às sulfidrilas, protegendo-as do ataque de ROS (BETTGER & O'DELL, 1981; GIBBS et al., 1985; POWELL, 2000); (2) induz a formação de metalotioneínas que estocam Zn para atividades antioxidantes na célula; (3) quando desloca o Fe, evitando que este forme ROS; e (4) participa da formação da Sod-1, estabilizando a sua estrutura (AHMAD, 1995). Apesar de se saber que algumas EMR induzem à geração de ROS e que o Zn tem todas essas ações antioxidantes, existem poucos trabalhos relacionando radiações eletromagnéticas com o conteúdo de zinco *in vivo*. Num deles, Kiliçalp et al. (2009) pesquisou a ação de UHF EMR de 900 MHz sobre o fígado e o testículo de *Cavia porcellus.* Neste estudo, o fígado não apresentou mudaçança na sua quantidade. Diferentemente, o testículo teve diminuição. Os níveis de Zn no fígado não alteraram, provavelmente, devido à alta quantidade e atividade de antioxidantes enzimático e não-enzimático, não

havendo necessidade do seu consumo. Já no testículo, houve diminuição do Zn porque este órgão não tem tanto antioxidante como o fígado, havendo necessidade de consumi-lo para defender esta gônoda contra danos oxidativos causados pela geração de ERO induzidas pela UHF-EMR.

Melatonina administrada após exposição à UHF-EMR diminui LPO (OKTEM et al., 2005; KÖYLU et al., 2006; SOKOLOVIC et al., 2008) por ser um "scavenger" de radicais livres e por induzir o aumento da expressão gênica da SOD e GPX (TAN et al., 2007).

As enzimas antioxidantes Sod (MOUSTAFA et al., 2001; OZGUNER et al., 2005; OKTEM et al., 2005; OZGUR et al., 2007; ELHAG et al., 2007; KESARI & BEHARI, 2009; ARENDASH et al., 2010; HASSAN et al., 2010), Cat (MERAL et al., 2007; SOKOLOVIC et al., 2008; OZGUNER et al., 2005; OKTEM et al., 2005; HASSAN et al., 2010) e GPx (KESARI & BEHARI, 2009; MOUSTAFA et al., 2001; HASSAN et al., 2010; OZGUNER et al., 2005; OKTEM et al., 2005) pesquisadas reduzem as suas atividades após exposição de mamíferos à UHF-EMR. Excetuando-se a glutationa peroxidase, Sod e Cat apresentam metais em suas estruturas. As ondas eletromagnéticas de ultra-alta frequência podem ter interagido com esses metais de transição, provocando alterações estruturais e, conseqüentemente, diminuição nas suas atividades. Já a GPx não apresenta metal, mas precisa do co-substrato GSH para as suas atividades. Como este tripeptídeo encontra-se reduzido após a exposição à UHF-EMR, a atividade da glutationa peroxidase também diminui.

Nos experimentos com fígado de ratos com diferentes idades (Capítulo 2) foram avaliados os efeitos da radiação eletromagnética de ultra-alta-freqüência sobre os marcadores de danos a lipídios, proteínas e DNA, bem como as concentraçãoes de ácidos graxos insaturados. Além disso, este estudo analisou a expressão da catalase. Estas análises foram realizadas nos tecidos do fígado de ratos com 0, 6, 15 e 30 dias de idade que foram expostos à UHF-EMR dos primeiros dias de gestação até o nascimento deles. Os filhotes continuaram a ser irradiados por 30 min até atingirem 30 dias de idade.

Nas análises de lipoperoxidação do fígado, os neonatos, surpreendentemente, apresentaram menores níveis de TBARS no grupo exposto

quando comparados aos níveis do grupo controle. Este resultado não era o esperado, uma vez que ratos neonatos são estruturalmente menos protegidos. Eles não têm pelo e nem uma estrutura óssea forte. Além disso, a quantidade de água nestes roedores é maior quando comparada aos animais mais velhos. O aumento de água é o principal fator na absorção das ondas eletromagnéticas pelos tecidos (ELBEM, 2006).

Baseando-se nos resultados diminuídos de TBARS e conhecendo que os ácidos graxos insaturados (UFA) são alvos das espécies reativas de oxigênio, analisou-se o perfil e a concentração de ácidos graxos insaturados. Como esperado, as concentrações da maioria dos ácidos graxos poliinsaturados (PUFA) diminuíram, levando a menores níveis de aldeídos nos neonatos expostos. Dos seis PUFA testados, somente a concentração do ácido linoléico não diminuiu no grupo exposto.

A diminuição nas concentrações dos ácidos eicosatrienóico, aracdônico e eicosapentanóico pode se explicada pela menor atividade da delta-5 dessaturase (D5D). Esta enzima catalisa reações que podem levar à formação dos ácidos graxos supracitados. A baixa atividade da D5D pode ser explicada pela interação da UHF-EMR com o ferro não-heme ligado aos resíduos de histidina presentes no centro catalítico desta enzima (NAKAMURA et al., 2004). A redução da concentração do ácido eicosapentanóico nos neonatos expostos levou a menor concentração do ácido docosahexanóico porque o primeiro é o precursor do segundo (MAYES et al., 1994).

Houve um equilíbrio entre os oxidantes e as atividades antioxidantes e entre as concentrações dos UFA nos grupos de ratos expostos e nos ratos controle de 6, 15 e 30 dias de idade (Capítulo 2). Cabe ressaltar que as concentrações dos UFA no fígado dos animais com 30 dias de idade do grupo exposto não se alteraram quando comparadas ao grupo controle (dados não mostrados).

Esperava-se que os danos oxidativos a lipídios aumentassem com o aumento de idade (SOHAL, 2002). Este aumento foi observado somente quando os neonatos foram comparados aos ratos com 6 dias de idade ($p<0,005$, teste pós-ANOVA SNK – Figura 1 do capítulo 2). No entanto, os ratos com 15 e 30

dias de idade diminuíram os níveis de TBARS quando comparados aos animais com 6 dias de idade (Figura 1 – Capítulo 2). O grande aumento dos níveis de TBARS dos animais com 6 dias, comparados a todos os outros grupos, pode ser, principalmente, devido à redução na expressão/atividade da aldeído desidrogenase. Esta enzima oxida aldeídos a ácidos carboxílicos, que, por sua vez, deixa o fígado e é subseqüentemente metabolizado nos músculos (CRABB et al., 2004). A diminuição na expressão ou atividade desta enzima, e não o aumento de ROS, pode levar ao aumento de TBARS.

Nas análises de proteínas carboniladas de fígado, apesar de uma tendência para a diminuição de dano oxidativo a proteínas dos neonatos expostos, os resultados não mostraram diferença significante na concentração de proteínas carboniladas. FERREIRA et al. (2006) também não observaram nenhuma mudança nos níveis de proteínas carboniladas em ratos jovens expostos à UHF-EMR durante toda a gestação. Semelhantemente aos ratos neonatos, os animais com 6, 15 e 30 dias de idade não mostraram nenhuma diferença de proteínas carboniladas entre os grupos de RC e RE. Assim como nos resultados de TBARS, os níveis de proteínas carboniladas não foram significativamente diferentes entre os grupos de RC e RE de ratos com 6, 15 e 30 dias. A formação de aldeídos tais como MDA e HNE pode induzir à carbonilação de proteínas (REFSGAARD et al., 2000). Então, a igualdade de proteínas carboniladas nos grupos de RC e RE pode ser explicada pelos resultados de TBARS.

Não houve diferença de idade (teste SNK) no acúmulo de proteínas carboniladas em ratos com 0, 6, 15 e 30 dias de idade. Este resultado foi provavelmente devido às atividades proteolíticas (STADTMAN, 1992) e proteassômicas (HERSHKO & CIECHANOVER, 1992) contra proteínas oxidadas que não se alteraram com o tempo.

Na análise de danos ao DNA do fígado, como em proteínas carboniladas, não houve diferença entre RC e RE neonatos. Uma diminuição nos danos em DNA era esperada porque os níveis de TBARS diminuíram no grupo de ratos expostos. Esta expectativa foi baseada no fato que os aldeídos, medidos em TBARS, diminuíram no grupo exposto. Estes produtos finais da lipoperoxidação

induzem danos ao DNA (ONUKI et al., 2002). Devido à diminuída quantidade de aldeídos, esperava-se que os danos ao DNA também diminuíssem, o que não aconteceu. Os animais com 6 dias de idade também não exibiram nenhuma diferença nos índices de danos.

Os valores dos índices de danos de ratos com 15 e 30 dias de idade foram bem peculiares. Os animais com 15 dias exibiram menos danos em DNA no grupo exposto do que o grupo controle. Já os animais com 30 dias mostraram um índice de danos de ratos expostos maiores que os ratos controle. GÜLER et al (2012) também encontraram uma maior sensibilidade ao DNA de coelhos expostos à radiação do que os não-expostos. Os ratos com 30 dias foram mais sensíveis à UHF EMR do que os animais mais novos. Esta sensibilidade pode ser devido a um declínio na atividade de BER (sistema de reparo por excisão de bases) em animais mais velhos (SWAIN & RAO, 2012), juntamente com maior contato do DNA com as ondas eletromagnéticas.

Não houve diferença (teste SNK) nos índices de danos de DNA na comparação entre as idades dos grupos de RC e RE. Isto aconteceu provavelmente devido à ação eficiente dos sistemas de reparo de danos ao DNA (principalmente BER e NER) que não diminuíram sua ação com o passar da idade.

Na análise da expressão da catalase do fígado, os neonatos expostos apresentaram menor expressão do que os neonatos do grupo controle. Esta redução da catalase pode ser explicada pela diminuição das concentrações de PUFA observada pela cromatografia gasosa. A beta-oxidação de ácidos graxos forma peróxido de hidrogênio (BROWNING & HORTON, 2004). Uma menor concentração de lipídios leva a menor beta-oxidação, formando menos peróxido de hidrogênio. Então, uma baixa concentração de peróxido de hidrogênio induziu a uma pequena expressão de catalase no grupo de neonatos expostos à UHF-EMR. Este fenômeno não foi observado nos animais com 6, 15 e 30 dias de idade.

Nos experimentos com córtex cerebral esquerdo e direito de ratos neonatos e com 6 dias de idade (Capítulo 3), avaliou-se o efeito da radiação eletromagnética de ultra-alta-freqüência sobre o metabolismo das espécies

reativas de oxigênio no córtex cerebral esquerdo e direito. Este objetivo foi atingido pela checagem da lipoperoxidação, carbonilação de proteínas e índice de danos ao DNA. Além disso, verificou-se também a concentração de glicose no sangue total.

Nas análises de lipoperoxidação do córtex cerebral direito e esquerdo, não houve diferenças nos níveis de lipoperoxidação quando o grupo RE foi comparado ao grupo RC dos animais neonatos e com 6 dias de idade (Figura 1 – capítulo 3). Isto mostra que 950 MHz UHF EMR não causa desequilíbrio entre oxidantes e antioxidantes no córtex cerebral esquerdo e direito de ratos neonatos e com 6 dias de idade. FERREIRA et al (2006) também não encontraram diferença no córtex cerebral de ratos jovens quando animais expostos foram comparados aos controles.

Nas análises de carbonilação de proteínas do córtex cerebral direito e esquerdo, não houve diferença entre os grupos de RE quando comparado ao grupo de RC em ratos neonatos. O córtex cerebral esquerdo de animais com 6 dias de idade também não mostrou diferenças significativas nessa comparação. Diferentemente, o córtex cerebral direito de ratos com 6 dias apresentaram maior carbonilação no grupo RE. Como esta diferença não foi observada nas metodologias de TBARS e cometa alcalino, é provável que este resultado não seja provocado por ROS. A formação de proteínas carboniladas pode não ser induzida somente por estresse oxidativo. Hiperglicemia é um fator que pode levar à formação de produtos finais de glicação avançada (AGE). Estas moléculas também induzem à carbonilação de proteínas (BARBOSA et al., 2008).

O córtex cerebral esquerdo e o direito não mostraram diferenças de danos ao DNA quando o grupo de RE foi comparado ao grupo de RC no mesmo órgão. MALYAPA et al. (1997) também não encontraram diferença em danos ao DNA de fibroblastos de ratos e de células de glioblastoma humano. Estes resultados demonstram que 950 MHz UHF EMR não induz quebras do tipo simples e duplas no DNA daqueles órgãos. No nosso trabalho, embora não tenha havido diferenças estatísticas (teste-T duas caldas) entre os grupos de RE e RC, o córtex cerebral direito do grupo exposto teve uma tendência ($p<0,1$ em teste T duas caldas) a seguir os danos de proteínas carboniladas. Esta tendência de

diferença dos danos ao DNA pode ser explicada pela carbonilação também das proteínas do sistema de reparo do DNA, o que as impediria de corrigir de forma eficiente os possíveis danos que possam ter ocorrido no córtex cerebral direito.

Os resultados de proteínas carboniladas e de índice de danos ao DNA do RCC levaram-nos a medir os níveis de glicose sangüínea nos ratos com 6 dias de idade, uma vez que as determinações de TBARS indicaram que o aumento de proteínas carboniladas em RCC não foi resultado de danos induzidos por ERO. Intrigantemente, a concentração de glicose em sangue total diminuiu no grupo de RE quando comparado ao grupo de RC. Esta diminuição da glicose sangüínea pode ser devido a uma maior captação deste glicício pelas células do grupo exposto. Conseqüentemente, o aumento de glicose dentro da célula pode ter levado ao aumentado dano a proteínas, por cabonilação, no RCC do grupo RE, tendo em vista que o aumento de glicose gera AGE, o qual inibe o sistema proteassômico (BULTEAU et al., 2001), levando ao acúmulo de proteínas carboniladas em RCC.

É possível que o RCC tenha acumulado mais danos a proteínas que o LCC devido à assimetria neuroquímica resultante da idade e do estado hormonal (DENENBERG, 1981; CHUYAN, 2004) combinado com o efeito da UHF EMR.

Nossos resultados mostraram que a UHF EMR causa algumas alterações moleculares e celulares no sistema biológico, dependendo da idade e das condições de exposição do organismo. Certamente eles não podem ser diretamente relacionados aos efeitos em humanos, mas servem de alerta para que mais estudos sejam realizados de maneira aprofundada nessa área para entendermos os possíveis efeitos dessas radiações aos organismos e ao meio ambiente. Abaixo, há duas tabelas que sumarizam os principais resultados.

Tabela 1 – Resumo dos resultados de danos oxidativos, expressão da catalase e concentração de glicose.

Metodologias	**0d**			**6d**				**15d**	**30d**
	F	**CCD**	**CCE**	**F**	**S**	**CCD**	**CCE**	**F**	**F**
TBARS	↓	↔	↔	↔	-	↔	↔	↔	↔
PC	↔	↔	↔	↔	-	↑	↔	↔	↔
Cometa	↔	-	-	↔	-	↔	↔	↓	↑
WB catalase	↓	-	-	↔	-	-	-	↔	↔
Glicose	-	-	-	-	↓	-	-	-	-

Legenda: F – fígado, CCD – córtex cerebral direito, CCE – córtex cerebral esquerdo, S – Sangue, ↑ **-** aumento, ↓ - diminuição, ↔ - igualdade, TBARS – substâncias reativas com ácido tiobarbitúrico, PC – proteínas carboniladas, WB – Western Blotting

Tabela 2 – Resumo dos resultados de ácidos graxos do fígado dos neonatos

SÉRIES	**OA**	**EA**	**LA**	**AA**	**ALA**	**EPA**	**DHA**
ω – 9	↔	↓	**-**	**-**	**-**	**-**	**-**
ω – 6	**-**	**-**	↓	↓	**-**	**-**	**-**
ω – 3	**-**	**-**	**-**	**-**	↔	↓	↓

Legenda: OA – ácido oleico, EA- ácido eicosatrienóico, LA- ácido linoleico, AA – ácido aracdônico, ALA- ácido alfa-linoleico, EPA – ácido eicosapentaenóico, DHA – ácido docosahexanóico ↑ **-** aumento,↓ - diminuição, ↔ - igualdade, - não correspondente.

Conclusões Finais

Os resultados deste trabalho demonstram que, dependendo da freqüência, SAR, do tempo de duração da idade e do sexo, a radiação eletromagnética de ultra-alta-freqüência, de onda contínua, pode alterar o estado redox celular em mamíferos. Mais especificamente que:

- A irradiação de ratos com UHF EMR, onda contínua, de 950 MHz de freqüência, 35 v/m de campo elétrico, 1 w de potência, polarização vertical, SAR 1,3 W / kg, meia hora por dia, por 51 dias (21 dias de gestação + 30 dias de idade) não causa estresse oxidativo e nem é genotóxica ao fígado de ratos neonatos, de 6 e 15 dias de idade. Entretanto, altera a concentração de ácidos graxos poliinsaturados e reduz a expressão da catalase em ratos neonatos. Em animais com 30 dias de idade, nossas condições experimentais também não causaram estresse oxidativo, mas foram genotóxicas ao fígado de ratos expostos corpo total.

- As diferenças de idade entre os grupos de ratos neonatos, 6, 15 e 30 dias não foram suficientes para os animais de 15 e 30 dias apresentarem maiores níveis de biomarcadores de danos oxidativos quando comparados aos mais jovens, neonatos e com 6 dias de idade. Não há alteração da expressão da catalase em relação aos controles à medida que os animais passam para 6, 15 e 30 dias de idade.

- A irradiação de ratos neonatos e com 6 dias de idade por 27 dias (21 dias de gestação e 6 dias de idade) quando avaliado o córtex cerebral, observa-se que não é genotóxica e nem causa estressse oxidativo ao LCC e RCC de ratos neonatos e nem de recém-nascidos expostos nestas condições. No entanto, RCC dos RE tem mais carbonilação a proteínas, o que não parece ser dependente da indução de espécies reativas de oxigênio e sim relacionada aos níveis de glicose como foi verificado nesse trabalho.

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ANEXOS

Nesta seção, são apresentados outros resultados que, apesar de não estarem no corpo principal da tese, forneceram sugestões para realização de futuros trabalhos. Alguns desses resultados ainda precisam ser complementados.

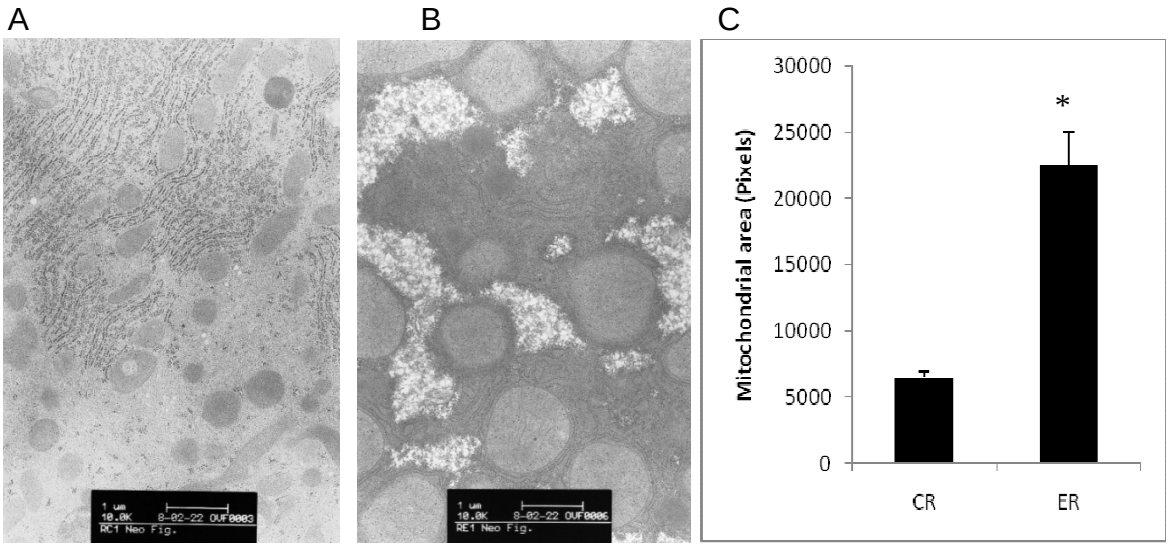

Figure 1. Electron micrographs of liver tissue. A, mitochondria from control rat; B, mitochondria of rats exposed to UHF EMR; C, Area mitochondria of rats exposed versus control rats. The results are presented as mean ± SEM, n= 16-20. *: Significantly different from the CR value, $p<0.001$ (two tailed t-test). Analysis of electron micrographs made with Image J software.Our experimental conditions led to morphological changes in liver mitochondria of 0 day rats. The organelles of the ER became swollen if compared to the CR. This result can be explained by the transient induction of mitochondrial permeability (MPT). Reduction of mitochondrial membrane potential, oxidative stress and increased calcium concentration results in MPT. The damages to lipids, proteins and DNA indicated that there was no oxidative stress. The decrease in concentration of PUFA led to lower membrane fluidity and, consequently, to changes in the permeability of the cell or organelle. Reduction of unsaturated fatty acids in rats from the exposed group may have caused an increase of the area in the mitochondria. However, there is still the need to investigate factors other than the oxidative stress that lead to mitochondrial swelling.

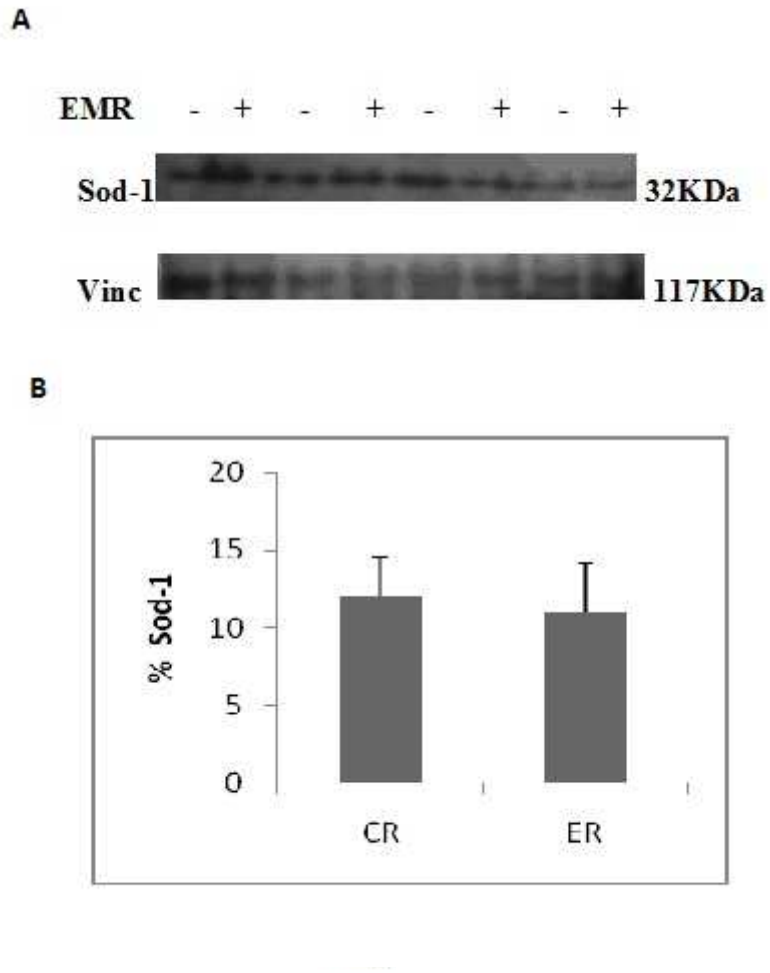

Figure 2. (A) Expression of Sod-1 by Western blotting. (B) Percentage of densitometric analysis of films made with Image J software. Results presented as mean ± SEM, n= 6. There was no difference from the CR values (two tailed t-test). CR, control rats and ER, exposed rats. Surprisingly, our results showed no change in expression of Sod-1 in the group of ER to UHF EMR. It was expected that expression of this enzyme would change after exposure to the UHF EMR. The literature has pointed out that the changes in biological systems depend on the type of electromagnetic wave (continuous or pulsed), the duration of exposure, frequency, power and SAR.

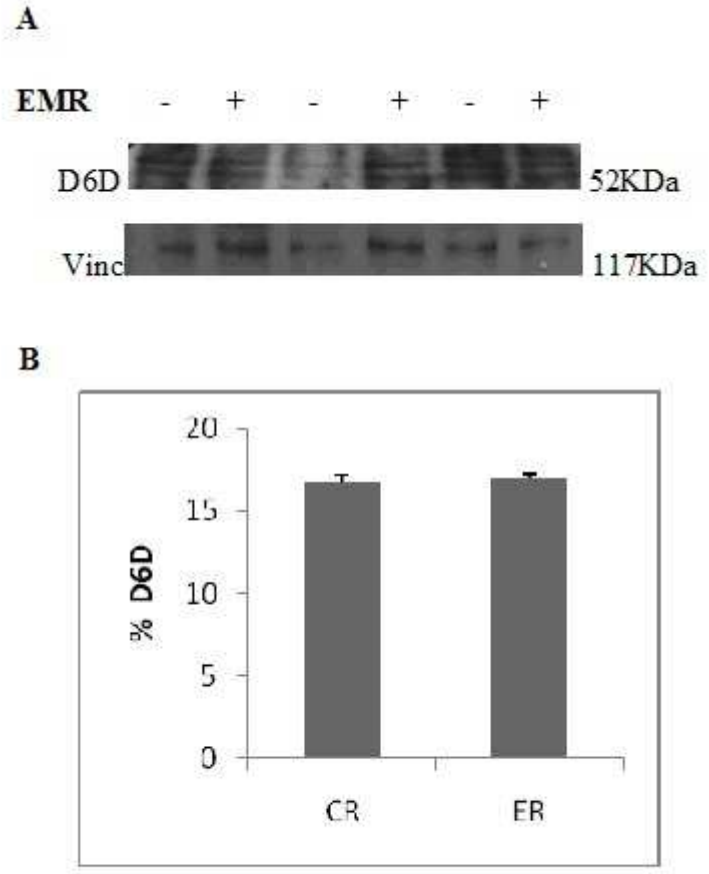

Figure 3. (A) Expression of D6D by Western blotting. (B) Percentage of densitometric analysis of films made with Image J software. Results presented as mean ± SEM, n= 3. There was no difference from the CR values (two tailed t-test). CR, control rats and ER, exposed rats. In our study, we did not detect any change in the expression of D6D, that is a key enzyme in the metabolism of PUFA. Moreover, we did not observed any induction of lipid peroxidation, as indicated by the TBARS levels of CR and ER groups. Besides the results of TBARS, the concentration of monounsaturated and polyunsaturated fatty acids (figue 4) did not change after exposure to UHF EMR, confirming our results of D6D expression.

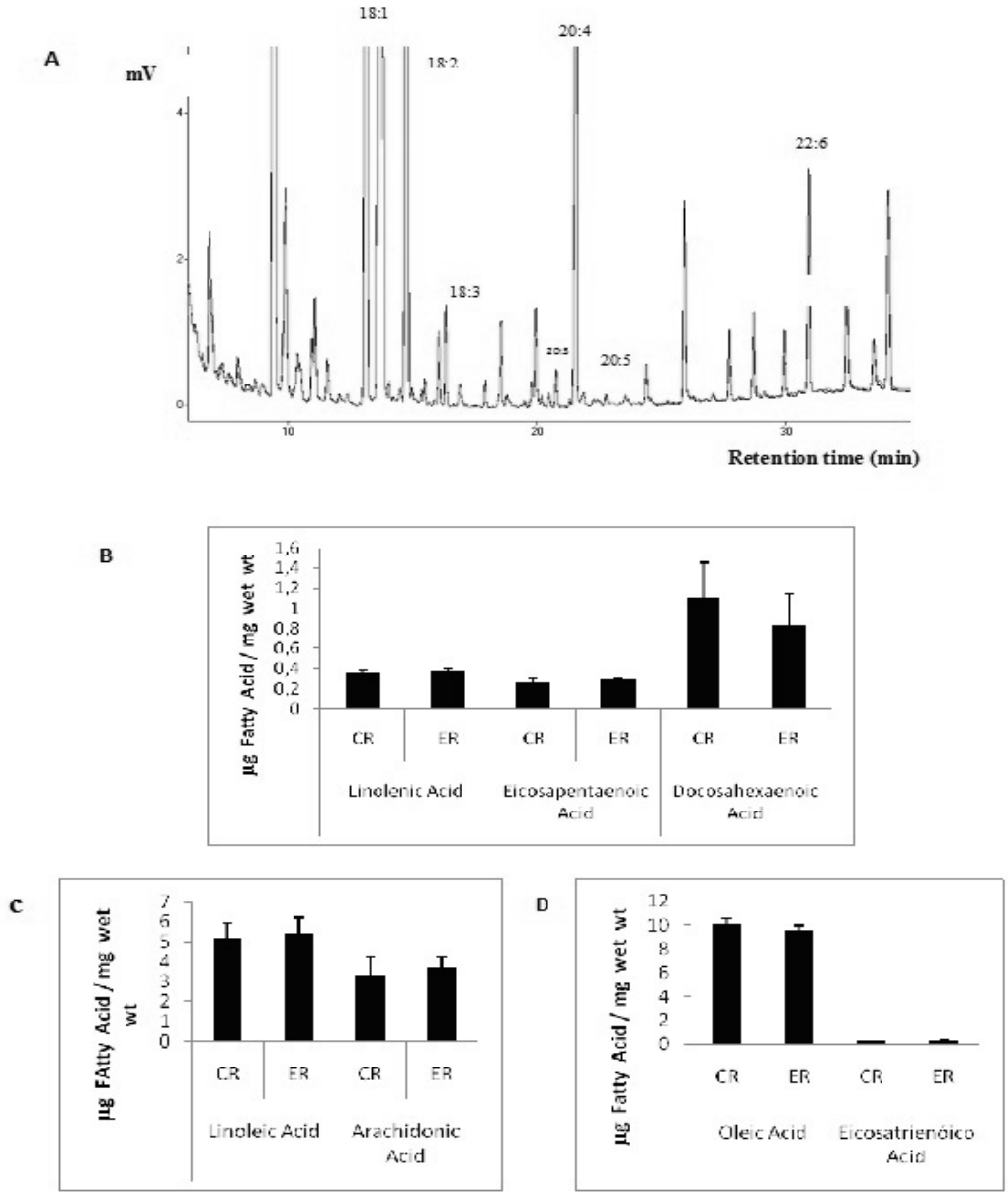

Figure 4. Determination of the concentration of fatty acids by gas chromatography of livers of rats exposed (ER) and unexposed (CR) to UHF EMR. The results are presented as mean ± SEM, n =6. There was no difference from the CR values (two tailed t-test).**A,** chromatogram of GC / FID of CR and ER. **B**, fatty acids ω–3 series; **C**, fatty acids ω–6 series; **D**, fatty acids ω–9 series. 18:1, Oleic acid; 18:2, Linoleic acid; 18:3, Linolenic acid; 20:3, eicosatrienoic acid; 20:4, Arachidonic acid; 20:5, Eicosapentaenoic acid; 22:6, Docosahexaenoic acid. The concentration of monounsaturated and polyunsaturated fatty acids did not change after exposure to UHF EMR, confirming our results of D6D expression and TBARS..

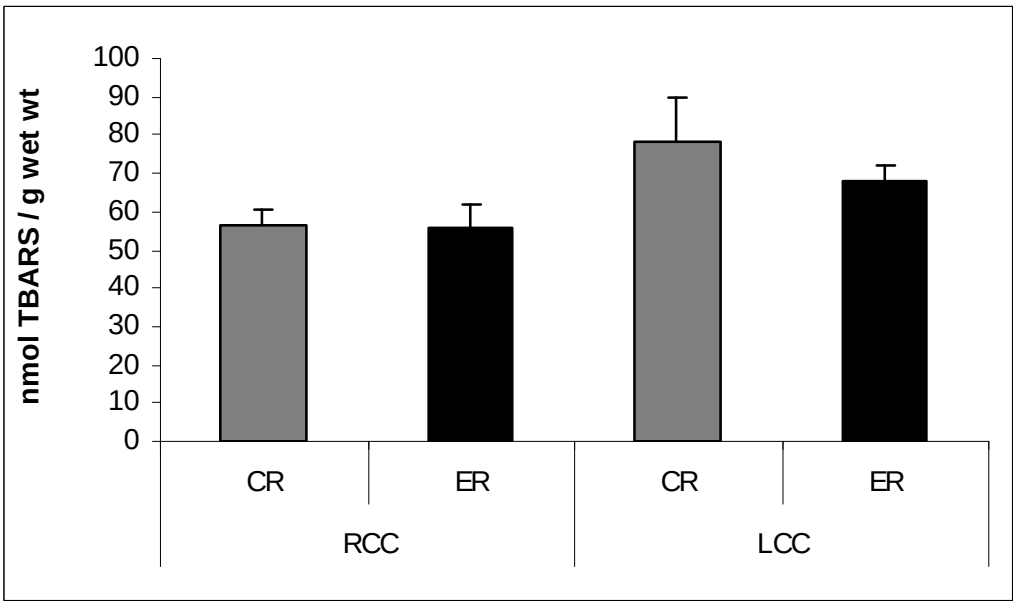

Figure 5. Determination of the levels of lipid peroxidation in left and right cerebral cortexes in rats with 15 days of age exposed (ER) and not exposed (CR) to UHF-EMW. The results are presented as mean ± SEM, n= 6. No different from the CR value(two tailed t-test and SNK test post-ANOVA). LCC, left cerebral cortex, RCC, right cerebral cortex.tThere is a balance between oxidant and antioxidant when comparing the group of CR with group ER of RCC and LCC.

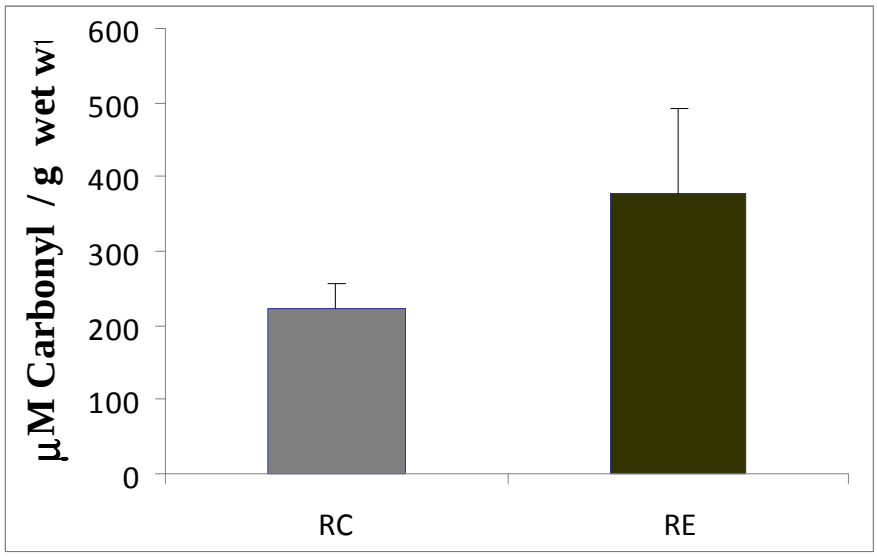

Figure 6. Determination of protein carbonyl concentrations in the right cerebral cortex in rats with 15 days of age exposed (ER) and not exposed (CR) to UHF-EMW.The results are presented as mean ± SEM, N= 6. No different from the CR value (two tailed t-test). Similarly the TBARS, no increase in oxidative damage, tracking levels of aldehydes.

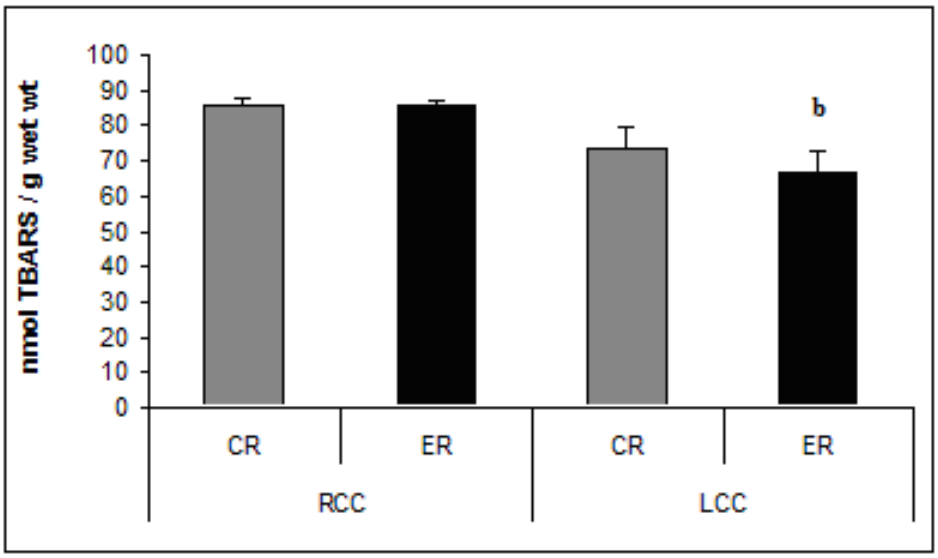

Figure 7. Determination of the levels of lipid peroxidation in left and right cerebral cortex in rats 30 days of age exposed and not exposed to UHF- EMR. The results are presented as mean ± SEM, n= 6. b: Significantly different from the ER value, $p<0.05$ (SNK test post-ANOVA). LCC, left cerebral cortex, RCC, right cerebral cortex.There was an oxidative asymmetry between the cerebral cortex where the RCC was more sensitive to lipid peroxidation than the LCC.

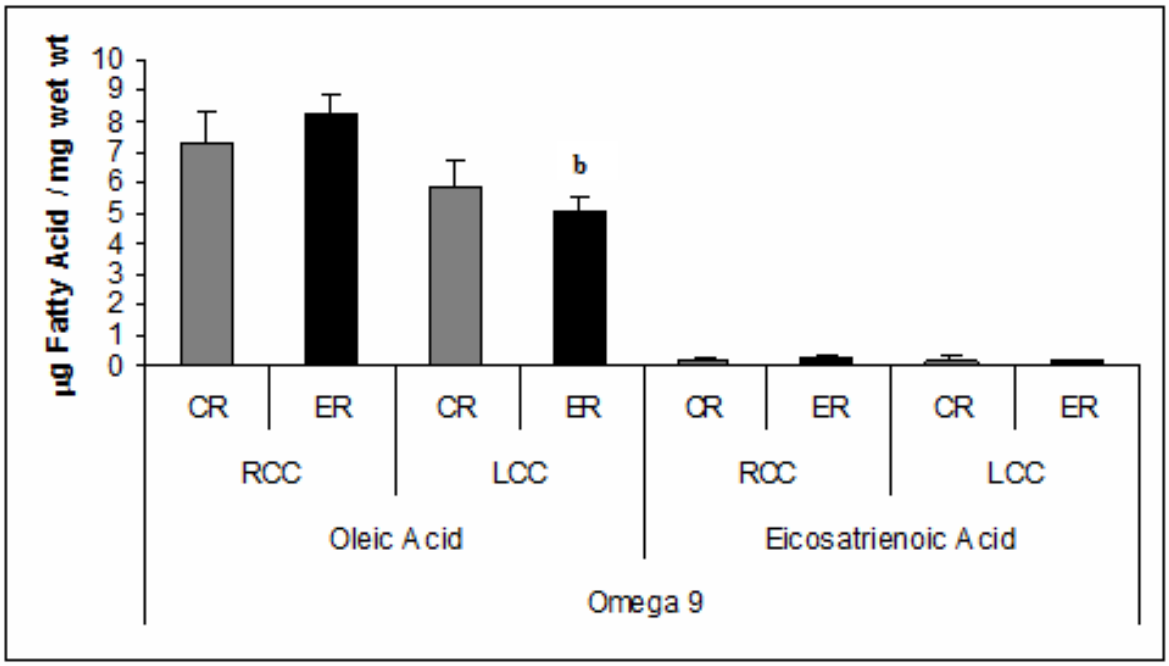

Figure 8. Determination of the concentration of oleic and eicosatrienoic fatty acids (omega 9) by gas chromatography (GC/FID) of left and right cerebral cortex in rats 30 days of age exposed and not exposed to UHF- EMR. The results are presented as mean ± SEM, n= 6. b: Significantly different from the ER value, $p<0.05$ (SNK test post-ANOVA). LCC, left cerebral cortex, RCC, right cerebral cortex. There was asymmetry in oleic acid concentration when comparing the group ER of RCC with ER of LCC.

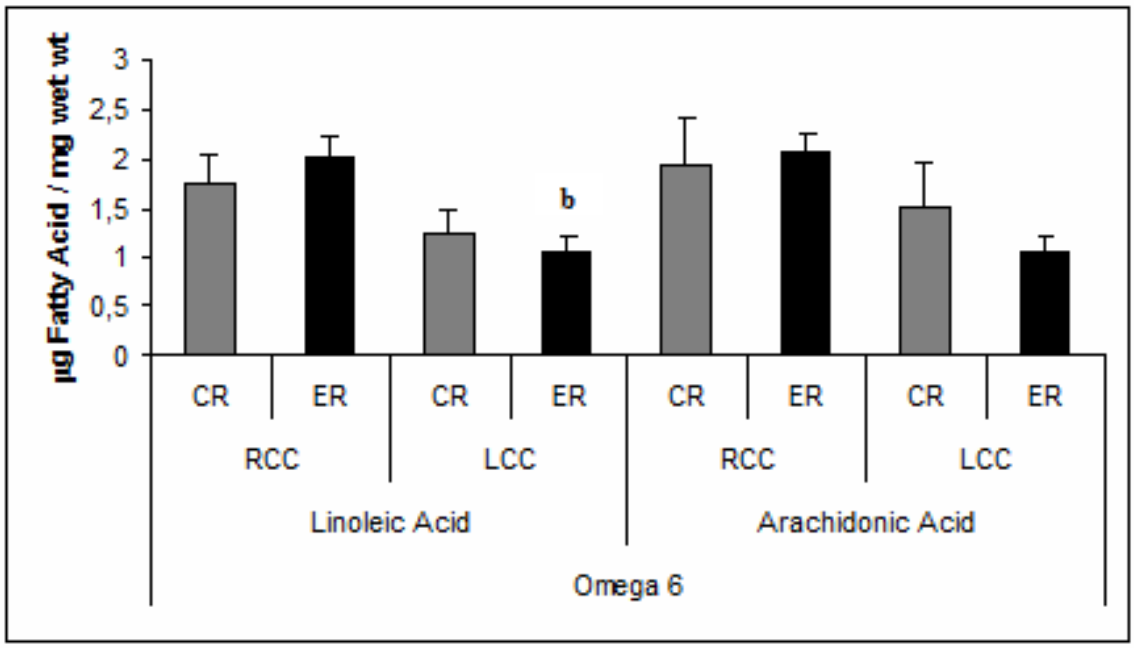

Figure 9. Determination of the concentration of linoleic and arachidonic fatty acids (omega 6) by gas chromatography (GC/FID) of left and right cerebral cortex in rats 30 days of age exposed and not exposed to UHF- EMR. The results are presented as mean ± SEM, n= 6. b: Significantly different from the ER value, $p<0.05$ (SNK test post-ANOVA). There was asymmetry in linoleic acid concentration when comparing the group ER of RCC with ER of LCC. LCC, left cerebral cortex, RCC, right cerebral cortex.

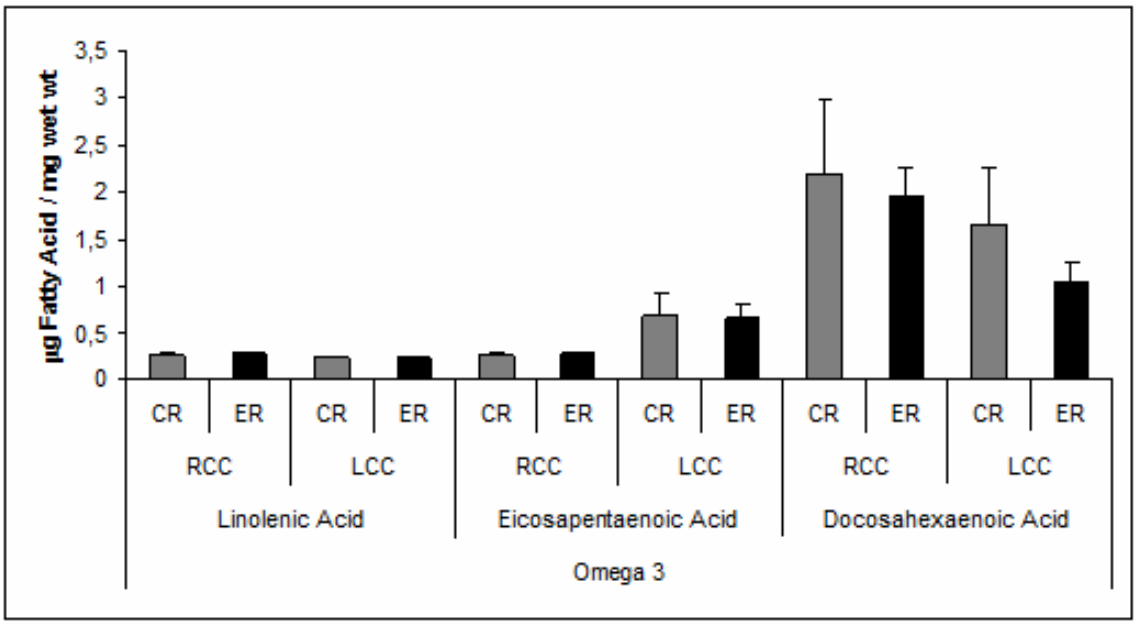

Figure 10. Determination of the concentration of linolenic, eicosapentaenoic and docosahexaenoic fatty acids (omega 3) by gas chromatography (GC/FID) of left and right cerebral cortex in rats 30 days of age exposed and not exposed to UHF- EMR. The results are presented as mean ± SEM, n= 6. No different in comparison between groups (SNK test post-ANOVA). LCC, left cerebral cortex, RCC, right cerebral cortex. The series 3 fatty acids do not show lateralization in the quantity of their components when RCC is compared to the LCC.

O conjunto destes resultados anexos revela que:

a. apesar da mitocôndria do fígado de ratos expostos apresentarem-se inchadas, não houve estresse oxidativo já que os danos a lipídios, proteínas e DNA não aumentaram. O inchaço é devido à mudança na concentração de ácidos graxos e, consequentemente, na fluidez da membrana;

b. diferentemente, o fígado dos animais com 30 dias de idade não apresentou alteração na concentração de UFA;

c. o córtex cerebral direito é mais sensível à lipoperoxidação;

d. há assimetria no metabolismo de ácidos graxos entre o RCC e LCC após exposição à radiação eletromagnética nas nossas condições experimentais.

Orlando Vieira Furtado Filho

Curriculum Vitae

Possui graduação em Ciências Biológicas pela Universidade da Região da Campanha (1996) e mestrado em Ciências Biológicas (Biologia Molecular) pela Universidade de Brasília (2003). Atualmente é doutorando em Biologia Celular e Molecular do Departamento de Biologia Molecular e Biotecnologia da UFRGS e professor de biologia do Colégio Militar de Porto Alegre do Ministério da Defesa - Exército. Tem experiência na área de Bioquímica e Biofísica com ênfase em Bioquímica de Radicais Livres, Metabolismo de Ácido Graxo, atuando principalmente nos seguintes temas: danos oxidativos, estresse oxidativo, antioxidantes, radicais livres, Caiman crocodilus yacare, Campo Eletromagnético de Ultra-Alta-Frequência e extrato vegetal.

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Título: Efeito Crônico não-térmico das Ondas Eletromagnéticas não-ionizantes de Ultra- Alta-Freqüência sobre Córtex Cerebral e Fígado de Ratos com Diferentes Idades.
Orientador: Jenifer Saffi

2001 - 2003 Mestrado em Ciências Biológicas (Biologia Molecular).
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Título: Relação entre danos oxidativos e processo de envelhecimento no jacaré do Pantanal, Ano de obtenção: 2003
Orientador: Marcelo Hermes Lima

1993 - 1996 Graduação em Ciências Biológicas.
Universidade da Região da Campanha, URCAMP, Bage, Brasil
Título: Licenciatura

Formação complementar

2005 - 2005 Extensão universitária em Biologia Molecular Básica.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2005 - 2005 Extensão universitária em Experimentação Animal.
Universidade Federal do Rio Grande do Sul, UFRGS, Porto Alegre, Brasil

2001 - 2001 Curso de curta duração em Fisiologia na Era da Biologia Molecular.
Federação de Sociedades de Biologia Experimental, FESBE, Brasil

2001 - 2001 Curso de curta duração em Estresse: mecanismos sitêmicos e moleculares.
Federação de Sociedades de Biologia Experimental, FESBE, Brasil

1999 - 1999 Extensão universitária em Atualização em Virologia Geral.
Universidade de Brasília, UNB, Brasília, Brasil

1995 - 1995 Extensão universitária em Educação, Ecologia e Meio Ambiente.
Fundação Educacional de Alegrete, FEA, Brasil

1995 - 1995 Curso de curta duração em Atualização em Biologia.
Fundação Educaional de Alegrete, FEA, Brasil

1994 - 1994 Curso de curta duração em Atualização em Bio:moléculas,evolução e energética.
Fundação Educacional de Alegrete, FEA, Brasil

Atuação profissional

1. Ministério da Defesa - Exército - MD - EX

Vínculo institucional

2005 - Atual Vínculo: Militar e Prof de Biologia , Enquadramento funcional: Prof de Biologia do Col Mil Porto Alegre, Regime: Dedicação exclusiva

Atividades

02/2005 - Atual Ensino médio
Especificação:
Biologia Geral: microbiologia, micologia, botânica e zoologia

02/2004 - 12/2004 Direção e Administração, Colégio Militar de Brasília
Cargos ocupados:
Chefe da Cadeira de Ciências Físicas e Biológicas

02/2001 - 12/2003 Direção e Administração, Colégio Militar de Brasília
Cargos ocupados:
Chefe da Cadeira de Biologia

02/2000 - 12/2004 Ensino fundamental
Especificação:
Ciências Físicas e Biológicas

02/1999 - 12/1999 Ensino médio
Especificação:
Biologia : Biologia Molecular, Citologia e Histologia

2. Universidade de Brasília - UNB

Vínculo institucional

2001 - 2003 Vínculo: Livre , Enquadramento funcional: Aluno de pós-graduação:mestrado , Carga horária: 40, Regime: Integral

2000 - 2000 Vínculo: Livre , Enquadramento funcional: Estagiário no Gp de Pesq de Radicais Livres , Carga horária: 20, Regime: Parcial

Atividades

07/2001 - 08/2001 Graduação, Medicina
Disciplinas ministradas:
Tópicos em Bioquímica

06/2001 - 09/2004 Treinamento, Biofísica - Depto Biologia Celular -Inst de Ciências Biológicas
Especificação:
Treinamento de métodos de danos oxidativos a alunos de iniciação científica, estagiários e pesquisadores visitantes ao laboratório de bioquímica de radicais livres.

09/2000 - 12/2000 Estágio, Gp Pesq Rad Livres Oxigênio - Biofísica
Estágio:
Manejo e dissecagem do caramujo Helix aspersa.

Áreas de atuação

1. Bioquímica de Radicais Livres.
2. Bioquímica Comparada
3. Fisiologia Comparada
4. Biologia Molecular

Projetos

Projetos de pesquisa: Estivacão e o metabolismo de H2O2 em caramujos Helix aspersa
Integrantes: Orlando Vieira Furtado Filho (Responsável);

Projetos de pesquisa: Mapeamento antioxidante/pró-oxidante em tecidos de jacarés-do-pantanal Caiman yacare
Integrantes: Orlando Vieira Furtado Filho (Responsável);

Idiomas

Inglês Compreende Razoavelmente , Fala Razoavelmente , Escreve Bem , Lê Bem
Espanhol Compreende Pouco , Fala Pouco , Escreve Pouco , Lê Razoavelmente
Francês Compreende Razoavelmente , Fala Razoavelmente , Escreve Bem , Lê Bem
Português Compreende Bem , Fala Bem , Escreve Bem , Lê Bem

Prêmios e títulos

2003 Medalha de Bronze em Desempenho Acadêmico-Científico, Grupo de Pesquisa em Radicais de Oxigênio - Biofísica - Depto Biologia Celular - UnB

2002 Menção Honrosa em Desempenho Acadêmico-Científico, Grupo de Pesquisa em Radicais de Oxigênio - Biofísica - Depto Biologia Celular - UnB

Producão

Produção bibliográfica

Artigos completos publicados em periódicos

1. FURTADO-FILHO, O. V., Polcheira, Machado, Mourão, Hermes-Lima
Selected oxidative stress markers in a South American crocodilian species. Comparative Biochemistry and Physiology. C, Toxicology & Pharmacology. , v.146, p.241 - 254, 2007.

Citações WEB OF SCIENCE™ 9 | SCOPUS 11

Trabalhos publicados em anais de eventos (resumo)

1. FURTADO-FILHO, O. V., Borba, J.B, Moreira, DALLEGRAVE, A., PIZZOLATO, T., Henriques, Saffi, J
Efeito das ondas eletromagnéticas de UHF de 950 MHz sobre biomarcadores de danos oxidativos e metabolismo de ácidos graxos insaturados do fígado de ratos neonatos In: Reunião Anual do PPGBCM da UFRGS, 2011, Porto Alegre.
Reunião Anual do PPGBCM da UFRGS. Porto Alegre: , 2011.

2. FURTADO-FILHO, O. V., Borba, J.B, MOREIRA, J. C. F., Henriques, Saffi, J
Estresse oxidativo em fígado de ratos expostos ao campo eletromagnético de UHF In: XII Reunião Anual do Programa de Pós-Graduação em Biologia Celular e Molecular - PPGBCM- CBIOT-UFRGS, 2010, Porto Alegre.
Estresse oxidativo em fígado de ratos expostos ao campo eletromagnético de UHF. , 2010.

3. FURTADO-FILHO, O. V., Borba, J.B, Henriques, Saffi, J
O efeito do campo eletromagnético de ultra-alta-freqüência sobre o fígado e o córtex cerebral de ratos In: XI Reunião anual Programa de Pós-Graduação em Biologia Celular e Molecular - PPGBCM - CBIOT - UFRGS, 2009, Porto alegre.
O efeito do campo eletromagnético de ultra-alta-freqüência sobre o fígado e o córtex cerebral de ratos. , 2009.

4. Borba, J.B, FURTADO-FILHO, O. V., Maraschin, T., Souza, L.M., Melecchi, F.S, Melecchi,M.I.S, Henriques, Saffi, J
O Efeito da Infusão de Hibiscus tiliaceus L. Sobre Ratas Gestantes e Lactantes Expostas ao Campo Eletromagnético de Ultra-Alta-Freqüência. In: X Reunião Anual Programa de Pós-Graduação em Biologia Celular e Molecular - PPGBCM-CBIOT-UFRGS, 2008, Porto Alegre.
O Efeito da Infusão de Hibiscus tiliaceus L. Sobre Ratas Gestantes e Lactantes Expostas ao Campo Eletromagnético de Ultra-Alta-Freqüência.. , 2008.

5. FURTADO-FILHO, O. V., Borba, J.B, Maraschin, T., Souza, L.M., Melecchi, F.S, Moreira, Henriques, Saffi, J
O Efeito do Campo Eletromagnético de Ultra-Alta-Freqüência Sobre Mitocôndrias e Metabolismo de Radicais Livres em Fígado de Ratos Neonatos. In: X Reunião Anual Programa de Pós-Graduação em Biologia Celular e Molecular- PPGBCM-CBIOT-UFRGS., 2008, Porto Alegre.
O Efeito do Campo Eletromagnético de Ultra-Alta-Freqüência Sobre Mitocôndrias e Metabolismo de Radicais Livres em Fígado de Ratos Neonatos.. , 2008.

6. Maraschin, T., Souza, L.M., Borba, J.B, FURTADO-FILHO, O. V., Melecchi, F.S, Melecchi,M.I.S, Henriques, Saffi, J
O Efeito do Extrato Aquoso de Hibiscus tiliaceus L. Sobre Ratas Gestantes/Lactantes Expostas ao Campo Eletromagnético de Ultra-Alta Freqüência. In: I Semana Científica da UFCSPA, 2008, Porto Alegre.
O Efeito do Extrato Aquoso de Hibiscus Tiliaceus L. Sobre Ratas Gestantes/Lactantes Expostas ao Campo Eletromagnético de Ultra-Alta Freqüência.. , 2008.

7. Melecchi,M.I.S, Borba, J.B, FURTADO-FILHO, O. V., Melecchi, F.S, Souza, L.M., Maraschin, T., Fanka,B.R., Saffi, J, Camarão, E.C
Ação Inibidora do Extrato das Flores de Hibiscus tiliaceus L. sobre Estresse Oxidativo Causado por Campo Eletromagnético de Ultra-Alta-Freqüência In: 14º Encontro Nacional de Química Analítica (ENQA), 2007, Joõ Pessoa.
Ação Inibidora do Extrato das Flores de Hibiscus tiliaceus L. sobre Estresse Oxidativo Causado por Campo Eletromagnético de Ultra-Alta-Freqüência. , 2007.

8. Machado, Ferreira, FURTADO-FILHO, O. V., Carreiro, Polcheira, Ito, Mourão, Hermes-Lima
Antioxidant defenses and oxidative stress in a Brazilian caiman: a developmental and environmental study. In: Second Workshop on Comparative Aspectis of oxidative Stress in Biological Systems, 2005, Baja California.
Workshop on Comparative Aspectis of Oxidative Stress in Biological Systems. Baja California: , 2005. v.II.

9. Machado, FURTADO-FILHO, O. V., Mourão, Hermes-Lima
Glutathione metabolism and oxidative stress in Caiman yacare In: Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular, 2004, Caxambu.
Radicais Livres e Espécies Ativas de Oxigênio. Caxambu: SBBq, 2004. v.XXXIII.

10. FURTADO-FILHO, O. V., Machado, Ferreira, Polcheira, Mourão, Hermes-Lima
Free radical metabolism in several development stages of a crocodilian species from the Pantanal wetlands. In: Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular, 2003, Caxambu.
Radicais Livres e Espécies Ativas de Oxigênio. Caxambu: SBBq, 2003. v.XXXII. p.241 -

11. Machado, Hermes-Lima, FURTADO-FILHO, O. V., Mourão
Determinação de enzimas antioxidantes e peroxidação lipídica em jacarés-do-pantanal Caiman yacare. In: VII Congresso de Iniciação Científica da UnB, 2002, Brasília.
VII Congresso de Iniciação Científica da UnB. Brasília: UnB, 2002. v.VII.

12. Machado, FURTADO-FILHO, O. V., Polcheira, Brandão, Ito, Ramos-Vasconcelos, Mourão, Hermes-Lima
Oxidative damage in organs of a crocodilian species from the Pantanal wetlands. In: Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular, 2002, Caxambu.
Radicais Livres e Espécies Ativas de Oxigênio. São Paulo: SBBq, 2002. v.XXXI. p.232 -

13. Hermes-Lima, Ferreira, Ramos-Vasconcelos, FURTADO-FILHO, O. V., Lushchak, Cardoso, Machado, Polcheira, Campos
Physiological oxidative stress in ovary, uterus and adrenal during the estrous cycle of rats In: 31 Annual Meeting of the Brazilian Biochemical Society - SBBQ, 2002
Anais do 31 Annual Meeting of the Brazilian Biochemical Society. , 2002.

14. Hermes-Lima, Ferreira, Ramos-Vasconcelos, FURTADO-FILHO, O. V., Lushchak, Cardoso, Machado, Polcheira, Campos, Torres
Physiological oxidative stress versus animal adaptability to wild variations in oxygen availability . In: Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular, 2002, Caxambu.
Radicais Livres e Espécies Ativas de Oxigênio. São Paulo: SBBq, 2002. v.XXXI. p.232 -

15. Ramos-Vasconcelos, FURTADO-FILHO, O. V., Hermes-Lima
Oxidative stress during summer-estivation and arousal in garden snails Helix aspersa In: Anais da 16ª Reunião Anual da FeSBE, 2001, Caxambu.
Anais da 16ª Reunião Anual da FeSBE. , 2001.

Apresentação de trabalho e palestra

1. Borba, J.B, FURTADO-FILHO, O. V., Maraschin, T., Souza, L.M., Melecchi, F.S, Melecchi,M.I.S, Henriques, Saffi, J
O efeito da infusão de Hibiscus tiliaceus L. sobre ratas gestantes e lactantes expostas ao campo eletromagnético de ultra-alta-frequência, 2008. (Outra,Apresentação de Trabalho)

2. FURTADO-FILHO, O. V., Borba, J.B, Maraschin, T., Souza, L.M., Melecchi, F.S, Moreira, Henriques, Saffi, J
O efeito do campo eletromagnético de ultra-alta-frequência sobre mitocôndrias e metabolismo de radicais livres em fígado de ratos neonatos, 2008. (Outra,Apresentação de Trabalho)

3. Souza, L.M., FURTADO-FILHO, O. V., Borba, J.B, Maraschin, T., Melecchi, F.S, Melecchi,M.I.S, Henriques, Saffi, J
O efeito do campo eletromagnético de ultra-alta-frequência sobre o peso de ratos neonatos, 2008. (Outra,Apresentação de Trabalho)

4. Maraschin, T., Souza, L.M., Borba, J.B, FURTADO-FILHO, O. V., Melecchi, F.S, Melecchi,M.I.S, Henriques, Saffi, J
O Efeito do Extrato Aquoso de Hibiscus tiliaceus L. sobre Ratas Gestantes/Lactantes Expostas ao Campo Eletromagnético de Ultra-Alta-Frequência, 2008. (Outra,Apresentação de Trabalho)

Produção técnica

Demais produções técnicas

1. FURTADO-FILHO, O. V., Borba, J.B
Curso Teórico-Prático: Danos Oxidativos e Defesas Antioxidantes, 2009. (Outro, Curso de curta duração ministrado)

Organização de eventos, congressos, exposições e feiras e olimpíadas

1. FURTADO-FILHO, O. V.
II Seminário de Educação do CMPA, 2012. (Outro, Organização de evento)

Eventos

Participação em eventos

1. **II Seminário de Educação do CMPA**, 2012. (Seminário)

2. **Seminário Internacional Sobre Radiações Não Ionizantes, a Saúde e o Ambiente**, 2009. (Seminário)

3. Apresentação Oral no(a) **Congresso Brasileiro de Zoologia**, 2004. (Congresso)
Sobrevivendo aos super-extremos da natureza.

4. Apresentação de Poster / Painel no(a) **Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular**, 2003. (Congresso)
Free radical metabolism in several development stages of a crocodilian species from the Pantanal wetlands..

5. Apresentação de Poster / Painel no(a) **XXXI Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular**, 2002. (Congresso)
Oxidative damage in organs of a crocodilian species from the Pantanal wetlands.

6. Apresentação de Poster / Painel no(a) **Reunião Anual de Sociedades de Biologia Experimental**, 2001. (Congresso)
Oxidative stress during summer-estivation and arousal in garden snails Helix aspersa..

Organização de evento

1. FURTADO-FILHO, O. V.
II Seminário de Educação do CMPA, 2012. (Outro, Organização de evento)

Bancas

Participação em banca de trabalhos de conclusão

Graduação

1. FURTADO-FILHO, O. V.
Participação em banca de Fernando Barella. **Estresse oxidativo em modelo experimental da doença de Canavan: efeito protetor do ácido lipóico**, 2011
(Biomedicina) Fundação Universidade Federal de Ciências da Saúde de Porto Alegre