

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:
ENDOCRINOLOGIA

**EFEITO DA N-ACETILCISTEÍNA SOBRE O METABOLISMO DOS HORMÔNIOS
TIREOIDIANOS, FUNÇÃO CARDÍACA E ESTRESSE OXIDATIVO EM MODELO
ANIMAL DE SÍNDROME DO T3 BAIXO.**

TATIANA EDERICH LEHNEN

Porto Alegre, outubro de 2017.

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TESE DE DOUTORADO

TATIANA EDERICH LEHNEN

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da Universidade Federal do Rio Grande do Sul como requisito parcial para obtenção do título de Doutor em endocrinologia.

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Esta tese de doutorado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, sendo apresentada na forma de 3 manuscritos sobre o tema da Tese:

- Artigo de revisão (1): Effects of Non Thyroidal Illness Syndrome in Cardiovascular Disease.
- Artigo original (2): N-acetylcysteine prevents low T3 syndrome and attenuates cardiac dysfunction in a male rat model of myocardial infarction (publicado na revista *Endocrinology* em 2017 - Fator de Impacto 4.286).
- Artigo original (3): Oxidative stress induces type 3 deiodinase in multiple tissues after myocardial infarction: implications to Low T3 Syndrome pathophysiology.

LISTA DE ABREVIATURAS E SIGLAS

ATP	Adenosine triphosphate
CHF	Chronic heart failure
D1	Type 1 iodothyronine deiodinase
D2	Type 2 iodothyronine deiodinase
D3	Type 3 iodothyronine deiodinase
EF	Ejection fraction
FT4	Free Thyroxine
GSH	Glutathione
GSSG	Oxidized glutathione
LV	Left ventricle
LVDd	Left ventricular diastolic
LVDs	Left ventricular systolic
MCT-10	Monocarboxylate transporters 10
MCT-8	Monocarboxylate transporters 8
MI	Myocardial infarction
mRNA	Messenger RNA
NAC	N-acetylcysteine
NTIS	Nonthyroidal illness syndrome
ROS	Reactive oxygen species
rT3	Reverse triiodothyronine
SERCA 2	Sarcoplasmic reticulum calcium adenosine triphosphatase
T2	Diiodothyronine
T3	Triiodothyronine
T4	Thyroxine
TH	Thyroid hormones
TR	Thyroid hormone receptors
Trx	Thioredoxin
TSH	Thyrotropin

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INTRODUÇÃO

A síndrome do T3 baixo se caracteriza por alterações no metabolismo dos hormônios tireoidianos. Independente da condição clínica existente, à medida que ocorre a piora da doença, ocorre mudanças na conversão periférica do T4 em T3, diminuindo os níveis séricos de T3. Esta síndrome ocorre em cerca de 75% dos pacientes em unidades de terapia intensiva, sendo também normalmente observada em pacientes com outras patologias como infarto agudo do miocárdio. Em todas as situações os baixos níveis séricos de T3 estão inversamente correlacionados com a mortalidade.

A conversão periférica dos hormônios tireoidianos é feita pelas enzimas iodotironinas desiodases tipo 1, 2 e 3 (D1, D2 e D3, respectivamente. As iodotironinas deiodinases são oxiredutases que catalisam a remoção de iodo do anel externo (D1 e D2) ou interno (D3) dos hormônios tireoidianos, ativando ou inativando esses hormônios.

A alteração na função das desiodases tem sido observada em várias doenças e está relacionada à presença do stress oxidativo. Trabalhos prévios do nosso grupo desenvolveram estudos em modelo celular demonstrando que o aumento da IL-6, observado em todas as situações de doença, estimula NADPH oxidase, que forma radical superóxido, levando a um aumento das espécies reativas de oxigênio, que por sua vez consome as reservas antioxidantes da célula, levando à disfunção das desiodases e síndrome do T3 baixo.

Foi observado que esse desequilíbrio redox resulta na alteração da atividade das desiodases, inibindo D1 e D2, diminuindo a conversão de T4 em T3, e aumentando D3, que inativa o T3. Recentemente, foram feitos estudos com antioxidantes na tentativa de corrigir a Síndrome do T3 baixo. E um dos antioxidantes usados tem sido a N-acetilcisteína (NAC), potente antioxidante que restaura os níveis de glutathiona evitando o desequilíbrio redox. Um estudo clínico randomizado realizado em pacientes com infarto

do miocárdio que receberam tratamento NAC e placebo mostrou que o tratamento com NAC reverte a Síndrome do T3 baixo, corrigindo o metabolismo dos hormônios tireodianos e a função cardíaca.

O infarto do miocárdio leva ao estresse oxidativo e altera a função das desidases, resultando na síndrome do T3 baixo. Demonstramos que a NAC corrige o metabolismo dos hormônios tireodianos. Considerando todos esses pontos, e devido à limitações de estudos que avaliem o efeito do stress oxidativo gerado pela doença em nível tecidual em humanos, o objetivo da nossa pesquisa foi avaliar o efeito da NAC sobre a função cardíaca, as enzimas que metabolizam os hormônios tireoidianos e o estresse oxidativo no sangue e em múltiplos tecidos na Síndrome do T3 baixo em ratos submetidos ao infarto do miocárdio.

ARTIGO I

Effects of Nonthyroidal Illness Syndrome in Cardiovascular Disease

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ABSTRACT

Non-thyroidal illness syndrome (NTIS) is characterized by changes in the metabolism of thyroid hormones (TH), regulated by enzymes called iodothyronine deiodinases (type 1, 2 and 3). Alterations on the function of deiodinases has been observed in several diseases, mainly in the cardiovascular diseases, being generally observed in patients with myocardial infarction (MI). These alterations leads to an inverse correlation of low T3 levels with poor prognosis and mortality. However, the mechanisms of this syndrome are still poorly understood. Thus, given the importance of TH metabolism in cardiovascular diseases, mechanisms involving the pathogenesis of NTIS are studied *in vivo* and *in vitro* models, and due to several lacks in the understanding of the pathophysiological mechanisms of NTIS, there is no currently approved treatment for these set of TH derangements.

Key-words: thyroid hormone, non-thyroidal illness syndrome, cardiovascular diseases.

1. INTRODUCTION

Thyroid hormones (TH) are essential for energy metabolism, cell growth and differentiation. Although Thyroxine (T4) is the main product of the thyroid gland, Thyroxine (T3) is the biologically active hormone. Most of the T3 production (80-90%) occurs in the peripheral tissues through the action of iodothyronine deiodinases types 1, 2 and 3 (D1, D2 and D3, respectively), which controls TH activation and inactivation.

The nonthyroidal illness syndrome (NTIS), also known as low T3 syndrome reflects alterations in TH levels that occur in almost every form of acute and chronic illness (Larsen and Baxter 2008). The acute phase of critical illness is marked by low T3 and free T3 (FT3) and high rT3 levels. T4 levels may be normal or reduced without increased thyroid-stimulating hormone (TSH) levels (Warner and Beckett 2010). While decreased T4 levels are associated with the severity of disease, low FT3 levels have been recognized as a marker of poor prognosis (Iervasi, Pingitore et al. 2003, Wang, Pan et al. 2012, Ozcan, Osmonov et al. 2014, Jabbar, Pingitore et al. 2017). The exact mechanisms leading to TH alterations in the acute phase of disease are unknown but changes in peripheral hormone metabolism are implicated. NTIS is commonly observed in patients with several pathologies, among them the majority of cardiovascular disease (Kimura, Kanda et al.

2000, Pavlou, Kliridis et al. 2002), such as myocardial infarction (MI) and chronic heart failure (CHF) (Li, Guo et al. 2011, Ozcan, Osmonov et al. 2014).

TH have a direct effect on the cardiac muscle and the impact of T3 levels on heart disease can be explained by the physiological actions of these hormones regulating cardiac function, having a large participation in myocardial contractility, systolic and diastolic function (Bengel, Nekolla et al. 2000). TH metabolism is altered in myocardial infarction (MI), cardiac hypertrophy and heart failure (Ojamaa, Kenessey et al. 2000, Henderson, Danzi et al. 2009, Pol, Muller et al. 2010).

TH also have a direct positive inotropic effect on the heart through genomic and nongenomic effects on components of the adrenergic receptor complex (Klein and Ojamaa 2001) and the sarcoplasmic reticulum calcium adenosine triphosphatase (SERCA2) channels has a genomic action, regulating contractile function and heart rhythm. Low levels of T3 stimulate phospholamban, inhibiting SERCA. This reduces calcium removal in the cytosol by decreasing systolic heart function (Carr and Kranias 2002). T3 enters the cardiomyocyte through membrane transporters and is also activated intracellularly by the conversion of T4. At the nucleus, T3 binds to thyroid hormone receptors (TR), regulating transcription. Elevations of TH levels increase oxidative capacity and fatty acid utilization in skeletal muscle, which is the main target of thyroid hormone signaling (Simonides, Mulcahey et al. 2008).

The changes in TH availability modulate the contractile and metabolic properties of skeletal muscle, increasing SERCA and indicating that these hormones are in dynamic equilibrium. This balance helps explain the muscle changes that characterize insufficiency and excess in TH. It is also suggested that there may be a temporally restricted expression of DIO2 and DIO3 in satellite cells during differentiation and repair of muscle cells (Salvatore, Simonides et al. 2014).

Although the severity of the disease is the main determinant of NTIS, the production and function of THs seems to be affected by the increased levels of oxidative stress and cytokines. Interleucin-6 (IL-6) has a negative correlation with serum T3 levels in hospitalized patients, especially after MI (Papanas, Papatheodorou et al. 2008). Augmented IL-6 increases oxidative stress, decreasing T3 and increasing rT3 levels (Pingitore, Iervasi et al. 2006, Wajner, Goemann et al. 2011, Vidart, Wajner et al. 2014). Given the importance of TH metabolism, mechanisms involving the pathogenesis of NTIS

are studied *in vivo* and *in vitro* models. Here we review the alterations on NTIS related to cardiovascular diseases.

2. NONTHYROIDAL ILLNESS SYNDROME

The pathophysiology of NTIS is complex and poorly understood. The relationship between changes in TH parameters and the severity of the disease is a *continuum* that seems to depend not on the type of disease but on the severity and prognosis of the underlying disease (Iervasi, Pingitore et al. 2003). NTIS is associated with an increase cardiovascular mortality and morbidity in patients with acute coronary syndrome (Adawiyah, Norasyikin et al. 2010). With the progression of the disease, serum T3 remains falling to extremely low levels, with concomitant increase in rT3. Changes in T4 are more complex. In mild forms, T4 tends to be normal, and the fall is observed only in very severe cases (Docter, Krenning et al. 1993). The decrease in T3 levels can be observed in up to 75% of patients admitted to the intensive care unit, while the T4 decrease occurs in up to 50% of cases (Ray, Macduff et al. 2002). Until now, it is unclear whether the observed changes correspond to an adaptive mechanism intended to reduce energy consumption, or it is a form of secondary hypothyroidism that could benefit with treatment (Mebis and van den Berghe 2009).

Inflammatory cytokines has an important pathophysiological role in NTIS, since the serum IL-6 levels are usually high and have an inverse relationship with T3 levels (Boelen, Platvoet-Ter Schiphorst et al. 1993). In the acute phase of the disease some mechanisms are implicated in the peripheral alterations of TH. Particularly, one of them is the direct effect of augmented systemic IL-6 on T3 production, decreasing the peripheral conversion of T4 to T3 and elevating rT3 (Peeters, Wouters et al. 2003). Interestingly, cardiovascular diseases, as myocardial infarction (MI), increases the production of pro-inflammatory cytokines, decreasing T3 levels in cardiomyocytes in the left ventricle (LV) and causing an intracellular hypothyroid state (Pol, Muller et al. 2011). Other mechanism is the monocarboxylate transporters 8 e 10 (MCT8 and MCT10), adenosine 5-triphosphate (ATP) dependent transporters (Visser, van Mullem et al. 2011). MCT8 carries T4 and T3 and is expressed in the liver, kidney, brain, heart and thyroid gland; and MCT10 transports T3 instead of T4 and is expressed in the liver, kidney, skeletal muscle and placenta (Heuer, Maier et al. 2005, Nishimura and Naito 2008, Visser, van Mullem et al. 2011). These transporters (MCT8 and MCT10) depend on adenosine 5-triphosphate (ATP) to

uptake TH by the cell membrane. It is known that the transport of TH in tissues may be reduced in NTIS (Boelen, van der Spek et al. 2017) but the association with decreased expression of transporters is controversial (Friesema, Jansen et al. 2008).

2.1 Role of peripheral thyroid hormone metabolism alterations on NTIS

The activation and inactivation process of thyroid hormones is dependent on the action of three enzymes known as iodothyronine deiodases types 1, 2 and 3 (D1, D2 and D3, respectively) (Baqui, Botero et al. 2003). The iodothyronine deiodinases (D1, D2 and D3) constitute a family of oxidoreductases, which removes a molecule of iodine from the outer (tyrosyl) and/or the inner (phenolic) ring of TH. D1 and D2 are hormone-activating enzymes, converting T4 to T3, although D1 also act as inactivating enzyme. On the other hand, D3 exclusively inactivates T3 and T4, leading to the formation of T2 and rT3, which are inactive compounds (Maia, Goemann et al. 2011, Wajner, Goemann et al. 2011). The majority of T3 production (80-90%) occurs in the peripheral tissues through the action of iodothyronine deiodinases (Maia, Kim et al. 2005) and T3 levels increase as it increases D2 activity in the heart, improving cardiac contractile function (Trivieri, Oudit et al. 2006).

While D1 is found in the plasma membrane, D2 is located in the endoplasmic reticulum. Both have the active site in the cytosol, thus allowing access to the yet unknown cofactor(s) (Bianco, Salvatore et al. 2002, Gereben, Zavacki et al. 2008). D3 is also found in the plasma membrane but the location of its active site is not still identified (Wajner, Goemann et al. 2011). D1 is located in the liver, kidney, thyroid and pituitary, while D2 is located in the thyroid, heart and skeletal muscle in humans and in animals is also located in the pituitary, brain and brown adipose tissue. D3 is located in the brain, skin, uterus, placenta and fetus (St Germain, Galton et al. 2009).

Besides converting T4 into T3 in peripheral tissues, these enzymes are highly expressed in the thyroid gland (Toyoda, Nishikawa et al. 1992, Salvatore, Tu et al. 1996). D2 and D3 are widely expressed during development (Galton 2005) and in the fetal period D3 regulates the degree of exposure of the fetus to TH (Cooper, Gibbens et al. 1983, Mortimer, Galligan et al. 1996) since the deficiency of these hormones can lead to neurological disturbances (Zoeller and Rovet 2004, Williams 2008).

The interaction between D2 activation and D3 inactivation in tissues expressing these enzymes regulates intracellular T3 concentration, providing flexibility for the tissue regeneration and development process (Larsen and Zavacki 2012). The function of deiodinases influences the levels and action of TH. Several diseases and fasting alter deiodinases function, decreasing T3 and T4 without a compensatory increase of serum TSH levels (Kmiec, Kotlarz et al. 1996, De Groot 2006, Boelen, Wiersinga et al. 2008).

Nevertheless, studies approaching the role of deiodinases in disease produce controversial results. An animal study using three NTIS models (acute systemic inflammation by intraperitoneal injection of bacterial, chronic local inflammation by a turpentine injection in the hind limb and severe pneumonia and sepsis by intranasal inoculation with *Streptococcus pneumoniae*) to study TH metabolism showed that peripheral TH metabolism is differentially regulated during disease, depending on the organ and the severity of the disease. This study showed that NTIS differentially affects the expression and activity of D1 and D3 in the liver, resulting in a decrease in D1 and D3 activity in acute inflammation. In muscle, the decrease in TH concentrations is less severe than the observed decrease in liver (Boelen, van der Spek et al. 2017). In addition, other studies have shown that fasted rats increased *in vitro* D2 activity in the rat hypothalamus, resulting in an increase in T3 levels in this tissue (Coppola, Hughes et al. 2005, Fekete, Sarkar et al. 2005). Nevertheless, D3 induction is observed *in vivo* in response to tissue injury and may influence healing or regenerative processes in tissue injury and inflammation under conditions of hypoxia and / or oxidative stress (Lamirand, Pallud-Mothre et al. 2008, Simonides, Mulcahey et al. 2008). Therefore, an increase in D3 has been observed in animal models at sites of inflammation and in the heart with MI (Olivares, Marassi et al. 2007). In contrast, a recent animal study showed that in response to stress, such as caloric restriction or disease, in the liver and kidneys there was a decrease in TH signaling, increasing D3 activity. However, in the brain, muscle and heart, the TH signaling was preserved (Visser, Heuer et al. 2016). Interestingly, the induction of post-MI D3 cardiomyocytes is associated with a 50% reduction in T3 levels in the tissue, leading to a local hypothyroid condition and affecting the remodeling of the LV in hearts of animals that were severely hypothyroid when compared to euthyroid controls (Pol, Muller et al. 2010).

NTIS is correlated with decreased D1 in liver and D3 in liver and skeletal muscle in critically ill patients (Peeters, van der Geyten et al. 2005, Peeters, Wouters et al. 2005). Studies in humans have shown a decrease in D1 in the liver and an increase in D3 in skeletal muscle and liver in states of fasting and/or illness (Chopra 1997, De Groot 2006).

3. ROLE OF OXIDATIVE STRESS ON NTIS PATHOPHYSIOLOGY IN CARDIOVASCULAR DISEASE

Oxidative stress is characterized by unbalance between the production of pro-oxidant substances and antioxidant defenses and one of the most important oxidants is the ROS (Breitkreuz and Hamdani 2015). Recent study showed that oxidative stress induces functional damage in tissues and plays an important role in the pathophysiology of NTIS and the synthesis, activity and metabolism of TH. One of the possible mechanisms related to oxidative stress is the induction of cytokines, since all of three deiodinases require a still undefined cofactor, probably a thiol, which acts as a reducing agent releasing iodine from the selenocysteine residue and regenerating the active enzyme (Wajner, Goemann et al. 2011).

The alteration of deiodinases in cardiovascular diseases is directly linked to the changes in TH and one of the mechanisms involved in the relationship between TH imbalance to MI is the oxidative stress (Friberg, Werner et al. 2002, Lamirand, Pallud-Mothre et al. 2008), being associated with a low ejection fraction, which may interfere with in the improvement of the patient (Rybin and Steinberg 1996, Jankauskiene, Orda et al. 2015, Wang, Guan et al. 2015). The redox balance of the heart is an important factor, since ROS concentration can be related with the promotion of cardiac damage (Schenkel, Tavares et al. 2010), which can lead to changes inactivation of T3 and T4. In addition, the increased ROS production can produce cell damage, contributing to the pathophysiology of myocardial infarction.

Stress state, such as MI, leads to an adrenergic overload by increasing intracellular calcium in cardiomyocytes, reducing SERCA and altering the efficiency of cardiomyocytes (Weber 2016). Deiodinases also seem to alter this equilibrium in disease. Moreover, TH exert profound effects on the heart and cardiovascular hemodynamics, maintaining the cardiac mass and stress of the wall while preserving the LV ellipsoidal geometry (Pol, Muller et al. 2010). This effects on cardiovascular hemodynamics includes reduction of cardiac output, increase in vascular resistance and decrease in diastolic filling rates (Klein and Ojamaa 2001).

Several clinical and experimental studies also report an important role of D3 in NTIS (Peeters, Wouters et al. 2003, Peeters, van der Geyten et al. 2005, Rodriguez-Perez, Palos-Paz et al. 2008). D3 activity is nearly undetectable in the healthy heart and is

induced in various models of heart failure and MI (Hamilton, Stevenson et al. 1990, Kinugawa, Minobe et al. 2001). It is known that cardiovascular diseases induce IL-6, increasing oxidative stress and decreasing GSH and other antioxidants (Wajner et al. 2012). However, the increase in IL-6 has an inverse correlation with T3 levels in patients with NTIS, reducing the activity of deiodases. Recent studies have shown that the inhibitory action of IL-6 is corrected by the administration of N-acetylcysteine (NAC), indicating that IL-6 inhibits the function of D1 and D2 by increasing cellular ROS. Differently, IL-6 stimulates endogenous D3-mediated inactivation of T3, which is probably secondary to the plasma membrane location of D3 that allows this enzyme to have ready access to extracellular GSH and other antioxidants (Wajner and Maia 2012, Vidart, Wajner et al. 2014) (Figure 1).

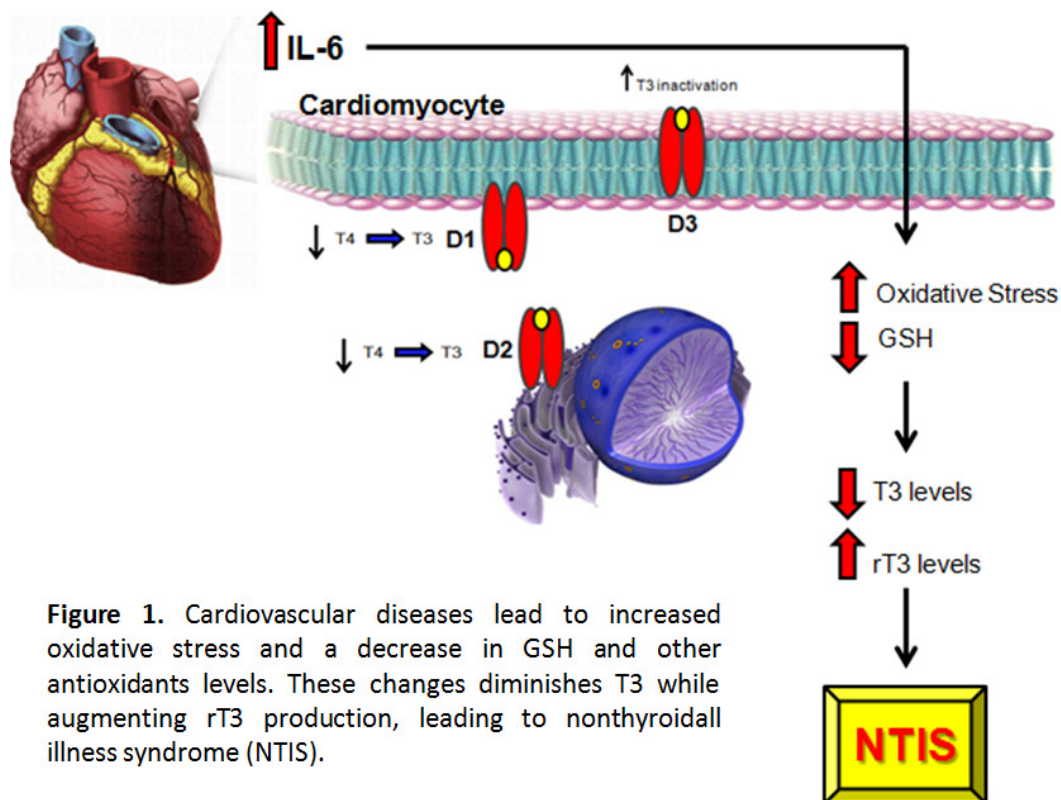


Figure 1. Cardiovascular diseases lead to increased oxidative stress and a decrease in GSH and other antioxidants levels. These changes diminishes T3 while augmenting rT3 production, leading to nonthyroidal illness syndrome (NTIS).

Changes in redox balance are known to disrupt deiodinases function and studies have made use of *N*-acetylcysteine (NAC) as a treatment in order to prevent these changes (Wajner, Goemann et al. 2011). *N*-acetylcysteine (NAC) is an antioxidant that increases intracellular cysteine levels and increase GSH levels. Thus, it can restore the deiodinases activity and restoring redox equilibrium, preventing the inhibitory effect of IL-6 on D1 and D2 mediated T4 to T3 conversion. NAC is a glutathione precursor that can correct the *upregulation* of D3 induced by ROS. The mechanism implicated in this phenomena is still not fully understood, but it is possible that D3 is protected by

extracellular GSH during the process of D3 endosomal internalization or that its active center is facing outward. After all, as previously described, GSH does not cross the plasma membrane. Thus explaining why its activity in intact cells parallels the D3 activity in sonicates of the same cells (Wajner, Goemann et al. 2011).

Lehnen et al (2017), demonstrated in animals submitted to AMI that NAC treatment prevents decreases in T3 levels after MI and preserves cardiac function in rats with NTIS, improving the diastolic and systolic diameters of the right and left ventricles, with a strong positive correlation between the ejection fraction and serum T3 levels. Moreover, NAC corrects the activity of D3 in cardiomyocytes after AMI through the improvement of the altered redox state (Lehnen, Santos et al. 2017). NAC also increases the antioxidant capacity, prevents apoptosis (Galang, Sasaki et al. 2000) and restores cardiomyocytes contractility (Kubin, Skoumal et al. 2011, Andre, Fauconnier et al. 2013). A recent study showed that NAC administration TH disorders that normally occurs in patients during the acute phase of MI (Vidart, Wajner et al. 2014). NAC rapidly reverses the acute thyroid disorders produced by oxidative stress to normal levels in patients with MI. In addition, the improvement of redox imbalance related to MI by NAC prevents abnormalities in TH metabolism and improves cardiac performance (Vidart, Wajner et al. 2014).

Due to its importance on heart metabolism, thyroid hormone administration was studied in order to improve cardiac function in cardiovascular disease. Animal studies showed that hormonal treatment with T3 and T4 promotes an improvement in cardiac function (de Castro, Tavares et al. 2014, Corssac, de Castro et al. 2016). The TH may have a protective effect after MI (Pantos, Mourouzis et al. 2007, Pantos, Mourouzis et al. 2008, Forini, Lionetti et al. 2011) and in CHF (Townsend, Nichols et al. 2015), since the concentrations of intracellular and circulating TH (mainly T3) decreases. Accordingly, low T3 administration ($\sim 6 \mu\text{g} / \text{kg} / \text{day}$) following MI improves cardiac performance and reverses adverse changes in the gene expression without negative effects (Rajagopalan, Zhang et al. 2016).

Although long-term thyroid hormone administration has been shown to improve chronic cardiac function in post-MI heart failure in rats, experience with hormone replacement in humans is limited. The lack of benefit in the long-term outcomes and concerns about adverse effects, such as increased myocardial demand, arrhythmias and suppression of the hypothalamic-pituitary-thyroid axis, have reduced enthusiasm for T3 administration in acute settings. In humans, a study with post-ischemic patients who received T3 by continuous infusion showed that a short-term T3 administration reduced the activation of

the neuroendocrine system and improved systolic volume in LV in patients with ventricular dysfunction and NTIS (Pingitore, Galli et al. 2008). According to some authors, the therapy of TH replacement should be used only in patients with NTIS or subclinical hypothyroidism (Jabbar, Pingitore et al. 2017). And although T3 treatment improves cardiac function in patients with CHF, the true functional significance of low T3 and the effect of chronic T3 therapy remain obscure (Wassner, Jugo et al. 2017). Moreover, as in NTIS one of the major alterations is in the peripheral conversion of T4 to T3 and not the availability of T4 itself, the oral administration of T4 might not be of benefit as a treatment option.

5. FINAL REMARKS

The mechanisms behind NTIS are complex and encompass all stages of hormonal production and metabolism. So far, clinical studies have shown that the hormonal changes as seen in NTIS are common to different illness and associated with worse prognosis. In the acute phase of critical disease, TH changes were once thought as adaptive, as it could reduce energy expenditure and protein intake. However, the increase in morbidity and mortality associated with NTIS and the persistence of TH alterations in the chronic phase of disease, in which catabolism is deleterious, has stimulated further studies and development of treatment. Whether these changes are a mere marker of poor health or a part of the pathophysiological circle that sustains systemic derangement, thus leading to an increased morbidity and mortality is still a matter of debate. Given the available evidence, and probably due to several lacks in the understanding of the pathophysiological mechanisms of NTIS, there is no currently approved treatment for this set of TH derangements.

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ARTIGO III

Oxidative stress induces type 3 deiodinase in multiple tissues after myocardial infarction: implications to T3 Low Syndrome pathophysiology

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ABSTRACT

Induction of type 3 deiodinase (D3) alters the metabolism of thyroid hormones in the low T3 syndrome. Imbalances in the redox status, existent in disease are involved in the change of deiodinases function. N-acetylcysteine (NAC), a potent antioxidant, corrects the oxidative damage and alterations seen in deiodinases in cell culture model. However, the effect of oxidative stress on D3 expression and activity in other tissues than the initially affected are unknown. Objective: Evaluate the oxidative stress and the D3 function and activity in liver, muscle and brain in an animal model of myocardial infarction (MI) and low T3 syndrome. Methods: Male Wistar rats submitted to left anterior coronary artery occlusion (MI) received NAC (10mg/kg, n=20) or placebo (NaCl; n=20). After 10 or 28 days post-MI the animals were sacrificed and tissues collected. The total carbonyl content and GSH were used as a parameter of intracellular redox balance. D3 expression and activity were measured. Results: As predicted, the formation of carbonyls, a marker of oxidative damage to proteins, was increased in heart ($P=0.0001$). Interestingly, liver, muscle and brain (all $P<0.001$) also showed increase in carbonyl levels in the placebo but not in NAC group. GSH levels were diminished and GSSG levels augmented in all tissues of the placebo group ($P<0.001$) while maintained in the NAC-treated animals. DIO3 expression and activity were increased in the placebo group in liver ($P=0.002$), muscle ($P=0.03$) and brain ($P=0.01$) as compared to the NAC group. Conclusion: Oxidative stress occurs after myocardial infarction and results in systemic induction of D3, which is related to the low T3 levels in disease. Antioxidant treatment prevents thyroid hormone derangement and corrects D3 activity to levels observed in controls.

Key-words: thyroid hormone, non-thyroidal illness syndrome, N-acetylcysteine, oxidative stress.

INTRODUCTION

The low T3 syndrome, also known as non-thyroidal illness syndrome (NTIS), is a clinical condition characterized by altered levels of thyroid hormones in critically ill patients (Larsen and Baxter, 2008). With worsening of the clinical condition, we can first observe a decrease in serum triiodothyronine (T3) levels, accompanied by increased reverse T3 (rT3), and subsequently a decrease in plasma thyroxine (T4), without elevated levels of thyroid stimulating hormone (TSH) (Warner and Beckett 2010). It is not yet known if these changes represent an attempt to protect metabolism in a state of disease or if it is a consequence of the disease process. However, the alterations in thyroid hormone levels are directly related to the prognosis of the patients (Iervasi, Pingitore et al. 2003, Wang, Pan et al. 2012, Ozcan, Osmonov et al. 2014). The pathophysiology of NTIS is characterized by profound changes in the peripheral conversion of T4 to T3. This process occurs by the action of a group of enzymes, the deiodinases types 1, 2 and 3 (D1, D2 and D3, respectively). D1 and D2 convert T4 to T3, while D3 exclusively inactivates both T4 and T3. These enzymes regulate the homeostasis of thyroid hormones (Maia, Goemann et al. 2011). All three deiodinases require an as-yet undefined cofactor, probably a thiol, which acts as a reducing agent releasing iodine from the selenocysteine residue and regenerating the active enzyme.

The low T3 syndrome is commonly observed in patients with myocardial infarction (MI). Although the pathophysiological process is yet unknown, one of the mechanisms involves the increase in the production of reactive oxygen species (ROS). Changes in the intracellular redox state can decrease deiodinase function by independent mechanisms. The decrease in intracellular glutathione (GSH) levels leads to redox imbalance. Oxidative stress compromises deiodinases function, altering the metabolism of thyroid hormones (Wajner, Goemann et al. 2011, Wajner, Rohenkohl et al. 2015). Upon oxidation, the cysteine residues within proteins can be modified to different products, including reversible inter- or intra-molecular disulfide bonds and GSH-mixed disulfides. This might be particularly relevant for deiodinase function (Kuiper, Klootwijk et al. 2002, Lu and Holmgren 2009).

Indeed, a recent randomized clinical study performed in patients with MI, showed that oxidative stress alters the thyroid hormone metabolism and that N-acetylcysteine

(NAC), a potent antioxidant that increases intracellular cysteine and GSH levels avoids thyroid hormone derangement. It was demonstrated that NAC administration in this scenario prevented the derangement in thyroid hormone concentrations commonly occurring in the acute phase of MI, indicating that oxidative stress is involved in the NTIS pathophysiology in humans (Vidart, Wajner et al. 2014).

The effect of NAC in type 3 deiodinase in the heart tissue was then evaluated in an animal model of low T3 syndrome and MI (Lehnen, Santos et al. 2017). The redox imbalance after MI altered D3 function, resulting in diminished T3 levels and both T3 and D3 function were corrected with the administration of NAC. Moreover, significant improvement in cardiac performance was shown (Lehnen, Santos et al. 2017). These results demonstrated that restoring redox balance by NAC treatment prevents low T3 syndrome-related thyroid hormone derangement and preserves heart function in rats subjected to MI. However, one can easily question whether only the heart tissue alterations on D3 could result in a systemic cascade of thyroid hormone modifications. Thus, in this study, we aimed to evaluate the effect of oxidative stress and D3 function in the liver, skeletal muscle and brain in an animal model of MI and NTIS.

MATERIAL AND METHODS

Reagents

Reagents were obtained from Invitrogen (Life Technologies Inc., NY, USA), Calbiochem-Novabiochem (St. Louis, MO, USA), or Sigma-Aldrich (St. Louis, MO, USA).

Animals and procedures

Male Wistar rats (weighing \pm 250g) were used in the experiments. All procedures and experiments involving animals were performed in accordance with the recommendations of the Brazilian College of Animal Experimentation (COBEA). Our study complied with the ethical principles of the Guide for the Care and Use of Laboratory Animals (NIH 1996) and the international standards for animal research of the Guidelines for Reporting Animal Research (Kilkenny, Browne et al. 2010). This study was

approved by the Research Ethics Committee of our institution (Protocol #130501). All animals received standard food and water *ad libitum* and were kept in cages under 12-hour light/dark cycles.

On the day of MI induction, the animals underwent basal echocardiography and blood sample collection (retro-orbital plexus) before being subjected to anterior descending artery ligation, as previously described (Pfeffer, Pfeffer et al. 1979, Lehnen, Lehnen et al. 2014), resulting in infarction of the left ventricle (LV) free wall. Briefly, the rats were anesthetized intraperitoneally (1 mL/kg of ketamine and 0.5 mL/kg of xylazine), intubated and connected to a mechanical ventilator with ambient air at a volume of 2.5 mL and ventilatory rate of 65 breaths per minute using a pressure fan for rodents (Harvard Apparatus, model 683, Massachusetts, USA). Anesthesia was administered through a vaporizer (Surgivet, Saint Louis, MN, USA) containing isoflurane (1 mL/mL, Isoforine, Cristália, Itapira, SP, Brazil). After MI or Sham procedure, it was administered Tramadol (20 mg/kg, every 6h for 48 h, Teuto, GO, Brazil) and animals were kept under medical oxygen at temperatures of 25-27°C for 6 hours. Twenty four hours after surgery, echocardiography was performed to measure cardiac parameters and to determine the MI extension. Blood was collected for troponin measurements to confirm successful MI induction. The animals were then divided into two groups (10 or 28 days of follow-up) and randomly assigned to receive either intraperitoneal NAC (10 mg/kg, 12/12 h for 48 h n=20) or the same volume of vehicle (saline, n=20; placebo group). Serial cardiac functional assessments and measurements of serum TSH, T4, T3 and rT3 levels were performed on the day of the procedure and at 10 or 28 days after surgery. Previous publications showed that neither T3 levels nor echocardiographic parameters changed after 28 days post-MI (Olivares, Marassi et al. 2007, Schenkel, Tavares et al. 2012).

At the conclusion of each experimental period (10 or 28 days), the animals were euthanized (anesthetized with vaporizer (Surgivet, Saint Louis, MN, USA) and tissues were rapidly removed and frozen in liquid nitrogen before being stored for subsequent analyses.

Carbonyl measurements

Duplicate aliquots of 0.3 mg of protein homogenate of each tissue were incubated with 500 μ L of 10 mM 2,4-dinitrophenylhydrazine or 1.0 mL of 2 M HCl (blank tube). After

30 min, 250 μ L of 50% trichloroacetic acid was added to the aliquots. The samples were subsequently centrifuged at 8000 g for 30 min to obtain the protein pellets, which were immediately washed with ethanol-ethyl acetate at a 1:1 (v/v) ratio. The final protein pellets were resuspended in 500 μ L of 8 M urea buffer and incubated at 50°C for 90 min. The difference between the 2,4-dinitrophenylhydrazine-treated and HCl-treated samples (blank) was used to calculate the carbonyl content determined at 370 nm. Carbonyl content was calculated using the millimolar absorption coefficient of hydrazine ($\epsilon_{370\text{ nm}}=21,000,000\text{ M}^{-1}\text{ cm}^{-1}$), and the results were expressed in nmol carbonyl/mg protein (Zanatta, Viegas et al. 2013).

Non-enzymatic antioxidant defenses

Reduced glutathione (GSH) levels were measured according to a standard method. Briefly, tissues were homogenized in the presence of 300 μ L of 20 mM sodium phosphate and 140 mM KCl buffer, pH 7.4. Proteins were precipitated by adding sodium metaphosphoric acid for a final ratio of 1:1. Samples were centrifuged for 10 min at 7,000 g . Fifteen microliters of tissue preparation was incubated with an equal volume of ophthalaldehyde (1 mg/mL methanol) at room temperature for 15 min in the presence of 20 volumes (1:20, v/v) of 100 mM sodium phosphate buffer, pH 8.0, containing 5 mM EDTA. Fluorescence was measured using excitation and emission wavelengths of 350 nm and 420 nm, respectively. A calibration curve was generated using standard GSH (0.001–0.1 mM), and GSH concentrations were calculated as nmol/mg protein. Total glutathione (tGS) and oxidized glutathione (GSSG) levels were determined using the enzymatic recycling method described elsewhere (Teare, Punched et al. 1993), with some modifications. Briefly, tissues were homogenized in 4 (w/v) volumes of a sulfosalicylic acid solution (11%) and Triton X-100 (0.11%) (1:1 ratio). After incubating for 5 min at 4°C with continuous shaking, the samples were centrifuged at 10,000 g for 10 min (4°C), and the supernatant was collected for analyses of glutathione levels. For GSSG measurement, 10 μ L of the supernatant was added to 110 μ L of a GSH masking buffer (100 mM phosphate buffer, 1 mM EDTA, 1.1% 2-vinylpyridine), pH 7.4, and incubated for 1 h at room temperature. The samples prepared for tGS and GSSG measurement were subjected to enzymatic analysis in a recycling buffer system containing 300 μ M NADPH, 225 μ M DTNB, 1.6 U/mL GR and 1.0 mM EDTA in 100 mM phosphate buffer (pH 7.4). The linear increase in absorbance at 405 nm over time was monitored using a microplate reader

(SpectraMax M5, Molecular Devices, California, US). A standard curve was generated using known amounts of GSH (100 μ M) and GSSG (3.47, 6.95, 13.89 μ M).

D3 activity assay

D3 activity in tissue samples was determined using paper chromatography as previously described (Huang, Mulcahey et al. 2005). Tissues were individually homogenized and sonicated with 10mM Tris-HCl, 0.25 sucrose buffer (pH 7.5), and 10mM dithiothreitol (DTT). Protein concentration was quantified by Bradford assay using BSA as a standard. The homogenates were incubated for an hour with 200,000 cpm ¹²⁵I-labeled T3, 2 nM T3, 20mM DTT, and 1mM propylthiouracil (PTU) in order to inhibit any D1 activity. The addition of 200nM of T3 completely abolished D3 activity in all samples. The reaction was stopped by adding 200 μ L ethanol 95%, 50 μ L NaOH (0.04 N), and 5mg PTU. Deiodination was determined based on the amount of ¹²⁵I-3, 3'-T2 produced after separation of reaction products by paper chromatography. Results were expressed as the fraction of T2 counts minus the nonspecific deiodination (always < 1.5%), obtained with the saturating concentration of T3 (200 nM). D3 activity was expressed as femtomoles T3 per minute per milligram protein. The quantity of protein assayed was adjusted to ensure that < 30% of the substrate was consumed. All experiments were performed in triplicate in at least two independent experiments.

Real Time PCR

Total RNA was extracted from tissues with an RNeasy kit (Qiagen) and used to synthesize cDNA (SuperScript First-Strand Synthesis System for RT-PCR; Invitrogen). The generated cDNAs were used in a real-time PCR with a SYBR Green PCR Master Mix (Applied Biosystems) in ABI Prism 7500 Sequence Detection System (Applied Biosystems). Standard curves representing 5-point serial dilution of cDNA of the experimental and control groups were analyzed and used as calibrators of the relative quantification of product generated in the exponential phase of the amplification curve. The r^2 was greater than 0.99, and the amplification efficiency varied between 80% and 100%. Samples were measured by relative quantification (change in expression in the experimental group versus control; untreated versus treated cells). The data generated by

ABI Prism 7500 system SDS software (Applied Bio systems) were then transferred to an Excel spreadsheet (Microsoft), and the experimental values were corrected to that of the cyclophilin A standard. The primers used for D3 were (5'-TCCAGAGCCAGCACATCCT-3' and 5'-ACGTCGCGCTGGTACTTAGTG-3'), with the internal controller cyclophilin A (5'-GTCAACCCACCGTGTTCTTC-3' and 5'-ACTTGCCACCAGTGCCATTATG-3').

Western Blotting

Tissues were collected and prepared by homogenization and lysis at 4°C. After centrifugation at 13,000 ×g for 15 min at 4°C, the total protein concentration was determined using the BCA assay. Protein extracts (30–50 mg protein of each sample) were fractionated by 8–12% SDS–PAGE and blotted onto an Immobilon PVDF membrane (Millipore, Billerica, MA, USA). Non-specific binding sites were blocked by incubation with 5% nonfat dry milk in Tris-buffered saline 0.1% Tween-20. The following primary antibodies were used: anti-D3 (1:400; Novus Biologicals), anti- α -tubulin B7 (1:500; Santa Cruz Biotechnology) and anti- β -actin (1:10 000; Sigma–Aldrich). The antigen–antibody complexes were visualized using HRP-conjugated secondary antibody and an enhanced chemiluminescence system (GE Healthcare, Pittsburgh, PA, USA). Expression was quantified using image densitometry with ImageJ analysis software.

Statistical analysis

Unless otherwise specified, the results are presented as the mean \pm standard deviation. Data were analyzed using 2-tailed Student's t tests or one-way analysis of variance (ANOVA) followed by post hoc Duncan multiple range tests when F was significant. Prism 6.0 software was used for statistical analyses and $P < 0.05$ was considered significant.

RESULTS

Plasma T3 levels and low T3 syndrome

Compared to baseline, serum T3 levels were decreased in MI-placebo animals at 10 (108 to 88.4 ng/dL, $P<0.01$) and 28 days (108.5 to 91.6 ng/dL, $P<0.03$) as previously shown (Lehnen, Santos et al. 2017). In contrast, no significant decreases in T3 levels were noted in MI-NAC animals at 10 or 28 days after MI.

NAC administration restores nonenzymatic antioxidant compounds and total carbonyl content in peripheral tissues

We next determined the concentrations of the non-enzymatic antioxidant GSH in all tissues. GSH content was diminished in the liver (37.2 to 32.3 nmol/mg prot, $P<0.001$, Figure 1A), muscle (30.8 to 25.4 nmol/mg prot, $P<0.001$, Figure 1B) and brain (30.8 to 25.8 nmol/mg prot, $P<0.001$, Figure 1C) tissue samples of MI-placebo animals at 10 days after MI. At 28 days no difference in GSH content was observed in all tissues (Figure 1A, 1B and 1C). The GSH/GSSG ratio was decreased in all tissue samples ($P<0.001$, Figure 1A, 1B and 1C) at 10 days in placebo group, but not in 28 days.

The tissue carbonyl content, a parameter of protein oxidation, was increased in the placebo group in 10 days in the liver (0.12 to 0.9 nmol/mg prot, $P<0.001$, Figure 2A), muscle (0.08 to 2.9 nmol/mg prot, $P<0.001$, Figure 2B) and brain (0.03 to 0.89 nmol/mg prot, $P<0.001$, Figure 2C). At 28 days, carbonyl content was still increased only in the liver (0.12 to 0.89 nmol/mg prot, $P<0.001$, Figure 2A) and in the brain (0.03 to 0.46 nmol/mg prot $P<0.001$, Figure 2C), but not in muscle (0.08 to 0.13 nmol/mg prot, $P>0.05$, Figure 2B) in the placebo group. In the NAC group, the carbonyl content was not different from controls in 10 or 28 days (Figure 2A, 2B and 2C).

D3 expression and activity in peripheral tissues is affected by NAC

D3 expression was increased in the Placebo group at 10 days post-MI in liver (~4.2, $P<0.001$, Figure 3A), muscle (~11 fold, $P<0.001$, Figure 3B) and brain (~3 fold, $P<0.001$, Figure 3C). At 28 days, D3 expression in the Placebo group was increased in liver (~2.5 fold, $P<0.001$, Figure 3A) and brain (~2 fold, $P<0.001$, Figure 3C), but not in the muscle ($P>0.05$, Figure 3C). We observed that NAC treatment prevented the increase of D3 expression at 10 and 28 days post MI in all tissues (Figure 3A, 3B and 3C). Accordingly, D3 activity levels were augmented at 10 days post-MI in all tissue samples of placebo

group when compared to sham (liver 1 to 4.7 fmol/ mg prot/min; muscle 1.8 to 8 fmol/ mg prot/min and brain 1.6 to 5.2 fmol/ mg prot/min, all $P < 0.001$, Figures 4A, 4B and 4C). At 28 days, D3 activity levels were not significantly increased ($P > 0.05$, Figures 4A, 4B and 4C). NAC treatment prevented the increase of D3 activity at 10 and 28 days after MI in all tissues ($P > 0.05$, Figures 4A, 4B and 4C). We next sought to determine the protein content of the samples at day 10 and observed that it was augmented in the placebo group in all tissues but not in the NAC treated animals (Figures 5A, 5B and 5C).

DISCUSSION

The present study aimed to investigate whether oxidative stress induced alterations in D3 in peripheral tissues in rats submitted to MI. The observed oxidative stress secondary to MI, here determined as augmented carbonyl and diminished GSH content decreased serum T3 levels and resulted in induced D3 function in all peripheral tissues. Treatment with NAC prevented redox imbalance, preserved T3 levels, and corrected D3 expression and activity. Taken together, these findings indicate that altered D3 function is observed systemically in oxidative stress- induced low T3 syndrome related to MI.

The pathophysiology of NTIS is characterized by several changes in the metabolism of thyroid hormones. Generally, NTIS is observed in patients after MI and the low serum T3 levels are inversely correlated with the mortality of these patients (Iervasi, Pingitore et al. 2003). Clinical and experimental studies showed increased inactivation of thyroid hormone by robust stimulation of D3 playing a significant role in the low T3 syndrome (Peeters, Wouters et al. 2003, Peeters, Wouters et al. 2005, Rodriguez-Perez, Palos-Paz et al. 2008). Previous studies also demonstrated that the correction of oxidative stress induced in disease prevented the low T3 syndrome, indicating that changes in redox balance, directly affect the peripheral metabolism of the thyroid hormones (Wajner, Goemann et al. 2011, Vidart, Wajner et al. 2014). NAC, a potent antioxidant that restores intracellular reduced glutathione (GSH) levels, prevents the deleterious effects of altered intracellular redox state on thyroid hormone metabolism.

The function of deiodinases is affected by changes in the redox balance (Wajner and Maia 2012). Type 3 deiodinase is a selenocysteines oxireductases enzyme that catalyzes the removal of iodine from the inner rings of thyroid hormones, inactivating them (Maia, Goemann et al. 2011). Although the effect of NAC on oxidative damage and D3

expression and activity is not yet totally understood, it is probably associated with the maintenance of intracellular GSH levels and with the correction of redox imbalance. NAC is the major cysteine donator, not only to GSH but also to several thiol enzymes which might be critical to proteins dependent on thiols (Peeters, Wouters et al. 2003). Moreover, GSH is known to have its antioxidant effect potentiated since small increases in GSH concentration result in large antioxidant effects (Lamirand, Pallud-Mothre et al. 2008, Wajner, Goemann et al. 2011, Wajner, Rohenkohl et al. 2015). All these mechanisms can be of importance to D3 function maintenance.

Importantly, studies with ill patients were mainly designed with septic patients or have analyzed only the primary affected organ. It was not demonstrated, until now that oxidative stress occurred in the course of MI was able to alter D3 function in tissues primarily unaffected by the disease, thus leading to low T3 syndrome. Additionally, the administration of NAC protects proteins from oxidative damage as well as corrects the impaired total body antioxidant capacity. These results further confirms that the T3 decrease is mainly due augmented thyroid hormone inactivation due to oxidative stress and that type 3 deiodinase might be the most important enzyme in the low T3 syndrome context.

In conclusion, our findings demonstrate that oxidative stress occurs after MI and results in systemic induction of D3. The improvement of MI-related redox imbalance by NAC prevents changes in thyroid hormone metabolism by preventing the increase in D3 expression and activity systemically. These results suggest that type 3 deiodinase might be the main altered enzyme that orchestrates the low T3 syndrome.

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FIGURES

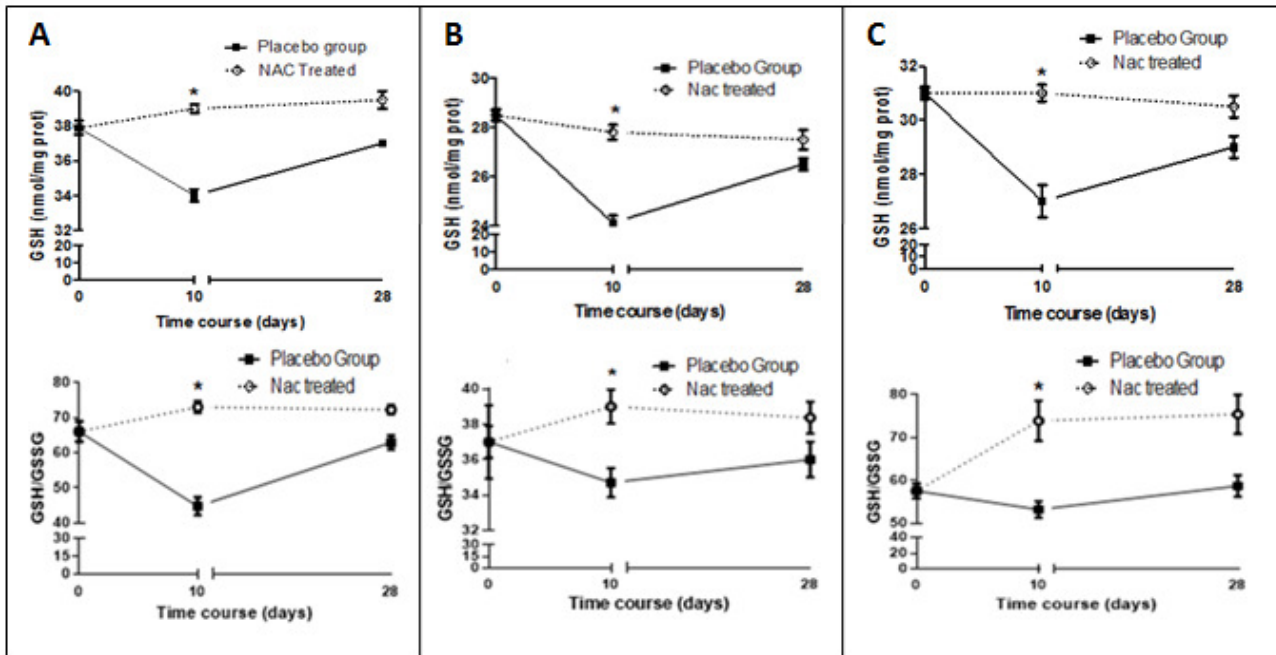


Figure 1. Changes in GSH levels and GSH/GSSG ratio in the liver (A), muscle (B) and brain (C) in rats subjected to myocardial infarction that received NAC or placebo. GSH content was diminished in the liver, muscle and brain tissue samples of MI-placebo animals, but not MI-NAC animals (Figures 1A, 1B and 1C) at 10 days after AMI. At 28 days we observed that the GSH content returned to initial levels. The GSH/GSSG ratio was decreased in both tissue samples (Figures 1A, 1B and 1C) at 10 days post-AMI, indicating augmented oxidation. All data with $P < 0.001$. * $P < 0.001$, vs Placebo group, $n = 20$ in each group.

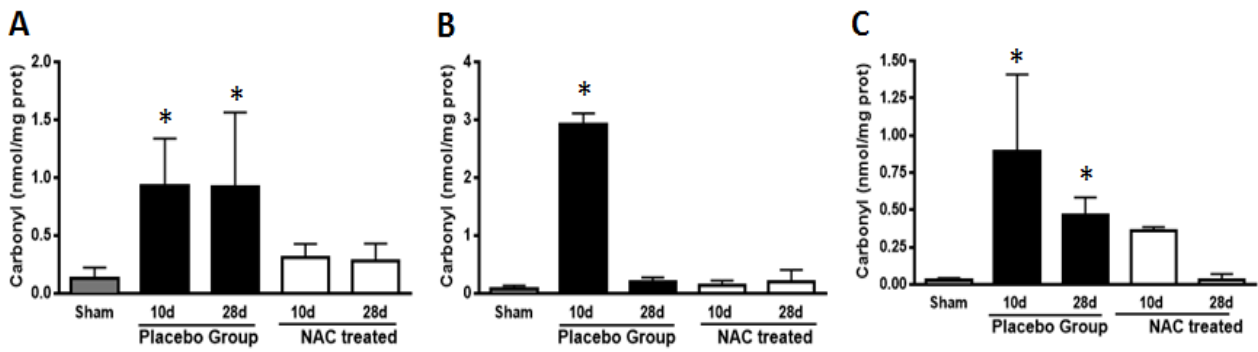


Figure 2. Oxidative stress biomarkers in rats subjected to myocardial infarction that received NAC or placebo. The carbonyl content was increased in the placebo group in 10 and 28 days in the liver (Figure 2A), muscle (Figure 2B) and brain (Figure 2C). At 28 days, carbonyl content increased only in the liver (Figure 2A) and in the brain (Figure 2C) in the placebo group. In the NAC group, the carbonyl content was decreased by 10 and 28 days (Figures 2A, 2B and 2C). All data with $P < 0.001$. * $P < 0.001$, vs Sham, $n = 20$ in each group.

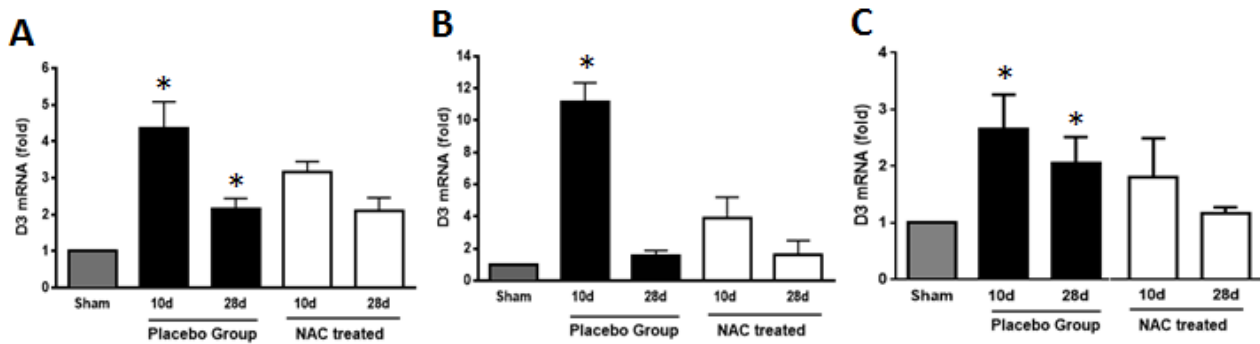


Figure 3. D3 mRNA in rats subjected to myocardial infarction that received NAC or placebo. D3 expression was increased in the Placebo group at 10 days post-MI in liver (Figure 3A), muscle (Figure 3B) and brain (Figure 3C). At 28 days, D3 expression in the Placebo group was increased just in liver (Figure 3A) and brain (Figure 3C). In muscle, this difference did not exist anymore at 28 days (Figure 3C). NAC treatment with NAC prevented the increase of D3 expression at 10 and 28 days post-MI in all tissues (Figures 3A, 3B and 3C). All data with $P < 0.001$. * $P < 0.001$, vs Sham, $n = 20$ in each group.

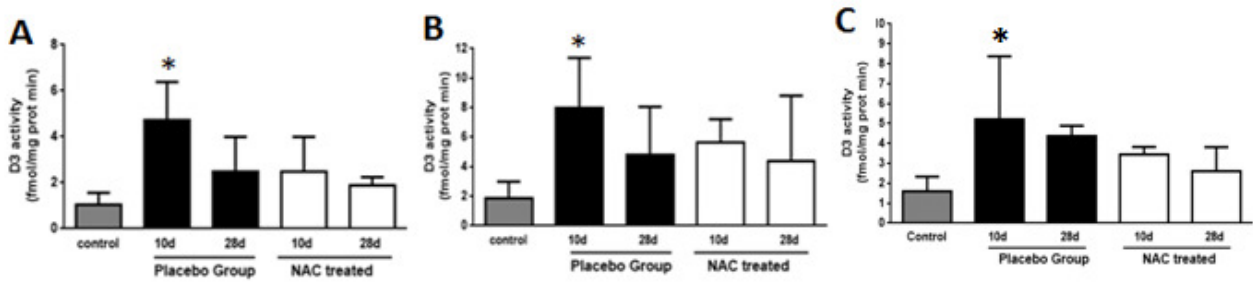


Figure 4. D3 activity and Western Blotting in rats subjected to myocardial infarction that received NAC or placebo. D3 activity levels was augmented at 10 days post-MI in all tissue in placebo group (Figures 4A, 4B and 4C). At 28 days, D3 activity levels were not significantly increased (Figures 4A, 4B and 4C). NAC treatment prevented the increase of D3 activity at 10 and 28 days after MI in all tissues (Figures 4A, 4B and 4C).

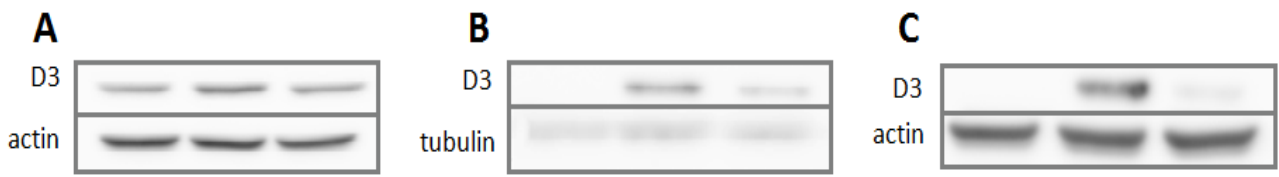


Figure 5. Illustrative western blot images demonstrating that the amount of protein was increased at 10 days in Placebo group in liver, muscle and brain (Figures 5A, 5B and 5C).

CONCLUSÃO GERAL

Concluimos que o estresse oxidativo induzido pelo infarto do miocárdio resulta em um aumento local e sistêmico da desidase tipo D3, contribuindo para a diminuição dos níveis circulantes de T3. A NAC, pela sua natureza antioxidante, restaura o equilíbrio redox e preserva a função cardíaca, evitando a síndrome do T3 baixo. Ainda, o tratamento com NAC previne a disfunção observada da desidase tipo D3 em vários tecidos (além do cardíaco), prevenindo a síndrome do T3 baixo. A correção do desequilíbrio redox pela NAC está associada à prevenção de anormalidades sistêmicas no metabolismo dos hormônios tireoidianos em ratos submetidos ao infarto do miocárdio.