UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE FARMÁCIA

DISCIPLINA DE TRABALHO DE CONCLUSÃO DE CURSO DE FARMÁCIA

OXIDATIVE STRESS IN HOMOCYSTINURIA: FINDINGS IN PATIENTS AND IN ANIMAL MODELS

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OXIDATIVE STRESS IN HOMOCYSTINURIA: FINDINGS IN PATIENTS AND IN ANIMAL MODELS

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Abstract

Homocystinuria is an inborn error of amino acid metabolism caused by deficiency of cystathionine ß-synthase (CBS) activity, leading to the accumulation of homocysteine (Hcy) and methionine (Met) in biological fluids and high urinary excretion of homocystine (Hci). This accumulation leads to a variety of clinical manifestations, in different organs and tissues, as thinning and lengthening of the long bones, osteoporosis, dislocation of the ocular lens, thromboembolism and mental retardation, but the pathophysiology of this disease is not completely understood. In this context, this review addresses some findings obtained from patients and animal studies indicating that oxidative stress plays an important role in the pathophysiology of homocystinuria. Several studies have shown that enzymatic and non-enzymatic antioxidant defenses are decreased, as well as markers of lipid, protein, and DNA oxidative damage have been reported increased in blood, brain, liver and muscle in the animal models studied, as well as in homocystinuric patients, which may be due to an increased free radical generation or secondary to the deprivation of micronutrients which are essential for these defenses. A considerable set of data from *in vitro* and *in vivo* animal studies have shown that Hcy induces reactive species formation in brain rodent. Considering these findings, it is well established that oxidative stress may contribute to the damage found in homocystinuric patients. This review offers new perspectives for the treatment in homocystinuria, which may include the use of appropriate antioxidants as a novel adjuvant therapy for the patients.

Short title

Oxidative stress in homocystinuria

Keywords

Homocystinuria, Oxidative stress, Homocysteine, Antioxidants, Homocystinuric patients, Animal models

Abbreviations

4-HNE, 4-hydroxyalkenal; AChE, Acetylcholinesterase; ALT, Alanine aminotransferase; ApoA-1, Apolipoprotein A1; AST, Aspartate aminotransferase; BuChE, Butyrylcholinesterase; CAT, Catalase; CBS, Cystathionine ß-synthase; DCF, 2'7'dichlorofluorescein; EC-SOD, Extracellular superoxide dismutase; G6PD, Glucose 6-phosphate dehydrogenase; GPx, Glutathione peroxidase; GSH, Glutathione; Hci, Homocystine; Hcy, Homocysteine; Hhcy, Hyperhomocysteinemia; IEM, inborn errors of metabolism; IFN-γ, Interferon-γ; IL-1β, Interleukin-1β; IL-6, Interleukin-6; MDA, Malondialdehyde; Met, Methionine; MTHFR, Methylenetetrahydrofolate reductase; PON1, Paraoxonase; SOD, Superoxide dismutase; TAR, Total antioxidant reactivity; TAS, Total antioxidant status; TBARS, Thiobarbituric acid reactive substances; TRAP, Total radical-trapping antioxidant potential; TUNEL, Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick end-labeling; Vitamin B6, Pyridoxine.

1. Introduction

Homocystinuria is an autosomal recessive disorder of amino acid metabolism caused in most cases by deficiency of cystathionine ß-synthase (CBS), the enzyme that catalyzes the transsulfuration of homocysteine (Hcy) to cystathionine. CBS is a pyridoxine (vitamin B6) dependent enzyme. Untreated patients have accumulation of Hcy and methionine (Met) in biological fluids and high urinary excretion of homocystine (Hci). Not only deficient activity of CBS, but other genetic defects may lead to increased of Hcy in the plasma, such as mutations that inactivate the enzymes methionine synthase and methylenetetrahydrofolate reductase (MTHFR) [1]. Mudd et al [1] found estimates of the frequency of homocystinuria ranging from 1 in 58,000 to 1 in 1,000,000 in countries that systematically screen newborns.

In CBS deficiency, there is a variety of clinical manifestations in different organs and tissues, as thinning and lengthening of the long bones, osteoporosis, dislocation of the ocular lens, thromboembolism and mental retardation [1]. There are two different clinical forms of CBS deficiency: pyridoxine-responsive and pyridoxine-non-responsive [2]. Treatment for B6-responsive patients consists in the use the cofactor of CBS, vitamin B6, at a dose of approximately 200 mg/day, and also protein-restricted diet. B6-non-responsive patients require restriction of Met intake and cystine supplementation [3]. An alternate therapy can be the use of betaine, 6 to 9 g/day [4]. Moreover, folate and vitamin B12 are also used, 5 mg/day for folic acid and 1 mg intramuscular per month for vitamin B12 in the form of hydroxocobalamin [3].

Mudd et al [2] considered that is more likely that the pathogenesis of homocystinuria is related to the Hcy excess or cysteine deficiency than to the accumulation of Met. Although the pathogenesis of this disease is not completely understood, it has been suggested that oxidative stress plays an important role in the pathophysiology in this disorder [5-11]. Besides, studies done in animal models have demonstrated an association between Hcy and oxidative stress [12-17].

Oxidative stress is defined as an imbalance between the production of reactive species and the decrease in the antioxidant defenses, in favor of the former, leading to cell damage. Increasing evidence has shown that damage caused by free radicals and reactive species are an important contributing factor in chronic-inflammatory, vascular, neoplasic and neurodegenerative diseases [18]. Regarding to important role of oxidative stress in neurodegenerative diseases, various findings have shown that oxidative stress participates in the pathophysiology of some inborn errors of metabolism (IEM) such as aminoacidopathies and organic acidemia [7,19]. Although the relation between oxidative stress and IEM pathophysiology is not well elucidated, the accumulation of toxic metabolites is appointed as the main reason for the increase of free radicals [19].

Therefore, in the present work we aim to review the parameters of oxidative stress in homocystinuric patients by CBS deficiency and the contribution from animal models studies for the understanding of oxidative damage in this disease.

2. Oxidative stress in homocystinuric patients

Oxidative damage to proteins, lipids and DNA, as well as antioxidant defenses and inflammatory profile have been investigated in homocystinuric patients (Table 1). It was verified by Vanzin and coworkers [9,10] that carbonyl content is increased in plasma of homocystinuric patients at diagnosis and partially reduced under treatment when compared with healthy individuals, indicating that there is protein oxidative

damage and that treatment partially protect against protein oxidation. Patient's treatment was based on protein restriction with supplementation of pyridoxine, folate, betaine and vitamin B12. It was determined by these authors the lipid peroxidation index on the basis of malondialdehyde (MDA) levels and it was observed that plasma MDA levels in patients at diagnosis were significantly higher when compared to patients under treatment and healthy individuals, implying that patients treated presented a significant decrease in MDA levels when compared with patients at diagnosis. The data indicated that treatment prevented the lipid damage found in homocystinuric patients at diagnosis, but did not reach the levels found in healthy individuals [9]. Furthermore, sulfhydryl content was significantly lower in homocystinuric patients at diagnosis and in patients under treatment when compared to healthy individuals [9,10]. The total antioxidant status (TAS), reflecting the quantity of tissues antioxidants, was significantly lower in patients at diagnosis and during dietary treatment when compared to healthy people, suggesting that treatment was not able to prevent the decrease in the antioxidant defenses found at diagnosis [9]. In the same study, it was examined plasma Hcy and Met levels: treated patients presented a significant decrease of both amino acids (Hcy: 140.3 ± 99.3 μmol/L; Met: 2.0 ± 0.6 μmol/L) when compared with patients at diagnosis (Hcy: 266.5 ± 66.7 μmol/L; Met: 2.6 ± 0.3 μmol/L), but still remained above the normal range (reference values: Hcy: 5 - 15 μmol/L; Met: 7 - 47 μmol/L). In order to investigate whether Hcy and Met levels were associated to oxidative stress, the parameters investigated were correlated with these two amino acids levels and it was verified a significant negative correlation between sulfhydryl group content and Hcy levels and a significant positive correlation between MDA levels and Hcy levels, suggesting a potential mechanistic role for Hcy in the oxidative damage. Regarding to Met, no correlation was found between this amino acid and the oxidative stress parameters, which reinforces the assumption that Met and its derivatives make little contribution to the oxidative manage of CBS deficiency [9,10].

The lipid [total cholesterol, HDL cholesterol, LDL cholesterol, oxidized LDL cholesterol, apolipoprotein A1 (ApoA-1)] and inflammatory [Interleukin-6 (IL-6), Interleukin-1β (IL-1β), Interferon-γ (IFN-γ)] profiles and the activities of enzymes paraoxonase (PON1) and butyrylcholinesterase (BuChE) in plasma of CBS-deficient patients at diagnosis and under treatment were also evaluated by Vanzin et al [10]. All those parameters measured were correlated with the Hcy, folic acid, and vitamin B12 concentrations. With regard to lipid profile, it was found a significant decrease in HDL and ApoA-1 levels in treated or not homocystinuric patients at diagnosis when compared to healthy individuals. LDL and oxidized LDL levels were statistically similar between patients and healthy individuals. Total cholesterol levels were significantly reduced in the CBS-deficient patients, probably due to the decrease in HDL levels. PON1 activity was decreased in homocystinuric patients at diagnosis and under treatment when compared with healthy people, differently from BuChE activity that was increased only in the untreated patients when compared the healthy individuals and patients under treatment. It was verified a significant positive correlations between PON1 activity and sulfhydryl groups content and between HDL and ApoA-1 levels. Besides, it was demonstrated that IL-6 was significantly higher in patients at diagnosis and there was a tendency to reduction in the IL-6 levels in patients under treatment when compared the healthy individuals. It was found a significant positive correlation between IL-6 levels and carbonyl groups content, indicating a possible association between inflammation and oxidative protein damage. Moreover, it was also evaluated the IL-1β and INF-γ levels which were statistically similar between CBS-deficient patients and healthy individuals. It was found a significant positive correlation between

vitamin B12 and ApoA-1 levels, as well as a significant positive correlation between vitamin B12 levels and PON1. These findings suggest that vitamin B12 could be essential to increase the ApoA-1 levels and PON1 activity in CBS-deficient patients, which is interesting since these components demonstrate important atheroprotective effects [20-22] that could, at least in part, decrease or revert the vascular alterations found in these patients. The vitamin B12 levels were similar in treated or not treated CBS-deficient patients, indicating a poor adherence to the treatment. Additionally, it was demonstrated a significant negative correlation between folic acid and total Hcy concentrations. Folic acid was increased in treated patients when compared with not treated patients [10].

Pullin and coworkers [23] verified what the vitamin C ameliorates endothelial dysfunction in patients with homocystinuria, independent of changes in Hcy concentration. They also concluded that vitamin C should therefore be considered as an additional adjunct to therapy to reduce the potential long-term risk of atherothrombotic disease.

F2-isoprostanes represent a family of bioactive prostaglandin F2–like compounds that are produced from arachidonic acid through a non-enzymatic process of lipid peroxidation. Among F2-isoprostanes, of particular interest is 8-isoprostaglandin F2α, which induces vasoconstriction and modulates the function of human platelets and can be increased in association with several cardiovascular risk factors. Davi and coworkers [24] verified a significant positive correlation between plasma Hcy levels and urinary 8-isoprostaglandin F2α in CBS-deficient patients. Urinary 8-isoprostaglandin F2α and 11-dehydrothromboxane B2 excretion was significantly higher in CBS-deficient patients when compared to healthy individuals and it was verified a significant positive correlation between these biomarkers. The vitamin E supplementation was associated with statistically significant reductions in urinary 8-isoprostaglandin F2α and 11-dehydrothromboxane B2 levels, but 2 weeks of vitamin E supplementation at 600 mg/day induced a reduction but failed to normalize enhanced lipid peroxidation. These preliminary observations suggest a potential role for antioxidant therapy in attenuating Hcy dependent oxidative changes that may promote atherothrombosis in this disease.

Another study of Vanzin and coworkers [11] showed that Hcy induces DNA damage *in vivo* and *in vitro* in a dose-dependent manner in white blood cells. It was verified that DNA damage was significantly higher in the CBS-deficient patients under treatment when compared the healthy individuals, suggesting that DNA damage in these patients can be correlated with the high plasma Hcy levels found, since the Hcy average level of treated patients included in this study was 166.5 ± 117.1 μmol/L (average ± standard deviation), remaining above of ideal levels.

An important component of the endogenous antioxidant defense opposing the deleterious vascular effects of free radicals is superoxide dismutase (SOD), present in the vascular wall. Among the existing SOD isoenzymes, more of 90% of interstitial SOD is extracellular superoxide dismutase (EC-SOD). It was demonstrated a significant positive correlation between EC-SOD and total Hcy in CBS-deficient patients, what could represent a protective antioxidant response to Hcy-induced oxidative damage and could contribute to reducing cardiovascular risk in homocystinuric patients. In contrast there were no correlations between EC-SOD and the Met concentrations [6].

Vilaseca-Buscà and coworkers [7] evaluated the antioxidant status in patients affected by inborn errors of metabolism (including CBS-deficient patients) through the measurement of erythrocyte antioxidant enzyme activities, as SOD. The SOD activity was significantly higher in the patients with protein-free diet when compared with the

patients on protein restricted diet or with healthy individuals, suggesting an induction of enzyme protein synthesis owing to an excess of free radical generation. The lower activities observed in patients on protein restriction may likely be due to a deficient bioavailability of antioxidant cofactors.

3. Oxidative stress in animal models of homocystinuria

Animal models are useful to better understand the pathophysiology of human diseases. In the study of Matté and coworkers [25] it was investigated the effect of chronic hyperhomocysteinemia (Hhcy) on some parameters of oxidative damage, catalase (CAT), SOD, glutathione peroxidase (GPx), total radical-trapping antioxidant potential (TRAP) and DNA damage in blood and parietal cortex of rats. In order to evaluate whether DNA damage was permanent after cell division, it was also investigated the effect of Hcy on the micronucleus test, and it was showed that Hhcy did not alter micronucleus frequency in blood of rats. It was also evaluated the effect of folic acid on biochemical alterations elicited by Hhcy. The results demonstrated that chronic Hcy administration increased DNA damage, evaluated by comet assay, and significantly reduced in TRAP, CAT and GPx activities in parietal cortex. SOD and CAT activities were increased, TRAP was decreased and GPx activity was not altered in blood of rats. It was also verified that folic acid concurrent administration per se did not alter the activities of CAT, SOD, GPx, TRAP and DNA damage but prevented the inhibition of these parameters caused by Hcy. The results allowed to propose that the supplementation with folic acid can be used as an adjuvant therapy in disorders that accumulate Hcy, possibly by its antioxidant and DNA stability maintenance properties.

Moreover, it was investigated the effect of chronic administration of Hcy on antioxidant enzymes CAT and SOD, on glutamate uptake and in the Na(+),K(+)-ATPase activity in hippocampus of rats. The results showed a significant reduction in these parameters. Besides, vitamin C alone did not alter these parameters, but when administered concomitantly with Hcy, it was able to prevent the inhibition caused by Hcy. It was also evaluated the levels of 2'7'dichlorofluorescein (DCF) that was increased in the hippocampus. Vitamin C, when administered concomitantly with Hcy, was able to prevent the damage caused by the amino acid [15].

Furthermore, it was evaluated the effect of acute Hcy administration in some parameters of oxidative stress, TRAP, CAT, SOD and GPx and on Na(+),K(+)-ATPase activity and the effect of chronic pretreatment with vitamins E and C in rat hippocampus. Results showed that Hcy significantly decreased TRAP, Na(+),K(+)-ATPase and CAT activities, without affecting the activities of SOD and GPx. The chronic pretreatment with vitamins E and C per se did not alter these parameters, but prevented the reduction of TRAP, Na(+),K(+)-ATPase and CAT activities caused by Hcy [12].

The effect of the acute administration of Hcy on memory in rats and the action of vitamins E and C on the effects produced by Hcy was investigated by Reis et al [26]. The memory was significantly impaired in Hcy-treated group, an effect probably mediated by oxidative stress, whereas the rats chronically treated with vitamins E and C had this effect prevented.

The *in vitro* effect of Hcy on some parameters of oxidative stress in the rat hippocampus were investigated by Streck et al [13]. The Hcy significantly increased thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation, and

decreased TRAP, but did not change *in vitro* the antioxidant enzymes SOD, CAT, and GPx in rat hippocampus.

Matté and coworkers [27] investigated the chronic Hcy administration on hepatic antioxidant status, TRAP and total antioxidant reactivity (TAR), suggesting a reduction on quantity and quality of non-enzymatic antioxidants in liver of rats. Lipid peroxidation was assessed by chemiluminescence and by TBARS and both were increased. Also, it was verified a reduction on total thiol content, such as sulfhydryl groups and antioxidant glutathione and a significant inhibition of hepatic CAT activity in rats. Histological analysis indicated the presence of inflammatory infiltrate, fibrosis and reduced content of glycogen/glycoprotein in liver tissue sections. The chronic Hcy administration did not altered alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in liver and in plasma of rats. The data showed a consistent profile of liver injury elicited by Hcy, which could contribute to explain, at least in part, the mechanisms involved in human liver diseases associated to Hhcy.

In another study, the histologic evaluation of liver CBS-deficient mice showed development inflammation, fibrosis and hepatic steatosis concomitant with an enhanced expression of tissue inhibitor of metalloproteinase-1, α-smooth muscle actin, pro(α)1 collagen type I, transforming growth factor-β1, and proinflammatory cytokines. The formation of carbonyl groups and the levels of MDA and 4-hydroxyalkenal (4-HNE) were increased in liver of mice, demonstrating an enhanced protein oxidation and lipid peroxidation. Moreover, the absence of caspase-3 activation, DNA fragmentation, and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate biotin nick end-labeling (TUNEL)-positive cells showed that protective signals may counteract apoptotic signals in liver of CBS-deficient mice [14].

The study of Kolling et al [17] investigated the effect of chronic Hhcy on some parameters of oxidative stress and the effect of creatine on biochemical alterations, but this time in skeletal muscle of rats. It was showed that chronic Hcy administration increased the DCF oxidation, an index of production of reactive species, and the TBARS levels, an index of lipid peroxidation. Antioxidant enzyme activities, SOD and CAT were also increased, but GPx activity was not altered. The content of glutathione (GSH), sulfhydryl and carbonyl were decreased, as well as nitrite levels. Moreover, the creatine concurrent administration prevented some Hcy effects probably by its antioxidant properties. Based on these findings, it was suggested that creatine may be used as adjunctive therapy for ameliorating the symptoms associated with oxidative insult that can be found in homocystinuric patients.

The effect of chronic Hhcy on some parameters of oxidative stress in the rat lung was evaluated by Cunha and coworkers [16]. The chronic Hhcy significantly increased TBARS and protein carbonyl content, suggesting that this amino acid causes lipid peroxidation and oxidative damage to protein. Also, the Hcy significantly increased the levels of reactive species in lung indicated by DCF fluorescence assay. Next, it was evaluated the enzymatic antioxidant defenses SOD, CAT and GPx, and it was observed that Hcy significantly increases SOD activity and reduced the CAT and GPx activities. Moreover, it was showed that chronic Hcy administration induced a decrease in the TRAP, suggesting that this amino acid cause a reduction in non-enzymatic antioxidants. It was also observed that Hcy significantly reduced the GSH content and glucose 6-phosphate dehydrogenase (G6PD) activity and not changed the nitrite levels. These findings showed a consistent profile of oxidative stress in the lung of rats elicited by Hcy.

Acetylcholine is a neurotransmitter hydrolyzed by two different types of cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE).

BuChE is present in all tissues, including serum, heart, vascular endothelia and nervous system [28]. Schulpis and coworkers [29] determined the *in vitro* effects of Hcy, Hci and Met and the mixture of these three amino acids simulating CBS-deficiency on AChE activity in hippocampus of rats. The preincubation with the amino acids Hcy or Met resulted in a significant activation of AChE, whereas the Hci inhibited the enzyme activity. The presence of SH-group in both the amino acids, Hcy and Met, could explain AChE activation. Furthermore, the *in vitro* CBS-deficiency resulted in a high activation AChE activity, since each one of the SH-group containing amino acids produced simultaneously significant enzyme stimulation.

In this context, in the study of Stefanello and coworkers [30] it was investigated the acute and chronic effect of Hcy administration on BuChE activity in serum of rats. The results showed that acute and chronic administration of Hcy significantly decreased BuChE activity. Furthermore, the rats were pretreated for one week with vitamins E and C. Per se, the vitamins not alter BuChE activity, but prevented the reduction of this enzyme activity caused by acute administration of Hcy, suggesting that the inhibitory effect of Hcy on BuChE activity is probably mediated by free radicals. Another study of Stefanello et al [31] investigated the *in vitro* effects of Hcy on platelet Na(+),K(+)-ATPase and serum BuChE activities of young rats. Results showed that Na(+),K(+)-ATPase and BuChE activities were significantly inhibited by Hcy. The inhibition of Na(+),K(+)-ATPase and BuChE activities might be one useful peripheral marker for the neurotoxic effects of Hcy.

Na(+),K(+)-ATPase is a fundamental enzyme responsible for maintaining the ionic gradient necessary for neuronal excitability and it is present at high concentrations in the brain cellular membrane [32] and it is known that the activity of this enzyme is decreased in various neurodegenerative disorders [33]. Streck and coworkers [34] studied the chronic Hcy administration in brain of young rats and Na(+),K(+)-ATPase and Mg(2+)-ATPase activities were determined in the hippocampus of treated Hcy rats. Chronic administration of Hcy significantly decreased Na(+),K(+)-ATPase activity but did not alter Mg(2+)-ATPase activity. In another study, Streck and coworkers [35] investigated the effects of preincubation of hippocampus homogenates in the presence of Hcy or Met on Na(+),K(+)-ATPase and Mg(2+)-ATPase activities in synaptic membranes of rats. Hcy significantly inhibited Na(+),K(+)-ATPase activity, whereas Met had no effect. Mg(2+)-ATPase activity was not altered by the metabolites. It was also evaluated the effect of incubating trolox (vitamin E analogue) alone or with Hcy on Na(+),K(+)-ATPase and Mg(2+)-ATPase activities in synaptic membranes from the hippocampus of rats. Trolox prevented the inhibitory effect of Hcy on Na(+),K(+)-ATPase activity. Besides, Streck et al [36] determined the *in vitro* effects of Hcy and Met on Na(+),K(+)-ATPase, and Mg(2+)-ATPase activities in synaptic membranes from the hippocampus of rats. The results showed that both metabolites significantly inhibit Na(+),K(+)-ATPase but not Mg(2+)-ATPase activity at concentrations usually observed in plasma of homocystinuric patients. Furthermore, incubation of hippocampal homogenates with Hcy also elicited an inhibition of the enzyme activity which was however prevented by the simultaneous addition of cysteine to the medium. In addition, cysteine or Met per se did not modify the two enzymatic activities. These findings indicate that oxidation of critical groups in the enzyme may possibly be involved in Hcy inhibitory effect. Considering that Na(+),K(+)-ATPase plays a crucial role in the central nervous system, these results suggest that the inhibition of this enzyme activity by Hcy is possible mediated by free radicals and may contribute to the neurological dysfunction found in homocystinuric patients.

Table 2 describes the oxidative stress parameters studied in tissues and biological fluids from animal models of homocystinuria. These findings showed a consistent profile of oxidative stress in the brain, blood, liver, muscle and lung of rats elicited by Hcy, which could explain, at least in part, the mechanisms involved in disease that is present in some homocystinuric patients.

4. Concluding remarks

Homocystinuric patients at diagnosis and during dietary therapy are susceptible to oxidative damage caused by an increase in free radical production and by a depletion in antioxidant capacity, with an increase of biomarkers reflecting lipid, protein, and DNA damage, probably secondary to increased formation of reactive species or secondary to the deprivation of micronutrients which are essential for these defenses. Also, mounting evidence obtained from experimental animal homocystinuric models indicated that oxidative stress may occur in the blood, brain, liver, lung and muscle of rats induced by Hcy. The data from literature, obtained from humans and experimental animal homocystinuric models, suggest that oxidative stress may be involved in the pathophysiology of the cardiovascular and neurological damage characteristic in this disease and, therefore, the administration of compounds with antioxidant properties could be considered as an adjuvant tool for being used in association with the present mainstay therapy when treating homocystinuric patients. Therefore, clinical studies should evaluate the use of antioxidants in further researches.

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6. Declarations of interest

The authors declare that they have no conflict of interest.

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8. Author contributions

Jéssica Lamberty Faverzani drafted the manuscript. Carmen Regla Vargas reviewed and revised the manuscript.

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Tables

Table 1 Oxidative stress and inflammatory parameters evaluated in biological fluids from homocystinuric patients at diagnosis and during dietary treatment.

Parameter	Diagnosis	Treatment	Reference
Lipid peroxidation			
MDA levels	Increased*	Increased	[9]
8-isoprostaglandin F2α	NM	Increased	[24]
11-dehydrothromboxane	NM	Increased	[24]
Protein oxidation			
Carbonyl content	Increased*	Increased	[9,10]
Sulfhydryl content	Decreased*	Decreased	[9,10]
DNA damage	NM	Increased	[11]
Antioxidant defense			
TAS levels	Decreased*	Decreased	[9]
Vitamin B12	Decreased*	Decreased	[10]
Vitamin E	NM	Increased	[24]
Folic acid	Decreased*	Increased	[10]
SOD	NM	Increased	[7]
EC-SOD	Increased	Increased	[6]
Inflammatory profile			
IL-6	Increased*	Increased	[10]
IFN-y	Similar*	Similar	[10]
IL-1β	Similar*	Similar	[10]

NM: not measured

*at late diagnosis

Table 2 Oxidative stress parameters evaluated in tissues and biological fluids from animal models of homocystinuria.

Parameter	*In vitro* / acute / chronic	Reference
Lipid peroxidation	Increased	[13,14,16,17,27]
Protein oxidation	Increased	[14,16]
DNA damage	Increased	[25]
Antioxidant defense	Decreased	[12,13,16,25,27]
Antioxidant enzyme	Altered	[12,13,15-17,25,27]
Activity of enzyme		
AChE	Increased	[29]
BuChE	Decreased	[30,31]
Na(+),K(+)-ATPase	Decreased	[12,15,31,34-36]

ANEXO I - NORMAS PARA PUBLICAÇÃO NA REVISTA *BIOSCIENCE REPORTS*

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