



Basic Study

Melatonin restores zinc levels, activates the Keap1/Nrf2 pathway, and modulates endoplasmic reticular stress and HSP in rats with chronic hepatotoxicity

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Abstract

BACKGROUND

Melatonin (MLT) is a potent antioxidant molecule that is shown to have a beneficial effect in various pathological situations, due to its action against free radicals.

AIM

To evaluate the effect of MLT on carbon tetrachloride (CCl₄) induced liver injury in rats in terms of oxidative stress, reticular stress, and cell damage.

METHODS

Twenty male Wistar rats (230-250 g) were divided into four groups: Control rats, rats treated with MLT alone, rats treated with CCl₄ alone, and rats treated with CCl₄ plus MLT. CCl₄ was administered as follows: Ten doses every 5 d, ten every 4 d, and seven every 3 d. MLT was administered intraperitoneally at a dose of 20 mg/kg from the 10th wk to the end of the experiment (16th wk).

RESULTS

MLT was able to reduce the release of liver enzymes in the bloodstream and to decrease oxidative stress in CCl₄ treated rats by decreasing the level of thiobarbituric acid reactive substances and increasing superoxide dismutase activity, with a lower reduction in serum zinc levels, guaranteeing a reduction in liver damage; additionally, it increased the expression of nuclear factor (erythroid-derived 2)-like 2 and decreased the expression of Kelch-like ECH-associated protein 1. MLT also decreased the expression of the proteins associated with endoplasmic reticulum stress, *i.e.*, glucose-regulated protein 78 and activating transcription factor 6, as well as of heat shock factor 1 and heat shock protein 70.

CONCLUSION

MLT has a hepatoprotective effect in an experimental model of CCl₄-induced liver injury, since it reduces oxidative stress, restores zinc levels, and modulates endoplasmic reticulum stress.

Key Words: Liver injury; Cell damage; Antioxidant; Melatonin; Carbon tetrachloride

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Core Tip: Liver cirrhosis is a chronic condition of the liver that is characterized by inflammation, steatosis, and formation of fibrotic tissue that impair liver function. Several studies have demonstrated the relationship between oxidative stress and the development of different diseases. As oxidative stress can damage lipids, proteins, and DNA, causing changes in cell homeostasis, it is important to study therapeutic substances that can minimize or delay the effects of the disease. Hepatotoxic drugs are commonly used as an experimental model to assess different stages of liver disease. Melatonin was used in this work as a therapeutic strategy and showed hepatoprotective action in a chronic hepatotoxicity model.

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INTRODUCTION

Chronic liver diseases are characterized by multistep processes that involve several molecules and cellular events to transform a normal parenchyma into a parenchyma with steatosis, increased collagen deposition, fibrosis, and cirrhosis[1]. Many studies have demonstrated the presence of overproduction of free radicals and reactive oxygen species (ROS) in inflammatory chronic diseases. ROS are able to oxidize macromolecules or activate transcription factors[2-4]. The relation between the development of chronic liver diseases and ROS has been widely discussed since oxidative stress may cause damage in lipid, protein, and DNA, producing alterations in cellular redox homeostasis[5].

Cellular homeostasis can be disrupted by a variety of stimuli, including metabolic imbalance, oxidative stress, and folding of malformed proteins. In response to these stressors, cells induce specific molecular pathways that usually involve the activation of signaling cascades or changes in gene expression. These responses allow cells to adapt to stress and to regain homeostasis. However, if stress is intense or prolonged, the cells are unable to reestablish homeostasis and, in turn, activate pathways that result in cell death[6].

Carbon tetrachloride (CCl₄) is a hepatotoxic drug used in experimental models to evaluate different stages of liver disease and thus define therapeutic strategies. Exposure to CCl₄ have a toxic effect on liver cells, promoting damage in tissue and causing changes in the antioxidant defense mechanism. This process results in an imbalance between ROS production and antioxidative enzymes release[5].

The antioxidant defense mechanism is dependent on some minerals, including zinc. This mineral is essential for a large number of structural proteins, enzymatic processes, and transcription factors. Its deficiency causes numerous clinical manifestations, such as appetite loss, smell and taste disturbances, cerebral and immune dysfunction, and reduced drug elimination capacity. These clinical characteristics have been observed in chronic liver diseases[7].

The alteration in cellular redox homeostasis either results in mitochondrial dysfunction or can affect other organelles, such as the endoplasmic reticulum (ER). The ER stress may impair protein synthesis, resulting in accumulation of misfolded proteins. ER stress induces the activation of the intracellular

signaling pathway called unfolded protein response (UPR), which contributes to the pathogenesis of several chronic diseases[8,9].

Melatonin (N-acetyl-5-methoxytryptamine; MLT) is an indoleamine lipophilic derivative of tryptophan. It is produced primarily by the pineal gland of vertebrates and is also detected in other organs[10-13]. MLT has effects on sleep, mood, sexual maturation and reproduction, immune function, aging, and the antioxidative defense system[14,15], and exhibits numerous actions, including anti-inflammatory, antioxidant, and oncostatic properties. In hepatocytes, it was observed that MLT protects from oxidative damage. Its action is based on scavenging the free radicals and stimulating antioxidant enzymes[15-17]. Reducing the generation of ROS would be a way to slow the progression of cell damage observed in liver diseases.

Our aim was to study the effects of MLT on biochemical indexes, serum zinc levels, and oxidative stress in rats exposed to CCl₄. Also, we evaluated the expression of proteins involved in cell damage, ER stress, and unfolded protein response in animals with liver injury induced by CCl₄ and treated with MLT.

MATERIALS AND METHODS

Animal experiments and drug treatment

Twenty male Wistar rats with an average weight of 230-250 g were used. The animals were housed at 22 °C with 12 h light-dark cycles, had free access to water, and received a restricted diet (16 g of chow per day for each animal).

All experiments were performed in accordance with the Guiding Principles for Research Involving Animals (NAS) and the Committee of Ethics and Research in Health of the Graduate and Research Group of Hospital de Clinicas de Porto Alegre under protocol number 100316.

The animals were divided into four groups: Control (CO) rats, rats treated with MLT alone (MLT), rats treated with CCl₄ alone (CCl₄), and rats treated with CCl₄ plus MLT (CCl₄ + MLT). The CCl₄ and CCl₄ + MLT groups received 27 intraperitoneal doses of 0.5 mL of CCl₄ dissolved in mineral oil (1:6). The first ten doses were given at an interval of 5 d, the following ten at an interval of 4 d, and the last seven at an interval of 3 d[18]. In order to promote cytochrome P450 enzyme induction, phenobarbital was added to the drinking water of each animal at a concentration of 0.3 g/L 7 d before the first application and throughout the experiment[19].

MLT (Sigma Aldrich, St. Louis, MO) was administered intraperitoneally to the MLT and CCl₄ + MLT groups at a dose of 20 mg/kg/day from the 10th wk to the end of the experiment (16th wk)[20].

At 24 h after the last administration of CCl₄, the animals were anesthetized with 1% xylazine and 10% ketamine, and then we collected blood samples from the retro-orbital plexus. Liver samples were obtained for the remaining analyses. At the end of the experiment, the animals were killed under deep anesthesia by exsanguination, as described in the guidelines of the American Veterinary Medical Association (AVMA) on Euthanasia (AVMA, 2007).

Biochemical analysis

Serum levels of alanine aminotransferase (ALT) (U/L) and aspartate aminotransferase (AST) (U/L) were determined by the kinetic UV test. Alkaline phosphatase (AP) (U/L) was quantified by the colorimetric kinetic test. The levels of these enzymes were measured using routine laboratory methods of the Hospital de Clinicas de Porto Alegre by enzymatic methods (automated - Siemens Advia 1800 Chemistry system).

Lipid peroxidation and cytosolic superoxide dismutase

Frozen tissue from each rat was homogenized in ice-cold phosphate buffer (KCl 140 mmol/L, phosphate 20 mmol/L, pH 7.4) and centrifuged at 3000 rpm for 10 min. Protein concentration in liver homogenates was determined using a bovine albumin solution[21]. Lipid peroxidation (LPO) was determined by measuring the concentration of thiobarbituric acid reactive substances (TBARS) (nmol/mg protein)[22]. Spectrophotometric absorbance in the supernatant was measured at 535 nm. Cytosolic superoxide dismutase (SOD) (EC 1.15.1.1) was assayed as described previously[23]. The auto-oxidation rate of epinephrine, which is progressively inhibited by increasing amounts of SOD in the homogenate, was monitored spectrophotometrically at 560 nm. The amount of enzyme that inhibited 50% of epinephrine auto-oxidation was defined as 1 U of SOD activity.

Western blot analysis

Western blot analysis was performed using cytosolic and nuclear extracts prepared from liver homogenates as previously described[24]. The supernatant fraction was collected and stored at -80 °C in aliquots until use. Protein concentration was measured as described previously[21]. Lysate proteins were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride membranes[25]. The membranes were then blocked with 5%

nonfat dry milk in Tris-buffered saline containing 0.05% Tween 20 (TTBS) for 1 h at room temperature and probed overnight at 4 °C with polyclonal antibodies against nuclear factor (erythroid-derived 2)-like 2 (Nrf2) (SC30915/57kDa), Kelch-like ECH-associated protein 1 (Keap1) (SC33569/69kDa), activating transcription factor 6 (ATF6) (SC166659/90kDa), and 78-kDa glucose-regulated protein (GRP78/BiP) (SC376768/78kDa) (Santa Cruz Biotechnology, Santa Cruz, CA, United States) at a dilution of 1:200-1000 with TTBS in 5% nonfat dry milk. Antibodies against heat shock factor 1 (HSF1) (H4163/75kDa) and heat shock protein 70 (HSP70) (H5147/73 and 72kDa) (Sigma Aldrich, St Louis, MO, United States) were used at a dilution of 1:5000 with TTBS in 5% nonfat dry milk, as well as antibodies against β -actin (A5060/42kDa) and anti-glyceraldehyde 3-phosphate dehydrogenase (G9545/37kDa) (Sigma Aldrich, St Louis, MO, United States) at a dilution of 1:2,000 with TTBS in 5% nonfat dry milk. After washing with TTBS, the membranes were incubated for 1 h at room temperature with secondary HRP conjugated antibody (Santa Cruz Biotechnology, Santa Cruz, CA, United States, 1:4000). Protein detection was performed *via* chemiluminescence using a commercial ECL kit (Amersham Pharmacia Biotech, Little Chalfont, UK)[24]. The density of the specific bands was quantified with imaging densitometer software (Scion Image, Maryland, MA).

Zinc measurement

The serum zinc concentration was measured using a Zinc Assay Kit (Abnova Corporation, Taipei City, Taiwan). The absorbance (425 nm) of the supernatant was measured by spectrophotometry, and the values are expressed in $\mu\text{g/dL}$.

Statistical analysis

The mean and standard deviation (SD) were calculated for all data. Significant differences between means were evaluated by one-way analysis of variance (ANOVA). Statistical significance was assessed using Tukey's test. *P* values < 0.05 were deemed significant. All analyses were carried out using Statistical Package for Social Sciences (SPSS), version 18.0 (SPSS Inc., Chicago, IL).

RESULTS

Effect of MLT on biochemical analysis

We evaluated the hepatoprotective activity of MLT against CCl_4 -induced liver injury in rats (Table 1). Analyses of transaminase enzymes showed that in the CCl_4 group, AST and ALT were increased compared to the CO, MLT, and CCl_4 + MLT groups. Also, AP demonstrated a significant increase in the CCl_4 group compared to the other groups, indicating the presence of hepatocellular injury. Animals treated with MLT showed reduced enzymatic levels when compared to the CCl_4 group ($P < 0.001$).

Effect of MLT on LPO and SOD activity

Determination of lipid peroxidation in liver tissue with liver injury induced by CCl_4 and treated with MLT was performed by the TBARS method, which showed an increase of malondialdehyde (MDA) formation in tissues exposed to CCl_4 . Lipid peroxidation was significantly increased in the CCl_4 group (+ 61%) *vs* control animals. The CCl_4 + MLT group had LPO levels similar to the controls (Table 1). SOD activity was lower in the CCl_4 group (- 27%) compared to animals of the control group. However, treatment with MLT reduced LPO and restored SOD activity in the CCl_4 + MLT group in comparison with the CCl_4 group (Table 1).

Effect of MLT on Keap1/Nrf2 pathway regulation

Protein markers related to oxidative stress were also evaluated. Animals in the CCl_4 group overexpressed Keap1 and underexpressed nuclear factor Nrf2. Conversely, animals in the CCl_4 + MLT group underexpressed Keap1 and overexpressed Nrf2 (Figure 1). The overexpression of Keap1 observed in the CCl_4 group suggested an inhibitory action on nuclear factor Nrf2. However, the underexpression of Keap1 evidenced in the CCl_4 + MLT group suggested that MLT shows a cytoprotective effect on hepatocytes by stimulating the action of Nrf2.

Effect of MLT on endoplasmic reticular stress

Concerning markers of ER stress, ATF6 and GRP78/BiP expression was strongly increased in animals treated with CCl_4 ($P < 0.05$), while that in the CCl_4 + MLT group had expression similar to the control group (Figure 2).

Effect of MLT on HSP70 and HSF1 expression

Stressful insults, such as exposure to toxic agents, stimulate HSF1 to act as a master activator of the response of HSPs, including HSP70[26]. HSF1 and HSP70 expression was significantly higher in animals from the CCl_4 group compared with the control group and significantly reduced in animals from the CCl_4 + MLT group compared with the CCl_4 group (Figure 3).

Table 1 Effect of melatonin on liver function, lipid peroxidation, superoxide dismutase activity, and zinc level

Parameter	CO	MLT	CCl ₄	CCl ₄ + MLT
AST (U/L)	175.4 ± 34.4	161.8 ± 20.3	1016.8 ± 340.8 ^a	519.6 ± 127.5
ALT (U/L)	50.2 ± 5.6	43.8 ± 6.6	270 ± 90.8 ^a	177 ± 42.7
AP (U/L)	80.2 ± 25.4	75 ± 14.3	395 ± 130.8 ^a	238 ± 24.5
TBARS (nmol/mg protein)	0.18 ± 0.01	0.15 ± 0.01	0.29 ± 0.03 ^a	0.18 ± 0.05
SOD (U/SOD/mg protein)	12.84 ± 1.08	11.43 ± 0.7	9.32 ± 0.3 ^a	13.18 ± 1.6
Zinc (µg/dL)	48.66 ± 4.9	0.62 ± 10.4	11.11 ± 4.31 ^a	28.96 ± 6.67

^a*P* < 0.001, carbon tetrachloride (CCl₄) group *vs* other groups. Data are expressed as the mean ± SD. CO: Control group; Melatonin (MLT): Rats receiving MLT alone; CCl₄: Rats treated with CCl₄ alone; CCl₄ + MLT: Rats treated with CCl₄ plus MLT; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AP: Alkaline phosphatase; TBARS: Thiobarbituric acid reactive substances; SOD: Superoxide dismutase.

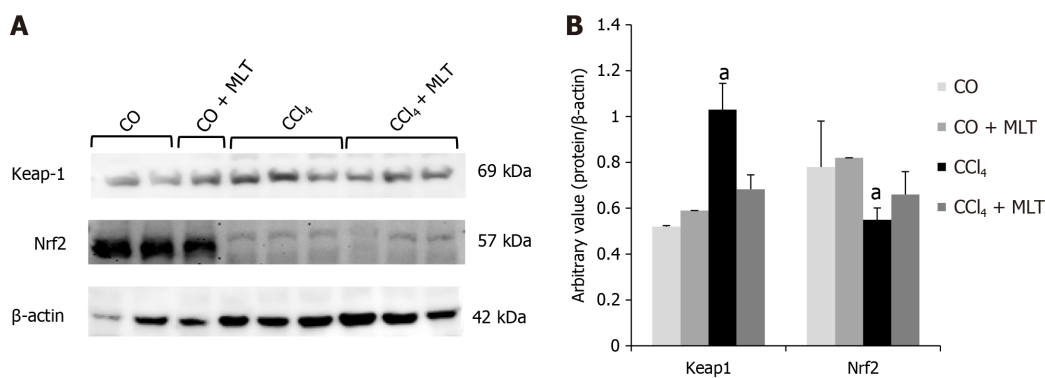


Figure 1 Western blot analysis. A: Cytoplasmic and nuclear fractions were analyzed by Western blot with antibodies against Kelchlike-ECH-associated protein 1, nuclear factor (erythroid-derived 2)-like 2, and β-actin; B: Arbitrary values expressed as the mean and standard deviation. ^a*P* < 0.05 CCl₄ *vs* other groups. MLT: Melatonin.

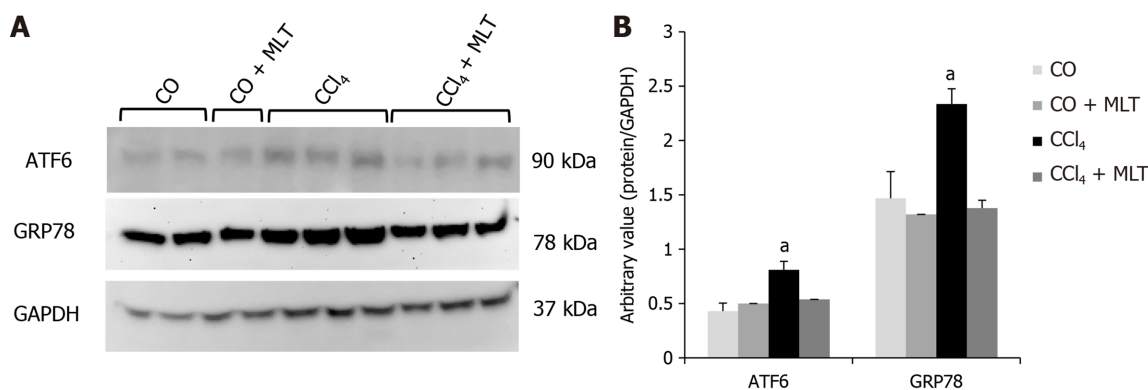


Figure 2 Western blot analysis. A: Nuclear and cytoplasmic fractions were analyzed by Western blot with antibodies against activating transcription factor 6, glucose-regulated protein 78, and glyceraldehyde 3-phosphate dehydrogenase; B: Arbitrary values expressed as the mean and standard deviation. ^a*P* < 0.05 CCl₄ *vs* other groups. MLT: Melatonin.

Effect of MLT on serum zinc levels

When assessing serum zinc levels in different groups, we observed that the induction of liver injury by CCl₄ caused damage to hepatocytes associated with a significant reduction in zinc levels. In contrast, animals treated with CCl₄ + MLT showed zinc levels similar to those in the control group, indicating that MLT has a hepatoprotective effect (Table 1).

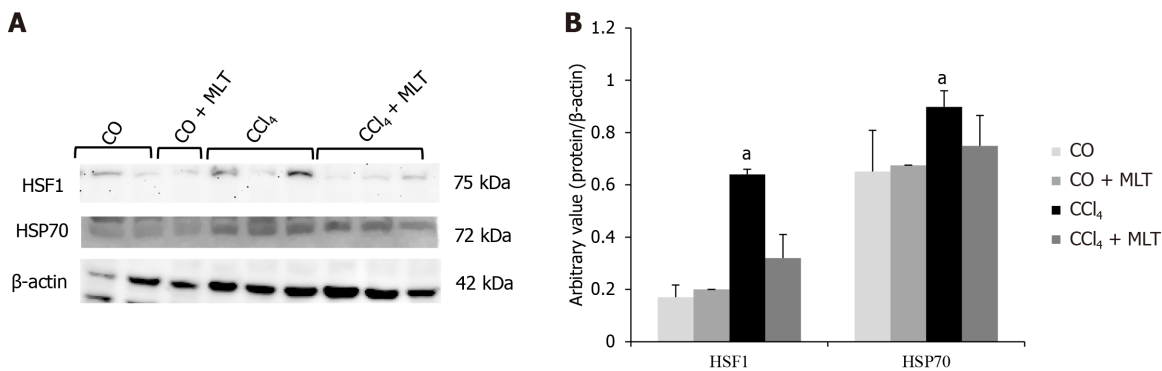


Figure 3 Western blot analysis. A: Nuclear and cytoplasmic fractions were analyzed by Western blot with antibodies against heat shock factor 1, heat-shock protein (HSP70), and β -actin; B: Arbitrary values expressed as the mean and standard deviation. ^a $P < 0.05$ CCl₄ vs other groups. MLT: Melatonin.

DISCUSSION

Many studies have demonstrated the presence of overproduction of free radicals and ROS in chronic liver diseases. ROS are able to oxidize macromolecules or activate transcription factors, resulting in oxidative stress and ER stress[27,28,29]. Our study evidenced that MLT treatment in rats with liver injury chemically induced by CCl₄ was promising. We observed that MLT was able to activate the Nrf2 pathway and modulate the unfolded protein response and ER stress in rats with liver injury. MLT plays a key role in hepatoprotection, which results in the reduction of hepatic enzymatic levels and oxidative stress.

We evaluated the efficacy of MLT regarding the protection of macromolecules against the oxidative damage during hepatotoxicity process in animals exposed to CCl₄. In general, MLT consistently improved the pattern of the hepatic enzymes. Also, this study observed a reduction of oxidative stress in animals that were treated with MLT. With respect to oxidative damage, MLT stimulated the Keap1/Nrf2 pathway and restored the normal expression of the proteins involved in the ER stress.

In the present study, chronic exposure to CCl₄ induced liver injury, as evidenced by the significant increase in the liver enzymes under study. MLT treatment protected the liver from toxicity promoted by the toxic agents (CCl₄). AST and ALT in the CCl₄ group increased significantly compared to the other groups. This analysis characterized a condition of necrosis or alteration of cell membrane permeability. This increase is indicative of acute liver injury and hepatocellular damage[30]. Animals treated with CCl₄ + MLT had significantly reduced AST and ALT activities, indicating a significant cytoprotective effect for MLT on hepatocytes[31]. Other studies reported that the use of hepatotoxic agents such as CCl₄ and thioacetamide to generate free radicals affects the permeability of hepatocyte membranes and increases serum levels of enzymatic biochemical parameters. The use of antioxidants such as quercetin was able to reduce the release of these enzymes, probably due to the restoration of liver parenchyma[32, 33].

The involvement of ROS in liver injury and in the death of hepatocytes exposed to toxic agents has been extensively documented both *in vitro* and *in vivo*[8]. It is believed that the changes in redox homeostasis may play a significant role in the pathogenesis of many diseases characterized by chronic inflammation, activation of wound healing, and fibrogenesis[27,28,32,34]. The mechanism of CCl₄ toxicity occurs through the generation of highly toxic free radicals. In the present study, this evidence is reinforced by the increase in TBARS levels in the CCl₄ group. Among many effects attributed to MLT, we highlight its potent antioxidant effect[20,35-37]. Our results showed that MLT significantly reduced liver LPO, which is consistent with previous reports indicating that MLT is capable of removing free radicals and has a protective effect in experimentally induced hepatotoxicity[20,37,38]. There is evidence that MLT increases the efficiency of the electron transport chain and, as a consequence, reduces the generation of ROS[39,40]. This antioxidant property is due to the double bond existing in its chemical structure, which allows electrons to be transferred to unstable chemical species[40].

The antioxidant enzyme system, represented in this study by SOD, prevents the accumulation of oxygen and hydrogen peroxide, and thus is considered the main line of defense of the body[41]. CCl₄ significantly decreased SOD activity, and MLT reversed this process. Reduced SOD activity, along with increased LPO in animals from the CCl₄ group, establishes a situation of oxidative stress[41-43]. MLT protects hepatocytes against damage from free radicals by directly scavenging free radicals and stimulating antioxidant enzymes[44,45]. Similar to previous studies, our study found that MLT increased the activity of the antioxidant enzyme SOD, which occurred in parallel with Nrf2 activation[46,47].

Nrf2 is a major transcriptional regulator that secures a vast range of tissues and cells from ROS mediated induction because of its various antioxidant and phase II detoxification enzymes[4]. Furthermore, several chemopreventive compounds can abate tissue fibrosis by enhancing nuclear

translocation of Nrf2 and induction of glutathione S-transferases (GSTs) and SOD expression[4]. One of these essential mechanisms responsible for the induction of enzymes in response to stress is the pathway of Nrf2 and its inhibitor Keap1. When translocated into the nucleus, Nrf2 binds to the antioxidant responsive element (ARE), regulating the expression of endogenous antioxidants and proteins involved in the regulation of cell cycle and death[46-50]. In an Nrf2-knockout mouse model after long term CCl₄ treatment, the liver fibrosis was aggravated[4].

In this study, Nrf2 expression was significantly reduced, whereas Keap1 expression was greater in animals from the CCl₄ group. Studies have shown that Nrf2 plays a protective role in liver disease, with Nrf2-deficient rats being more sensitive and susceptible to liver injury and fibrosis induced by hepatotoxins[51]. Our study showed that the improvement in liver damage was due to MLT treatment. This improvement was due to MLT that is able to activate the nuclear factor Nrf2, resulting in less oxidative stress[51-54]. It has also been documented that Nrf2 activators, a wide variety of compounds such as sulfur-containing compounds, polyphenols, terpenoids, carotenoids, and selenium, exert the cytoprotective effect and emerge to play a part in the antioxidative response, presumably providing a series of protections needed for normal cellular activities. Furthermore, it is widely recognized that reduced Nrf2-mediated antioxidant defense plays a key role in the process of CCl₄-induced liver fibrosis. Nrf2 activators thus far exhibit hepatoprotective effects and significantly attenuate liver injury and fibrosis in clinical studies[4]. Kaufman *et al* in 2020 related that the modulation of the Nrf2 is dependent of zinc [55]. They evaluated cells incubated in zinc deficient medium and evidenced a reduction of the nuclear Nrf2 levels[55]. Zinc deficits aggravated the oxidative stress and reduced Nrf2 nuclear translocation[55-58].

As already observed in the study by Fernandes *et al*[59], cirrhotic animals showed significantly lower serum levels of zinc when compared to controls, showing an association of zinc deficiency with liver damage in sick animals.

Studies linking zinc and liver diseases often described that the replacement of the zinc element results in an improvement in the clinical and morphological condition[7,59]. We determined the zinc levels in the liver of rats exposed to CCl₄. Our results demonstrated reduced levels of zinc in injured livers. MLT treatment was effective in restoring normal zinc levels. A study with old rats treated with MLT showed a link between zinc and MLT. It is believed that MLT is able to modulate zinc turnover, due to the synchronization of circadian patterns of zinc and MLT and the concomitant increase in the levels of both in animals treated with MLT[56-58].

Our results show that MLT treatment in animals treated with CCl₄ resulted in an improvement in the liver enzyme pattern, lower oxidative stress, activation of Nrf2, and normalization of serum zinc levels. In addition to these findings, we measured the expression of ATF6 and GRP78/BiP, two important proteins involved in ER stress.

In the presence of stressors, GRP78/BiP is released, leading to the activation of the UPR signaling pathway, including ATF6[60]. Under the action of released GRP78/BiP, ATF6 decouples from the ER and undergoes cleavage in the Golgi system. At that moment, the activation of the nuclear factor (ATF6 50-kDa) that regulates the GRP78/ BiP and GRP94 proteins occurs, and the maintenance of this activation results in ER stress[61].

Physiological or pathological processes that disturb ER homeostasis led to a pathologic response called ER stress, causing the activation of the intracellular signaling pathway called UPR, thereby contributing to the pathogenesis of several conditions, including liver diseases[8,9,29,62]. A growing amount of evidence reinforces that increased ROS production is strongly related to induction of ER stress[6]. Our findings suggest the presence of ER stress in the animals from the CCl₄ group, since there was an increase in the expression of ATF6 and GRP78/BiP. The transcription of these chaperones is increased in response to various stimuli that disturb or overload ER function, including exposure to xenobiotics[29]. A significant decrease in the expression of proteins that predict ER stress was observed in animals receiving MLT. Our data are consistent with recent findings showing that MLT reduces ER stress in different models of cell injury[24]. For example, treatment with MLT reduced ER stress and modulated UPR in rabbits with fulminant hepatitis of viral origin, an effect that was associated with a reduction in apoptosis, cell death, and liver damage[24]. MLT also showed a neuroprotective effect through the reduction of ER stress in neuronal cells of newborn rats after hypoxia-ischemia[63]. Typically, ROS are controlled by intracellular antioxidants such as SOD; however, the excess demand in protein folding can overload antioxidant response. In support of this theory, the use of antioxidants has been shown to enhance protein folding and reduce apoptosis in response to ER stress[29].

Another highly regulatory mechanism essential for cellular redox homeostasis and protein folding involves HSPs and the nuclear factor HSF1[29]. Among HSPs, HSP70 is one of the protein families that has been more conserved in evolution, being expressed in the cell both constitutively and inductively. Our data show that animals exposed to CCl₄ showed higher expression of HSP70 and HSF1. One of the cellular responses to stress is HSP activation[64]. Several lines of evidence highlight the deleterious effects of HSPs on various human diseases, including cancer, in which case it promotes survival and proliferation of tumor cells and drug resistance[65]. Members of the HSP70 family have been particularly implicated in the pathophysiology and pathogenesis of several liver diseases such as hepatitis B and C, non-alcoholic steatohepatitis, autoimmune hepatitis, primary biliary cirrhosis, and others[26]. Treatment with MLT reduced HSP70 and HSF1 expression.

The exact mechanism leading to xenobiotic-induced cellular stress is still not well understood and may involve multiple factors, such as xenobiotic concentration, time of exposure, mechanism of action, cell type affected, among others. The combination of different cell damages, including oxidative stress, ER stress, UPR, and cytosolic responses, may lead to cell apoptosis[29].

CONCLUSION

We conclude that MLT acts as a potent antioxidant, promoting the activation of the nuclear factor Nrf2, which allows the reduction in oxidative stress. Concomitantly, we observed the restoration of serum zinc levels, which contributes to numerous hepatic cytoprotective processes. With the reduction of oxidative stress, it is possible to attenuate the ER stress and the unfolded protein response, as well as the cell damage caused by the toxic agent CCl₄.

The evaluation of new markers in this model may contribute to a better understanding of other pathophysiological mechanisms of cirrhosis and its complications. MLT may offer a promising therapeutic approach to liver diseases, given its effectiveness in the attenuation of oxidative stress. A major limitation in the application of MLT is that most of the studies were designed especially on rats. More research is needed in clinical trials to approve the potentials of this indolamine in humans.

ARTICLE HIGHLIGHTS

Research background

Chronic liver diseases are characterized by a multistep process that involves several molecules and cellular events to transform a normal parenchyma into a parenchyma with steatosis, increased collagen deposition, fibrosis, and cirrhosis. Melatonin (MLT) is a potent antioxidant molecule that is shown to have a beneficial effect in various pathological situations, due to its action against free radicals.

Research motivation

To reduce the generation of reactive oxygen species would be a way to slow the progression of cell damage observed in liver diseases.

Research objectives

To study the effects of MLT on biochemical analysis, on serum zinc levels, and on oxidative stress in rats exposed to carbon tetrachloride (CCl₄), and to evaluate the expression of proteins involved in cell damage, endoplasmic reticular stress, and unfolded protein response in animals with liver injury induced by CCl₄ and treated with MLT.

Research methods

Twenty male Wistar rats (230-250 g) were divided into four groups: Control rats, rats treated with MLT alone, rats treated with CCl₄ alone, and rats treated with CCl₄ plus MLT. CCl₄ was administered as follows: Ten doses every 5 d, ten every 4 d, and 7 every 3 d. MLT was administered intraperitoneally at a dose of 20 mg/kg from the 10th wk to the end of the experiment (16th wk).

Research results

Administration of CCl₄ caused an increase in liver enzyme levels, a reduction in serum zinc levels, and greater oxidative stress and ER stress. MLT treatment was able to reverse the changes promoted by the toxic agent CCl₄.

Research conclusions

We conclude that MLT acts as a potent antioxidant, promoting activation of the nuclear factor Nrf2, which allows the reduction in oxidative stress. Concomitantly, we observed the restoration of serum zinc levels, which contributes to numerous hepatic cytoprotective processes. With the reduction of oxidative stress, it is possible to attenuate the ER stress and the unfolded protein response, as well as the cell damage caused by the toxic agent CCl₄.

Research perspectives

MLT may offer a promising therapeutic approach to liver diseases, given its effectiveness in the attenuation of oxidative stress. A major limitation in the application of MLT is that most of the studies were designed especially on rats. More research is needed in clinical trials to approve the potentials of this indolamine in humans.

FOOTNOTES

Author contributions: Marroni NP contributed to conceptualization; Bona S, Rodrigues G, and Moreira AJ contributed to data collection and review of the formal analysis of the databases; Di Naso F, Fernandes SA, Schemitt EG, and Marroni CA contributed to original preparation of the manuscript; Marroni NP, Bona S, Rodrigues G, Moreira AJ, Di Naso F, Fernandes SA, Schemitt EG, and Marroni CA have read and agreed to the published version of the manuscript.

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