EXPERIMENTAL STUDY

Melatonin treatment prevents carbon tetrachloride-induced acute lung injury in rats by mitigating tissue antioxidant capacity and inflammatory response

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ABSTRACT
AIM: Carbon tetrachloride (CCl4) is an organic chemical that produces different tissue-damaging effects when ingested or inhaled. Present study aims to determine whether the application of exogenous melatonin, a neurohormone with numerous biological properties, can prevent disturbances in lung tissue antioxidant capacities and arginine metabolism, tissue inflammation and oxidative damage induced by exposure to CCl4 in rats. METHODS: The effects of melatonin on the changes occurring in rat lung tissue after an acute exposure to CCl4 were studied by monitoring alterations in antioxidant capacities, inflammatory parameters, parameters of arginine metabolism, and lipid and protein oxidative damage. RESULTS: The results indicated that melatonin prevents CCl4-induced lung damage by mitigating tissue antioxidant capacity and preventing nitric oxide production through a shift from nitric oxide synthase to arginase. Also, melatonin partially prevented tissue inflammation and molecules' oxidative modification seen after exposure to CCl4. CONCLUSIONS: The protective activity of melatonin can be attributed to its ability to scavenge both free radicals, as well as to its potential to increase tissue antioxidant capacity. The modulation of inflammatory response through both decrease in tissue inflammatory parameters and influence on arginine-nitric oxide metabolism might be an additional mechanism of action (Tab. 1, Fig. 2, Ref. 33). Text in PDF www.elis.sk.

KEY WORDS: lung injury, carbon tetrachloride, melatonin, tissue antioxidant capacity, arginine metabolism.

Introduction

Carbon tetrachloride (CCl4) is an organic industrial chemical most frequently generated in chemical production facilities that produce chlorine, hydrogen, and alkali, and while its toxic potential is well known, its production and consumption for emissive uses is believed to be fully controlled (1). In humans (e.g. workers exposed to CCl4) and in experimental animals administered with CCl4, it causes massive damage to the liver, kidneys, respiratory system, skin, etc. (2). Since around 4% of CCl4 is eliminated by exhalation, one can expect that lung tissue might be a potential target organ for the damage caused by the exposure to this highly toxic chemical (3).

Shortly after its absorption, CCl4 is metabolized by cytochrome P-450 which is predominantly localized in liver tissue. Nevertheless, its expression is not negligible in other tissues such as lungs (4), where the products of this metabolism, highly reactive trichloromethyl (CCl3-) and trichloromethyl peroxy radical (CCl3O2-) induce intra-alveolar septal ruptures and interstitial cell degenerations. This sequence of events takes place due to the activity of inflammatory cells (neutrophils and macrophages) attracted to the damaged tissue (6). One could also argue that the lung tissue damage after CCl4 application could be incurred by kidney damage, since a decrease in water excretion leads to lung oedema (7). Besides the increase in reactive oxygen species (ROS) production, CCl4 causes a release of inflammatory cytokines (8) and suppression in antioxidant capacities (decrease in reduced glutathione (GSH), vitamin C and E, and antioxidant enzymes), as well as brings about an increase in lipid peroxidation, protein carbonylation, and DNA damage, which lead to cellular caspase-mediated apoptosis or necrosis (9).

Melatonin (MLT, N-acetyl-5-methoxytryptamine) is a neurohormone produced mainly by pineal gland cells, but also by other tissues/cells (10). Studies revealed that MLT is able to scavenge ox-
ygen- and nitrogen-based reactants that are known to damage cell macromolecules, as well as to increase tissue antioxidant capacity (6, 10, 11). It was found that MLT acts by increasing the expression of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) mRNA, and mRNA of enzymes related to GSH synthesis (11). Melatonin was previously proven to ameliorate lung tissue injury induced by bleomycin (11), acute pancreatitis (12), aortic occlusion (13), lipopolysaccharide (14), phosgene (15), etc.

Having in mind that CCl₄ is a dangerous industrial pollutant still present in the working environments of many undeveloped countries, and that its inhalation might lead to lung tissue injury, we aimed to evaluate whether the application of MLT would prevent lung tissue damage induced by this hazardous chemical. The effects of MLT would be estimated based on its potential in preventing the disturbances in pulmonary non-enzymatic tissue antioxidant capacity (TAC; vitamin C and GSH) and enzymatic antioxidant capacities (GPx and CAT) produced by CCl₄. The effect of MLT on inflammatory cell infiltration would be estimated indirectly through the myeloperoxidase (MPO) activity, which represents an enzyme located within macrophages/neutrophils. The changes in arginine metabolism that follow CCl₄ application and the impact of MLT treatment are going to be assessed based on nitrate/nitrite and citrulline concentrations, as well as based on changes in arginase and iNOS activities. In the end, the potential of MLT in the prevention of lung tissue damage induced by CCl₄, was performed according to previous descriptions (6, 10, 11). It was found that MLT acts by increasing the expression of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) mRNA, and mRNA of enzymes related to GSH synthesis (11). Melatonin was previously proven to ameliorate lung tissue injury induced by bleomycin (11), acute pancreatitis (12), aortic occlusion (13), lipopolysaccharide (14), phosgene (15), etc.

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Lipid and protein damage determination

The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17). The amount of lipid peroxidation was estimated through the levels of MDA that was measured in reaction with thiobarbituric acid (17).
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Acid (10). Protein carbonyl content was used for quantifying the oxidatively modified proteins and their content was determined spectrophotometrically using 2,4-dinitrophenylhydrazine as a colour reagent (22).

Statistical analysis

The obtained variables are presented as means ± SD and were compared using one-way ANOVA and appropriate post-hoc tests (GraphPad Prism, ver. 5.03; San Diego, CA). Probability values were taken to be statistically significant if the p value was lower or equal to 0.05.

Results

Although the exposure of animals to CCl₄ led to a decrease in relative lung tissue mass, this decrease was not found to be statistically significant when compared to group I (control group). Interestingly, the mean value for the relative lung tissue weight in group IV was almost identical to that in the control groups. Nevertheless, no significant difference was observed (data not shown).

Total antioxidant capacity in lung tissue was significantly increased in healthy animals treated with MLT, i.e. in group II (Tab. 1), while a single dose of CCl₄ led to a decrease in TAC (group III). Such a dramatic decrease in TAC was prevented by the treatment with MLT in group IV (Tab. 1).

Compared to untreated animals, the acute application of CCl₄ significantly affected all studied participating species of arginine metabolism (enzymes and their products) (Tab. 1). The metabolism of arginine in animals treated with MLT after CCl₄ was only slightly disturbed, and in majority of parameters, MLT’s effect appeared to be significant compared to the parameters obtained from animals treated with CCl₄ only (Tab. 1).

When compared to the untreated animals from group I, the applied CCl₄ produced a significant increase in MDA and lung tissue concentrations of oxidized proteins (p < 0.001) (Figs 2A and B). The treatment with MLT taking place 1 h after CCl₄ administration prevented such a significant increase in these two parameters. However, its potential was limited since the detected concentrations of MDA and carbonylated proteins were still higher than those in group I (Figs 2A and B).

Discussion

Free radicals generated in the liver as well as in other tissues after the application of CCl₄ (4) react with polyunsaturated fatty acids present in cell and organelle membranes. Such enhancement in lipid peroxidation leads to the formation of products that can be...
measured in the reaction with thiobarbituric acid (23). The process of lipid peroxidation is tightly connected with the activity of GPx which uses GSH to terminate this reaction. A significant increase in the amount of lipid peroxidation products (MDA and 4-hydroxy-2-nonenal) causes enzyme inhibition (GPx and others) and decreases protein synthesis (24). Generally, due to excessive ROS caused by alveolar macrophage-borne enzyme to act and cause cell oxidative damage. MPO activity was slight, it leaves a “window” for this neutrophil/macrophage inflammation based on MPO activity and NO concentrations (Fig. 1 and Tab. 1), where a significant increase in inflammatory parameters in rat lungs was observed after CCl4 application. As in the case of studied antioxidant capacities, exogenous MLT partially ameliorated the inflammatory reaction in rat lung tissue (Fig. 1 and Tab. 1). Previous studies showed that MLT application prevents lung tissue neutrophil infiltration estimated based on MPO activity in mice with pulmonary fibrosis induced by bleomycin (11). This partial discrepancy in the findings might be attributed to different models of lung tissue damage, as well as to a different treatment regime, which in the mentioned study was sub-chronic while in ours, it was acute. Released MPO was found to be acting as a pro-inflammatory cytokine on alveolar and bronchial epithelial cells and leading to an increase in haemoxgenase-1 expression, thus causing lung tissue oxidative damage (29). Since the effect of MLT on MPO activity was slight, it leaves a “window” for this neutrophil/macrophage-borne enzyme to act and cause cell oxidative damage.

Besides lipid damage, the increased concentration of ROS oxidatively modifies cell proteins (forming carbonyl groups on protein side chains), thus leading to their conformational changes and impairing their function (26). This marker is suggested to be related to acute respiratory distress syndrome, where an increased concentration of carbonylated proteins are found in bronchoalveolar lavages (27). Previous studies suggest that when found in alveoli, these proteins cause a disturbance in protease/antiprotease balance and/or surfactant, or lead to mucus disorganization (27). This study is not the first one to show the potential of MLT in preventing the formation of carbonylated proteins (28). However, to the best of our knowledge, this is the first study showing the toxic potential of CCl4 in inducing protein oxidation in lung tissue (Fig. 2B).

The results related to CAT activity in lung tissue of animals exposed to CCl4 and treated with MLT contribute to the understanding of our previous findings. The increase in CAT activity previously reported to be seen in animals treated with MLT (11), or the preservation of its activity (spend) could lead to the expectation that the activity of GPx and GSH concentrations would be only partially affected. Apart from the disturbances in tissue oxidative capacities, CCl4 is known to cause tissue inflammatory response, and the lungs are not an exception (8). In the present work, we estimated lung tissue inflammation based on MPO activity and NO concentrations (Fig. 1 and Tab. 1), where a significant increase in inflammatory parameters in rat lungs was observed after CCl4 application. As in the case of studied antioxidant capacities, exogenous MLT partially ameliorated the inflammatory reaction in rat lung tissue (Fig. 1 and Tab. 1). Previous studies showed that MLT application prevents lung tissue neutrophil infiltration estimated based on MPO activity in mice with pulmonary fibrosis induced by bleomycin (11). This partial discrepancy in the findings might be attributed to different models of lung tissue damage, as well as to a different treatment regime, which in the mentioned study was sub-chronic while in ours, it was acute. Released MPO was found to be acting as a pro-inflammatory cytokine on alveolar and bronchial epithelial cells and leading to an increase in haemoxgenase-1 expression, thus causing lung tissue oxidative damage (29). Since the effect of MLT on MPO activity was slight, it leaves a “window” for this neutrophil/macrophage-borne enzyme to act and cause cell oxidative damage.

In the pathophysiological response of lungs to CCl4, macrophages seem to play an important role since they release numerous different lytic enzymes (MPO) and inflammatory signalling molecules (NO, TNF-α and IL-1 β) (8). An increase in these signalling molecules is known to be tightly connected to the increase in calcium-independent iNOS activity and NO which increases tissue blood flow by causing profound vasodilation (30, 31). An application of MLT in rats with damaged lung tissue effectively inhibited NO production and iNOS activity which is in accordance with a previously observed decrease in NO production by inhibition of iNOS expression via inhibition p300 histone acetyltransferase in macrophages (32). The hereby obtained results of the lung tissue arginase activity in rats treated with CCl4 and MLT are in accordance with previous studies, where possibly due to an increase in arginine concentrations following iNOS inhibition, the substrate upregulates the activity of the enzyme (arginase) by itself (33).

The activity of MLT in CCl4-mediated lung tissue damage model can occur in consequence of several factors. Having in mind that MLT was administered after intoxication with CCl4 in a single dose of 50 mg/kg, we can conclude that MLT most certainly possesses a significant protective potential. Since the application of CCl4 is causing multiorgan damage via a very similar, one may say identical mechanism, it is unrealistic to expect that a single dose of MLT would completely prevent multiorgan damage. Generally
speaking, MLT predominantly improved tissue antioxidant capacities, or prevented its decline, and decreased oxidative tissue damage that was estimated through the levels of MDA and carboxyl-modified proteins. Also, MLT inhibited the inflammatory response by mitigating the increase in MPO activity produced by the application of CCl₄.

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