Research Journal of Biological Sciences 6 (8): 381-385, 2011
ISSN: 1815-8846

Hepatoprotective Actions of Melatonin Against Methotrexate Induced Hepatic Injury in Animal Model

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Abstract: Methotrexate is one of the acid folic antagonists which are used widely as chemotherapy agent to treatment of kinds of leukemia and other malignancies. It has been suggested that the substance melatonin (5-methoxy-N-acetyltryptamine), discovered by Aaron Lerner in 1958, exists in almost every animal species and possibly even in all plants. In this study, 30 wistar rats were allocated into the 3 groups of 10 rats. Group 1 as control group received normal saline. Group 2 received MTX at the dose of 20 mg kg^{-1} as ip. Group 3 beside of MTX received melatonin at the dose of 25 mg kg^{-1} as oral. Results showed that AST and ALT serum levels and hepatic injury in that group received methotrexate were higher than control group and ALT serum level in that group received melatonin plus methotrexate was more than control group and was less than group 2. In this study revealed that melatonin has hepatoprotective effect against methotrexate induced hepatotoxicity in rats.

Key words: Hepatoprotection, melatonin, MTX, hepatotoxicity, rats, Iran

INTRODUCTION

Methotrexate is one of the acid folic antagonists which are used widely as chemotherapy agent to treatment of kinds of leukemia and other malignancies. One of its side effects is hepatotoxicity. Of its action mechanism in creation of the damages can be mention to the production of the free radicals consequence use of methotrexate therefore, several antioxidant agents have been used to reduce its side effects.

Methotrexate, a folic acid antagonist, interferes among other actions with the methylation of deoxyuridylate to form thyuridylate. This inhibition is brought about by blocking the action of the enzyme dihydrofolate reductase and preventing the formation of tetrahydrofolate, the latter being the coenzyme (as 5,10-methylene tetrahydrofolate) in the conversion of deoxyuridylate to thyuridylate.

Tetrahydrofolate is also essential for the de novo synthesis of the purine moiety of inosinic acid, the precursor for adenylic and guanylic acid. Folinic acid (5-formyltetrahydrofolate) will counteract the inhibitions caused by methotrexate. It has been suggested that the substance melatonin (5-methoxy-N-acetyltryptamine), discovered by Aaron Lerner in 1958, exists in almost every animal species and possibly even in all plants (Reiter *et al*., 2007; Pandi-Perumal *et al*., 2006). Its physiological functions are said to be diverse while melatonin may be involved in modifications of vasomotor tone (Doolen *et al*., 1998; Ting *et al*., 1997) and thermoregulation (Viswanathan *et al*., 1990), it is primarily known as the signal of darkness (Arendt, 1998). In vertebrates, melatonin is synthesized in the pineal gland and secreted during darkness as a hormonal message of the photoperiod (Korf *et al*., 1998).

The rhythm of melatonin synthesis is mainly driven by an oscillator which is situated in the hypothalamic Suprachiasmatic Nucleus (SCN) (Klein and Moore, 1979). This oscillator is usually entrained to a 24 h rhythm by environmental lighting conditions which are perceived in the retina by rods, cones and intrinsically photosensitive retinal ganglion cells (Reppert and Weaver, 2002). Based on the photoperiodic information transduced from the retina via the SCN to the pineal gland, melatonin is secreted during darkness after de-novo synthesis from Tryptophan (Sugden, 1989). This nocturnal melatonin signal is proportional to the length of the night thus, encoding not only circadian but also seasonal variations in the photoperiod (Goldman, 2001). In so-called photoperiodic animals like the Siberian hamster, these seasonal variations in melatonin output may have a profound influence on the regulation of reproduction

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(Reiter, 1980, 1993), prolactin secretion (Lincoln *et al*., 2003) as well as coat color (Niklowitz *et al*., 1994). The nocturnal secretion of melatonin is generally independent of an animal's active period in both nocturnal and diurnal species, melatonin levels rise during darkness (Arendt, 1998).

Melatonin synthesis is not exclusively located in the pineal gland but has also been described in numerous peripheral organs such as theretina (Tosini and Menaker, 1998), bone marrow (Conti *et al*., 2000), skin (Slominski *et al*., 2008), Harderian gland (Djeridane and Touitou, 2001), platelets (Champier *et al*., 1997), lymphocytes (Carrillo-Vico *et al*., 2004), testes (Tijmes *et al*., 1996) and in the gastrointestinal tract (Bubenik, 2002). Data on messenger RNA expression of two key enzymes responsible for melatonin synthesis, arylalkyamine-N-acetyltransferase and hydoxyindole-O-methyltransferase, suggest that even more peripheral organs may be able to produce this hormone (Stefulj *et al*., 2001).

So far, the physiological significance of extrapineal sites of melatonin synthesis remains unclear. However, besides its relevance in the time-keeping system, melatonin has been demonstrated to be a powerful radical scavenger (Reiter *et al*., 1997) it is tempting to assume that extrapineal melatonin may serve as a tissue protective agent.

MATERIALS AND METHODS

In this study, 30 wistar rats were allocated into the 3 groups of 10 rats. Group 1 as control group received normal saline. Group 2 received MTX at the dose of 20 mg kg^{-1} as ip. Group 3 beside of MTX received melatonin at the dose of 25 mg kg^{-1} as oral. This group, 3 days before administration of the MTX received melatonin after 6 days rats were euthanized and their liver was achieved to pathologic studies. Also, serum samples to measurement of AST and ALT were obtained. The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), Version 13.0 was used for statistical analysis. All data are presented as mean±SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Results showed that AST and ALT serum levels and hepatic injury in that group received MTX were higher than control group and ALT serum level in that group received melatonin plus MTX was more than control group and was less than group 2 ($p<0.05$) (Table 1, Fig. 1). AST serum value in that group received methotrexate was higher than control group and AST serum value in group 3 was more than control group and was less than group 2 ($p<0.05$) (Table 2, Fig. 2).

Table 1: ALT serum value in groups at the end of the experiment

Groups	ALT mg dL^{-1}
Control	23.40±3.120
MTX only	118.22±9.210
Melatonin plus MTX	72.90±5.380

ALT: Alanine Amino Transferase

Table 2: AST serum value in groups at the end of the experiment

Groups	AST mg dL^{-1}
Control	29.18±5.880
MTX only	126.59±11.21
Melatonin plus MTX	84.70±6.220

AST: Aspartate Amino Transferase

Table 3: Hepatic injury index in groups at the end of the experiment

Groups	Hepatic injury index
Control	0.36±0.26
MTX only	9.98±2.39
Melatonin plus MTX	5.56±1.88

Finally, results showed that hepatic injury in group 2 was more than control group and in that group received methotrexate plus melatonin however, was more than the group had not received methotrexate but was less than the group that received methotrexate alone ($p<0.05$) (Table 3, Fig. 3). Processes of acute inflammation, e.g., sepsis, hemorrhagic shock or ischemia/reperfusion, typically result in an imbalance of oxidative homeostasis with excess generation of Reactive Oxygen Species (ROS) and a relative deficiency of endogenous antioxidants this state is called oxidative stress. ROS include oxidants such as peroxynitrite and free radicals such as hydroxyl radicals and superoxide these substances are toxic and may induce Lipid Peroxidation (LPO) as well as protein, sugar and DNA degradation (Cuzzocrea and Reiter, 2002). The powerful antioxidant capacity of melatonin is usually attributed to its potential to eliminate free radicals by the donation of electrons (Poeggeler *et al*., 1994; Hardeland, 2005). For example, melatonin may neutralize hydroxyl radicals by forming 3-hydroxymelatonin which is excreted in the urine (Tan *et al*., 1998). Furthermore, melatonin was demonstrated to interact with toxic reactants like peroxyl radicals (Pieri *et al*., 1944), singlet oxygen species (Cagnoli *et al*., 1995) and hydrogen peroxide (Tan *et al*., 2001). Metabolites of melatonin, including the major hepatic metabolite 6-hydroxymelatonin as well as N-acety l-N-formy l-5-methoxykynuramine and N-acetyl-5-methoxykynuramine

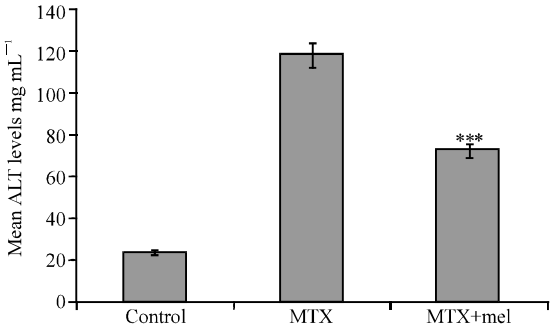

Fig. 1: The comparison amount of ALT between control group, MTX group, MTX+MEL group. Results are expressed as mean±SE. ***p<0.001 significantly different from the control group

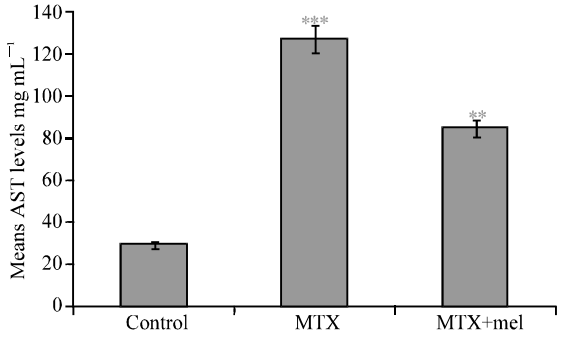

Fig. 2: The comparison amount of AST between control group, MTX group, MTX+MEL group. Results are expressed as mean±SE. ***p<0.001, **p<0.01 significantly different from the control group

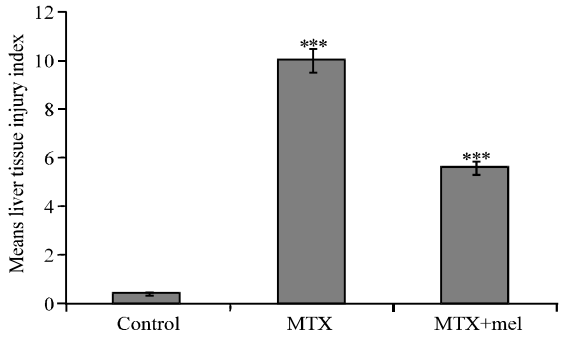

Fig. 3: The comparison rate of hepatic injury between control group, MTX group, MTX+MEL group. Results are expressed as mean±SE. ***p<0.001 significantly different from the control group

have been shown to detoxify radicals themselves (Guenther *et al*., 2005; Tan *et al*., 2007). This powerful pyramid scheme of radical scavenging has been named the antioxidant cascade of melatonin (Reiter *et al*., 2007; Tan *et al*., 2007). In addition to these direct interactions with ROS, melatonin may induce upregulation of the activity of antioxidants and antioxidant enzymes such as Superoxide Dismutase (SOD), Glutathione (GSH), Glutathione Peroxidase (GPx) and Glutathione Reductase (GSR) in the environment of oxidative stress (Rodriguez *et al*., 2004; Tomas-Zapico and Coto-Montes, 2005). In addition, the pineal hormone may induce downregulation of pro-oxidant enzymes like Nitric Oxide Synthase (NOS) (Bettahi *et al*., 1996; Pozo *et al*., 1997) and lipoxygenases (Zhang *et al*., 1999) thus, reducing the formation of Nitric Oxide (NO), superoxide anions and subsequently peroxynitrite anions. Both the direct detoxification of radicals as well as the modification of pro and antioxidative enzyme activities are thought to be relevant for the pineal hormone to act as a protective substance, for example when administered in models of oxidative stress.

This valuable effect appears to be independent of the type of injury and the species investigated. Exogenous melatonin may exhibit beneficial actions in a myriad of models of organ damage this is especially true for the liver. With respect to its hepatoprotective effects, countless publications have demonstrated that exogenous melatonin may be used successfully to treat a great variety of different pathophysiological conditions (Crespo *et al*., 1999).

Table 1 shows an overview of the hepatoprotective effects of exogenous melatonin administration without the pretension of being complete. Included in this summary are investigations mainly presenting a model of liver damage *in vivo*, evaluating parameters of hepatic integrity as a major endpoint and the administration of melatonin as the primary therapeutic agent. Studies on chronic disease development, aging, investigations on nutritional or dietary changes, exercise-induced stress, remote organ injuries with the liver as a secondary target as well as investigations on tumor development, cancer progression and liver metastases were excluded.

Based on this extraordinary pool of data, treatment with melatonin appears to be a versatile hepatoprotective strategy in models of experimental liver injury as demonstrated *in vivo* for rats, mice and chicks. There are remarkable variations concerning both the route of melatonin administration as well as the dose given, the latter ranging a thousand old from 100 μg kg^{-1} to 100 mg kg^{-1} melatonin. Only limited data are available on dose-response relationships and most studies did not include measurements of plasma melatonin levels. Furthermore, it should be mentioned that in some investigations, melatonin was given either as a single dose or repetitively in some publications for weeks as a pretreatment before or while the damage was induced.

CONCLUSION

Unfortunately, not all researchers used melatonin as a therapeutic substance following the infliction of damage although, this would be of high relevance for the evaluation of its clinical use. Nevertheless, all these studies show similar or even identical results concerning the hepatoprotective effects of treatment with melatonin. Improvements are consistently demonstrated for but not limited to parameters of antioxidant enzymes, hepatocellular integrity, interleukin response, NO signaling and survival.

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