PROTECTIVE EFFECT OF MELATONIN AGAINST METHOTREXATE INDUCED HEPATOTOXICITY IN MICE

Aamna Khokhar, Aisha Qayyum*, Muhammad Waqar Aslam Khan

Army Medical College/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Fazaia Medical College Islamabad Pakistan

ABSTRACT

Objective: To evaluate protective role of melatonin against methotrexate induced hepatotoxicity.
Study Design: Randomized controlled trial.
Place and Duration of Study: Department of Pharmacology Army Medical College, duration of the study was, from Apr to Aug 2016.
Material and Methods: Eighteen mice were randomly divided into three groups (n=6). Group (Gp)-1 received normal saline. Gp-2 received single intraperitoneal injection of methotrexate (MTX) while Gp-3 received melatonin along with MTX. Blood samples for measuring serum alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) along with liver samples for hepatic histological examination were taken after 24 hours of last dose.
Results: In Gp-2 MTX there was significant rise in serum ALT, AST and ALP as compared to its control gp (p<0.05). There was significant attenuation of serum ALT, AST and ALP with protective Gp-3 (MTX + Melatonin) when compared with Gp-2 (p<0.05). The histopathological findings in the liver of mice of Gp-2 MTX showed mild fatty changes which were markedly reduced in mice treated with melatonin along with MTX though minimal inflammation was seen.
Conclusion: Melatonin has hepatoprotective potential when administered along with methotrexate.

Keywords: Hepatotoxicity, Melatonin, Methotrexate.

INTRODUCTION

Methotrexate (MTX) is an antifolate subclass of antimetabolites. It is widely used in cancer chemotherapy as well as in autoimmune diseases, alone or in combination with other drugs. It is used in low doses (<50 mg/m2) in rheumatoid arthritis and in high doses (>500 mg/m2) in leukemias and in severe psoriasis resulting in hepatic fibrosis and cirrhosis[1].

The incidence of hepatotoxicity with mean dose of 12.5 mg/wk MTX when used for 3.5 to 4 years has been calculated for both elevated liver enzymes and liver biopsies. The incidence of Liver function tests (LFTs) above upper normal limit is 49% and 17% of rheumatoid arthritis patients have raised LFTs 2 to 3 times the upper normal limit. Liver biopsies showed 15.3% of patients with mild fibrosis, 1.3% with moderate to severe fibrosis and 0.5% with cirrhosis showing chronic liver damage[2,3].

Melatonin, a methoxyindole derivative of amino acid tryptophan, is a neuro-hormone that is secreted by the pineal gland. It is a potent antioxidant with immunomodulatory, anti-inflammatory, anti-cancerous, sleep inducing and anticonvulsant properties[4,5]. Therapeutically efficacy of melatonin has been shown in treatment of sleep disorders, acute pancreatitis, irritable bowel syndrome, ulcerative colitis, myocardial ischemia, neurodegenerative diseases, epilepsy, depression, aging, diabetes mellitus, rheumatoid arthritis, chronic pain disorders, infectious diseases and drug induced toxicities[6,7]. It is used as adjunct to cancer therapy due to its oncostatic, chemoprotective and

Correspondence: Dr Aamna Khokhar, Department of Pharmacology Army Medical College Rawalpindi Pakistan
Email: dr.aamnakhokhar@yahoo.com
Received: 14 Nov 2016; revised received: 11 Jan 2017; accepted: 18 Jan 2017

radioprotective properties[8]. In a review of melatonin role in hepatic damage, Chojnacki et al confirmed the beneficial effects of melatonin in non-alcoholic fatty liver disease (NAFLD) and the complications associated with partial resection of the liver in humans[9]. Moreover, Melatonin, due to its antioxidant properties, was used during the Fukushima Daiichi nuclear disaster in Japan in 2011 to prevent the damage caused by ionizing radiation[10].

The purpose of this study is to establish the hepatoprotective role of melatonin in MTX induced hepatotoxicity in mice.

MATERIAL AND METHODS

Methotrexate, Melatonin and other chemicals used were of the analytical grade from standard companies.

The study was a laboratory based randomized controlled trial which was carried out in animal house of the department of pharmacology and therapeutics, Army Medical College, Rawalpindi. The biochemical analysis of serum was performed with the collaboration of Department of Chemical Pathology, Army Medical College, Rawalpindi. Study protocol was approved by Ethical Committee of Centre for Research in Experimental and Applied Medicine (CREAM) Army Medical College, Rawalpindi. Eighteen (18) Balb/c male mice of age 8-12 weeks and weighing 30-40 grams were obtained from NIH National Institute of Health (NIH) Islamabad, Pakistan. The animals were kept in the animal house of Army medical college, Rawalpindi, for two weeks before the commencement of actual study for acclimatization to the new environment. Standard laboratory conditions of twelve hour light and dark cycle, 20-25^{o} C temperature and 70 ± 15% humidity were maintained. Mice were given same rodent pellet diet and tap water ad *libitum* for the entire time period.

Table: Comparative analysis of serum LFTs between group 1 (control) and group 2 (MTX) and between group 2 (MTX) and group 3 (MTX + Melatonin) mice.

Comparative groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Gp-1 (normal saline)	31.33 ± 3.28	87.83 ± 3.57	94.67 ± 4.91
Gp- 2 (MTX)	73.67 ± 3.67	128.50 ± 7.77	315.33 ± 12.44
p value	0.001*	0.001*	0.001*
Gp- 2 (MTX)	73.67 ± 3.67	128.50 ± 7.77	315.33 ± 12.44
Gp- 3 (MTX + Melatonin)	44.50 ± 4.46	95.17 ± 3.80	155.33 ± 8.83
p value	0.001*	0.002*	0.001*

p <0.05 highly significant (*)
p >0.05 non-significant (NS)

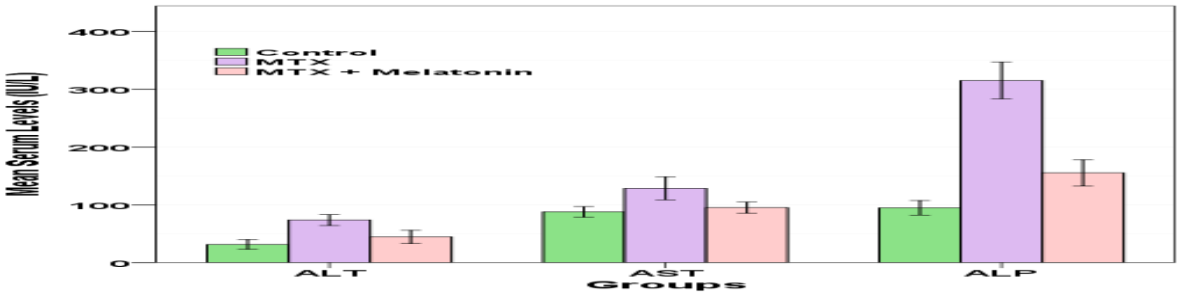

Figure-1: Graph showing comparison among different groups.

The animals were selected by non-probability convenience sampling method and divided randomly into 3 groups by lottery method. All groups contained 6 animals each. Group (Gp) 1 served as control and was given 0.2 ml normal saline intraperitoneally. Gp 2 served as toxic group and received single intraperitoneal injection of MTX 20 mg/kg[11]. Gp 3 received melatonin 10 mg/kg orally via oral gavage for 7 days[12] with MTX at day 4. Blood samples for measuring serum alanine aminotransferase test (ALT), aspartate aminotransferase test (AST) and alkaline phosphatase level test (ALP) along with liver samples for histopathological analysis were taken after 24 hours of last dose. Liver was fixed in 10% buffered formalin, processed, imbued with paraffin wax and sections were cut less than 3µ thick rotatory microtome. Tissue sections were then stained with Eosin and Haematoxylin dyes and examined thoroughly under light microscopy for histopathological changes.

Results were expressed as mean ± S.E.M. Statistical analysis was done on SPSS 23. One way ANOVA followed by Post Hoc Tukey Test was used for multiple comparisons of biochemical markers between the groups. The difference between two observations were considered as significant if the *p*-value was <0.05.

RESULTS

Total number of study animals was eighteen (each group n=6). One way Analysis of Variance (ANOVA) of serum ALT, AST, ALP among all the three groups was highly significant (p =0.001). Post Hoc Tukey Test showed significant rise in serum ALT, AST and ALP in Gp-2 (MTX) as compared to its control group (table, fig-1). There was significant attenuation of serum ALT, AST and ALP with protective Gp-3 (MTX + Melatonin) when compared with Gp-2 (MTX) (table-I, fig-1).

The histopathological findings in the liver of mice of Gp 2 (MTX) showed mild fatty changes which were markedly reduced in mice treated with melatonin along with MTX though minimal inflammation was seen (fig-2,3 & 4).

DISCUSSION

Rheumatoid arthritis (RA) is the third most common type of arthritis with the global prevalence of approximately 1 percent[13]. MTX is a commonly used disease modifying antirheumatic drug (DMARD), used in low doses. MTX, though relatively safe in low doses in RA, can cause myelosuppression, hepatotoxicity, gastrointestinal and mucocutaneous adverse effects[14]. The pooled data of 21 studies showed hepatotoxicity on low dose of MTX is the second common cause (18.5%) after gastrointestinal adverse effects resulting in 10–37% cases of treatment discontinuation[15].

The outcome of our study suggests that MTX treatment causes hepatic damage in mice after single intra peritoneal injection. Serum ALT, AST and ALP raised significantly as compared to its control group. ALT is a cytosolic enzyme of

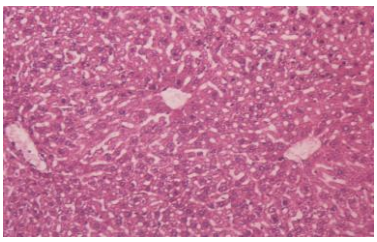

Figure-2: Photomicrograph of the liver tissue in group-I (Normal saline) showing normal hepatic architecture (H&E 100X).

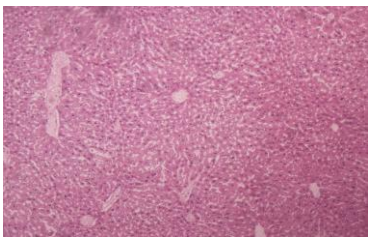

Figure-3: Photomicrograph of the liver tissue in group-2 (MTX) showing mild steatosis and focal nuclear pleomorphism (H&E 100X).

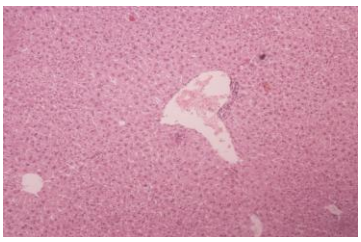

Figure-4: Photomicrograph of the liver tissue in group-3 (MTX + Melatonin) showing minimal inflammation around central vein (H&E 100X).

liver cells and its raised serum levels depict distortion in membrane permeability. It is the best marker of hepatic necrosis[11]. The raised levels of ALP more than that of ALT shows obstructive liver disease though no cholestasis is seen on histopathological analysis. It may be because of the single dose of MTX along with early sample collection. The findings are consistent with the Akbulut et al[16] and Tag[11] who also demonstrated elevation of LFTs with rodents treated with MTX in same dose and route of administration. The exact pathogenesis of MTX induced hepatotoxicity is still unclear though different mechanisms are thought to participate in causing liver damage like accumulation of polyglutamates in hepatocytes, depletion of hepatic folate store resulting in decrease nucleic acid synthesis, inhibition of methionine biosynthesis and resultant increased homocysteine leading to increase cellular sensitization to reactive oxygen species (ROS) and reactive nitrogen species (RNS) along with lipid peroxidation of biological membranes and microvascular derangement. High homocysteine levels, in addition to the oxidative stress, can also mediate endoplasmic reticulum (ER) stress which disturbs the metabolism of cholesterol and triglycerides resulting in fatty infiltration of liver[17-19]. MTX also decreases the availability of intracellular NADPH leading to depletion of cellular glutathione making cells more vulnerable to damage by ROS[20].

In our study, melatonin administration with MTX in mice showed hepatoprotective effects as depicted by significant attenuation of serum ALT, AST and ALP. The findings are in agreement with the study of Issabeagloo et al[21] which showed significant decrease in the levels of liver enzymes on administration of these two drugs to experimental rodents.

Melatonin exhibits its intrinsic free radical scavenging property by donating electron and hydrogen atom[22]. It is a suicidal antioxidant without any pro-oxidant potential because of stable, non-reactive end product formation, which is its unique characteristic being an antioxidant[23]. Melatonin also potentiates various anti-oxidant enzymes. Moreover, melatonin, stabilizes the mitochondrial electron transport chain boosting mitochondrial respiration and ATP synthesis and preventing the intra-mitochondrial glutathione loss and free radical generation[22]. These may be the mechanisms by which melatonin is effective for treating MTX induced hepatotoxicity.

Our study suggests that MTX causes substantial hepatic damage in mice which may be avoided by melatonin administration due to its anti-oxidant potential. However, further research is necessary to understand the mechanisms by which melatonin prevents liver damage against MTx toxicity.

CONCLUSION

Melatonin has hepatoprotective potential when administered along with methotrexate

ACKNOWLEDGEMENT

We are sincerely thankful to Pathology department, Army medical college for their support in running biochemical markers.

CONFLICT OF INTEREST

No conflict of interest to be declared by any author regarding this study.

REFERENCES

1. Bath RK, Brar NK, Forouhar FA, Wu GY. A review of methotrexate associated hepatotoxicity. Journal of digestive diseases 2014; 15(10): 517-24.
2. Visser K, Van der Heijde D. Risk and management of liver toxicity during methotrexate treatment in rheumatoid and psoriatic arthritis: a systematic review of the literature. Clin Exp Rheumatol 2009; 27(6): 1017-25.
3. Romão VC, Lima A, Bernardes M, Canhão H, Fonseca JE. Three decades of low-dose methotrexate in rheumatoid arthritis: Can we predict toxicity? Immunologic research 2014; 60(2-3): 289-310.
4. Hardeland R, Cardinali DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR. Melatonin - A pleiotropic, orchestrating regulator molecule. Progress in neurobiology 2011; 93(3): 350-84.
5. Jung KH, Hong SW, Zheng HM, Lee DH, Hong SS. Melatonin downregulates nuclear erythroid 2-related factor 2 and nuclear

factor-kappaB during prevention of oxidative liver injury in a dimethylnitrosamine model. Journal of pineal research 2009; 47(2): 173-83.
6. Carpentieri A, de Barboza GD, Areco V, López MP, de Talamoni NT. New perspectives in melatonin uses. Pharmacological Research 2012; 65(4): 437-44.
7. Reiter RJ, Paredes SD, Manchester LC, Tan DX. Reducing oxidative/nitrosative stress: a newly-discovered genre for melatonin. Critical reviews in biochemistry and molecular biology 2009; 44(4): 175-200.
8. Kostoglou-Athanassiou I. Therapeutic applications of melatonin. Therapeutic advances in endocrinology and metabolism 2013; 4(1): 13-24.
9. Chojnacki C, Walecka-Kapica E, Romanowski M, Chojnacki J, Klupinska G. Protective role of melatonin in liver damage. Current pharmaceutical design 2014; 20(30): 4828-33.
10. Reiter RJ, Tan DX, Korkmaz A, Manchester LC. The disaster in Japan: utility of melatonin in providing protection against ionizing radiation. Journal of pineal research 2011; 50(4): 357-8.
11. Tag HM. Hepatoprotective effect of mulberry (Morus nigra) leaves extract against methotrexate induced hepatotoxicity in male albino rat. BMC complementary and alternative medicine 2015; 15(1): 1.
12. Shokrzadeh M, Ahmadi A, Naghshvar F, Chabra A, Jafarinejhad M. Prophylactic efficacy of melatonin on cyclophosphamide-induced liver toxicity in mice. BioMed research international 2014.
13. Gibofsky A. Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. The American journal of managed care 2012; 18(13 Suppl): S295-302.
14. Albrecht K, M ler-Ladner U. Side effects and management of side effects of methotrexate in rheumatoid arthritis. Clinical and Experimental Rheumatology-Incl Supplements 2010; 28(5): S95.
15. Salliot C, van der Heijde D. Long term safety of methotrexate monotherapy in rheumatoid arthritis patients: a systematic literature research. Annals of the rheumatic diseases 2008.
16. Akbulut S, Elbe H, Eris C, Dogan Z, Toprak G, Otan E, et al. Cytoprotective effects of amifostine, ascorbic acid and N-acetylcysteine against methotrexate-induced hepatotoxicity in rats. World journal of gastroenterology: WJG 2014; 20(29): 10158.
17. Matte C, Stefanello FM, Mackedanz V, Pederzolli CD, Lamers ML, Dutra-Filho CS, et al. Homocysteine induces oxidative stress, inflammatory infiltration, fibrosis and reduces glycogen/glycoprotein content in liver of rats. International Journal of Developmental Neuroscience 2009; 27(4): 337-44.
18. De Carvalho SCR, Muniz MTC, Siqueira MDV, Siqueira ERF, Gomes AV, Silva KA, et al. Plasmatic higher levels of homocysteine in non-alcoholic fatty liver disease (NAFLD). Nutrition journal 2013; 12(1): 1.
19. Dai Y, Zhu J, Meng D, Yu C, Li Y. Association of homocysteine level with biopsy-proven non-alcoholic fatty liver disease: a meta-analysis. Journal of clinical biochemistry and nutrition 2016; 58(1): 76.
20. Paul M, Hemshekhar M, Thushara RM, Sundaram MS, NaveenKumar SK, Naveen S, et al. Methotrexate promotes platelet apoptosis via JNK-mediated mitochondrial damage: alleviation by N-acetylcysteine and N-acetylcysteine amide. PloS one 2015; 10(6): e0127558.
21. Issabeagloo E, Ahmadpoor F, Kermanizadeh P, Taghizadiyeh M. Hepatoprotective actions of melatonin against methotrexate induced hepatic injury in animal model. Res J Biol Sci 2011; 6: 381-5.
22. Reiter RJ, Tan DX, Galano A. Melatonin: exceeding expectations. Physiology 2014; 29(5): 325-33.
23. Pohanka M, Ruttkay-Nedecký B, Fusek J, Adam V, Kizek R. Melatonin regulates oxidative stress initiated by freund's complete adjuvant. Acta medica (Hradec Králové) 2015; 58(1): 21-4.