Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

Effect of Melatonin and Vitamin E on Amphotericin-B Induced Morphological Changes of Liver in Rabbits

Hossein Najafzadeh-Varzi,*[1] Hassan Morovvati[2]

1. Department of Pharmacology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran
2. Department of Histology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahwaz, Iran

Article information

Article history:
Received: 20 July 2011
Accepted: 26 Apr 2012
Available online: 18 Nov 2012
ZJRMS 2013; 15(5): 30-34

Keywords:
Amphotericin B
Melatonin
Vitamin E
Hepatotoxicity
Rabbit

*Corresponding author at:
Department of pharmacology and toxicology-faculty of Veterinary Medicine- Shahid Chamran University- Ahwaz-Iran
E-mail:
najafzadeh@scu.ac.ir

Abstract

***Background*:** Since melatonin has been effective in some studies to improve liver failure and yet its effect in reducing liver toxicity of amphotericin B was not evaluated, in the present study the effects of melatonin and vitamin E were compared in reducing liver toxicity caused by amphotericin B in rabbits.

***Materials and Methods*:** The study was performed on five groups of rabbits; one control group, and 4 amphotericin (1 mg/kg) receiving groups (2 to 5), group 3 received melatonin (1 mg/kg), group 4 received vitamin E (80 mg/kg), and group 5 received melatonin together with vitamin E. Alterations in enzyme activity of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase in serum and liver tissue were evaluated.

***Results*:** Results showed that administration of amphotericin had significantly increased the activity level of liver function related enzymes. But consumption of vitamin E and melatonin prevented this increase, while the combination of vitamin E and melatonin had a more significant effect on lactate dehydrogenase. Also compared with the control group, liver tissue damage and cellular damage were significant after amphotericin consumption and these changes were decreased by melatonin and vitamin E.

***Conclusion*:** Melatonin and vitamin E may prevent liver damage caused by amphotericin through their antioxidant properties.

Introduction

Amphotericin B binds to existing ergosterol in fungal cell membrane and provides pores and channels in the membrane that lead to leaking the cell contents out and death. Renal toxicity is considered as a serious side effect of amphotericin B. It causes renal vasoconstriction, decreased renal filtration, and tubular epithelial damage [1, 2]. But there are also some reports about liver amphotericin toxicity; for instance, Miller reported liver toxicity of amphotericin in a patient. Amphotericin consumption by this patient significantly increased alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and bilirubin levels while following drug withdrawal, their levels were returned to normal [3]. Also Ellis et al. reported severe liver damage after taking amphotericin [4].

Vitamin E protects cell membranes and acts as an antioxidant at cellular level. Its presence in lipid membrane prevents fatty acids oxidation by peroxides and free radicals that were produced during metabolic processes [2, 5].

Melatonin is the main hormone of pineal gland. This hormone has various physiological roles such as regulation of circadian rhythm, sleep and wake, mood, motor function, reproduction, tumor growth and so on [6, 7]. Melatonin has been considered as a pharmacological drug and has been used in the treatment of some diseases. Therefore, further understanding of physiological and pharmacological effects of this hormone as well as its chemotherapeutical, immunological and toxicological aspects is important (especially in terms of its antioxidant properties) [6].

Since melatonin has been effective in some studies to improve liver failure and yet its effect in reducing liver toxicity of amphotericin B was not evaluated, in the present study the effects of melatonin and vitamin E were compared in reducing liver amphotericin B toxicity in rabbits.

Materials and Methods

In this basic-applied experimental research, five groups of healthy rabbits weighing about 2 kg (6 rabbits in each group) were studied for 14 days as follows:

Group 1 or the control group was kept under almost identical conditions with the other groups but without any medication. Group 2 received 1 mg/kg amphotericin B intraperitoneally every other day [8, 9]. Group 3 received 1 mg/kg amphotericin B and 1 mg/kg melatonin intraperitoneally every other day [10, 11]. Normal saline was used as the solvent of melatonin. Group 4 received 1 mg/kg amphotericin B and 80 mg/kg vitamin E intraperitoneally every other day [12]. And group 5

received 1 mg/kg amphotericin B, 80 mg/kg vitamin E, and 1 mg/kg melatonin intraperitoneally every other day. It should be noted that the interval between administration of these drugs was short (less than 10 minutes) and when prepared the next drug was administered.

After 14 days, rabbits were easily killed and the liver samples were separated in maximum 0.5 cm dimensions and were fixed in 10% formalin-saline. They were then processed through routine histological preparations, including washing with running water, dehydration with ascending concentrations of ethanol, clearing with xylene solution, and embedding. After molding the samples with liquid paraffin, the casts were trimmed and cut up to 5 micrometer thick with a microtome, then stained through routine method of eosin-hematoxylin staining and the

At the end of the study, the rabbits were anesthetized with ketamine, the blood was sampled from the heart and its serum was separated and maintained at -20˚C until measurement of the enzymes. The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) enzymes were measured with a commercial kit (Pars Azmoon Iran Co.). The changes in different groups were statistically analyzed with SPSS-16 software. One-way variance analysis and LSD posttest were used for evaluating significant differences between groups.

Results

Data analysis showed that administration of amphotericin led to a significant increase in mean activity of liver function related enzymes (p=0.001). So that, the mean ALT was raised from 18 IU per liter in the control group to 40 units per liter. But vitamin E and melatonin consumption had prevented this increase (Fig. 1).

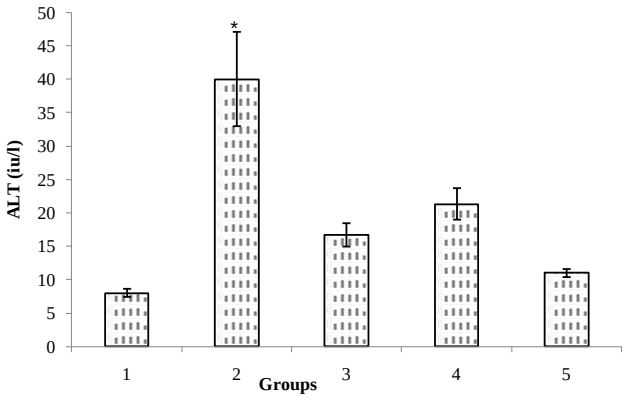

Figure 1. The Mean±SD of serum ALT levels in different groups of rabbits: 1- control, 2- amphotericin, 3- amphotericin+melatonin, 4- amphotericin+vitamin E, 5- amphotericin+melatonin+vitamin E. * Indicates significant difference with p=0.001 (N=6)

Results showed that administration of amphotericin significantly increased the mean AST enzyme activity in serum of the rabbits. So that, this mean was raised from 23 units per liter in control group to 56 units per liter in only amphotericin receiving group (p=0.0001). But vitamin E and melatonin consumption had prevented this increase (Fig. 2)

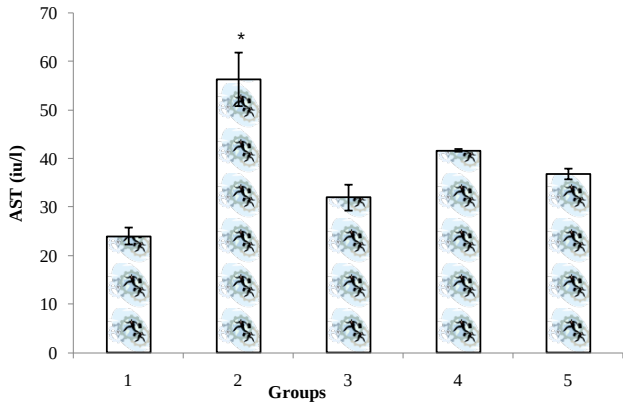

Figure 2. The Mean±SD of serum AST levels in different groups of rabbits: 1- control, 2- amphotericin, 3- amphotericin+melatonin, 4- amphotericin+vitamin E, 5- amphotericin+melatonin+vitamin E. * Indicates significant difference with p=0.0001 (N=6)

Also, analysis of the mean of ALP enzyme activity in rabbit serum revealed that amphotericin injection led to the increase of this enzyme. So that this mean was raised from 130 units per liter in control group to 490 units per liter in only amphotericin receiving group (p=0.0001). But vitamin E and melatonin consumption had prevented this increase (Fig. 3).

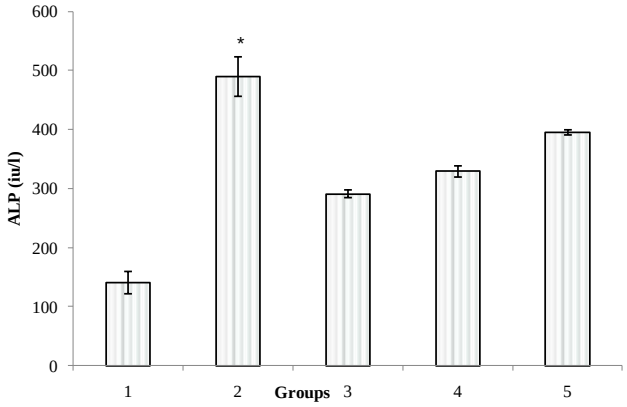

Figure 3. The mean±SD of serum ALT levels in different groups of rabbits: 1- control, 2- amphotericin, 3- amphotericin+melatonin, 4- amphotericin+vitamin E, 5- amphotericin+melatonin+vitamin E. * Indicates significant difference with p=0.0001 (N=6)

In addition, the results showed that administration of amphotericin significantly increased the mean AST enzyme activity in serum of the rabbits (p=0.009). So that, this mean was raised from 100 units per liter in the control group to 1200 units per liter in only amphotericin receiving group. But vitamin E and melatonin consumption had prevented this increase (Fig. 4).

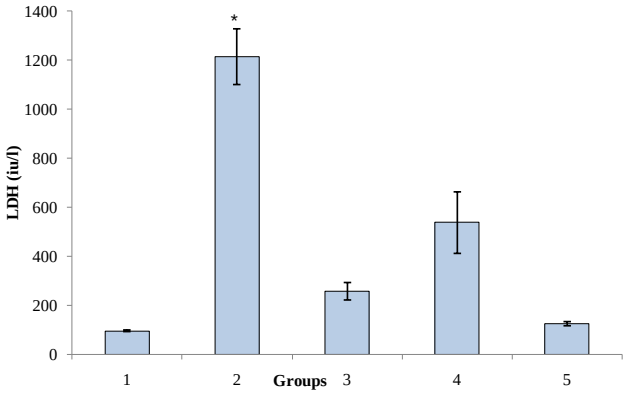

Figure 4. The Mean±SD of serum LDH levels in different groups of rabbits: 1- control, 2- amphotericin, 3- amphotericin+melatonin, 4- amphotericin+vitamin E, 5- amphotericin+melatonin+vitamin E. * Indicates significant difference between group 2 with other groups, with p=0.0001 (N=6)

Microscopic examination of rabbits' liver tissue sections in the control group (group 1) showed normal liver tissue, a view is depicted in figure 5.

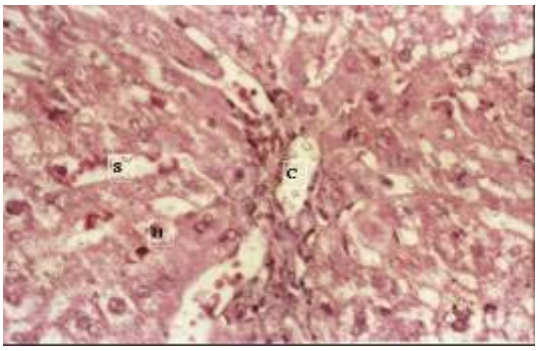

Figure 5. View of the liver tissue in the control group; 240-fold magnification and H & E stained (C: central vein, H: liver cells, and S: sinusoids)

Administration of amphotericin B in group 2 led to liver damage; disorganization of tissue structure and increased necrotic cells are shown in figure 6.

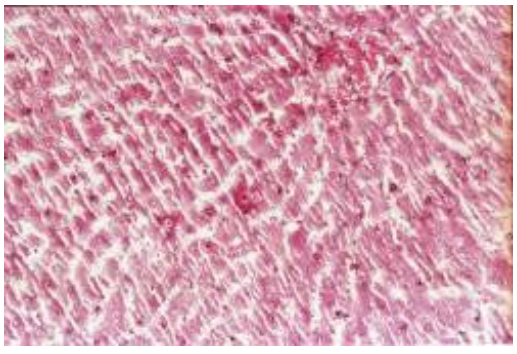

Figure 6. View of the liver tissue in group 2 (receiving amphotericin); 240-fold magnification and H & E stained

The use of melatonin as a protective substance prevented the liver tissue from severe damage in group 3 and could keep it in normal condition. Microscopic view of liver tissue in rabbits of group 3 is shown in figure 7.

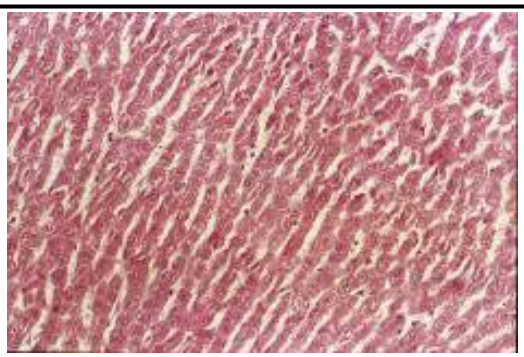

Figure 7. Microscopic view of the liver tissue in group 3 (receiving amphotericin plus melatonin); 240-fold magnification and H & E stained

The use of vitamin E as an antioxidant substance failed to prevent severe damage to liver tissue in group 4 and could not keep it in normal condition. Microscopic view of liver tissue in rabbits of group 4 is shown in Figure 8.

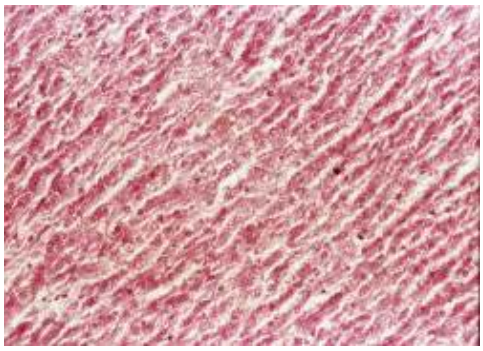

Figure 8. Microscopic view of the liver tissue in group 4 (receiving amphotericin plus vitamin E); 240-fold magnification and H & E stained

Combined consumption of melatonin and vitamin E in group 5 led to the most protective effect against liver damage caused by amphotericin; so that the severity of lesions compared with group 2 (the group receiving amphotericin alone) was lower.

Counting the number of necrotic cells in damaged liver tissue in rabbits with graticuled lens showed that amphotericin administration increased the number of these cells while melatonin reduced them. However, vitamin E failed to increase the number of cells damaged by amphotericin.

Discussion

The results of the present study showed that amphotericin B can damage the liver, and vitamin E and melatonin with their antioxidant properties can reduce this damage, although the relationship of these effects requires a review of the factors involved in oxidative stress and enzymes participated in antioxidative defense. Amphotericin is highly effective antifungal substance against systemic fungal infections but its side effects have limited its potential use. Studies indicated that amphotericin interferes with cytochrome P_{450} enzyme and therefore can reduce the metabolic capacity of the liver. The incidence of acute and subacute liver toxicity by amphotericin is very low [13].

In the literature review there are not enough studies regarding the effects of amphotericin on the liver, thus we will refer to some research done in this regard. In addition, a similar research to our empirical study was not found for further comparison. In a research, Chamilos et al. investigated the histopathology of the liver in amphotericin recipients. Histopathological findings in these individuals were similar to findings that previously described in animal and included multifocal necrosis of hepatocytes, fat accumulation and vacuolization of macrophages [14]. The above results are similar with the results of this study.

Gill et al. studied the concomitant consumption of amphotericin and itraconazole in a patient and observed a large increase in the levels of liver function related enzymes, this change was corrected after discontinuation of amphotericin, and they concluded that amphotericin can potentially create liver toxicity, especially if interfered with other medicines [15]. Lee et al. reviewed the effect of amphotericin on rabbits liver and observed that amphotericin in different doses of 0.5 to 10 mg/kg of body weight can cause a moderate liver toxicity [8].

Miller reported amphotericin liver toxicity in a patient. After receiving amphotericin B by this patient, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and bilirubin levels were significantly increased. With drug withdrawal, the levels of these factors were returned to normal [3]. Ellis et al. also reported severe liver damage after amphotericin consumption [4]. The results of mentioned studies are consistent with the results of the present study [4].

Ramaswam et al. studied the relationship between plasma cholesterol level and amphotericin toxicity and concluded that cholesterol affects the pharmacokinetics of amphotericin and reduces its toxicity [9]. In another study, Groll et al. evaluated the kinetics of liposomal amphotericin in rabbits [16].

When investigating liver toxicity of cyclosporine in rabbits, it was observed that vitamin E reduced liver damage through its antioxidant activity [17]. In their study, Tayal et al. observed that 100 mg/kg tocopherol had more protective effects than 50 mg/kg against the hepatotoxicity of isoniazid in rabbits [12]. The present study is consistent with the results of these two studies.

In a survey conducted by Abd-Elghaffar et al. antioxidant effects of melatonin were observed in reducing the neurological toxicity of aluminum [18].Winiarska et al. study showed the beneficial effect of melatonin in reducing oxidative stress and diabetes in rabbits [19]. Effect of melatonin on carbon tetrachloride hepatotoxicity in rats was observed by Ogeturk et al [10]. The result of the present study is similar to the results of mentioned studies, that melatonin and vitamin E, have a protective effect against liver amphotericin toxicity, possibly through their antioxidant properties.

The results of the present study showed that administration of amphotericin leads to a significant increase of activity levels of liver function related enzymes. But this increase was prevented by consumption of vitamin E and melatonin. Also, liver tissue alterations and cellular damage were significant after consumption of amphotericin, compared with the control group and these changes were reduced by melatonin and vitamin E.

Acknowledgements

The authors hereby appreciate and thank the Shahid Chamran University Research Deputy for grant provision.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Shahid Chamran University- Ahwaz-Iran.

References

1. Najafzadehvarzi H. [Antibiotics and antimicrobial agents] Persian. 1st
2. Najafzadehvarzi H, Minaiyan M. [Importance of physiological and pharmacological and therapeutic application of melatonin] Persian. Razi J 2003; 11(1): 10-13.
3. Abd-Elghaffar SK, El-Sokkary GH, Sharkawy AA. Aluminum-induced neurotoxicity and oxidative damage in rabbits: Protective effect of melatonin. Neurol Endocrinol Let. 2005; 26(5): 609-16.
4. Adam's, HR. Veterinary pharmacology and therapeutics. 8th ed. Iowa State University Press/ Ames; 2001: 955-956.
5. Burton GW, Traber MG. Vitamin E: Antioxidant activity, biokinetics and bioavailabity. Annu Rev Nutr 1990; 10: 357-82.
6. Chamilos G, Luna M, Lewis RE, et al. Effects of liposomal amphotericin B versus an amphotericin B lipid complex on liver histopathology in patients with hematologic malignancies and invasive fungal infections: A retrospective, nonrandomized autopsy study. Clin Ther 2007; 29(9): 1980-6.
7. Durak I, Ozbek H, Elgun S. Cyclosporine reduces hepatic antioxidant capacity: Protective roles of antioxidants. Int Immunopharmacol 2004; 4(3): 469-73.
8. Ellis M, Shamoon A, Gorka W, et al. Severe hepatic injury associated with lipid formulations of amphotericin B. Clin Infect Dis 2001; 32(5): E87-9.
9. Falcon J. Cellular circadian clocks in the pineal. Prog Neurobiol 1999; 58(2): 121-162.
10. Gill J, Sprenger HR, Ralph ED, and Sharpe MD. Hepatotoxicity possibly caused by amphotericin B. Ann Pharmacother 1999; 33(6): 683-5.
11. Groll AH, Mickiene D, Petraitis V, et al. Comparative drug disposition, urinary pharmacokinetics, and renal effects of multilamellar liposomal nystatin and amphotericin B deoxycholate in rabbits. Antimicrob Agents Chemother 2003; 47(12): 3917-3925.
12. Inselmann G, Inselmann U, Heidemann HT. Amphotericin B and liver function. Eur J Intern Med 2002; 13(5): 288-292.
13. Lee JW, Amantea MA, Francis PA, et al. Pharmacokinetics and safety of a unilamellar liposomal

formulation of amphotericin B (AmBisome) in rabbits. Antimicrob Agents Chemother. 1994; 38(4): 713-8.
14. Macchi M, Bruce J. Human pineal physiology and functional significance of melatonin. Front Neuroendocrinol 2004; 25(3-4): 177-95.
15. Miller MA. Reversible hepatotoxicity related to amphotericin B. Can Med Assoc J 1984; 131(10): 1245-7.
16. Ogeturk M, Kus I, Kavakli A, et al. Effects of melatonin on carbon tetrachloride-induced changes in rat serum. J Physiol Biochem 2004; 60(3): 205-10.
17. Ramaswamy M, Peteherych KD, Kennedy AL and Wasan KM. Amphotericin B Lipid complex or amphotericin b multiple-dose administration to rabbits with elevated plasma cholesterol levels: pharmacokinetics in plasma and blood, plasma lipoprotein levels, distribution in tissues, and renal toxicity. Antimicr Agents Chemother. 2001; 45(4): 1184-1191.
18. Tayal V, Kalra BS, Agarwal S, et al. Hepatoprotective effect of tocopherol against isoniazid and rifampicin induced hepatotoxicity in albino rabbits. Indian J Exp Biol 2007; 45(12): 1031-6.
19. Winiarska K, Fraczyk T, Malinska D, et al. Melatonin attenuates diabetes-induced oxidative stress in rabbits. J Pineal Res 2006; 40(2): 168-76.

Please cite this article as: Najafzadeh-Varzi H, Morovvati H. Effect of melatonin and vitamin E on amphotericin-B induced morphological changes of liver in rabbits. Zahedan J Res Med Sci (ZJRMS) 2013; 15(5): 30-34.