ORIGINAL ARTICLE

Protective Effect of Montelukast Sodium in Acute Ethyl Alcohol-induced Hepatic Injury in Rats

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ABSTRACT

Objective: *Ethyl alcohol (EA) is a substance that is used commonly worldwide and known to have toxic effects on the liver. The aim of this study was to investigate the effect of montelukast sodium (MK) on acute hepatopathy induced by a single dose of EA in rats.*

Methods: *The study consisted of four groups each containing eight Wistar albino male rats. The groups were classified as follows: the control group received distilled water; the EA group received 6 g/kg EA diluted with distilled water orally by gavage; the MK group received 30 mg/kg MK orally by gavage; the EA + MK group received, 2 hours after the EA administration, ie 30 mg/kg MK orally by gavage. After 24 hours, all the rats were sacrificed, and their blood and liver tissue samples were taken for biochemical and histopathological examinations.*

Results: *The administration of EA caused a statistically significant increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels compared with the control group (220.50 ± 66.90 and 92.38 ± 5.90 versus 84.88 ± 15.66 and 43.75 ± 10.22). The administration of EA + MK caused a statistically significant decrease in the AST and ALT levels compared with the EA alone group. Ethyl alcohol administered to the rats caused lesion in the liver including congestions, hydropic degeneration and irregular shaped area caused coagulation necrosis. The histopathological changes seen in the EA group were not detected in the EA + MK group.*

Conclusion: *Consequently, these data suggested that MK had beneficial effects in alleviating EA-induced hepatotoxicity in rats.*

Keywords: Ethyl alcohol, hepatotoxicity, montelukast sodium, rat.

INTRODUCTION

Acute alcohol poisoning has been diagnosed with increasing frequency in recent years in emergency departments (1). The first-line treatment for ethyl alcohol (EA) poisoning involved accelerating the elimination and excretion of EA, which was similar to the treatments for other drug intoxications. The goal of the treatment of EA poisoning is to protect the organs, especially the liver, as EA is mostly metabolized by the hepatic enzyme, alcohol dehydrogenase. The treatment is often supportive and aimed at reducing symptoms (2).

Montelukast sodium (MK) is a prototypic pharmacologic antagonist of selective type-1 cysteinyl leukotriene (CysLT) receptors (3). Human studies had shown that CysLTs played significant roles in the pathogenesis of alcohol intoxication (4), bile duct obstruction (5), hepatitis B (6), hepatorenal syndrome (7), liver cirrhosis, and other diseases (8). Additionally, the data from experimental studies had shown that the production of CysLT increased in certain situations, such as CCl4-induced hepatopathy (9), alcoholic hepatopathy (10), polysaccharide-induced liver injury (11), hepatic ischemia

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reperfusion (12), liver cirrhosis (13), and liver allograft rejection (14). Montelukast sodium antagonized the pathways activated by CysLTs, such as the proasthmatic, proinflammatory and priming pathways adequately (15). Furthermore, there is evidence that MK acted as an anti-inflammatory in a manner that was independent of CysLT antagonism, through its interactions with corticosteroid-insensitive neutrophils (16–19).

The aim of this study was to determine whether MK supplementation could attenuate acute EA-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals and experimental procedures

Experiments were carried out using male Wistar albino rats (n = 32) weighing 240–280 g, which were obtained from the Dicle University Health Sciences and Research Centre (Diyarbakır, Turkey). The animals were placed in timbered lattices that were 14 × 9 × 8 cm in size. Before and throughout the experimental procedure, all the animals were fed standard lab rat chow and water *ad libitum* and were kept in a temperature-controlled room (21˚C) with 12 hours light/dark cycles. All the food, except the water, was withheld 6 hours prior to the experiment. This study was performed in accordance with the guidelines for animal research and approved by the Ethical Committee of the Dicle University (2012/33). The rats were classified into four groups of eight animals each. The experimental groups were designed as follows: the control group received distilled water; the EA group received 6 g/kg EA diluted with distilled water orally by gavage; the MK group received 30 mg/kg MK orally by gavage; EA + MK group received, 2 hours after EA administration, the 30 mg/kg MK orally by gavage. Ethyl alcohol was obtained from Merck Chemical, Inc. (Darmstadt, Germany). Montelukast sodium was purchased from Sanovel Drug Company, Turkey. Distilled water was given to the control group via gastric gavage. Ethyl alcohol diluted with distilled water (6 g/kg) was given to rats by gastric gavage (20, 21). This particular EA dose was chosen because it was the maximum tolerated dose, based on clear signs of toxicity with little or no lethality. Two hours after the EA administration, MK (30 mg/kg; Notta tb 10 mg, Sanovel, Turkey) diluted with distilled water was given by gastric gavage (22).

Ketamine hydrochloride (50 mg/kg intramuscularly) was used for anaesthesia 24 hours after the experiment. All the surgical procedures were performed with rats in the supine position. A midline incision was used for the laparotomy. The liver and blood samples were taken from all rats using 20 gauge injection syringes. The exsanguination method was used to sacrifice the rats at the end of the procedure.

Biochemical analyses

The rats' food was obtained via the intracardiac route and was centrifuged at 3000 rpm for 10 minutes in order to separate it, and the samples were stored at –70ºC for later analyses. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), direct bilirubin and total bilirubin levels were determined with Abbott Architect c16000 Autoanalyzer (Abbott Diagnostics, Abbott Park, IL, USA), and the assay results were expressed in U/L.

Histopathological analyses of liver tissue

In order to perform the histopathological evaluation, the hepatic samples were fixed in a 10% formalin solution for 48 hours. Then the samples were embedded in paraffin and the sections (with thickness of 4–5 μm) were stained with haematoxylin–eosin (H&E). The histopathological examinations were done by a pathologist who was blinded to the study groups.

Statistical analyses

Statistical analyses were performed using SPSS for Windows Version 11.0 (SPSS Inc., Chicago, IL, USA). The data were presented as means (minimum, maximum) for the biochemical variables. The Chi-square test was used for the categorical variables. The groups were compared using the non-parametric Kruskal–Wallis test. The Mann–Whitney *U* test was used for the binary comparisons. The Spearman correlation test was used to evaluate the relationships between the numerical variables. A p value of less than 0.05 was considered statistically significant.

RESULTS

Biochemical analyses

The serum AST, ALT and ALP levels in all the groups are shown in the Table. The serum AST and ALT levels were statistically significantly higher in the EA group compared with the other groups ($p < 0.05$ and $p < 0.001$, respectively). The administration of the MK in the EA + MK group led to the lower increase in the serum AST and ALT levels compared with the EA group. There were no statistically significant differences in the serum ALP activity and the levels of direct and total bilirubin between any of the groups ($p > 0.05$).

Table: Effect of montelukast sodium on serum liver enzymes in control and experimental groups (n = 8)

Parameters	Groups				
	Control	Ethyl alcohol	Montelukast sodium	Ethyl alcohol + Montelukast sodium	*p*
AST (U/L)	84.88 ± 15.66[a]	220.50 ± 66.90[b]	129.13 ± 39.86[a]	115.63 ± 27.38[a]	0.005
ALT (U/L)	43.75 ± 10.22[a]	92.38 ± 5.90[c]	53.13 ± 12.97[a]	65.38 ± 9.03[b]	0.001
ALP (U/L)	169.88 ± 32.90	199.75 ± 56.95	147.88 ± 30.09	159.13 ± 34.26	NS

AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, NS = not significant.
The data were expressed as mean ± standard deviation. Mean values within the same row with different superscript letters a, b and c are significantly different.

Histopathological analyses

Histopathological studies showed that the control group animals showed no pathology (Fig. 1A). Only the MK administrated group had similar appearance with the control group's animals' liver (Fig. 1B). The EA administered rat caused lesions in the rats' liver including congestions, hydropic degeneration and irregular shaped area caused by coagulation necrosis (Fig. 1C, 1D). The liver was almost normal in appearance with light necrosis of the rats treated with EA + MK (Fig. 1E).

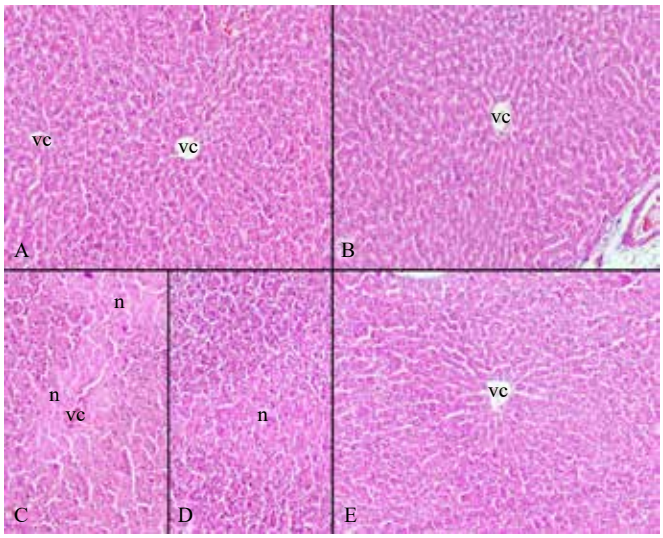

Figure: (A) Normal structure of liver in control group. Vc: Vena centralis. Heamatoxylin and eosin. Magnification ×200. (B) Stained sections of liver of montelukast sodium group. Heamatoxylin and eosin. Vc: Vena centralis. Magnification ×200. (C and D) Ethyl alcohol administered rat liver. Severe necrosis (n) were seen. Heamatoxylin and eosin. Magnification ×200. (E) Ethyl alcohol + montelukast sodium group. Structures of liver were similar to the control group. Heamatoxylin and eosin. Magnification ×100.

DISCUSSION

Alcohol is widely used, especially in Western countries. It also represents the oldest and the most diffuse substance of abuse (23). Acute alcohol intoxication is a clinically harmful condition that usually follows the ingestion of a large quantity of alcohol (24). Therefore, acute EA intoxication and EA-related diseases such as acute alcoholic hepatitis are serious problems for the emergency services departments (25). The quantity of alcohol in a standard drink varies from country to country (26). Naimi *et al* (27) defined binge drinking as the consumption of 5 ≥ alcoholic drinks on a single occasion within 2 hours, which usually results in acute intoxication. In humans and experimental animal models, acute alcohol consumption can lead to a variety of pathologies, including the motility disorders of the oesophagus, stomach and duodenum, acute alcoholic hepatitis and even death due to central nervous system depression, depending on the quantity of the alcohol consumed (24, 28, 29).

The liver is the main site of alcohol metabolism and the major target of alcohol-induced organ damage. The sensitivity of the liver to alcohol-related toxicity depends on both the concentration of the alcohol in the portal blood and the metabolism of EA (28). Cell damage in any one of numerous organs is followed by the release of a number of cytoplasmic enzymes into the blood, providing a basis for clinical diagnosis (30). The increases in plasma AST, ALT and ALP activities are mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (31), indicating the hepatotoxic effect of EA. Some biochemical parameters, such as ALT activity, are correlated with hepatic necrosis in rats and indicate the alteration in hepatic functions (32). Both AST and ALT are released into the circulation from damaged hepatocytes, resulting in the elevations in AST and ALT which are classic findings of hepatotoxicity (33). In many experimental rat studies, an increase in the ALT level was seen after 5–6 mg/kg of ethanol was administered, indicating liver damage had occurred (20, 21, 28, 29). Similar to the literature, we found significantly increased levels of ALT and AST in the ethyl alcohol given group.

It is important to develop a better understanding of the mechanism of alcoholic liver damage in order to develop new drugs that can decrease alcoholic liver disease (ALD), improve past liver injury and potentially cure the livers of chronic alcoholics. Peters-Golden *et al* (3) found that MK antagonized the alcoholic hepatopathy mediated by CysLT. In an experimental rat study of CCl4-induced hepatotoxicity, it was found that MK reversed the increase in the biochemical parameters such as AST and ALT (34). In our study, we similarly found that the EA plus MK given group had lower AST and ALT levels compared with the EA group.

Alcohol causes ALD, which is characterized by steatosis (fatty liver), steatohepatitis and, in severe cases, fibrosis and/or cirrhosis in both humans and experimental animal models (35–37). However, some pathological changes were seen in liver tissue samples of the EA group, such as increased sinusoidal congestion and haemorrhage and degenerated hepatocytes. Furthermore, the present study found that liver tissue was generally normal in structure in the EA plus MK treated group, in contrast to the abnormal changes seen in the EA group.

This study was limited because it did not have a large sample size and was conducted in an artificial laboratory setting.

CONCLUSION

Our study showed that MK administration alleviated in some biochemical and histopathologic parameters changed by the EA administration. However, further, randomized clinical trials are necessary to verify the beneficial effects of MK in preventing EA-mediated hepatotoxicity before its use in clinical practice. We believe that this is a cornerstone study that will help provide the direction for future experimental animal models of EA-induced liver injury.

AUTHORS' NOTE

The authors declare that they have no conflicts of interest.

REFERENCES

1. Alikaşifoğlu M, Ercan O. Ergenlerde madde kullanımı. Turk Pediatri Arşivi 2002; **37:** 66–73.
2. Marco CA, Kelen GD. Acute intoxication. Emerg Clin North Am 1990; **8:** 731–48.
3. Peters-Golden M, Henderson WR. Leukotrienes. N Engl J Med 2007; **357:** 1841–54.
4. Uemura M, Lehmann WD, Schneider W, Seitz HK, Benner A, Keppler-Hafkemeyer A. Enhanced urinary excretion of cysteinyl leukotrienes in patients with acute alcohol intoxication. Gastroenterology 2000; **118:** 1140–8.
5. Richter L, Hesselbarth N, Eitner K, Schubert K, Bosseckert H, Krell H. Increased biliary secretion of cysteinyl-leukotrienes in human bile duct obstruction. J Hepatol 1996; **25:** 725–32.
6. Kasirga E, Coker I, Aydogdu S, Yagci RV, Taneli B, Gousseinov A. Blood levels of leukotrienes (LTC4, D4, E4, B4) and synthesis of leukotriene B4 by peripheral leukocytes in children with acute A and B hepatitis. Turk J Pediatr 1999; **41:** 457–65.
7. Capella GL. Anti-leukotriene drugs in the prevention and treatment of hepatorenal syndrome. Prostaglandins Leukot Essent Fatty Acids 2003; **68:** 263–5.
8. Huber M, Kastner S, Scholmerich J, Gerok W, Keppler D. Analysis of cysteinyl leukotrienes in human urine: enhanced excretion in patients with liver cirrhosis and hepatorenal syndrome. Eur J Clin Invest 1989; **19:** 53–60.
9. Nagai H, Shimazawa T, Yakuo I, Aoki M, Koda A, Kasahara M. Role of peptideleukotrienes in liver injury in mice. Inflammation 1989; **13:** 673–80.
10. Satoh S, Uetake S, Ohata M, Nakajima H, Yamauchi M. Effect of type of dietary fat and ethanol on hepatic leukotriene level in experimental alcoholic liver disease. Nihon Arukoru Yakubutsu Igakkai Zasshi 2003; **38:** 350–63.
11. Kawada N, Mizoguchi Y, Sakagami Y, Kobayashi K, Yamamoto S, Morisawa S. Changes in leukotrienes and prostaglandins in the liver tissue of rats in the experimental massive hepatic cell necrosis model. Prostaglandins Leukot Essent Fatty Acids 1990; **40:** 149–55.
12. Takamatsu Y, Shimada K, Chijiiwa K, Kuroki S, Yamaguchi K, Tanaka M. Role of leukotrienes on hepatic ischemia/reperfusion injury in rats. J Surg Res 2004; **119:** 14–20.
13. Graupera M, Garcia-Pagan JC, Titos E, Clara J, Massaguer A, Bosch J. 5-Lipoxygenase inhibition reduces intrahepatic vascular resistance of cirrhotic rat livers: a possible role of cysteinyl-leukotrienes. Gastroenterology 2002; **122:** 387–93.
14. Gonzalez R, Ancheta O, Marquez M, Rodriguez S. Hepatoprotective effects of diethylcarbamazine in acute liver damage induced by carbon tetrachloride in rats. Zhongguo Yao Li Xue Bao 1994; **15:** 495–7.
15. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M et al. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008; **31:** 143–78.
16. Ramires R, Caiaffa ME, Tarsi A, Haeggström JZ, Macchia L. Novel inhibitory effects on 5-lipoxygenase activity by the anti-asthma drug montelukast. Biochem Biophys Res Commun 2004; **324:** 815–21.
17. Tahan F, Jazrawi T, Rovati GE, Adcock IM. Montelukast inhibits tumour necrosis factor-α-mediated interleukin-8 expression through inhibition of nuclear factor-κB p65-associated histone acetyltransferase activity. Clin Exp Allergy 2008; **38:** 805–11.
18. Anderson R, Theron AJ, Gravett CM, Steel HC, Tintinger GR, Feldman C. Montelukast inhibits neutrophil pro-inflammatory activity by a cyclic AMP-dependent mechanism. Br J Pharmacol 2009; **156:** 105–15.
19. Robinson AJ, Kashanin D, O'Dowd F, Williams V, Walsh GM. Montelukast inhibition of resting and GM-CSF-stimulated eosinophil adhesion to VCAM-1 under flow conditions appears independent of CysLT1R antagonism. J Leukoc Biol 2008; **83:** 1522–9.
20. Gershman H, Steeper J. Rate of clearance of ethanol from the blood of intoxicated patients in the emergency department. J Emerg Med 1991; **9:** 307–11.
21. Carson EJ, Pruett SB. Development and characterization of a binge drinking model in mice for evaluation of the immunological effects of ethanol. Alcohol Clin Exp Res 1996; **20:** 132–8.
22. Jones TR, Labelle M, Belley M, Champion E, Charette L, Evans J et al. Pharmacology of montelukast sodium (Singulair™), a potent and selective leukotriene D4 receptor antagonist. Can J Physiol Pharmacol 1995; **73:** 191–201.
23. Lieber CS. Medical disorders of alcoholics. N Engl J Med 1995; **333:** 1058–65.
24. Vonghia L, Leggio L, Ferrulli A, Bertini M, Gasbarrini G, Addolorato G. Acute alcohol intoxication. Eur J Intern Med 2008; **19:** 561–7.

25. Wildt BT, Andreis C, Auffahrt I, Tettenborn C, Kropp S, Ohlmeier M. Alcohol related conditions represent a major psychiatric problem in emergency departments. J Emerg Med 2006; **23:** 428–30.
26. Altıntoprak AE, Kayahan B, Tezcanlı B, Kosova B, Coşkunol H. Catechol-O-methyltransferase Val108/158Met gene and alcoholism in Turkish subjects. Turk J Med Sci 2012; **42:** 289–97.
27. Naimi TS, Brewer RD, Mokdad A, Denny C, Serdula MK, Marks JS. Binge drinking among US adults. JAMA 2003; **289:** 70–5.
28. Massey VL and Arteel GE. Acute alcohol-induced liver injury. Front Physiol 2012; **3:** 193–200.
29. Siegmund S, Haas S, Schneider A, Singer MV. Animal models in gastrointestinal alcohol research—a short appraisal of the different models and their results. Best Pract Res Clin Gastroenterol 2003; **17:** 519–42.
30. Sundberg A, Appelkwist EL, Dallner G, Nilsson R. Glutathione transferase in urine: sensitive methods for detection of kidney damage induced by nephrotoxic agents in human. Environ Health Persp 1994; **102:** 293–6.
31. Navarro CM, Montilla PM, Martin A, Jimenez J, Utrilla PM. Free radicals scavenger and antihepatotoxic activity of Rosmarinus. Plant Med 1993; **59:** 312–4.
32. Thrall MA, Weiser G, Allison R, Campbell T. Mammalian hematology: laboratory animals and miscellaneous species, in Campbell TW, ed. Veterinary hematology and clinical chemistry. Malden, MA: Blackwell Publishing; 2012: 225–37.
33. Hung OL, Nelson LS. Acetaminophen. In: Tintinalli JE, Kelen GD and Stapczynski JS, eds. Emergency medicine: a comprehensive study guide. New York, NY: McGraw-Hill; 2004: 1088–94.
34. Cuciureanu M, Caruntu ID, Paduraru O, Stoica B, Jerca L, Crauciuc E et al. The protective effect of montelukast sodium on carbon tetrachloride induced hepatopathy in rat. Prostaglandins Other Lipid Mediat 2009; **88:** 82–8.
35. Crabb DW. Pathogenesis of alcoholic liver disease: newer mechanisms of injury. Keio J Med 1999; **48:** 184–8.
36. Kaiser JP, Beier JI, Zhang J, David HJ, Montfort C, Guo L et al. PKC epsilon plays a causal role in acute ethanol- induced steatosis. Arch Biochem Biophys 2009; **482:** 104–11.
37. Donohue TM, Osna NA, Trambly CS, Whitaker NP, Thomas PG, Todero SL et al. Early growth response-1 contributes to steatosis development after acute ethanol administration. Alcohol Clin Exp Res 2012; **36:** 759–67.

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