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: Control group received distilled water; ethyl alcohol group received 6 g/kg ethyl alcohol diluted with distilled water orally by gavage; montelukast sodium group received 30 mg/kg montelukast sodium orally by gavage; ethyl alcohol+montelukast sodium group received, two hours after ethyl alcohol administration, 30 mg/kg montelukast sodium orally by gavage. After 24 hours, all rats were sacrificed and blood and liver tissue samples were taken for biochemical and histopathological examination.

Results: The administration of EA caused a statistically significant increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels compared to 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 ethyl alcohol+montelukast sodium caused a statistically significant decrease in AST and ALT levels compared to the EA alone group. Ethyl alcohol administered rat caused lesion in liver including congestions, hydropic degeneration and irregular shaped area caused coagulation necrosis. The histopathological changes seen in ethyl alcohol group were not detected in ethyl alcohol+montelukast sodium group.

Conclusion: Consequently, these data suggested that MK had beneficial effects in alleviating ethyl alcohol-induced hepatotoxicity.

Keywords: Ethyl alcohol; hepatotoxicity; montelukast sodium; rat

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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 involves accelerating the elimination and excretion of EA, which is similar to the treatments for other drug intoxications. The goal of treatment of EA poisoning is to protect the organs, especially the liver, as EA is mostly metabolized by the hepatic enzyme, alcohol dehydrogenase. 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 have shown that CysLTs play 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, data from experimental studies have shown that the production of CysLT increases in certain situations, such as CCl4-induced hepatopathy (9), alcoholic hepatopathy (10), polysaccharide-induced liver injury (11), hepatic ischemia reperfusion (12), liver cirrhosis (13) and liver allograft rejection (14). Montelukast sodium antagonizes pathways activated by CysLTs, such as the proasthmatic, proinflammatory and priming pathways adequately (15). Furthermore, there is evidence that MK acts as an anti-inflammatory in a manner that is independent of CysLT-antagonism, through interaction with corticosteroid-insensitive neutrophils (16-19).

The aim of this study was to determine whether montelukast sodium supplementation could attenuate acute ethyl alcohol-induced hepatotoxicity in rats.

METHODS

Animals and experimental procedures

Experiments were carried out using male Wistar albino rats (n=32) weighing 240-280 g, were obtained from the Dicle University Health Sciences and Research Center (Diyarbakır, Turkey). Animals were placed in timbered lattices that were 14×9×8 cm in size. Before and throughout the experimental procedure, all animals were fed standard lab rat chow and water *ad lib* and were kept in a temperature-controlled room (21 ◦C) with 12h light/dark cycles. All food, except for water, was withheld 6 h 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. Experimental groups were designed as follows: Control group received distilled water; ethyl alcohol group received 6 g/kg ethyl alcohol diluted with distilled water orally by gavage; montelukast sodium group received 30 mg/kg montelukast sodium orally by gavage; ethyl alcohol+montelukast sodium group received, two hours after ethyl alcohol administration, 30 mg/kg montelukast sodium 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 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 anesthesia 24 hours after the experiment. All surgical procedures were performed with rats in the supine position. A midline incision was used for the laparotomy. 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

Blood was obtained via the intracardiac route and was centrifuged at 3000 rpm for 10 minutes in order to separate it and samples were stored at -70 ºC 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 and the assay results were expressed in U/L.

Histopathological analysis of liver tissue

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

Statistical Analysis

Statistical analysis was performed using SPSS for Windows 11.0 (SPSS Inc., Chicago, IL, USA). Data were presented as mean (minimum, maximum) for biochemical variables. The Chi-square test was used for categorical variables. Groups were compared using the nonparametric Kruskal-Wallis test. The Mann-Whitney U test was used for binary comparisons. The Spearman correlation test was used to evaluate relationships between numerical variables. A p value of less than 0.05 was considered significant.

RESULTS

Biochemical analyses

The serum AST, ALT and ALP levels in all groups are shown in Table 1. The serum AST and ALT levels were significantly higher in the ethyl alcohol group compared to the other groups ($p<0.05$ and $p<0.001$, respectively). Administration of MK in the ethyl alcohol+montelukast sodium group led to less rise in serum AST and ALT levels compared to the ethyl alcohol group. There were no statistically significant differences in serum ALP activity and levels of direct and total bilirubin between any of the groups ($p>0.05$).

Histopathological analyses

Histopathological studies showed that control group animal showed no pathology (Figure 1A). And only montelukast sodium administrated group had similar appearance with control group animal liver (Figure 1B). Ethyl alcohol administered rat caused lesion in liver including congestions, hydropic degeneration and irregular shaped area caused coagulation necrosis (Figure 1C, 1D). The liver was almost has normal appearance with light necrosis of rats treated with ethyl alcohol+montelukast sodium (Figure 1E).

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 amount of alcohol (24). For this reason, acute EA intoxication and EA-related diseases such as acute alcoholic hepatitis are serious problems for emergency services departments (25). The amount of alcohol in a standard drink varies from country to country (26). Naimi *et al.* (27) defined binge drinking as consumption of ≥5 alcoholic drinks on a single occasion within 2 hours, which usually results in acute

intoxication. In human and experimental animal models, acute alcohol consumption can lead to a variety of pathology, including motility disorders of the esophagus, stomach and duodenum, acute alcoholic hepatitis and even death due to central nervous system depression, depending on the amount of 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 alcohol in the portal blood and the metabolism of EA (28). Cell damage in any one of several organs is followed by 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 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, which is correlated with hepatic necrosis in rats and indicates alteration in hepatic functions (32). AST and ALT are released into the circulation from damaged hepatocytes, thus elevations in AST and ALT 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 montelukast sodium antagonizes the alcoholic hepatopathy mediated by CysLT. In an experimental rat study of CCl4-induced hepatotoxicity, it was found that MK reversed the increase in biochemical parameters such as AST and ALT (34). In our study we

similarly found that the ethyl alcohol plus montelukast sodium given group had lower AST and ALT levels compared to the ethyl alcohol group.

Alcohol causes alcoholic liver disease, 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). Although some pathological changes were seen in liver tissue samples of the ethyl alcohol group, such as increased sinusoidal congestion and hemorrhage and degenerated hepatocytes. Furthermore, the present study found that liver tissue was generally normal structure in the ethyl alcohol plus montelukast sodium treated group, in contrast to the abnormal changes seen in ethyl alcohol group.

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

CONCLUSION

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

AUTHORS' NOTE

The authors have declared no conflict of interest.

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Table 1: Effect of montelukast sodium on serum liver enzymes in control and experimental groups (n=8)

Parameters	**Groups**				**p**
	Control	Ethyl alcohol	Montelukast sodium	Ethyl alcohol+ Montelukast sodium	
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

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. NS: not significant.

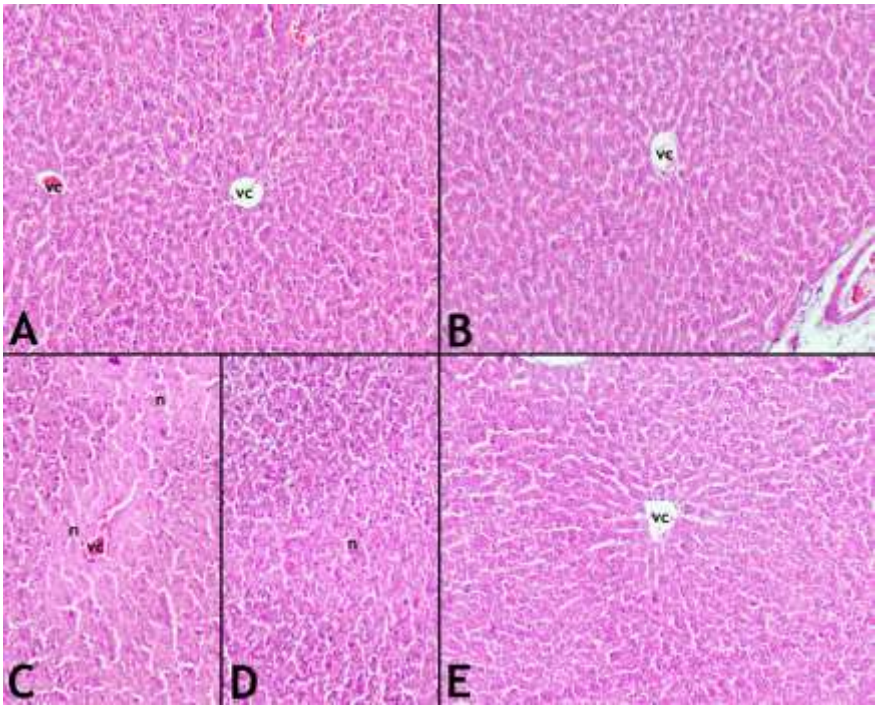

Figure 1: (A) Normal structure of liver in control group. Vc: Vena centralis. Heamatoxylin and eosin.Magnification 200X. (B) Stained sections of liver of montelukast sodium group. Heamatoxylin and eosin. Vc: Vena centralis. Magnification 200X. (C and D) Ethyl alcohol administered rat liver. Severe necrosis (n) were seen. Heamatoxylin and eosin. Magnification 200X. (E) Ethyl alcohol+montelukast sodium group. Structures of liver were similar to the control group. Heamatoxylin and eosin. Magnification 100X.