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Is montelukast as effective as *N*-acetylcysteine in hepatic injury due to acetaminophen intoxication in rats?

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ABSTRACT

This study aims to investigate the acute protective effect of montelukast sodium in hepatic injury secondary to acetaminophen (APAP) intoxication.

This study used 60 rats. The rats were grouped into 6 groups. The control group was administered oral distilled water 10 ml/kg, the APAP group oral APAP 1 g/kg, the montelukast sodium (MK) group oral MK 30 mg/kg, the acetaminophen + *N*-acetylcysteine (APAP + NAC) group oral APAP 1 g/kg, followed by a single dose of intraperitoneal NAC 1.5 g/kg three hours later, the acetaminophen + montelukast sodium (APAP+MK) group oral APAP 1 g/kg, followed by oral MK 30 mg/kg 3 h later, the acetaminophen + *N*-acetylcysteine + montelukast sodium (APAP + NAC + MK) group oral APAP 1 g/kg, followed by a single intraperitoneal NAC 1.5 g/kg plus oral MK 30 mg/kg 3 h later. Blood and liver tissue samples were taken 24 h after drug administration. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin were studied from the blood samples. Liver tissue samples were used for histopathological examination.

Compared with the control group, serum AST and ALT activities were higher in the APAP and APAP + NAC groups. APAP + NAC, APAP + MK, and APAP + NAC + MK groups had reduced serum ALT and AST activities than the group administered APAP alone. APAP + MK and APAP + NAC + MK groups had a lower serum ALP activity than the control group. Histopathologically, there was a difference between the group administered APAP alone and the APAP + MK and APAP + NAC + MK groups.

MK is as protective as NAC in liver tissue in APAP intoxication in rats.

1. Introduction

Acetaminophen (paracetamol, *N*-acetyl-*p*-aminophenol; APAP) is a drug commonly used as an analgesic and antipyretic (Bessems and Vermeulen, 2001). However, it may lead to severe liver injury in high doses in humans and experimental animal models (Kalsi et al., 2011). In therapeutic doses, a large part of APAP is metabolized by sulfation and glucuronidation pathways while 5-9% is transformed to a highly reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI), by cytochrome p450 (CYP 450) enzymes (Moyer et al., 2011). NAPQI is responsible for acetaminophen-induced hepatotoxicity (AIH) (Jaeschke and Bajt 2006). NAPQI is transformed to a non-toxic inactive form by reduced glutathione (GSH) (Mitchell et al., 1973). However, as glucuronidation and sulfation pathways are saturated in APAP overdose, APAP is largely metabolized to NAPQI by CYP 450 system (Mitchell et al., 1973). This leads to depletion of intracellular GSH depots, which in turn increases the amount of NAPQI to bind intracellular and mitochondrial proteins with resultant tissue damage (Jaeschke and Bajt 2006; Kon et al., 2004; Terneus et al., 2007). In acetaminophen-induced hepatotoxicity, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities exceed 1000 IU/L (Kalsi et al., 2011).

N-acetylcysteine (NAC) is a drug that is effective in APAP overdose. It is a glutathione precursor that increases the amount of GSH. NAC reduces APAP toxicity and NAPQI-induced oxidative stress by increasing GSH production (Terneus et al., 2007; Tan et al., 2008).

Montelukast sodium (MK) is a selective antagonist of the type 1 cysteinyl leukotriene (CysLT1) receptor (Sener et al., 2006). It is usually used in exercise- and aspirin-induced asthma (Scow et al.,

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2007). Cysteinyl leukotrienes (CysLTs) that belong to the eicosanoid lipid mediator family, namely LTC4, LTD4, and LTE4, are synthesized from arachidonic acid by the metabolic pathways initiated by the 5-lipooxygenase (5-LO) enzyme and act via CysLT1 and CysLT2 receptors bound to G protein (Capra et al., 2007). CysLTs are mainly synthesized by mast cells, basophils, eosinophils, and macrophages; however, they are also produced by neutrophils, thrombocytes, lymphocytes, endothelial cells, and erythrocytes (Henderson 1994).

Many human and animal studies have reported that cysteinyl leukotrienes play a role in hepatitis (Kasirga et al., 1999) liver cirrhosis (Huber et al., 1989; Graupera et al., 2002), hepatic ischemic reperfusion (Takamatsu et al., 2004), lipopolysaccharide-induced (Kawada et al., 1990) and alcohol-induced (Satoh et al., 2003) liver injury.

There exist some experimental studies showing the hepatoprotective effects of montelukast sodium (MK) (Cuciureanu et al., 2009; Ozkan et al., 2010). On the other hand, some other studies have mentioned hepatotoxicity among the adverse drug reactions induced by MK (Calapai et al., 2014; Lebensztejn et al., 2014).

A literature scan yielded no study investigating the protective effect of montelukast sodium in preventing hepatic injury after paracetamol intoxication. Under the light of this information, this study aimed to investigate the acute protective effect of montelukast sodium in hepatic injury secondary to paracetamol intoxication.

2. Materials and method

2.1. Animals

This study used 60 Wistor albino male rats weighing 250–300 g cared in Dicle University Medical Sciences and Research Center (Diyarbakır, Turkey), which were randomly selected for this experimental study. The rats were grouped into 6 groups. Each group were placed into glass cages with a sawdust-covered floor. The animals were housed in a medium with a 12 h/12 h light–dark cycle, a relative humidity of 42 ± 5%, and a temperature of 20 ± 1 °C. They were subjected to fasting for 6 h before the experiment, with ad libitum access to annulus water. This study was approved by Dicle University Animal Experiments Local Ethics Committee (Diyarbakır, Turkey) (Ethics committee protocol no: 2012/34).

2.2. Experimental procedures

Six groups each containing 10 rats were formed. The control group was administered oral distilled water 10 ml/kg via a gastric tube. The acetaminophen (APAP) group was orally administered a single dose of acetaminophen 1 g/kg (Parol tablet 500 mg, Atabay®/Turkey) via gastric tube after being diluted in distilled water (10 ml/kg). The APAP dose administered was toxic but non-lethal (Jin et al., 2012). The montelukast sodium (MK) group received a single dose of montelukast 30 mg/kg (Onceair tablet 10 mg, Abdi İbrahim®/Turkey) via gastric tube after being diluted in distilled water (10 ml/kg) (Hele et al., 2001). The acetaminophen + *N*-acetylcysteine (APAP + NAC) group was administered a single dose of APAP 1 g/kg via gastric tube after being diluted in distilled water (10 ml/kg). Three hours later, a single intraperitoneal dose of NAC (ACT ampoules 300 mg/3 ml,Adeka®/Turkey) 1.5 g/kg was administered (Xia et al., 2007). The acetaminophen + montelukast sodium (APAP + MK) group was administered a single dose of APAP 1 g/kg via gastric tube after being diluted in distilled water (10 ml/kg). Three hours later, a single oral dose of MK 30 mg/kg was administered orally via a gastric tube after diluting it distilled water (10 ml/kg). The acetaminophen + *N*-acetylcysteine + montelukast sodium (APAP + NAC + MK) group was administered a single dose of APAP 1 g/kg via gastric tube after being diluted in distilled water (10 ml/kg). Three hours after APAP administration, a single dose of NAC 1.5 g/kg was administered via intraperitoneal route and a single oral dose of MK 30 mg/kg was administered orally via a gastric tube after diluting it distilled water (10 ml/kg). All rats were anesthetized with ketamine hydrochloride (50 mg/kg, via intramuscular route) 24 h after the treatment. All rats were placed to supine position for surgical intervention. Each rats was operated with laparotomy with a midline incision. This was followed by liver tissue and blood sampling. All animals were sacrificed via exsanguination after the procedure.

2.3. Biochemical analysis

Blood samples of maximum amount taken via intracardiac route were centrifuged at 3000 rpm for 10 min. This was followed by storage of sera at −70 °C until biochemical tests. Serum AST, ALT, alkaline phosphatase (ALP) activities and total bilirubin level were measured with Abbott Architect c16000 Autoanalyzer and the results were expressed in U/L.

2.4. Histopathological examination of hepatic tissue

The livers were fixed in 10% buffered formalin solution for 48 h. After tissue sampling and routine histological tissue preparation, paraffin blocks were sectioned and the sections stained with hematoxylin-eosin (H&E). Histopathological evaluation was performed under light microscopy. Hepatotoxicity were scored as none (0), mild (1), moderate (2), severe (3), according to the area of cell death, degeneration (ballooning), and inflammation around the central veins (0; 0%, 1; less than 20%, 2; 20 with 70%, 3; more than 70% of hepatic lobules) (Naiko-Ito et al., 2010).

2.5. Statistical analysis

SPSS for Windows 11.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Study data were expressed as mean (minimum, maximum) values for biochemical values. The categorical variables were compared by the chi-square test. The study groups were compared with the nonparametric Kruskal–Wallis test. Paired comparisons were carried out using Mann–Whitney *U* test. The

Table 1
Serum AST, ALT, ALP, and total bilirubin levels of the control and experimental groups.

Groups	AST(U/L)	ALT(U/L)	ALP(U/L)	Total bilirubin
Control	75.50 ± 12.32^{a}	51.5 ± 17.73^{a}	171.4 ± 45.86^{a}	0.1 ± 0.01^{a}
APAP	3350.10 ± 922.83^{b}	3193.30 ± 987.78^{a}	134.60 ± 25.39^{abc}	0.41 ± 0.28^{b}
MK	143.20 ± 80.17^{a}	46.7 ± 8.87^{a}	155.00 ± 29.60^{ab}	0.1 ± 0.01^{a}
APAP + NAC	1757.20 ± 1620.58^{c}	1349.30 ± 1525.25^{c}	146.60 ± 34.86^{ab}	0.19 ± 0.03^{a}
APAP + MK	393.30 ± 273.56^{a}	145.8 ± 85.10^{a}	116.10 ± 16.05^{bc}	0.14 ± 0.06^{a}
APAP + NAC + MK	326.50 ± 197.61^{a}	$110,30 \pm 72.92^{a}$	95.50 ± 20.45^{a}	0.11 ± 0.03^{a}

a, b, c: There was a significant difference between the groups that were in the same column but carried different letters.

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