Liver injury induced by the non-steroidal anti-inflammatory drug mefenamic acid

N Somchit, F Sanat, E H Gan, I A W Shahrin, A Zuraini

ABSTRACT

Introduction: Non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat musculoskeletal disorders, inflammation and to control pain. Virtually all NSAIDs are capable of producing liver injury ranging from mild reversible elevation of liver enzymes to severe hepatic necrosis.

Methods: Mice were dosed intraperitoneally with mefenamic acid either one day at 100mg/kg and 200mg/kg, or 14 days dosing at 50mg/kg/day and 100mg/kg/day. Plasma was taken for alanine aminotransferase activity. Mice were sacrificed at the end of the study. Livers were removed and weighed. Liver samples were taken for histology.

Results: One-day doses of mefenamic acid revealed dose-dependent hepatocyte degeneration in the liver parenchyma. There were no significant changes in plasma alanine aminotransferase activity. Interestingly, 14-day daily doses induced hepatocellular necrosis, massive degeneration and inflammation. This was accompanied by a significant increase in plasma alanine aminotransferase activity and significant increase in the liver weight in the 100mg/kg/day mefenamic acid-dosed mice.

Conclusion: Results from this study suggest that mefenamic acid is capable of producing hepatotoxicity and care should be taken when prescribing or using this drug.

Keywords: hepatotoxicity, liver, mefenamic acid, non-steroidal anti-inflammatory drug

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Pharmacology and Toxicology Unit
Department of Biomedical Sciences
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
43400 Serdang
Malaysia

N Somchit, DVM, PhD
Lecturer in Pharmacology & Toxicology

F Sanat, BSc, MSc
PhD Candidate

E H Gan, BSc
MD Candidate

A Zuraini, DVM, PhD
Lecturer in Physiology

Department of Clinical Laboratories

I A W Shahrin, MD, MSc
Lecturer/Consultant Histopathologist

Correspondence to:
Dr Nhareet Somchit
Tel: (60) 3 8946 8464
Fax: (60) 3 8943 6178
Email: nhareet@medic.upm.edu.my

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely- used therapeutic agents[1]. More than 100 million NSAIDs are prescribed throughout the world, costing over US $5 billion[2]. The analgesic efficacy of mefenamic acid or "Ponstan" (2',3'-dimethyl-N-phenyl-anthranilic acid) has been documented for inflammatory and non-inflammatory pain such as pain after dental interventions or after trauma. Mefenamic acid is also used to treat dysmenorrhoea. Despite its wide usage, it can cause serious toxicity such as severe gastrointestinal tract disorders, hepatotxicity and nephrotoxicity[3].

Like other NSAIDs, mefenamic acid inhibits prostaglandin biosynthesis, a fundamental element of the analgesic, anti-pyretic and anti-inflammatory properties of all drugs in this group[4]. However, NSAIDs have recently gained attention as an effective therapy for tumour patients[5]. Prospective cohort studies indicated a protective effect of NSAIDs on the incidence or mortality of colon cancer[6,7]. However, the adverse drug reactions caused by NSAIDs limit the usage of these drugs as anti-tumour therapy. We aimed to evaluate the hepatotoxicity caused by mefenamic acid in mice by focusing on the histopathological and biochemical changes.

METHODS

Male Balb/C mice (25g, n=8 animals/group) were housed in plastic cages (eight mice/cage) with wood shavings as bedding. Mice were adapted to laboratory conditions for four days prior to use and fed pellets and tap water *ad libitum*. The experimental procedures were carried out in strict compliance with the Animal Ethics Committee's rules and regulations of this institute.

The mice received single intraperitoneal (IP) injections of mefenamic acid (Ponstan, United Pharma Limited, Baar, Switzerland) at 100mg/kg or 200mg/kg in 10% dimethyl sulfoxide/palm oil, or were treated daily with the drug IP (at 50mg/kg or 100mg/kg in 10% dimethyl sulfoxide/palm oil) for 14 days. Control animals received equivalent amount of dosed vehicle (10% dimethyl sulfoxide/palm oil).

Venous blood samples were collected from the tail vein of the mice in the subchronic dosing study (50 μL) prior to and 14 days post-dosing, and placed into heparinised 0.3ml vials. Blood samples were centrifuged at 4000 rpm for 15 minutes and the plasma was transferred to 0.5ml plastic eppendorf tubes where

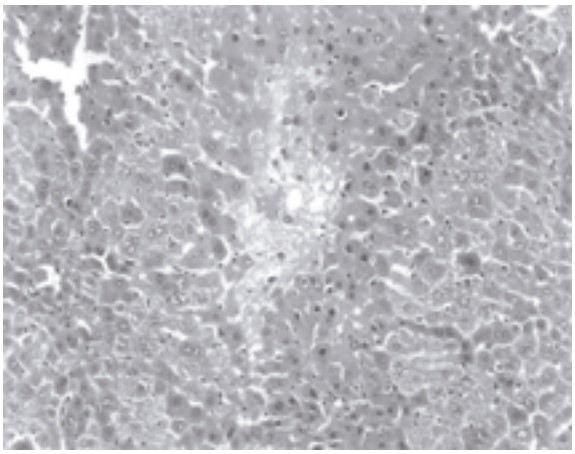

Fig. 1 Photomicrograph of liver of mouse treated with mefenamic acid shows hepatocellular necrosis and massive hepatocyte degeneration.(Haematoxylin & eosin, x 200).

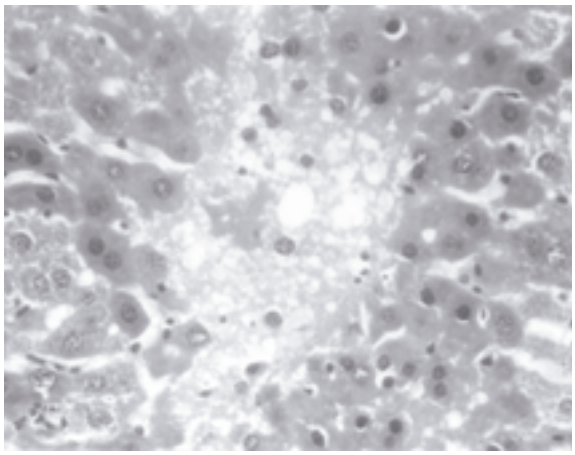

Fig. 2 Photomicrograph of liver of mouse treated with mefenamic acid shows hepatocellular necrosis. (Haematoxylin & eosin, x 400).

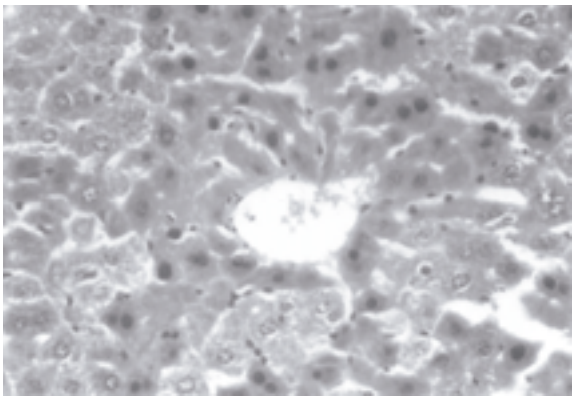

Fig. 3 Photomicrograph of liver of mouse treated with mefenamic acid shows centrilobular hepatocyte degeneration. (Haematoxylin & eosin, x 400).

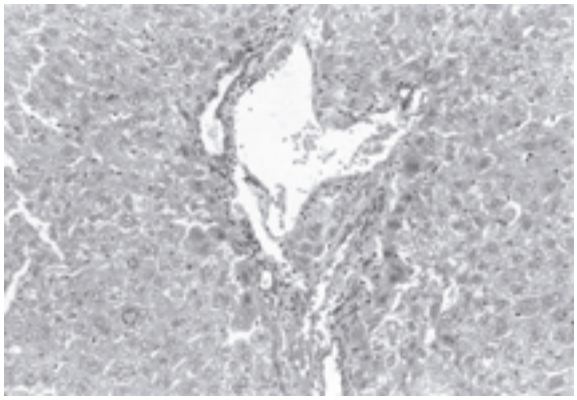

Fig. 4 Photomicrograph of liver of mouse treated with mefenamic acid shows periportal inflammation and hepatocyte degeneration. (Haematoxylin & eosin, x 200).

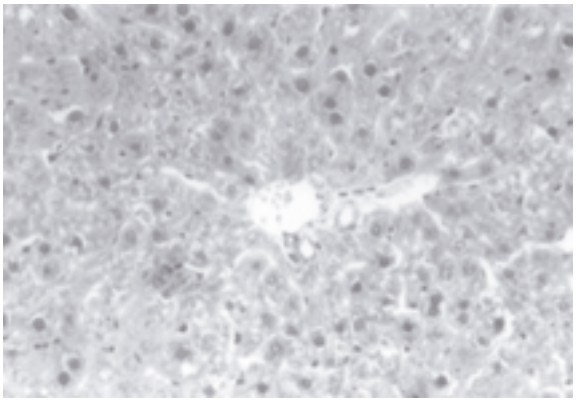

Fig. 5 Photomicrograph of liver of mouse treated with mefenamic acid shows hepatocellular degeneration. (Haematoxylin & eosin, x 400).

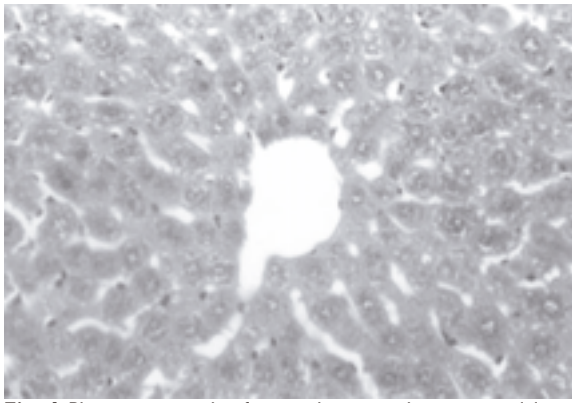

Fig. 6 Photomicrograph of control mouse shows normal liver histology. (Haematoxylin & eosin, x 400).

they were stored at minus 20ºC until analysis. Plasma alanine aminotransferase (ALT) activity was measured using a commercial kit (Sigma Chemicals, Dorset, England).

Six hours after the final dose (single and subchronic doses), mice were sacrificed by cervical dislocation. The livers were removed, blotted dry on filter paper and weighed. Sections from the left lobe of the liver were fixed in 10% buffered formalin for 48 hours, dehydrated in graded ethanol solutions, embedded in paraffin, sectioned at 5μm and stained with haematoxylin and eosin. Data are expressed as mean + standard deviation and were analysed for statistical significance ($p<0.05$) using analysis of variance (ANOVA), with Duncan multiple post-test or Student's t test.

RESULTS

Plasma ALT of mice treated with 50mg/kg/day and 100mg/kg/day mefenamic acid for 14 days showed a significant increase compared to that of controls (136.4 + 23.7 U/L, 298.3 + 19.2 U/L and 64.9 + 7.5 U/L, respectively; $p<0.05$). Only mice treated with 100mg/kg/day mefenamic acid showed a significant increase in liver weight compared to the controls (4.3 + 0.4mg/kg body weight for 50mg/kg/day, 6.2 + 0.3mg/kg body weight for 100mg/kg/day and 4.01 + 0.3mg/kg body weight for the controls, $p<0.05$).

The liver of mice treated with a high dose of mefenamic acid (100mg/kg for 14 days) showed massive degeneration involving the periportal, mid-zonal and centrilobular region (Figs. 1 & 2). Scattered areas of necrosis were noted with some focal inflammatory reactions. There were many hepatocytes with pyknotic nuclei (Figs. 3 & 4). Hepatocyte degeneration was detected in the periportal and mid-zonal regions of livers from the chronic 50mg/kg mefenamic acid-treated mice. Abundance of pyknotic nuclei were observed (not shown).

The liver of mice treated with a single dose of 100mg/kg mefenamic acid showed mild focal degenerative changes at the periportal region. Mid-zonal degeneration with pycnosis of hepatocytes was observed in the liver as a result of the high dose (200mg/kg) mefenamic acid treatment (Fig. 5). Liver sections of the control mice showed normal histology at the periportal, centrilobular and mid-zonal regions (Fig. 6).

DISCUSSION

With repeated daily treatment of mefenamic acid, there is an increase in plasma ALT activity, marked histopathological changes with inflammation and mild hepatocellular necrosis, and an increase in liver weight. However, a single dose of mefenamic acid did not result in an alteration of plasma activity or liver weight. Only mild degeneration of hepatocytes was observed.

The mechanism of mefenamic acid-induced hepatotoxicity is complex. Others have shown that NSAIDs such as diclofenac, sulindac and ibuprofen are capable of producing protein adducts that may be a key process in the pathogenesis of NSAID-induced liver injury[8]. NSAIDs are believed to cause hepatotoxicity via immunological idiosyncrasy[9]. It is difficult to reproduce immunological hepatotoxicity in an animal model. However, our model shows many similarities with cases of hepatotoxicity seen in humans. However, care must be taken in drawing this conclusion because the doses used in this current study is approximately 10 to 60 times more than the human therapeutic dose. The human therapeutic dose for mefenamic acid is 1.5mg/kg to 5mg/kg[10].

Minggato et al[11] reported the *in vitro* interference of mefenamic acid in the respiration of mitochondria and adenosine triphosphate synthesis. They subsequently reported the ability of NSAIDs to induce calcium-sensitive mitochondrial membrane permeability transition[12]. Although these are kidney mitochondria, similar processes may occur in the mitochondria of hepatocytes. This may be due to the effects of protein adducts on biochemical processes of cells in the liver, leading to hepatotoxicity.

As observed in the histological findings of single doses and repeated doses of mefenamic acid, there is a dose-dependent injury to the liver of mice. The injuries progressed from mild reversible degeneration to irreversible cellular necrosis. Repeated doses of mefenamic acid cause more severe hepatocyte lesions compared to treatment with a single dose. In conclusion, mefenamic acid exerts a dose-dependent toxicity on the liver of mice. Therefore, special care should be taken if this drug is prescribed over a long period of time.

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