

Zaltoprofen induces histological changes in albino rat liver

Ramanathan Shalini*, Salem S. Rajasekar and Kazhumaram V.P. Suriyakumari

Department of Anatomy, Sri Manakula Vinayagar Medical College and Hospital, Kalitheerthalkuppam, Madagadipet, Puducherry, India

SUMMARY

Hepatic injury associated with nonsteroidal anti-inflammatory drugs (NSAIDs) was found to be variable ranging from mild cholestasis to severe hepatocellular injury. Zaltoprofen is a NSAID used commonly as an analgesic in the treatment of painful disorders. The aim of this study was to determine the histological changes in the liver of albino rats after varying periods of oral zaltoprofen administration. Thirty-six adult male albino Wistar rats and the drug zaltoprofen at a dose of 40 mg/kg were employed, and 10% dimethyl sulphoxide was used as the solvent for the drug. Groups A1, B1 and C1 - each comprising 6 rats served as control groups and were treated with 10% dimethyl sulphoxide, orally via an oro-gastric tube, for 7, 14 and 21 days respectively. Groups A2, B2 and C2 - each comprising 6 rats served as experimental groups and were treated with the drug zaltoprofen dissolved in 10% dimethyl sulphoxide orally via an oro-gastric tube, for 7, 14 and 21 days respectively. Blood samples were collected from the tail vein of rats for analysis of serum alanine transaminase and serum aspartate transaminase before the sacrifice. The rats were sacrificed 24 hours after the last dose of drug and their liver was collected. The histological changes noted at the end of 7 days of zaltoprofen administration were central vein dilatation and congestion, periportal lymphocytic infiltration, and necrosis in the peripheral region. After 14 days of drug administration, there was extensive necrosis up to the central region. At the end of 21 days of drug administration, there was dilation of bile ducts as well. There was a significant increase in

the levels of serum alanine transaminase at the end of 14 and 21 days. Zaltoprofen should be used cautiously in patients with liver diseases.

Key words: NSAIDs – Zaltoprofen – Liver histology – Necrosis – Central vein

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce pain in patients with painful disorders like Rheumatoid arthritis and osteoarthritis. Ehrlich (2004) has reported that these agents also have unwanted effects on the gastrointestinal tract, liver and kidneys. Scarpingnato and Hunt (2010) have reported that the gastrointestinal side effects of NSAIDs are ulceration, bleeding and perforation of the stomach, as well as the small and large intestine.

Aithal and Day (2007) reported that NSAIDs are among the most common drugs associated with drug-induced liver injury with an estimated incidence between 3 and 23 per 100,000 patient years. Lewis et al. (1998) reported that the hepatic injury associated with NSAIDs was found to be variable ranging from mild cholestasis to severe hepatocellular injury. NSAIDs like Rofecoxib and Valdecoxib were also associated with hepatotoxicity (Yan et al., 2006; Niranjan et al., 2010).

Zaltoprofen, chemically 2-(10,11-dihydro-10-oxodibenzo[b,f]thiepin-2-yl)propionic acid (Nirogi et al., 2006) is a potent NSAID with preferential Cyclooxygenase-2 inhibition (Hirate et al., 2006). It is used in the treatment of rheumatoid arthritis (Hatori and Kokubun, 1998), osteoarthritis (Pareek et al., 2011), as well as to relieve pain

* **Corresponding author:** R. Shalini. Department of Anatomy, Sri Manakula Vinayagar Medical College and Hospital, Kalitheerthalkuppam, Madagadipet, Puducherry- 605107, India. Tel: 00 91 9841657895. E-mail: liniram80@yahoo.com

Submitted: November 2, 2012 Accepted: March 14, 2013

after surgery, injury and tooth extraction (Muratani et al., 2005; Komiya et al., 2009). After oral administration of zaltoprofen to humans, 62% of the dose is excreted as conjugates in the urine and only 3% is excreted unchanged (Furuta et al., 2002). Also the involvement of liver microsome CYP2C9 and UGT2B7 in the metabolism of zaltoprofen is established (Furuta et al., 2002).

The aim of the study was to observe the histologic changes in liver of albino rats treated with varying periods of zaltoprofen.

MATERIALS AND METHODS

Design

Thirty-six adult male albino Wistar rats, weighing 180-220 g were obtained from animal house of Sri Manakula Vinayagar Medical College and Hospital, Puducherry, India. The rats were acclimatized for a period of 7 days before starting the study. Standard environmental conditions such as temperature ($24 \pm 2^\circ\text{C}$), humidity (60-70%) and 12 hours of light/dark cycle were maintained. Food and water were allowed *ad libitum* under strict hygienic conditions.

Rats were divided into six groups. Groups A1, B1 and C1, each comprising 6 rats, served as the control groups and were treated with isovolumetric quantities of 10% dimethyl sulphoxide, orally via an oro-gastric tube, for 7, 14 and 21 days respectively. Groups A2, B2 and C2, each comprising 6 rats, served as the experimental group and were treated with the drug zaltoprofen (Ipca Laboratories Ltd) in a dose of 40 mg/kg body weight/day (Kiso to Rinsho, 1990) dissolved in 10% dimethyl sulphoxide, orally via an oro-gastric tube, for 7, 14 and 21 days respectively.

Blood samples were collected from the tail vein of the rats, before they were sacrificed, for biochemical analysis of serum alanine transaminase (ALT) and serum aspartate transaminase (AST). The rats were sacrificed 24 hours after the last dose of the drug by cervical dislocation and their liver was collected. The organs were preserved in

10% formalin, processed and stained with eosin and hematoxylin stains.

The experimental protocol was approved by the Institutional Animal Ethics Committee following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals for laboratory animal facility).

Statistical analyses

The program Epi info version 3.4.3 was used in the statistical evaluation of the biochemical results. Mean and standard deviation was calculated for each of the groups. 'F' test was used to determine the 'p' value for each of the parameters. A 'p' value < 0.05 was taken to be statistically significant.

RESULTS

On histological examination, the liver of the control groups were normal (Fig. 1A and Fig. 1B). The liver of the rats treated with the drug zaltoprofen for 7 days (A2 group) showed histological changes. There was central vein dilatation and congestion (Fig. 1C), periportal lymphocytic infiltration (Fig. 1C) and coagulative necrosis of hepatocytes in the peripheral region (Fig. 1D). On biochemical evaluation serum aspartate transaminase levels were significantly increased with a 'p' value of 0.04 (Table 1).

The histological changes observed in the liver of the rats treated with the drug Zaltoprofen for 14 days (B2 group) were central vein dilatation and congestion, periportal lymphocytic infiltration (Fig. 2A), and extensive coagulative necrosis of hepatocytes extending upto the central region (Fig. 2B). On biochemical evaluation serum alanine transaminase levels were significantly increased with a 'p' value of 0.03 (Table 1).

The histological changes observed in the liver of the rats treated with the drug Zaltoprofen for 21 days (C2 group) were central vein dilatation and congestion (Fig. 2C), periportal lymphocytic infiltration, dilatation of bile ducts (Fig. 2D) and

Table 1. Biochemical parameters

	Group A1 n= 6	Group A2 n= 6	'p' value	Group B1 n= 6	Group B2 n= 6	'p' value	Group C1 n= 6	Group C2 n= 6	'p' value
Serum alanine transaminase (ALT) U/lit Mean \pm SD	33.67 \pm 7.81	36.5 \pm 8.53	0.558	41.5 \pm 7.69	53.17 \pm 9.49	0.034	42.5 \pm 4.67	54.33 \pm 5.85	0.030
Serum aspartate transaminase (AST) U/lit Mean \pmSD	169.17 \pm 50.4	229 \pm 40.68	0.040	171.33 \pm 26.34	194.33 \pm 11.97	0.071	147 \pm 53.31	157 \pm 40.58	0.720

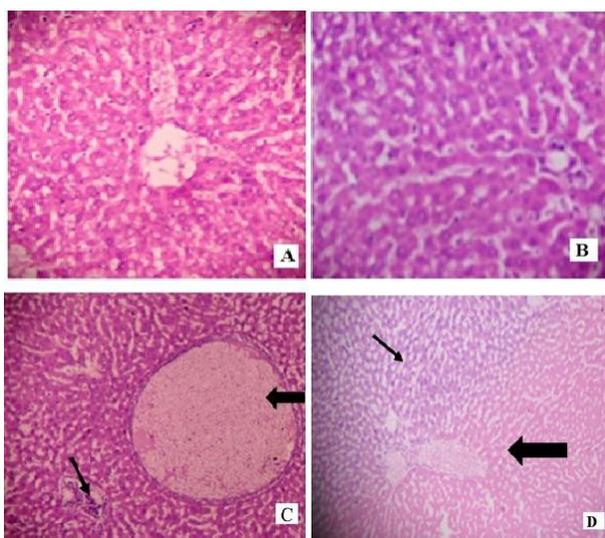


Fig. 1. (A) Liver of control group rat (x10). (B) Liver of control group rat (x40). (C) Liver of A2 group rat. The thick arrow represents dilated and congested central vein and the thin arrow points to lymphocytic infiltration (x10). (D) Liver of A2 group rat. The thick arrow indicates necrotic hepatocytes and the thin arrow indicates normal hepatocytes (x10).

extensive coagulative necrosis of hepatocytes up to the central region (Fig. 2C). On biochemical evaluation serum alanine transaminase levels were significantly increased with a 'p' value of 0.03 (Table 1).

DISCUSSION

Hepatic dysfunction has been associated with the clinical use of NSAIDs. The present study of the use of varying periods of zaltoprofen administration to Albino rats showed that there was a borderline increase in the level of liver enzymes and the histological changes observed were periportal lymphocytic infiltration, central vein dilatation and congestion, bile duct dilatation and hepatocellular necrosis. These histological changes have also been observed in previous studies using older NSAIDs.

Niranjan et al. (2010), from India, compared the adverse effects of diclofenac sodium and valdecoxib in liver of Albino rats. Diclofenac sodium treated group showed hepatocyte enlargement with vacuolation, reduced sinusoidal spaces with congested vessels in the interstitium. Valdecoxib treated group showed enlarged hepatocytes with cytoplasmic blebbing, central vein dilatation and periportal lymphocytic infiltration.

Ebaid et al. (2007), from Egypt, conducted a similar study with piroxicam and observed the histological changes in mice. They have observed inflammatory cellular infiltrates, vacuolated hepatocytes, dilated sinusoids and increased number of Kupffer cells.

Abatan et al. (2006), from Nigeria, studied the

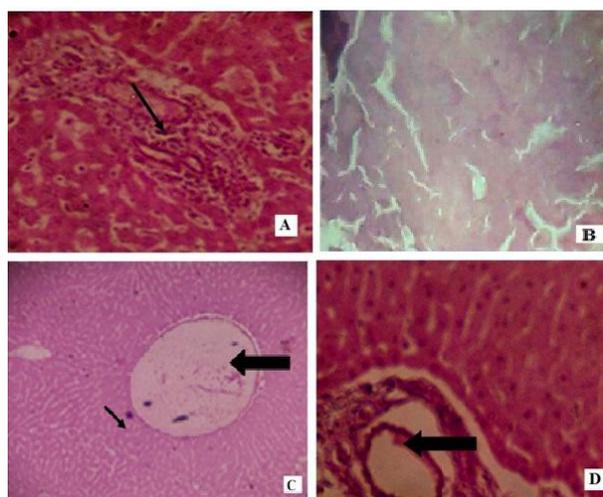


Fig. 2. (A) Liver of B2 group rat. The thin arrow points to periportal lymphocytic infiltration (x40). (B) Liver of B2 group rat. This slide indicates extensive necrosis of hepatocytes (x40). (C) Liver of C2 group rat. The thick arrow indicates dilated and congested central vein and thin arrow indicates necrotic hepatocytes near the central vein (x10). (D) Liver of C2 group rat. The thick arrow points to dilated bile duct (x40).

toxic effects of indomethacin, piroxicam, aspirin and phenylbutazone in the liver of albino rats. They observed increased levels of serum aspartate transaminase and periportal hepatic necrosis and kuppfer cell proliferation in the indomethacin treated group.

Hummdi and Habashi (2010) have reported that lornoxicam caused-hepatocyte hypertrophy with pyknosis of nuclei, blood sinusoidal collapse and cellular infiltration.

Somchit et al. (2004), from Malaysia, observed the histological changes in liver of mice after administration of mefenamic acid for 14 days. They observed- hepatocyte degeneration, hepatocellular infiltration and focal hepatic necrosis.

Aydin et al. (2003), from Turkey, studied the histological effects of diclofenac sodium in the liver of albino rats. They observed cloudy swelling and hydropic degeneration of the liver cells, focal sinusoidal and central vein dilatation, proliferation of the bile duct in portal areas, enlargement of the periportal area with mononuclear cell infiltration and focal necrosis.

In conclusion, zaltoprofen caused histological changes in the liver of albino rats similar to previously introduced NSAIDs. Hence they should be used cautiously in patients with hepatic diseases.

ACKNOWLEDGEMENTS

Thanks to Ipca Laboratories Ltd., Mumbai, India, for providing the drug Zaltoprofen for this study. Institution responsible for research support: Sri Manakula Vinayagar, Medical College and Hospital, Puducherry, India.

REFERENCES

- ABATAN MO, LATEEF I, TAIWO VO (2006) Toxic effects of non steroidal anti inflammatory agents in rats. *Afr J Biomed Res*, 9: 219-223.
- AITHAL GP, DAY CP (2007) Nonsteroidal anti-inflammatory drug induced hepatotoxicity. *Clin Liver Dis*, 11: 563-575.
- AYDIN G, GOKCIMEN A, ONCU M, CICEK E, KARAHAN N, GOKALP O (2003) Histopathologic changes in liver and renal tissues induced by different doses of diclofenac sodium in rats. *Turk J Vet Anim Sci*, 27: 1131-1140.
- EBAID H, MOHAMED A DKHIL, MOHAMED A DANFOUR, AMANY TOHAMY, MOHAMED S GABRY (2007) Piroxicam induced hepatic and renal histopathologic changes in mice. *Libyan J Med*, 2: 82-89.
- EHRlich GE (2004) A benefit / risk assessment of existing therapeutic alternatives for the treatment of painful inflammatory conditions. *Int J Clin Pract*, Suppl, 144: 20-26.
- FURUTA S, AKAGAWA N, KAMADA E, HIYAMA A, KAWABATA Y, KOWATA N, INABA A, MATTHEWS A, HALL M, KURIMOTO T (2002) Involvement of CYP2C9 and UGT2B7 in the metabolism of Zaltoprofen a NSAID and its lack of clinically significant CYP inhibitor potential. *Brit J Clin Pharmacol*, 54: 295-303.
- HATORI M, KOKUBUN S (1998) The long term efficacy and tolerability of the new anti inflammatory agent Zaltoprofen in rheumatoid arthritis. *Current Med Res Opin*, 14: 79-87.
- HIRATE K, UCHIDA A, OGANAYAMA Y, ARAI T, YODA K (2006) Zaltoprofen, a NSAID drug, inhibits bradykinin induced pain response without blocking bradykinin receptors. *Neurosci Res*, 54: 289-294.
- HUMMDI LA, HABASHI SH (2010) Histological and ultrastructure studies on the liver changes induced by xefo drug (lornoxicam) of adult female mice. Proceedings of the 3rd Scientific Conference of Animal Wealth Research in the Middle East and North Africa, Foreign Agricultural Relations (FAR), Egypt, 29 November - 1 December 2010.
- KISO TO RINSHO (1990) Clinical report 24, 5979.
- KOMIYA A, ENDO T, KOBAYASHI M, KIM W, ARAKI K, NAYA Y, SUZUKI H, TOBE T, ICHIKAWA T, FUSE H (2009) Oral analgesia by non steroidal anti inflammatory drug Zaltoprofen to manage cystoscopy related pain – a prospective study. *Int J Urol*, 16: 874-880.
- LEWIS JH (1998) Nonsteroidal anti-inflammatory drug induced hepatotoxicity. *Clin Liv Dis*, 2: 543-561.
- MURATANI T, DOI Y, NISHIMURA W, NISHIZAWA M, MINAMI T, ITO S (2005) Preemptive analgesia by Zaltoprofen that inhibits bradykinin action and cyclooxygenase in a post operative pain model. *Neurosci Res*, 51: 427-433.
- NIRANJAN R, MANIK P, SRIVASTAVA AK, PALIT G, NATU SM (2010) Comparative adverse effects of COX-1 and COX-2 inhibitors in rat liver: an experimental study. *J Anat Soc India*, 59: 182-186.
- NIROGI RVS, KOTA S, PERURI BG, KANDIKERE VN, MUDIGONDA K (2006) Chiral high performance liquid chromatographic method for enantioselective analysis of Zaltoprofen. *Acta Chromatographica*, 17: 202-208.
- PAREEK A, GUPTA AK, CHANDURKAR NB, SIRSIKAR AD, AMBADE RE, JESALPURA BH, SWAMY AP (2011) Zaltoprofen a non inferior alternative to Diclofenac for the treatment of primary knee osteoarthritis – a comparative evaluation of efficacy and safety in a 4 week multicentric, randomised, double blind, double dummy trial. *Expert Opin Pharmacother*, 7: 1007-1015.
- SCARPINGNATO C, HUNT RH (2010) Nonsteroidal anti inflammatory drug related injury to the gastrointestinal tract: clinical picture, pathogenesis, and prevention. *Gastroenterol Clin North Am*, 39: 433-464.
- SOMCHIT N, SANAT F, GAN EH, SHAHRIN IA, ZURAINI A (2004) Liver injury induced by the non-steroidal anti-inflammatory drug mefenamic acid. *Singapore Med J*, 45: 530-533.
- YAN B, LEUNG Y, URBANSKI SJ, MYERS RP (2006) Rofecoxib induced hepatotoxicity. A forgotten complication of coxibs. *Can J Gastroenterol*, 20: 351-355.