STUDY ON HAEMATO-BIOCHEMICAL AND OXIDATIVE STRESS IN EXPERIMENTALLY INDUCED KETOPROFEN TOXICITY IN WISTAR RATS

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ABSTRACT

The present research work was conducted to study the haemato-biochemical and oxidative stress effect of subacute intramuscular administration of ketoprofen for 14 days on 24 female Wistar rats. The animals were equally divided into 4 different groups A, B, C, D during randomization and dosed with vehicle control, 4 mg/kg as low dose, 8 mg/kg as mid dose and 12 mg/kg as high dose of ketoprofen, respectively. The drug was administered by intramuscular route every day for 14 days. At the end of 14 days treatment, all the animals from group A to D were subjected to blood collection and haemato-biochemical and oxidative stress investigation. The haematological study revealed dose dependent decrease in Hb, PCV,TEC, lymphocyte per cent and MCHC in Group C and Group D rats, while increase in MCV and neutrophil per cent in Group C and Group D rats.Administration of ketoprofen caused reduction in serum total protein (Group C and Group D), albumin (Group C and Group D) and globulin (Group D) in different groups. The result suggested that long term administration of ketoprofen at dose rate of 8 mg/kg and 12 mg/kg leads to macrocytic hypochromic anaemia possibly because of gastrointestinal bleeding or micro-bleeding and it is also evident that ketoprofen at the dose rate of 8 mg/kg b.wt. and 12 mg/kg b.wt. results into oxidative stress.

KEY WORDS: Ketoprofen, Haemato-biochemical, Oxidative stress, Mice

INTRODUCTION

Inflammation serves to destroy, dilute, or otherwise neutralize harmful agents (microbes, toxins) and repair the damaged tissues. It is basically a protective response however, under certain circumstances, inflammation may divert from its beneficial effect and may become considerably more harmful to the body. Inflammation of various tissues is the most common problem faced by practicing veterinarians. Administration of anti-inflammatory drugs like Non-Sterodial Anti inflammatory drugs (NSAID) or Steroidal Anti-inflammatory drug used to alleviate signs of inflammation is a standard therapeutic approach. Most of NSAID are acting through blocking the activity of various enzymes which helps to make chemicals mediators during inflammation such as prostaglandins, thromboxane and other inflammatory mediators. Ketoprofen is widely used NSAID in the treatment of inflammatory and painful conditions of the bones, joints and musculo-skeletal systems in cattle, horses, dogs and cats in veterinary practice, but their use in the treatment of pain, fever and inflammation is associated with significant untoward effects on the gastro-intestinal tract (Byoung et al., 2007). In veterinary therapeutics, ketoprofen is given by oral and parenteral (intravenous, intramuscular or subcutaneous) routs in cattle, cats, dogs and horses. As the drug is used parentally in domestic animals, toxicity study following long term intramuscular administration in laboratory animals is lacking. Very limited information is available regarding haemato-biochemical changes and oxidative stress in ketoprofen toxicity in laboratory animals especially rats. Hence, looking to the paucity of information, the present study was carried out.

MATERIALS AND METHODS

The study was conducted on 24 colony-bred adult female Wistar rats. Rats were procured from Zydus Research Center, Ahmadabad, Gujarat, and were maintained under standard management conditions. Before the start of the experiment, rats were kept in laboratory conditions for a period of 7 days for acclimatization. Rats were provided with standard pellet diet. The Institutional Animals Ethical Committee (IAEC) approved the experimental protocol. All the rats were randomly divided into 4 different groups (A, B, C and D), each comprised of 6 female rats. Ketoprofen injection (Neoprofen 10%, vetnex-Ranbaxy, India) was obtained from open market and used as test substance in the study. Dose volume was fixed to 1 ml/kg for easy administration. For that 1 ml of keteoprofen formulation was taken in sterilized bottle and 7.34 ml of water for injection was mixed with it to achieve concentration of 12mg/ml (solution IV) for group D. From Solution IV, 6 ml solution mixed with 3 ml of water for injection to achieve concentration of 8 mg/ml (Solution III) for group C. From solution III, 3 ml solution mixed with 3 ml of water for injection to achieve concentration of 4 mg/ml (Solution II) for group B. Group I was given only water for injection as control as per dose volume. Vehicle used for diluting ketoprofen to obtain the desired concentration was water for injection. Rats were anaesthetized by using diethyl ether during blood collection and blood was collected in K3EDTA vials for hematology and in heparin for biochemical estimations on day 0 and 14 of experiment. The serum was collected in sterile serum collecting vial and kept at -20^0 c till further analysis. The hematological parameters, viz., hemoglobin (g/dl), packed cell volume (%), total erythrocyte count (106/cumm), total leukocyte count (103/cumm), differential leukocyte count (%),mean corpuscular volume (fl), mean corpuscular hemoglobin (pg), and mean corpuscular hemoglobin concentration (gm/dl) were obtained with the auto-blood analyzer (Calltac MEK 6450 Nihon Kohden, Japan) by impedance method.

The biochemical profile like aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase, BUN, plasma albumin, plasma globulin, plasma total protein & creatinine were evaluated as per the method described by Teitz (1976) using of Merck Kits (Merck Specialties Pvt. Ltd., Ambernath) on Clinical Analyzer (Systronics, Ahmedabad). Oxidative stress was studied by assessment of lipid peroxidation (LPO) as per the method of Rehman (1984) and superoxide dismutase (SOD) in erythrocytes as per the method described by Madhesh and Balasubramanian (1998). The data generated on various parameters was subjected to statistical analysis using completely randomized design (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

The present study on subacute exposure of ketoprofen at the dose rate of 4 mg/kg (Group B), 8 mg/kg (Group C) and 12 mg/kg (Group D) body weight intramuscularly once daily for 14 days was carried out to elucidate the haemato-biochemical and oxidative stress. The results on haematological parameters are presented in Table 1. Significant reduction in Hb, PCV, TEC and MCHC as well as significant increase in MCV and neutrophil in rats belonging to high dose Group (Group D) was observed. The reduction in Hb, PCV and TEC suggested the existence of anemia. The increase in MCV as observed in the study indicated macrocytic anemia and decrease in Hb is suggestive of hypochromic anaemia. The decrease in parameters like hematocrit (PCV), Hb and TEC values may be possibly because of gastro-intestinal bleeding or micro-bleeding and/or hemodilution caused by fluid retention (Klasco, 2003). No significant difference in TLC and MCH was observed in ketoprofen treated rats, whereas there was significant increase in neutrophil and decrease in lymphocyte percent in rats of high dose group. Similarly, no significant changes were observed in dogs treated with aspirin (100mg/kg), diclofenac sodium (50 mg/kg), ibuprofen (10mg/kg) and nimesulide (5mg/kg) daily orally for 16 days by Sharma (2002). Velankar (1999) and Muchhara (2007) reported significant reduction in Hb and PCV as well as no significant change in TEC, MCV, MCH and MCHC in rats administered with NSAIDS like diclofenac sodium, ibuprofen and aspirin.

The findings of biochemical parameters are summarized in Table 2. Biochemical changes revealed significant decrease in total protein (Group C and Group D), albumin (Group C and Group D), and

Table 2:Effect of ketoprofen on haemogram in Wistar rats after daily intramuscular administration for 14 days (Mean ± SE, n=6)

Parameters	Group-A Control	Group-B Low dose	Group-C Mid dose	Group-D High dose
Hb(g/dl)	15.82±0.277^{a}	15.47±0.265^{ab}	14.60±0.280^{b}	10.00±0.351^{c}
PCV (%)	41.68±0.451^{a}	40.42±0.311^{a}	39.73±0.584^{a}	31.97±2.339^{b}
TEC(millions/cumm)	7.58±0.102^{a}	7.37±0.329^{a}	6.85±0.203^{a}	4.65±0.398^{b}
MCV (fl)	53.61±0.399^{c}	55.34±1.205^{bc}	59.88±2.416^{ab}	61.38±2.032^{a}
MCH (pg)	19.65±0.231^{a}	19.68±0.458^{a}	20.50±0.500^{a}	20.93±0.497^{a}
MCHC (%)	37.08±0.230^{a}	36.57±0.215^{ab}	36.03±0.247^{b}	33.67±0.543^{c}
TLC(thousand/cumm)	16.07±1.613^{a}	16.74±2.363^{a}	18.61±1.365^{a}	18.07±1.182^{a}
Neutrophils (%)	18.17±0.307^{c}	19.00±0.365^{bc}	20.00±0.577^{b}	28.00±0.577^{a}
Eosinophils (%)	2.17±0.166^{a}	2.33±0.210^{a}	2.67±0.333^{a}	2.50±0.428^{a}
Basophils (%)	1.00±0.000^{a}	1.00±0.000^{a}	1.00±0.000^{a}	1.00±0.000^{a}
Lymphocytes (%)	78.33±0.333^{a}	77.17±0.600^{a}	76.50±0.619^{a}	62.67±0.881^{b}
Monocytes (%)	2.17±0.166^{a}	2.33±0.333^{a}	2.33±0.333^{a}	2.83±0.477^{a}

Means with similar superscripts in a row do not differ significantly (P < 0.05).

Table 3: Effect of ketoprofen on biochemical constituents and oxidative alterations in Wistar rats after daily intramuscular administration for 14 days (Mean ± SE, n=6)

Parameters	Group-A Control	Group-B Low dose	Group-C Mid dose	Group-D High dose
AST (IU/L)	125.59±3.568^{a}	121.68±1.468^{a}	123.80±1.656^{a}	124.86±1.340^{a}
ALT (IU/L)	48.15±3.084^{a}	45.19±1.014^{a}	46.80±0.969^{a}	46.11±1.226^{a}
Total Protein (g/dl)	7.31±0.514^{a}	7.03±0.158^{ab}	6.67±0.235^{b}	5.23±0.110^{c}
Albumin (g/dl)	4.39±0.169^{a}	4.15±0.293^{ab}	3.64±0.226^{bc}	3.19±0.940^{c}
Globulin (g/dl)	2.94±0.144^{a}	2.88±0.258^{a}	3.01±0.225^{a}	2.03±0.921^{b}
Creatinine (mg/dl)	0.60±0.220^{a}	0.60±0.144^{a}	0.57±0.238^{a}	0.56±0.451^{a}
BUN (mg/dl)	38.14±2.526^{a}	39.40±1.145^{a}	42.78±2.817^{a}	40.84±2.740^{a}
Amylase (IU/L)	664.82±63.890^{a}	682.10±34.660^{a}	690.20±36.494^{a}	782.99±41.361^{a}
LPO (mg/dl)	6.33±0.144^{b}	6.56±0.841^{b}	7.04±0..149^{a}	8.10±0.232^{a}
SOD (mg/dl)	9.42±0.426^{a}	8.54 0.214^{b}	8.01±0.213^{b}	7.00±0.242^{c}

Means with similar superscripts in a row do not differ significantly (P < 0.05).

globulin (Group D), whereas, no significant difference was observed in the values of ALT, AST, creatinine, BUN and amylase in various treatment groups. These findings indicated that ketoprofen has no any adverse effect on liver and kidney function. The present findings were in the close conformity with the findings of Muchhara (2007), however, Ramesh *et al.* (2001) reported progressive increase in creatinine level in dogs treated with nimesulide at the dose of 2 mg/kg body weight twice daily for 4 days. The reduction in total plasma protein, albumin and globulin as observed in the present study has also been reported in domestic and laboratory animals following exposure to various NSAIDS (Misraulia and Kaskhedikar; 2010; Peter *et al.*, 2003).

Lipid peroxidase (mg/dl) increased significantly ($P<0.05$) in Group C (mid dose) and Group D (high dose) as compared to Group A (control) rats. On 14th day post- treatment, Group D rats showed the highest (8.10 ± 0.232) plasma lipid peroxidase activity and revealed significant ($P < 0.05$) increase when compared with Group B (6.56 ± 0.841) and Group A (6.33 ± 0.144) rats. SOD values (mg/dl) decreased significantly ($P < 0.05$) in various groups as compared to Group A rats. On 14th day post-treatment, Group D rats showed significant ($P<0.05$) decrease in SOD values (7.00 ± 0.242) as compared to Group B (8.54 ± 0.214),Group C (8.01± 0.213) and Group A (9.42 ± 0.426) rats. The increase in lipid peroxidation level and decrease in superoxide dismutase activity in rats belonging to Group C and D as observed in the present study was also reported earlier by Lastra *et al*. (2000, 2002). These results suggested that reactive oxygen metabolites contribute significantly in the development of gastro-intestinal lesions. Further, it is also evident that ketoprofen at the dose rate of 8 mg/kg body weight and 12 mg/kg body weight results into oxidative stress.

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