PHARMA SCIENCE MONITOR
AN INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES

SUB-CHRONIC TOXICOLOGICAL EVALUATION OF ACECLOFENAC IN RATS

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ABSTRACT
Non-steroidal anti-inflammatory drugs (NSAIDs) are mixed group of compounds that differ in chemical structure but share similar pharmacological action. Aceclofenac is NSAID used for relief of pain and inflammation in osteoarthritis, rheumatid arthritis and ankylosing spondylitis. Whenever aceclofenac is administered into the biological system, different types of interaction occur resulting into different dose related responses. In most cases these responses are desired and useful, but there are number of other effects which are not advantageous. The objective of the present study was to evaluate the sub-chronic toxicity study of aceclofenac in wistar rats (male and female), at different dose levels, ranging from 5 to 20 mg/kg body weight. Various physiological, hematological, biochemical parameters were studied and found not to be changed significantly. Aceclofenac did not show any significant behaviors toxicity except some sort of irritability in high and middle dose.
Key words: Aceclofenac, Sub-chronic toxicity

INTRODUCTION

The treatment of inflammation and pain is an important area of therapeutics. In the last decade, nonsteroidal anti-inflammatory drugs (NSAIDs) have played a central role in these indications and they are currently considered as the first choice, being one of the most widely prescribed drugs.[1,2] Prostaglandins are important mediators of inflammation. They are a family of chemicals that are produced by the cells of the body and have several important functions. They promote inflammation, pain and fever; support the blood clotting function of platelets; and protect the lining of the stomach from the damaging effects of acid.[3,4] The effect of NSAIDs is mediated to large extent by inhibition of prostaglandin synthesis through cyclo-oxygenase (COX). COX has two isoenzymes in humans: COX-1 has cytoprotective function in the gastric mucosa and COX-2 is detected in several tissues when an inflammatory reaction takes place.[5]

Cycloxygenase plays a key role in controlling inflammation. NSAIDs have been demonstrated to inhibit COX-2 activity and suppress the prostaglandin E_2 production by inflammatory cells.[6] NSAIDs are well accepted as a therapy for a variety of chronic arthritis pain syndromes and inflammatory conditions, most commonly rheumatid arthritis and osteoarthritis.[7,8] The popularity of these agents is largely attributable to their success in relieving pain and maintaining functional status. NSAIDs are primarily used for reducing the pain, disability and reduced quality of life associated with pain and inflammation.[9,10]

Aceclofenac is 2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl] oxyacetic acid. It exhibits a multifactor mechanism of action which is mediated by selective inhibition of E2.[11] Aceclofenac is available as oral, rectal and injectable formulation.[12] A number of toxic effects of aceclofenac are cited and includes gastrointestinal disorders, dermatological disorders and other allergic reactions.[13] Initial assessments of toxic manifestations provide information on health hazards likely to arise from short term exposure to aceclofenac. Data from the sub-chronic toxicity study may serve as a basis of classification and labeling, provide initial information on the mode of toxic action of aceclofenac, dose determination and determine the LD_{50} values that provide many indices of potential types of drug activity.[14] Hepatotoxicity is the most remarkable feature of aceclofenac overdose.[15] Acute overdoses of aceclofenac can cause potentially fatal liver damage, and in rare individuals, a normal dose can do the same. Aceclofenac toxicity is foremost cause of acute liver damage.[16] Renal effects of aceclofenac is less commonly seen than diclofenac.[17]

Despite the long usage of aceclofenac for various purposes, little information is available on the toxicity profile of aceclofenac. The present study was designed to evaluate sub-chronic toxicity of aceclofenac in wistar rats.

MATERIALS AND METHODS

Experimental animals and housing conditions

The protocol of this study was approved by Institutional Animal Ethical Committee (IAEC), Jadavpur University, Kolkata. Specific pathogen free wistar male and female rats (6 to 8 week old) were obtained from animal house, Indian Institute of Chemical Biology, Kolkata. Forty eight (24 male and 24 female) wistar rats were selected

for study as this rodent species is one of the recommended and is most widely used throughout the study as test system for the evaluation of toxicity of various products. Also, this test system has been demonstrated to be sensitive to toxins. Animals were obtained from random breeding in a closed colony, because the aim was to discover new and unexpected effects of aceclofenac in group of animals of wider variability. Each animal was assigned a unique identification number by individual marking on fur. Animals were acclimated for 7 days prior to commencement of the test. The animals were randomly allocated to four groups, each consisting of 6 males and 6 females, housed in a room with barrier system and maintained under the following conditions: temperature of 20 to 24 °C, relative humidity 30 to 70 %, ventilation frequency of 18 times/h and a 12 h light/dark cycle. The animals were caged in polycarbonate cages (six rats/cage) on soft chip bedding. Throughout the experiment the chips were removed every 3 days and rats were given aquaguard pure water in glass bottles ad libitum. The animals were fed with pelleted feed supplied by M/s Ghosh Enterprise, Kolkata.

Chemicals and administration dose levels

A range of doses to be administered was chosen for study. Aceclofenac was suspended in water was injected to rats at the dose levels of 5mg/kg, 10 mg/kg and 20 mg/kg in the dose volume of 1ml/100g body weight. The test article suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle only. Intraperitoneal route of administration was selected as it is one of the proposed routes for toxicity testing and dosage can be administered accurately.

Experimental treatment

Animals were divided into four groups of 6 animals each. Group I treated with vehicle (sterile water) was kept as control. Group II, III and IV were treated with 5 mg/kg, 10 mg/kg and 20 mg/kg body weight corresponding to low, intermediate and high dose, respectively for 28 days according to body weight of each group rats. Sterile water was injected intravenously to the animals of control group of mice. Treatment was done once daily for 28 days. At the treatment, overnight fasted animals were sacrificed and blood and tissue samples were collected on 29th day. Hematological, biochemical and histological parameters were measured in all treated groups as well as in control group.

The organs were quickly blotted, weighed in digital balance and processed for histological studies.

Observation and examination methods

Clinical signs and general appearance were observed once a day and body weights and food intake were measured once a week. An autopsy was performed at the end of the experiment. All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Physical parameters

Physical parameters (body weight, food and water intake) and local injury were studied during the treatment of animals. Mortality was also recorded during the treatment of all groups. Autopsy was done during the course of treatment.

Hematological parameters

Blood samples were analyzed for routine hematological parameters. Blood samples were collected from orbital sinus following morning using heparin as anticoagulant. Blood cell count was done using blood smears. Hematological parameters were studied using Sysmax-K1000 Cell Counter. Parameters studied were Hemoglobin (Hb), Total Red Blood Corpuscles (RBC), Reticulocyte (Rt), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelets, Total White Blood Corpuscles (WHC), Neutrophils (N), Lymphocytes (L), Eosinophils (E) and Monocytes (M).

Biochemical parameters

Serum biochemical examinations were performed using Robonik ASP-300. Blood urea nitrogen (BUN) (mg%), Serum Glutamic Pyruvic Transaminase (SGPT) (IU/L), Serum Glutamic Oxaloacetic Transaminase (SGOT) (IU/L) and Serum Alkaline Phosphatase (SAP) (IU/L) and blood sugar levels were estimated in plasma sample.

Necroscopy

All animals were sacrificed on day 29, using CO_2 asphyxiation technique. Necroscopy of all animals were carried out and the weights of the following organs were recorded: liver, kidney and heart. The organ weights were recorded as absolute values and their relative values (i.e. per cent of the body weight) were calculated.

Histopathology

Following tissue samples of organs from control and animals treated at the highest dose level of 20 mg/kg, were preserved in 10% formalin for histopathological examination. Heart; Kidneys; Liver; Lungs; Stomach.

Heart, Kidneys, Liver, Lungs and Stomach of low and intermediate dose group animals were preserved for possible histopathological examination, in case the histopathological examination of high dose group animals is indicative of abnormalities associated with the treatment.

Statistical analysis

The data for body and relative organ weights, hematology and serum biochemistry were analyzed statistically and resulting data were expressed as mean±SD. Statistical data was analyzed by Dennett's test, between control vs all treated groups. $p<0.05$ was considered statistically significant.

RESULTS

Body weight and food intake

There were no significant changes observed in the body weight in all the group of rats throughout the dosing period. However, animals from control and low dose groups exhibited normal body weight but in case of high and middle dose treated animals, rate of weight gain decreased in comparison with control group during the dosing period of 28 days. Data for food consumption are shown in figure 1 and 2. During the dosing period and at the termination, the food intake, in case of high and middle dose treated animals decreased gradually in comparison with the control group. Average intake per day of food was approximately 7.77 in both male and female rats.

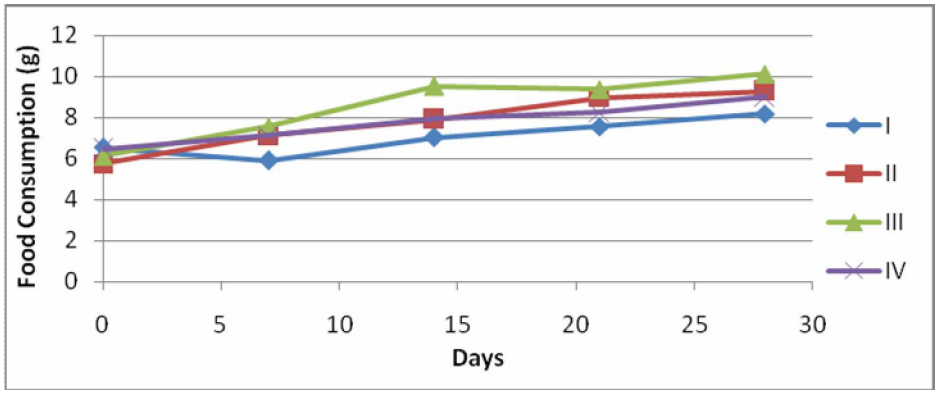

Figure 1
Curve showing food consumption by male rats

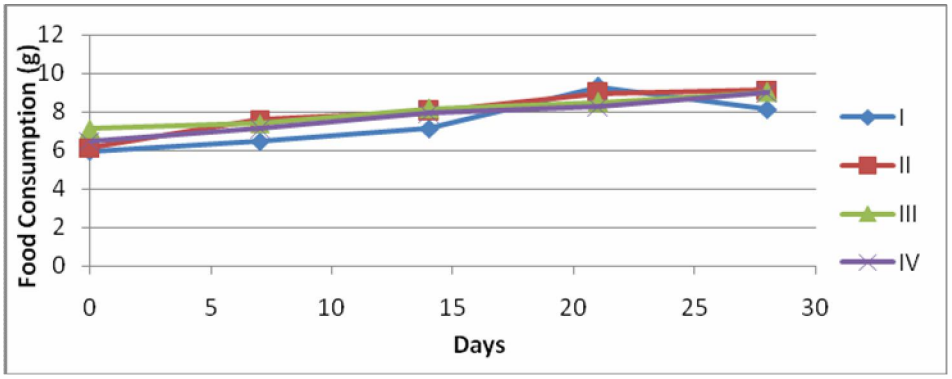

Figure 2
Curve showing food consumption by female rats

Hematology

At the termination of dosing on day 29, no significant changes were observed in the values of different hematological parameters studied when compared with control group and values obtained were within normal biological and laboratory limits for both male and female rats. Results of the hematological parameters are given in table 1 and 2.

Table 1: Hematological parameters in male rats

Gr. No.	I	II	III	IV
Hb (g%)	13.57 ±1.04	13.95±0.89	13.90±0.85	13.95±0.33
RBC (x10^6/cmm)	6.48±0.56	6.40±0.52	6.50±0.58	6.43±0.61
Rt (%)	1.25±0.19	1.18±0.21	1.22±0.23	1.33±0.17
HCT (%)	40.50±3.42	41.12±3.09	40.63±2.13	41.50±1.69
MCV (µm^3)	62.72±6.06	64.67±7.81	63.02±7.32	64.91±5.00
MCH (pg)	21.02±2.05	21.94±2.46	21.52±2.21	21.83±1.75
MCHC (%)	40.50±0.61	33.96±0.85	34.23±1.68	33.64±0.83
Platelets	6.53±0.56	6.50±0.55	6.42±0.58	4.88±2.73
WBC (x10^5/cmm)	6.38±0.58	6.65±1.05	6.78±0.85	5.32±0.3.05

Table 2: Hematological parameters in female rats

Sr. No.	I	II	III	IV
Hb (g%)	15.05±0.56	13.78±0.84	14.48±0.75	14.13±1.24
RBC (x10^6/cmm)	6.72±0.59	6.52±0.53	6.42±0.51	6.45±0.59
Rt (%)	1.27±0.16	1.58±0.34	1.48±0.37	1.13±0.22
HCT (%)	43.95±4.65	40.38±3.04	42.83±2.71	41.25±1.84
MCV (µm^3)	66.14±11.29	62.32±7.26	67.02±5.66	64.17±3.28
MCH (pg)	22.61±3.44	21.27±2.24	22.67±1.75	21.95±1.51
MCHC (%)	34.31±1.86	34.16±1.86	33.84±0.65	34.21±1.91
Platelets	6.42±0.51	6.30±0.68	6.75±0.54	6.63±0.63
WBC (x10^5/cmm)	6.55±0.71	6.43±0.54	6.43±0.50	6.80±1.01

Biochemical parameters

At the termination of day 29, all biochemical parameters studied i.e. Total serum protein, SAP, BUN and blood sugar were found to be comparable with control group and were within normal biological and laboratory limits but SGPT, SGOT value was high in case of high and middle dose treated animals. Results of biochemical parameters are given in table 3 and 4.

Table 3: Biochemical parameters in male rats

Sr. No.	I	II	III	IV
Total Serum Protein (g%)	6.77±0.66	6.68±0.62	6.70±0.84	5.44±3.09
BUN (mg%)	28.00±4.10	25.33±5.47	33.67±5.61	24.60±14.17
SGPT (IU/L)	45.00±9.03	48.00±6.45	54.67±11.24	42.80±24.77
SGOT (IU/L)	105.67±14.71	115.67±8.85	106.00±14.31	79.60±46.57
SAP (IU/L)	395.33±79.80	350.33±54.24	310.33±18.48	221.40±133.03
Blood sugar (mg%)	104.50±13.71	105.00±12.47	95.17±7.83	85.40±50.53

Table 4: Biochemical parameters in female rats

Sr. No.	I	II	III	IV
Total Serum Protein (g%)	6.38±0.78	6.77±0.61	6.38±0.55	6.45±0.50
BUN (mg%)	25.50±3.94	22.50±2.88	46.00±8.99	26.75±2.22
SGPT (IU/L)	49.00±8.58	55.50±3.73	75.33±10.01	52.75±2.63
SGOT (IU/L)	110.83±11.00	109.50±13.88	132.83±29.54	114.00±10.68
SAP (IU/L)	361.00±43.69	421.00±45.55	305.50±17.13	340.00±51.70
Blood sugar (mg%)	106.00±10.14	113.67±9.54	103.83±11.94	98.25±10.87

DISCUSSION

Aceclofenac is an effective simple analgesic and antipyretic drug. Intravenous administration of aceclofenac is found to be more effective and the target concentration achieved more rapidly and with less variability in plasma concentrations compared with enteral formulations.[18] Aceclofenac has been proven to be more superior to paracetamol and naproxen in pain reduction and functional improvement in symptomatic patients with osteoarthritis of knee, with no significant difference in tolerability.[19,20]

In the present investigation, there were no signs of local injury and inflammatory response at site of injection in the treated group of rats. No behavioral changes were observed during the study period in all the treatment groups. Blood was evaluated for hematological toxicity of aceclofenac infusion. Hemogram was estimated and no deleterious effects were observed on blood cell count, hemoglobin and other related parameters. Liver is the vital organ which is involved in the maintenance of metabolic function and detoxification of drugs. Liver damage is associated with cellular necrosis, increase in liquid tissue peroxidation and depletion in the tissue GSH levels. In addition, serum levels of many biochemical markers like SGOT, SGPT, BUN and total serum protein levels are elevated. At the termination of day 29, all biochemical parameters studied i.e. total serum protein, SAP, BUN and blood sugar were found to be comparable with controls and were within the normal biological and laboratory limits but SGPT, SGOT value was high in case of high and middle dose treated animals. In the present study, biochemical parameters related to kidney function were observed in blood urea,

creatinine, glucose and proteins with respect to control. Organ weight analysis showed no change in the weights of organs of rats of different dose groups as compared to control.

There were no signs of significant toxicity observed in any of the organ in histopathological analysis. Thus histological studies provide support to the safety data of other physiological, biochemical and hematological parameters of aceclofenac infusion at low dose. However, after dissection of high dose animals the stomach showed hyperemic patchy areas mainly in the antral region. At the middle doses (10 mg/kg) all animals showed same signs of toxicity during same duration but no mortality was found whereas at 5 mg/kg no significant toxicity was found. It was observed that 4 animals out of 12 (6 male and 6 female) have died at high dose of 20 mg/kg after 12 days of administration of drugs. With this dose (20 mg/kg) animals showed bleeding per rectum as well as black stool.

CONCLUSION

In summary, our data suggested that aceclofenac infusion is safe at low dose and can be intended for human use as it indicated no clinically relevant alterations of any of physiological and biochemical parameters. In conclusion, our study provides data for the safe use of aceclofenac. Aceclofenac is an effective safe antibiotic and possess wide clinical application and worth for wide use.

ACKNOWLEDGEMENT

We are grateful to Bioequivalence Study Centre, Jadavpur University, Kolkata for providing the necessary facilities and conducting animal testing for the study. Authors are thankful to financial department of Research and Development Centre, Health Biotech limited for providing the financial support.

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