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Evaluation of Toxicological Profile of Ibuprofen in Wistar Albino Rats

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Abstract

Ibuprofen is an effective, cheap, and frequently used non-steroidal anti-inflammatory drug. The present study investigated the dose- and time-dependent effects of ibuprofen on hepatic, renal, and hematological functions in rats. Groups of rats (n=6) were given ibuprofen (20, 40 $mgkg^{-1}day^{-1}$) for 7, 14 or 28 days; or vehicle (control), orally. Blood samples were obtained, and hematological indices and biochemical markers of hepatic and renal functions were measured. Ibuprofen significantly increased, $P < 0.001$ serum alkaline phosphatase level at all doses and durations of exposure. Serum uric acid level was dose- and time-dependently decreased by ibuprofen, but alanine transaminase was increased, $P < 0.05$ by ibuprofen, only at 40 $mgkg^{-1}$ and following subchronic (28 days) exposure. In addition, at 40 $mgkg^{-1}$, ibuprofen increased creatinine and urea levels at all durations of exposure; but at 20 $mgkg^{-1}$, creatinine and urea were increased only in rats that were exposed for 28 days. Furthermore, subchronic exposure of 40 $mgkg^{-1}$ ibuprofen increased, $P < 0.01$ WBC count, but it caused no significant effect on WBC at the lower durations of exposure and dose. Also, while RBC and hematocrit were not affected, ibuprofen significantly, $P < 0.01$,$P < 0.001$ decreased platelet counts in all treated rats except those that were exposed for 7 days. The implication of this research is that chronic use of ibuprofen could affect hepatic, renal and hematological functions in the rat; and duration of exposure may promote ibuprofen toxicity relative to dose.

Keywords: Aminotransferases, ibuprofen, platelets, renal toxicity, subchronic

1. Introduction

Ibuprofen, a propionic acid derivative, is an example of the non-steroidal anti-inflammatory drugs (NSAIDs), which are among the most frequently prescribed medications worldwide [1,2]. Ibuprofen is one of the most commonly used NSAIDs for the relief of fever, pains and inflammatory conditions. The drug is reported to be better and preferred for joint and muscle pain than most other analgesics and has been used by patients with arthritis for years [3]. The mechanism of action of ibuprofen, like other NSAIDs, has been established to be via inhibition of cyclooxygenase (COX) enzyme activity [4]. Inhibition of COX enzyme by NSAIDs results in prevention of the synthesis of prostaglandins which mediate vital physiological functions, including gastric cytoprotection, maintenance of renal blood flow, and platelet activation [5].

Although, NSAIDs are generally considered to have high safety profiles, the frequent and widespread use of ibuprofen and other NSAIDs is likely to increase the prevalence of their adverse effects. Ibuprofen and other NSAIDs are commonly associated with gastrointestinal (GI) toxicity [6,7]. In addition, NSAIDs have been shown in previous studies to alter renal function [8,9]. However, most of such reports are on high dose levels of the agents (> clinical doses) and existing data on ibuprofen-mediated renal toxicity in relation to duration of exposure is not exhaustive. Furthermore, NSAIDs are known to have antiplatelet activities [10], however, the antiplatelet effects of ibuprofen in relation to dose and duration of exposure is not fully established. Importantly, ibuprofen is an over-the-counter NSAID, with the consequence of an increase in its usage and toxicological potentials. We hypothesize that prolong exposure of clinical dose levels of ibuprofen would increase its adverse effects on biological systems. Herein, we investigated the effects of acute and subchronic exposure of clinical and double clinical dose equivalents of ibuprofen on hepatic, renal, and hematological functions in rats.

2. Materials and methods

Materials

Ibuprofen (Tabufen^{R}) tablets (Fidson Pharm Ltd, Nigeria) was purchased from the Pharmacy Unit of the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria. The drug was powdered and mixed with distilled water in a glass mortar and administered as aqueous suspension by oral gavage. The drug was continuously agitated during administration in order to deliver the drug homogeneously to the animals.

Animals

Male Wistar rats weighing between 210-220 g, obtained from the Animal House of the Department of Pharmacology, University of Port Harcourt, were used for the study. The animals were fed with standard rodent chow (Topfeeds Ltd, Sapele, Nigeria) and allowed free access to tap water *ad libitum*. They were maintained at a room temperature of 28.0±2.0^{o}C under natural lighting condition and handled in accordance with international guidelines for Care and Use of Laboratory Animals as promulgated by the Canadian Council of Animal Care [11].

Experimental design

The rats were divided into 7 groups containing 6 animals each. The first, second and third groups were given standard therapeutic dose equivalent of ibuprofen [12]: 20 mgkg^{-1} daily (in two divided doses) for 7, 14 and 28 days, respectively. The fourth, fifth and sixth groups were given 40 mgkg^{-1} of ibuprofen daily (in two divided doses) for 7, 14 and 28 days, respectively. The seventh group served as control and was given distilled water daily for 28 days. At the end of each administration, the animals were anesthetized with deep diethylether and blood samples were collected separately into clean specimen bottles and EDTA bottles for biochemical and hematological analyses, respectively.

Biochemical analysis

Blood samples in specimen bottles were centrifuged for 15 min at 3,000 rpm and clear

sera were separated from the cells and stored at −80°C. Serum was assayed for alkaline phosphatase (ALP) using the phenolphthalein method [13]; acid phosphatase (ACP) using the colorimetric method [14]; urea using the Urease-Berthelot method [15]; creatinine using the alkaline picrate method [16]; protein using the biuret method [17]; uric acid using the enzymatic colorimetric method [18]; and total cholesterol using the enzymatic endpoint method [19]. Serum aspartate transaminase (AST) and alanine transaminase (ALT) levels were also measured according to the method described by Reitman and Frankel [20].

Hematological analysis

Whole blood collected into EDTA bottle was assayed for hematocrit or packed cell volume (PCV). White blood cell (WBC), red blood cell (RBC) and platelet counts were also determined using an autoanalizer.

Statistical analysis

Data are expressed as mean ± SEM. Comparisons between control values and experimental values were performed with one-way analysis of variance (ANOVA), followed by Dunnett comparison test. Statistical significance was set at $P \leq 0.05$.

Results

Biochemical parameters

Serum alkaline phosphatase (ALP) levels in all ibuprofen exposed rats were significantly, $P < 0.001$ increased, when compared to control rats (Table 1). In addition, while acid phosphatase (ACP) levels were significantly, $P < 0.001$ increased in rats that received 40 $mgkg^{-1}$ ibuprofen for 14 and 28 days, ACP levels in all 20 $mgkg^{-1}$ ibuprofen treated rats, as well as those that received 40 $mgkg^{-1}$ ibuprofen for 7 days were not significantly different from control (Table 1). Average serum level of alanine transaminase (ALT) in rats that received 40 $mgkg^{-1}$ ibuprofen for 28 days was significantly, $P < 0.05$ increased, while there were no changes in ALT levels in all other treated rats, when compared to control rats (Table 2).

Table 1: Serum levels of phosphatase enzymes following different dose and time administration of ibuprofen in rats

Dose	ALP (IU L^{-1})			ACP (IU L^{-1})		
	7 Days	14 Days	28 Days	7 Days	14 Days	28 Days
Control	40.75±8.46	40.75±8.46	40.75±8.46	2.75±0.50	2.75±0.50	2.75±0.5
20 mg/kg	81.00±6.38*	82.75±3.30*	80.25±5.79*	3.25±1.89	4.00±0.82	5.00±2.16
40 mg/kg	87.75±7.00*	108.80±18.87*	95.25±10.56*	5.00±1.16	10.75±2.63*	10.50±2.65*

Data expressed as mean ± SEM. * Significant at $P < 0.001$. ALP- alkaline phosphatase; ACP- acid phosphatase.

Furthermore, while serum creatinine levels in 7 days treated rats were not affected, it was elevated, $P < 0.001$ in rats that received ibuprofen for 28 days, when compared to control (Table 3). In the 14 days treated rats, creatinine level was significantly, $P < 0.001$ increased in those that received 40 $mgkg^{-1}$ ibuprofen, while it was not affected in those that received 20 $mgkg^{-1}$ ibuprofen (Table 3). In addition, there were no changes in serum levels of urea in all 20 $mgkg^{-1}$ ibuprofen treated rats, but it was significantly, $P < 0.01$,$P < 0.001$ and time-dependently increased in 40 $mgkg^{-1}$ ibuprofen treated rats, compared to control (Table 3). Furthermore, when compared to control, serum uric acid levels obtained in ibuprofen exposed rats were significantly lower

at all doses and durations of exposure. The effects of ibuprofen on uric acid were also dose- and time-dependent (Table 5). There were no significant ($P > 0.05$) changes in serum AST, protein and cholesterol levels in ibuprofen exposed rats, when compared to their corresponding levels that were obtained in control rats (Table 2, Table 4).

Table 2: Serum levels of transaminase enzymes following different dose and time administration of ibuprofen in rats

Dose	AST (IU L^{-1})			ALT (IU L^{-1})		
	7 Days	14 Days	28 Days	7 Days	14 Days	28 Days
Control	22.75±2.06	22.75±2.06	22.75±2.06	10.50±0.65	10.50±0.65	10.50±0.65
20 mg/kg	25.00±2.31	24.25±4.86	26.25±5.62	13.00±5.29	11.50±4.20	16.00±1.41
40 mg/kg	27.50±1.73	27.25±2.22	28.25±1.50	13.25±4.72	15.50±6.14	18.50±1.73*

Data expressed as mean ± SEM. * Significant at $P < 0.05$. AST- aspartate transaminase; ALT- alanine transaminase.

Table 3: Serum levels of creatinine and urea following different dose and time administration of ibuprofen in rats

Dose	Creatinine (μmol L^{-1})			Urea (mmol L^{-1})		
	7 Days	14 Days	28 Days	7 Days	14 Days	28 Days
Control	35.00±4.76	35.00±4.76	35.00±4.76	4.75±0.29	4.75±0.29	4.75±0.29
20 mg/kg	33.00±3.56	47.00±3.46	81.75±6.70**	5.35±0.53	5.50±0.49	6.03±0.45
40 mg/kg	38.50±2.52	79.75±10.37**	93.00±11.34**	6.65±0.65*	7.23±1.49**	8.23±0.32**

Data expressed as mean ± SEM. * Significant at $P < 0.01$; ** Significant at $P < 0.001$.

Table 4: Serum levels of total protein and cholesterol following different dose and time administration of ibuprofen in rats

Dose	Total protein (g L^{-1})			Total cholesterol (mmol L^{-1})		
	7 Days	14 Days	28 Days	7 Days	14 Days	28 Days
Control	59.00±5.77	59.00±5.77	59.00±5.77	1.08±0.13	1.08±0.13	1.08±0.13
20 mg/kg	57.50±3.00	57.00±3.83	57.75±2.63	1.33±0.30	1.30±0.18	1.10±0.29
40 mg/kg	61.00±9.31	57.50±2.52	55.00±3.46	1.07±0.22	1.18±0.21	4.15±5.90

Data expressed as mean ± SEM.

Table 5: Serum levels of uric acid following different dose and time administration of ibuprofen in rats

Dose	Uric acid (mmol L^{-1})		
	7 Days	14 Days	28 Days
Control	0.46±0.24	0.46±0.24	0.46±0.24
20 mg/kg	0.19±0.12*	0.15±0.00**	0.18±0.10**
40 mg/kg	0.09±0.04***	0.07±0.00***	0.05±0.02***

Data expressed as mean ± SEM. * Significant at P < 0.05; ** Significant at P < 0.01; *** Significant at P < 0.001.

Hematological parameters

There were no significant changes in hematocrit levels between ibuprofen exposed and control rats (Table 6). Red blood cell (RBC) counts in rats that had 14 and 28 days exposure to ibuprofen were not significantly different, compared to control rats, but there was a significant, P < 0.01 reduction in RBC count in rats that received 40 $mgkg^{-1}$ ibuprofen for 7 days (Table 6). Additionally, white blood cell (WBC) counts in rats that had 7 and 14 days exposure to ibuprofen were not significantly different, compared to control rats, but there was significant, P < 0.01,P < 0.001 elevation in WBC counts in the rats that were exposed to ibuprofen for 28 days (Table 7). Furthermore, there were no changes in platelet counts in rats that were exposed to ibuprofen for 7 days, but counts obtained in rats that received ibuprofen for 14 and 28 days were significantly, P <0.01,P < 0.001 decreased when compared to control rats. The ibuprofen-induced platelet reduction was dose-dependent in the 14 days exposed group, but non-dose-dependent in the 28 days exposed group (Table 7).

Table 6: Hematocrit levels and red blood cell (RBC) counts following different dose and time administration of ibuprofen in rats

Dose	Hematocrit (%)			RBC (x 10^6 μL^{-1})		
	7 Days	14 Days	28 Days	7 Days	14 Days	28 Days
Control	49.00±5.48	49.00±5.48	49.00±5.48	6.99±0.53	6.99±0.53	6.99±0.53
20 mg/kg	42.75±5.56	44.50±3.70	48.00±8.91	6.12±0.41	6.69±1.18	6.13±0.71
40 mg/kg	37.50±1.29	45.50±8.39	49.75±4.92	5.24±0.18*	6.80±0.63	5.78±0.24

Data expressed as mean ± SEM. * Significant at P < 0.01.

Table 7: White blood cell (WBC) and platelet counts following different dose and time administration of ibuprofen in rats

Dose	WBC (x 10^9 μL^{-1})			Platelet (x 10^3 μL^{-1})		
	7 Days	14 Days	28 Days	7 Days	14 Days	28 Days
Control	4.35±0.26	4.35±0.26	4.35±0.26	445.00±47.26	445.00±47.26	445.00±47.26
20 mg/kg	4.25±0.60	4.20±0.33	5.55±0.52*	400.00±16.33	345.00±37.86*	300.00±27.08**
40 mg/kg	3.93±0.54	4.43±0.48	6.23±0.40**	420.00±32.66	312.50±9.574**	259.00±34.51**

Data expressed as mean ± SEM. * Significant at P < 0.01; ** Significant at P < 0.001.

Discussion

Ibuprofen is an effective, cheap, and frequently prescribed nonsteroidal anti-inflammatory drug (NSAID). It is an over-the-counter (OTC) drug, and is widely used as an analgesic, antipyretic, and anti-inflammatory drug globally [3,21]. Because of its widespread and frequent usage, regular toxicological evaluation of ibuprofen becomes essential. We report the effects of ibuprofen (20, 40 $mgkg^{-1}$) on hepatic, renal, and hematological indices in relation to dose and duration of exposure. The concentrations of ibuprofen used were equivalent to standard therapeutic dose and double of the standard dose of the drug [12].

Serum phosphatase and transaminase levels are generally used in toxicological studies to evaluate hepatic function [22,23]. Elevation in serum levels of alanine transaminase (ALT) and alkaline phosphatase (ALP) by ibuprofen in this study is indicative of cellular injury to the liver. Aspartate aminotransferase (AST) was also increased dose- and time-dependently, although not significantly. As ALT was affected only after 28 days of exposure of the high dose of ibuprofen (40 $mgkg^{-1}$) used in this study, the effect of ibuprofen on liver function strongly correlates positively with the dose and duration of exposure. Accordingly, higher dose levels and/or longer durations of ibuprofen exposure would increase hepatic toxicity, and the elevation of serum AST levels may become significant, as a result of increased leakage of the enzyme from damaged hepatocytes.

NSAIDs are known to alter renal function at high dosages [8,9], partly due to their inhibition of prostaglandin synthesis [4]. However, there is no much information on ibuprofen mediated renal toxicity at clinical dose levels. Urea and creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys [24], and their serum concentrations are commonly used as surrogate markers of renal toxicity [25,26]. From our results, ibuprofen would affect renal function as it increased urea and creatinine levels. The result also showed that urea and creatinine were increased by ibuprofen mostly at the high dose (40 $mgkg^{-1}$) and following subchronic exposure. This observation indicates that the renal effects of ibuprofen correlates with its dose and duration of exposure, which is consistent with previous reports on NSAIDs [27]. In addition, the results also show that prolong use of standard dose levels of ibuprofen may alter renal function.

Uric acid is an antioxidant which scavenges reactive oxygen radicals in the blood [28]; ibuprofen-induced reduction of uric acid in this study may alter cellular redox balance and affect biochemical functions. By this observation, ibuprofen may affect antioxidative actions of blood with consequent increase in oxidative stress. This may contribute to some of the toxicological effects of ibuprofen as oxidative stress has been linked to the pathogenesis of most conditions [29]. In addition, since ibuprofen did not affect cholesterol levels, clinical doses of the drug may not affect cholesterol metabolism and may not therefore have direct cardiovascular effects in the rat.

Alteration of hematological function is a common observation in the administration of many therapeutic agents. The present study shows that ibuprofen may not affect hematocrit levels over the dose range and duration of exposure used, but subchronic exposure would increase white blood cell counts. This observation is consistent with the result of a similar study on aquatic animals [30]. Although, NSAIDs are known to have antiplatelet activities [10], the antiplatelet effects of ibuprofen in relation to dose and duration of exposure is not fully established. Our observation of the reduction of platelet counts after 14 and 28 days of ibuprofen exposure, and a non-effect after 7 days of exposure in this study, strongly suggests that the antiplatelet effect of ibuprofen is more important with duration of exposure than dose. Furthermore, the antiplatelet effect of ibuprofen was observed to be dose-dependent during the 14 days treatment, but not dose-dependent during the 28 days treatment. This provides more evidence that the inhibitory effect of ibuprofen on platelet increases more with duration of exposure relative to dose.

Conclusion

The implication of this research is that subchronic exposure of standard dose levels of ibuprofen could affect hepatic, renal and hematological functions in the rat; and duration of exposure may increase ibuprofen toxicity relative to dose.

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