RENAL EFFECTS OF SOME NSAIDS IN ALBINO RATS: A COMPARATIVE STUDY

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ABSTRACT

Background: Non steroidal anti-inflammatory drugs (NSAIDs) are cyclooxygenase enzyme inhibitors used widely and frequently as analgesics, antipyretics and anti-inflammatory agents. This study investigated the comparative effects of aspirin (ASA), ibuprofen (IBF) and diclofenac sodium (DCF) on kidney function in albino rats, using biochemical parameters as indices.

Method: Animals were divided into 7 groups (n=5) and administered daily with ASA (50 & 100mg/kg), IBF (20 & 40mg/kg), DCF (2 & 4mg/kg) and vehicle by oral lavage for 28 days. Blood samples were collected and the serum levels of urea, creatinine, aspartate transaminase (AST) and total protein were measured using standard methods.

Results: The results showed that ASA, IBU and DCF caused significant (p<0.05) and dose-dependent increases in serum levels of urea (39.79, 47.58 and 73.89%, respectively), creatinine (104.29, 128.00 and 133.57%, respectively) and AST (63.74, 24.18 and 32.97%, respectively) without significant (p>0.05) effect on total protein, compared to the control.

Conclusion: The results obtained indicate that long administration of the NSAIDs will cause renal toxicity in a rank order of DCF > IBU > ASA, which may be partly due to their inhibitory effects on prostaglandins.

Keywords: Creatinine, diclofenac, ibuprofen, kidney and toxicity.
1. INTRODUCTION

Non steroidal anti-inflammatory drugs (NSAIDs) are clinically very useful drugs which are relied upon for the relief of pain, fever and treatment of inflammatory conditions (Green, 2001; Burke et al., 2006). These drugs are effective in the treatment of both acute and chronic conditions of pain and inflammation, including osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout, sprains, toothache and dysmenorrhoea (Gøtzsche, 1989; Ekman et al., 2005; Burke et al., 2006).

Although NSAIDs have wide therapeutic indices, their widespread and chronic use has increased the prevalence of the adverse effects of these drugs (Henry et al., 1996; Cannon et al., 2006). The pharmacological actions of these drugs have long been established to be via inhibition of cyclooxygenase (COX) enzyme activity (Vane, 1971; Reynolds, 1982). The inhibition of COX enzyme, which exists in two isoforms (COX-1 and COX-2) by NSAIDs results in prevention of the synthesis of prostanoids which mediate several vital physiological functions, including gastric cytoprotection, maintenance of renal blood flow and platelet activation (Capone et al., 2007). Two most common adverse effects associated with NSAIDs are gastrointestinal (GI) toxicity- especially dyspepsia and gastric ulceration (Larkai et al., 1987; Traversa et al., 1995; Ofman et al., 2003) and alteration in renal function (Bennett et al., 1996; Brater, 1999). The GI effects have been observed to be more prevalent with the COX-1 inhibitors (Mitchell et al., 1993), however, the relative risk of renal toxicity among NSAIDs is not very known.

Furthermore, NSAIDs are excreted by the kidneys, thus existing renal pathology in patients will increase their toxicities. It is therefore necessary to select NSAIDs with respect to patients’ pathophysiological status. In view of the wide range of indications and consequent frequent
usage of NSAIDs, knowledge on the relative hepatic effects of these agents will enhance their rational selection for patients and reduce their toxicities.

We evaluated the comparative adverse effects of prolong administration of aspirin, ibuprofen and diclofenac (three widely used NSAIDs) on renal function in male albino rats, using biochemical markers as indices.

2. MATERIALS AND METHODS

Materials

Aspirin (Acetylsalicylic acid) tablets (May & Baker Nigeria PLC); ibuprofen (TabufenR) tablets (Fidson Pharm Ltd, Nigeria); and diclofenac sodium (ClofenacR) tablets (Hovid Bhd, Malaysia) were obtained from the Pharmacy Department of the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria.

The drugs were powdered separately in a glass mortar mixed with distilled water and were administered as aqueous suspensions by oral gavage. The drug suspensions were continuously agitated during administration in order to deliver the drugs homogeneously to the animals.

Animals

Male albino rats weighing between 210-220 g, were obtained from the animal house of the Department of Pharmacology, University of Port Harcourt, Nigeria. The animals were allowed to acclimatize for 14 days in a well ventilated room at a room temperature of 28.0±2.0°C under natural lighting condition. The animals were fed with standard rodent chow (Topfeeds Ltd, Sapele, Nigeria) and allowed free access to tap water ad libitum. The animals were handled in
accordance with the international, national and institutional guidelines for Care and Use of Laboratory Animals as promulgated by the Canadian Council of Animal Care, (2009).

Methods

A total number of thirty-five (35) animals were divided into 7 groups (A, B, C, D, E, F and G) containing 5 animals each. Groups A and B were given 25 and 50 mg/kg of aspirin twice daily, respectively; C and D were given 10 and 20 mg/kg of ibuprofen twice daily, respectively; E and F had 2 and 4mg/kg of diclofenac sodium once daily, respectively. Group G animals (control) received only distilled water (0.1ml) twice daily. All drugs were administered for 28 days. At the end of the drug treatments, animals were sacrificed by decapitation under pentobarbitone anaesthesia, 37mg/kg ip (Flecknell, 2009) and blood samples were collected into clean specimen bottles.

Biochemical analysis

Blood samples were centrifuged for 15 min at 3,000 rpm and clear sera were separated from the cells and stored at −80°C. Urea was assayed using Urease-Berthelot method (Kaplan, 2006); creatinine assay was done using alkaline picrate method (Tietz et al., 1986), and protein was assayed using biuret method as described by Henry et al. (1974). In addition, aspartate transaminase (AST) level was measured according to the method described by Reitman and Frankel (1957).

Statistical analysis
Data were expressed as mean ± standard error of mean. Comparisons between control values and
the values obtained in treated groups were performed with one-way analysis of variance
(ANOVA). Statistical significance was set at p<0.05.

3. RESULTS

The results showed that aspirin (ASA), ibuprofen (IBU) and diclofenac sodium (DCF)
significantly (p<0.05) increased serum urea, creatinine and aspartate transaminase (AST) levels,
compared to the control (Figs. 1 - 9). The effects of the drugs on creatinine and AST were also
dose-dependent (Figs. 4 - 9), but they caused no significant (p>0.05) effects on total protein (Fig.
10, Fig. 11 and Fig. 12). The serum urea levels obtained in the ASA-administered animals were
6.53±0.65 and 6.64±0.28 mmol/L, respectively (Fig. 1); the levels in the IBF-administered
animals were 6.65±0.32 and 7.01±0.16 mmol/L, respectively (Fig. 2), while the levels in the
DCF-administered animals were 7.43±0.35 and 8.26±0.20 mmol/L, respectively (Fig. 3). These
values were all significantly (p<0.05) higher compared to the basal urea level (4.75±0.14
mmol/L) obtained in the control animals (Fig. 1, Fig. 2 and Fig. 3) and the ASA- IBF- and DCF-
induced maximum serum levels were equivalent to 39.79, 47.58 and 73.89 % increases,
respectively. In addition, the serum creatinine values obtained in the animals that received ASA
(100 mg/kg), IBF (40 mg/kg) and DCF (4 mg/kg) were 71.50±4.45, 79.80±3.49 and 81.75±3.35
µmol/L, respectively (Fig. 4, Fig. 5 and Fig. 6). These values were significantly (p<0.05) higher
than the control serum level (35.00±2.83 µmol/L) and represented percentage increases of
104.29, 128.00 and 133.57 %, respectively. Furthermore, the serum levels of AST induced by
ASA (100 mg/kg), IBF (40 mg/kg) and DCF (4 mg/kg): 37.25±2.43, 28.25±0.75 and 30.25±2.25
IU/L, respectively were significantly (p<0.05) higher than the serum level of 22.75±1.03 IU/L.
obtained in the control (Fig. 7, Fig. 8 and Fig. 9). These values were equivalent to 63.74, 24.18
and 32.97% increases, respectively.

The serum levels of protein in animals treated with ASA, IBF and DCF were not significantly
(p>0.05) different from the control (Fig. 10, Fig. 11 and Fig. 12).

4. DISCUSSION

In this study, the effects of subchronic administration of different dose levels of aspirin (50 – 100
mg/kg), ibuprofen (20 – 40 mg/kg) and diclofenac (2 – 4 mg/kg) on serum urea, creatinine,
aspartate transaminase (AST) and total protein levels were evaluated in rats. Aspirin, ibuprofen
and diclofenac, which are derivatives of salicylic, propionic and phenylacetic acids, respectively
are commonly used as analgesic, anti-inflammatory and antipyretic agents. The drugs are among
the most prominent and commonly used non steroidal anti-inflammatory drugs (NSAIDs) and
are available over-the-counter in most countries (Warden, 2010). The primary mechanism of
action of these drugs, like all other NSAIDs is the inhibition of cyclooxygenase (COX), a
heme protein that exists in two isoforms (COX-1 and COX-2). Although, a variant of the COX-1
enzyme has been described and identified as COX-3 recently, it is reported to be without any
COX activity in humans (Kis et al., 2005). Cyclooxygenase enzyme converts arachidonic acid to
prostanoids such as prostaglandin (PG) E₂, PGF₂α, PGD₂, prostacyclin I₂ (PGI₂), and
thromboxane (TX) A₂ (Vane 1971; Capone et al., 2007). Furthermore, aspirin is classified as
COX-1 selective, while ibuprofen and diclofenac are nonselective COX inhibitors because the
last two drugs have equal inhibitory effects on both COX isoforms.
Inhibition of the synthesis of renal prostaglandins by NSAIDs may affect renal function, as prostaglandins are involved in the regulation of solute homeostasis, glomerular filtration and vascular tone, which are vital processes for normal kidney function. Although, the actions of prostaglandins may play minimal role in renal physiology under normal conditions, renal function becomes increasingly dependent on renal prostaglandin synthesis in certain conditions such as reduced renal perfusion and decreased circulating blood volume (Bennet et al., 1996; Brater, 1999). Accordingly, NSAIDs, when taken at therapeutic doses in healthy individuals may have little adverse effect on kidney function, but in susceptible patients (e.g. elderly patients) or high doses and prolong use, they can cause renal toxicity. Clinical manifestations of NSAIDs-induced renal adverse effects have been reported to include acute renal insufficiency, hypertension, peripheral edema, hyperkalemia, congestive heart failure, and papillary necrosis (Whelton and Hamilton, 1991; Brater 2001). Whelton and Hamilton (1991) have also shown in their study that the drug effects were dose-dependent, independent of COX selectivity and occur in 15% of the test population.

Urea and creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys (Gaspari et al., 1998) and in most clinical and toxicological investigations, their serum concentrations are commonly used as surrogate markers of renal toxicity (Perrone et al., 1992; Mouton and Holder, 2006; Traynor et al., 2006). Furthermore, AST, also known as Serum Glutamic Oxaloacetic Transaminase (SGOT) is present in the liver and several other organs and is used as a marker of general toxicity (Wakade et al., 2008; Halima et al., 2010). In the present study, aspirin, ibuprofen and diclofenac caused significant (p<0.05) elevations in the serum levels of urea, creatinine and AST, compared to the control, indicating that these drugs may
adversely affect renal function. This is consistent with previous results and reports (Bennett et al., 1996; Brater, 2001). Also, the effects of the drugs were dose-dependent, which agrees with the findings of Brater (2001).

Furthermore, our results show that aspirin, ibuprofen and diclofenac significantly (p<0.05) increased urea by 39.79, 47.58 and 73.89%, respectively; creatinine by 104.29, 128.00 and 133.57%, respectively and AST by 63.74, 24.18 and 32.97%, respectively. This indicates that the drugs under investigations may have different propensities in causing renal toxicity, viz diclofenac > ibuprofen > aspirin, which makes this study novel. NSAIDs have been commonly associated with GI toxicity (Higuchi et al., 2009) and platelet dysfunction (Capone et al., 2007). Both conditions result from inhibition of COX-1 and COX-2 (especially COX-1) in the GI tract and platelets, respectively. In addition, previous studies have shown that COX-2 selective NSAIDs (the coxibs) have fewer GI effects than the nonselective NSAIDs (Bombardier et al., 2000; Laine et al., 2007). Also, indomethacin, ketoprofen and piroxicam have been reported to have the highest prevalence of gastric adverse effects, while ibuprofen and diclofenac produce lesser GI effects among the traditional NSAIDs (Traversa et al., 1995). Similarly, it has been shown that aspirin inhibits platelet function longer than the nonselective NSAIDs (Russell and Jobes, 2002). However, there is no similar data on the relative renal toxicities of NSAIDs prior to this study, which makes the finding of this study useful. Additionally, the result of this study will enhance rational selection among the NSAIDs and consequently reduce the prevalence of drug-induced toxicities, particularly as these drugs are widely and frequently used.

CONCLUSION
The results obtained in this study indicate that prolong administration of aspirin, ibuprofen and
diclofenac sodium will cause renal toxicity in a rank order of *diclofenac sodium* > *ibuprofen* >
*aspirin*, which may be partly due to their inhibitory effects on prostaglandins. Individualized use
of these drugs with respect to patients’ pathological state based on the results of this study will
reduce drug-induced toxicities.

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**FIGURE 1**: Aspirin-induced serum levels of urea in rats. Data expressed as mean ± SEM. *Significantly different from control at p<0.05.
FIGURE 2: Ibuprofen-induced serum levels of urea in rats. Data expressed as mean ± SEM.

* Significantly different from control at p<0.001.
FIGURE 3: Diclofenac-induced serum levels of urea in rats. Data expressed as mean ± SEM.

* Significantly different from control at p<0.001.
FIGURE 4: Aspirin-induced serum levels of creatinine in rats. Data expressed as mean ± SEM.

* Significantly different from control at p<0.05. ** Significantly different from control at p<0.001.
FIGURE 5: Ibuprofen-induced serum levels of creatinine in rats. Data expressed as mean ± SEM. * Significantly different from control at p<0.05. ** Significantly different from control at p<0.001.
FIGURE 6: Diclofenac-induced serum levels of creatinine in rats. Data expressed as mean ± SEM. * Significantly different from control at p<0.05. ** Significantly different from control at p<0.001.
FIGURE 7: Aspirin-induced serum levels of aspartate transaminase (AST) in rats. Data expressed as mean ± SEM. * Significant values at p<0.05 ANOVA.
FIGURE 8: Ibuprofen-induced serum levels of aspartate transaminase (AST) in rats. Data expressed as mean ± SEM. * Significant values at p<0.05 ANOVA.
FIGURE 9: Diclofenac-induced serum levels of aspartate transaminase (AST) in rats. Data expressed as mean ± SEM. * Significant values at p<0.05 ANOVA.
FIGURE 10: Aspirin-induced serum levels of total protein in rats. Data expressed as mean ± SEM.
FIGURE 11: Ibuprofen-induced serum levels of total protein in rats. Data expressed as mean ± SEM.
FIGURE 12: Diclofenac-induced serum levels of total protein in rats. Data expressed as mean ± SEM.