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Effects of Ciprofloxacin and Levofloxacin Administration on Some Oxidative Stress Markers in the Rat

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***Abstract*—**Fluoroquinolones are a group of antibiotics widely used because of their broad spectrum activity against both Gram-positive and Gram-negative bacteria. In this study, ciprofloxacin and levofloxacin were administered to rats at therapeutic doses to evaluate their effects on plasma arylesterase activity, as well as, on hepatic advanced oxidized protein products (AOPPs) and malondialdehyde (MDA) levels, as measures of oxidative stress. Ciprofloxacin (80 mg/kg body weight) and levofloxacin (40 mg/kg body weight) were administered to male albino rats for 7 and 14 days. The data obtained demonstrated that plasma arylesterase activity was significantly decreased by both drugs with ciprofloxacin administration inhibiting the activity by 29% and 30% while Levofloxacin treatment resulted in 35% and 30% inhibition, after 7 and 14 days treatment respectively. Hepatic AOPP and MDA levels were both elevated by these antibiotics. This study supplies further evidence that fluoroquinolones at therapeutic doses promote oxidative stress.

***Keywords*—**Arylesterase, Ciprofloxacin, Levofloxacin, Oxidative Stress.

I. Introduction

FLUOROQUINOLONES are anti-microbial agents, with broad spectrum of bacterial activity against both Gram-positive and Gram-negative bacteria [1], [2]. They are bacterial agents that exert their bactericidal action by inhibiting the action of bacterial enzymes DNA gyrase, a type II topoisomerase and topoisomerase IV, thereby, preventing cell division [3]. They are effective against urinary, gastrointestinal, skin, respiratory, bone and joint infections [4] and are the most commonly prescribed class of antibiotics, being utilized widely in the treatment of respiratory, urinary tract, gastrointestinal and abdominal infections [5], [6].

Fluoroquinolones are well tolerated in patients but their uses have been associated with some adverse effects, including gastrointestinal discomfort, cutaneous reactions e.g. phototoxicity, juvenile joint toxicity and adverse central nervous system (CNS) effects [7], [8]. Although the incidence of these side effects is relatively low, the high prescription rates of these antibiotics may pose serious health effects on the general population.

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Oxidative stress results when the antioxidant system is overwhelmed by the generation of excess reactive oxygen species (ROS) [9]. These reactive species like superoxide radical anion (O^{-2}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (HO^-) cause severe damage to macromolecules, tissues and organs through the process of lipid peroxidation (LPO), protein modification and DNA strand breaks [9], [10]. Oxidative stress resulting from the generation of these free radicals is known to contribute immensely to several pathological conditions like aging, cancer, cardiovascular disorder, neurodegenerative diseases among others [11], [12].

In this study, ciprofloxacin and levofloxacin were evaluated for their potential to induce oxidative stress based on their effects on arylesterase activity of plasma paraoxonase, hepatic malondialdehyde (MDA) and advanced oxidative protein products (AOPP) levels which are enzymatic, lipid peroxidation and protein oxidation parameters respectively.

II. Materials and Method

A. Animals and Treatment

Thirty male Wistar rats with body weights ranging from 150-170g were used for the study. They were allowed to acclimatize under the laboratory conditions two weeks before the experiments. The animals were allowed free access to food and water throughout the period of the experiment. The animals were randomly assigned to three main groups, each containing 10 rats. Group 1 received distilled water for the period of experiment. Group 2 (Ciprofloxacin-treated) animals were administered 80 mg/kg body weight of Ciprofloxacin in 1ml of distilled water daily. Group 3 (Levofloxacin-treated) animals administered 40 mg/kg body weight of Levofloxacin in 1ml of distilled water daily. The drugs were administered by gastric intubation and administered doses were calculated equivalent of human therapeutic dose according to the FDA [13]. At the end of each treatment regimen, 7 and 14 days, 5 animals were sacrificed from each group after 24 hours from last dose.

B. Drugs

Ciprofloxacin was manufactured by Fidson Healthcare Pharmaceuticals, Nigeria and Levofloxacin was a product of Bayer Healthcare.

C. Sample Preparation

Blood were collected by cardiac puncture and plasma was collected after centrifugation at 2000g for 10 minutes and stored at -20^{o}C.

D. Determination of Plasma Arylesterase Activity

Plasma Paraoxonase activity towards p-nitrophenol was determined as described by Junge and Klees [14]. Phenylacetate solution (1ml) was added to 1.0ml of 100mM Tris/acetate buffer pH 7.4 containing 10mM calcium chloride. This was allowed to stand for 10 minutes at room temperature. The mixture was then poured into a cuvette, 20µl of plasma was added and mixed. The rate of phenol generation was monitored at 270nm, at 30 seconds interval for 3 minutes. A molar extinction co-efficient of 1480 $M^{-1}cm^{-1}$ was used to calculate enzyme activity. 1 unit of arylesterase activity was defined as the enzyme quantity that disintegrated 1 milimole of phenylacetate substrate in 1 minute.

E. Determination of Lipid Peroxidation Level

Level of lipid peroxidation was measured by the method of Okhawa et al. [15] in liver homogenate. Briefly, 0.2ml of liver homogenate was added to the reaction mixture containing 0.2 ml 8% SDS, 1.5ml 20% acetic acid and 0.6ml distilled water. Reaction was initiated by adding 1.5 ml of 1% TBA and terminated by 10% TCA. The mixture was then centrifuged and absorbance of the supernatant was read at 532nm. LPO was expressed in terms of nmoles MDA formed/mg tissue using an extinction coefficient of $1.56\times10^{5}M^{-1}$ cm^{-1}.

F. Determination of Advanced Oxidized Protein Products Concentration

AOPP was determined by the method described by Witko et al. [16], also in the liver homogenate. Liver homogenate (400ml) was added to 1600ml of PBS solution and 100ml 1.16M potassium iodide was then added followed 2min later by 200ml of acetic acid. The absorbance of the reaction mixture was immediately read at 340nm against a blank containing 2000ml of PBS, 100ml of KI, and 200ml of acetic acid. Concentrations of AOPP were calculated by using the extinction coefficient of 26 lmM^{-1} cm^{-1}.

G. Statistical Analysis

Statistical analyses were performed with Graph Pad Prism, version 5. One way analysis of variance was used for the assessment of significance between groups. Significant groups (means ― standard deviations) were ascertained by Duncan's test

III. Results

The administration of either ciprofloxacin or levofloxacin resulted in significant ($p < 0.05$) inhibition of plasma arylesterase activity in the animals throughout the experimental periods. Plasma arylesterase activity was depressed in Ciprofloxacin administered groups by 29% and 30% while Levofloxacin treatment resulted in 35% and 30% inhibition, after 7 and 14 days treatment respectively (Fig. 1).

In the liver, the data revealed significant elevation of the advanced oxidized protein products (AOPP) as induced by the administrations of either fluoroquinolones (Fig. 2). The 7-day treatment resulted in 13% and 14% increases in AOPP concentrations in rats administered ciprofloxacin and levofloxacin respectively. Increasing the period of administration to 14 days produced increases of 31% and 32% respectively in AOPP levels of these animals, increases that were significantly more than the 7-day treatments ($p < 0.05$).

The hepatic MDA concentrations in the 7- and the 14-day ciprofloxacin-treated groups were significantly higher ($p < 0.05$) compared to the controls (Fig. 3). There was significant difference between the two groups with the 14-day ciprofloxacin treated animals increasing the MDA concentration in the liver by 7% above the 7-day treated rats. Levofloxacin also significantly ($p < 0.05$) increased hepatic MDA concentrations after 7- and 14- day administrations. The MDA level rose by 29% and 32% in the 7- and 14- day groups respectively. The MDA levels did not, however, differ significantly between these groups ($p > 0.05$).

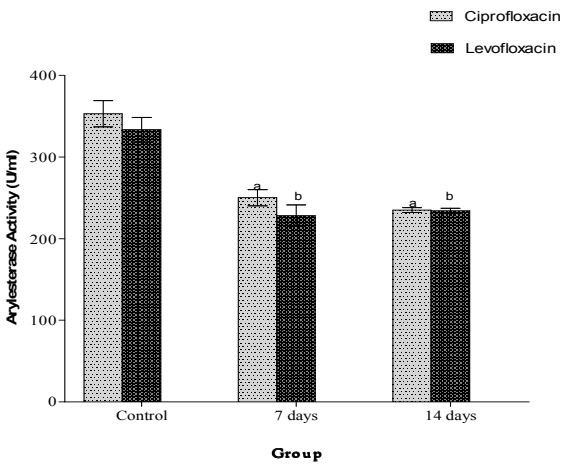

Fig. 1 Effects of ciprofloxacin and levofloxacin administration on plasma arylesterase of rats. Each column represents mean ± SD, n=5 'a' indicates a significant difference between ciprofloxacin control and ciprofloxacin-treated groups, and 'b' indicates a significant difference between levofloxacin control and levofloxacin-treated groups ($p < 0.05$)

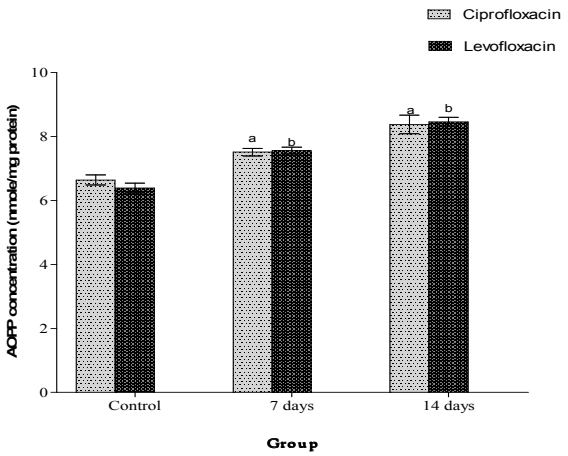

Fig. 2 Effects of ciprofloxacin and levofloxacin administration on hepatic AOPP of rats. Each column represents mean ± SD, n=5 'a' indicates a significant difference between ciprofloxacin control and ciprofloxacin-treated groups, and 'b' indicates a significant difference between levofloxacin control and levofloxacin-treated groups ($p < 0.05$)

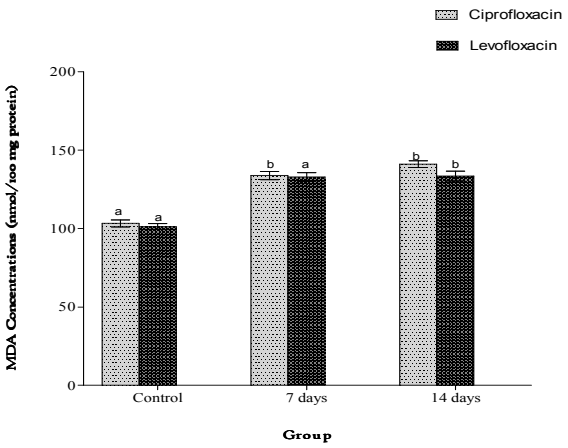

Fig. 3 Effects of ciprofloxacin and levofloxacin administration on hepatic MDA of rats Each column represents mean ± SD, n=5 'a' indicates a significant difference between ciprofloxacin control and ciprofloxacin-treated groups, and 'b' indicates a significant difference between levofloxacin control and levofloxacin-treated groups ($p < 0.05$).

IV. Discussion

An imbalance between intracellular production of free radicals and the cellular defense mechanisms results in increased oxidative stress. One of the manifestations of oxidative damage is lipid peroxidation which has been known to play important role in the toxicity of many chemical agents [17]. Lipid peroxidation is a significant determinant of the degree of free radical generation with MDA being one of the products, as well as, an important marker of the process of the oxidative stress induced as a result [18]-[20]. Fluoroquinolones are known to display hepatotoxic effect [21]. The studies of [22] and [23] reported that the generation of reactive oxygen species by fluoroquinolones resulted in cellular damage to liver and kidney. In this study, ciprofloxacin and levofloxacin at therapeutic doses used over a therapeutic period increased MDA concentrations in the liver of rats, indicating increased lipid peroxidation in these animals. A higher increase in MDA concentration was observed in the ciprofloxacin treated groups with significant increase in the 14-day treatment over the 7-day treated group, suggesting a progressive increase in lipid peroxides production with time of exposure. Increased hepatic lipid peroxidation as evidenced by the increased production of MDA in this study, indicates the involvement of free radical induced oxidative cell injury in mediating the toxicity of fluoroquinolones.

Other investigators have reported the induction of reactive oxygen species (ROS) by fluoroquinolones, as well as, some other antibiotics [24]. Fluoroquinolones as well as some other antibiotics have been demonstrated to have the ability to oxidized macromolecules in some bacteria, resulting in increased lipid peroxidation [25], which may be part of their antibacterial action [26]. Ciprofloxacin has been reported to cause a rise in advanced oxidative protein products (AOPP) in these bacteria [25]. AOPP are dityrosine-containing cross-linked protein products and are considered to be reliable markers for the estimation of the degree of oxidant-mediated protein damage [27]. Our results show significant elevation of hepatic AOPPs in the fluoroquinolone-treated animals. Fluoroquinolone treatments resulted in a progressive increase of these oxidative products over time. The generation of intracellular oxidized proteins is associated with increased production of ROS which result from a disruption in the balance between pro-oxidants and antioxidants [28]. The accumulation of these oxidized proteins could lead to the formation of cytotoxic protein aggregates, which are significant pathological factors involved in cellular damage [29]. The generation of AOPP in this study, therefore, suggests the induction of oxidative stress as a result of fluoroquinolones treatment, with subsequent damage to proteins in the animals.

Free radical generation induced by exogenous chemicals often occurs during direct redox cycling of the agent or its metabolism by cytochrome P450 [30]. In the case of fluoroquinolones, notably ciprofloxacin, it has been suggested that that the generation of ROS may occur during its oxidative metabolism [31].

Paraoxonase (PON1) is a HDL-associated antioxidant enzyme that has been reported to metabolize and detoxify biologically active lipid peroxides and has been reported to have antioxidant properties, especially against both LDL and HDL oxidation [32], [33]. Its primary physiological function is the protection of low-density lipoproteins (LDL) from oxidative modifications [34]. It has also been shown to play a significant role in the metabolism of pharmaceutical drugs [33] and given paraoxonase physiological importance, it is important to study the effect of fluoroquinolones, which are the most widely prescribed antibiotics, on its activity. Few

studies have been carried out to determine the effects of antibiotics on paraoxonase activity [35], [36] and these have majorly been *in vitro* studies in cell lines. A study by [36] reported a dose-dependent and time-dependent decrease of paraoxonase activity by some antibiotics, including ciprofloxacin. Our study showed the inhibition of paraoxonase activity towards phenyl acetate by the fluoroquinolones ciprofloxacin and levofloxacin, which is in agreement with the report of [36]. It has been implied that PON ability to protect against oxidation is usually accompanied by an inactivation of the enzyme [37]. The decrease in activity may, thus, be due to the increased oxidative stress induced by these antibiotics, as seen in the elevated hepatic MDA and AOPP concentrations in the animals. The increased generation of reactive oxygen species by ciprofloxacin and levofloxacin might have caused the inactivation of PON observed. With reports of PON1 activity been inhibited by oxidative stress [38], reduction in the enzyme activity could be evidence of lipid peroxidation activity of these drugs.

The inhibition of paraoxonase by these fluoroquinolones may also represent a biochemical event leading to the involvement of this class of antibiotics in the induction of atherosclerosis. This is because PON1 is known to protect against atherosclerosis [39]. It inactivates phospholipid hydroperoxides formed during early events of lipoprotein oxidation [40]. Fluoroquinolones may thus be atherogenic, an assertion that needs further investigation.

V. Conclusion

Our study supports the reports that fluoroquinolones generate oxidative stress which is known to produce multifactorial response in living systems. The oxidative stress resulted in an increase in lipid peroxidation as evidence in elevation of the hepatic MDA concentration, as well as, the inhibition of the enzyme, paraoxonase. The increase in advanced oxidative protein products, however, suggests a direct oxidant damage of the enzyme, paraoxonase and that its inhibition may not only just be an indirect effect through the formation of lipid peroxidation. In summary, we conclude that fluoroquinolone administration promotes oxidative damage which is highly significant as it may be means by which it exerts its toxic effects.

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