Journal of Investigational Biochemistry
www.scopemed.org
DOI: 10.5455/jib.20141119115356

Original Research

GESDAV

Alterations in some organ function indices in rats administered metal complexes of ofloxacin and norfloxacin

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Received: July 23, 2014
Accepted: November 19, 2014
Published: January 04, 2015

ABSTRACT

Background: Some metal complexes of norfloxacin and ofloxacin have been synthesized and found to be more potent than their free ligands, but have not been evaluated for their toxicities. **Objective:** Effects of ofloxacin, norfloxacin and their metal complexes (nickel, copper, and iron complexes) on kidney and liver function indices in rats were investigated. **Materials and Methods:** Ofloxacin and its metal complexes (2.86 mg/kg body weight twice daily) and norfloxacin and its nickel complex (5.71 mg/kg body weight twice daily) were orally administered to rats whereas the control animals received sterile distilled water for 7 days. Afterward, concentrations of selected serum electrolytes and biomolecules were determined. **Results:** Ofloxacin significantly reduced ($P < 0.05$) serum calcium ion, urea and total protein concentrations while it significantly increased ($P < 0.05$) the atherogenic index compared to controls. Cuprate (II) hydrate complex of ofloxacin significantly increased ($P < 0.05$) serum glucose concentration while it significantly reduced ($P < 0.05$) serum inorganic phosphate concentration compared to controls. Iron (III) complex of ofloxacin had no significant effect ($P > 0.05$) on all the parameters studied compared with controls. Norfloxacin significantly increased ($P < 0.05$) serum glucose concentration while it significantly reduced ($P < 0.05$) serum potassium ion concentration compared to controls. Nickel (II) complex of norfloxacin significantly reduced ($P < 0.05$) serum creatinine and inorganic phosphate concentrations compared to controls. **Conclusion:** The results of this study suggest that Iron (III) complex of ofloxacin may be a less toxic therapeutic option compared with ofloxacin.

KEY WORDS: Cardiovascular, fluoroquinolones, kidney, liver, toxicity

INTRODUCTION

The development of fluoroquinolones has resulted in antimicrobial agents with enhanced gram-negative activity [1,2]. Ofloxacin and norfloxacin are synthetic fluoroquinolones that inhibit the supercoiling activity of bacterial DNA gyrases, thus halting DNA replication [3,4]. The affinity of quinolones to metal ions generally seems to be an important pre-requisite for their antibacterial activity. They probably bind to the DNA gyrases-complex via a magnesium ion [5]. However, many of these conventionally used drugs have been rendered ineffective due to drug resistance by bacteria. Therefore, there is a need to develop new drugs against diseases caused by these organisms.

One of the approaches to drug discovery is to administer currently used drugs for the treatment of other diseases with the aim of evaluating their potency against the disease in question [6]. Another way is to structurally modify the already existing drug to obtain new molecules [6]. Fluoroquinolones generally form stable metal chelate complexes [7]. There have been reports that the metal complexes of some antimicrobial drugs demonstrated comparable efficacies with their parent compounds [8-11]. In our previous studies, metal complexes of norfloxacin and ofloxacin were synthesized and evaluated for their antibacterial activities [12]. Some of them were found to be as potent as the parent compound against some strains of bacteria while some even exhibited better antibacterial activities. However, it is necessary to test such new drugs for their probable toxicities.

Fluoroquinolones are generally well-tolerated and safe [13]. Though the most common adverse effects reported for all quinolones involve those associated with the gastrointestinal tract, skin and central nervous system [2], some have been reported to cause liver toxicity, phototoxicity, cardiotoxicity, arthropathy, tendinitis and hypoglycemia [14-16]. Some fluoroquinolones have been reported to cause nephrotoxicity, which manifests in elevated levels of creatinine and serum urea nitrogen e.g. ciprofloxacin, ofloxacin, norfloxacin and pefloxacin [13,17]. Furthermore, renal failure, nephritis and renal tubular disorder have been reported for ciprofloxacin [13]. The possibility of these adverse effects being reduced or aggravated when complexed with metals cannot be ruled out.

The present study was, therefore, carried out to evaluate the toxicities of these newly synthesized potent metal complexes of norfloxacin and ofloxacin in rat cellular systems using selected organ function indices.

MATERIALS AND METHODS

Chemicals and Reagents

Norfloxacin and ofloxacin were obtained from Sigma-Aldrich Chemie, Germany. All the other reagents used for this study were of analytical grade and were prepared in all glass-distilled water.

Animals

Thirty-five albino rats (*Rattus novergicus*) used for this study, with an average weight of 150 g, were obtained from the small Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

Animal Handling and Drug Administration

The experimental animals were handled and used in accordance with the international guide for the care and use of laboratory animals [18]. They were kept in standard laboratory conditions under natural light-dark cycle. The animals had access to rat chow (Bendel Feeds, Ewu, Delta State, Nigeria) and water *ad libitum* throughout the period of the experiment. The animals were randomly divided into seven groups (of five rats each), which were designated A (control), B, C, D, E F and G. Sterile distilled water was orally administered to control rats in group A for 7 days while rats in Groups B, C, D and E received 2.86 mg/kg body weight of ofloxacin (the therapeutic dose), copper (II) dehydrate-ofloxacin complex, iron (III)-ofloxacin complex and cuprate (II) hydrate-ofloxacin complex [Figures 1-4] respectively twice daily for 7 days. Norfloxacin (the therapeutic dose) and nickel-norfloxacin complex [Figures 5 and 6] at the dose of 5.71 mg/kg body weight were administered orally to animals in Groups F and G respectively twice daily for 7 days.

Sample Preparation

At the end of the experimental period, venous blood was collected from the experimental animals according to the method of Narayanan *et al.* [19]. The serum was prepared by centrifuging the clotted blood samples at 3000 rpm for 5 min [20], after which the serum was pipetted out and stored frozen until required for analysis.

Assay of Biochemical Parameters

Serum concentrations of sodium and potassium ions were determined by flame photometry using the Jenway Clinical PFP7 flame photometer [21]. Total cholesterol concentration in the serum was assayed using the CHOD-PAP method reported by Fredrickson *et al.* [22]. Serum high-density lipoprotein (HDL)-cholesterol concentration was assayed using the dextran method as described by Albers *et al.* [23]. The atherogenic index was also calculated using the Friedwald equation [24] Protein concentration in the serum was determined using the Biuret method [25]. Serum urea concentration was assayed as per Veniamin and Vakirtzi-Lemonias [26] while serum creatinine concentration was determined by the method of Cook [27]. Serum albumin concentration was estimated using the albumin-bromocresol green reaction method [28]. Serum phosphate ion and calcium ion concentrations were determined by the methods of Goldenberg and Fernandez [29] and Sarkar and Chauhan [30] respectively.

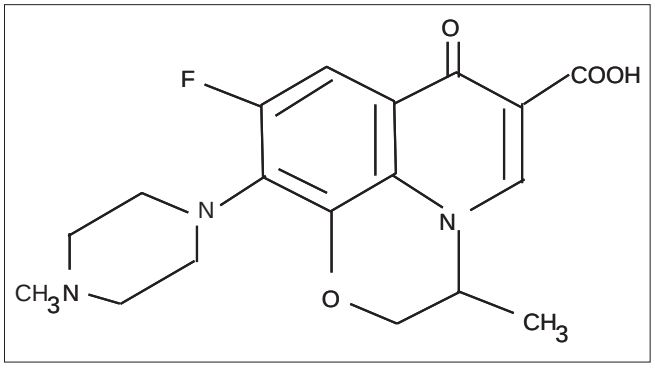

Figure 1: Ofloxacin

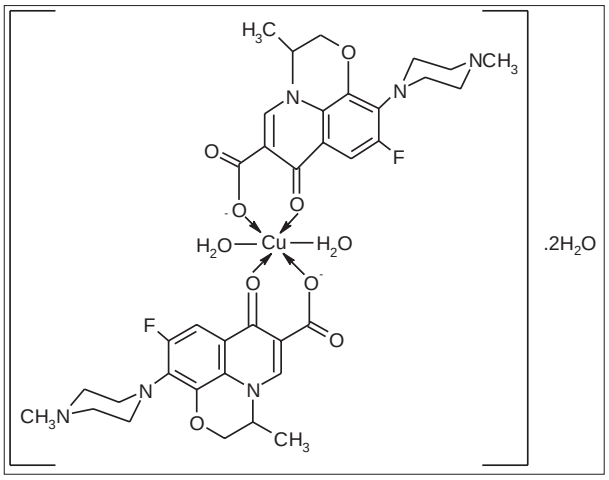

Figure 2: [$Cu(Ofl)_2(H_2O)_2.2H_2O$-[Di-aquodi(ofloxacinato)copper(II) dihydrate]

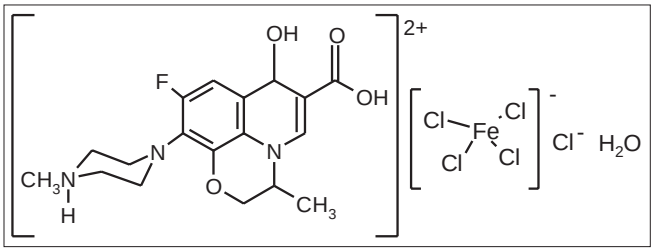

Figure 3: (H_3Ofl)[$FeCl_4$]Cl.H_2O-[Ofloxacinium(2+) tetrachloroferrate(II) hydrate]

Statistical Analysis

The data were statistically analyzed using one-way analysis of variance and Duncan Multiple Range test [31]. In all cases, probability level of 95% was taken as significant.

RESULTS

Ofloxacin significantly reduced ($P < 0.05$) serum calcium ion, urea, total cholesterol and total protein concentrations while it significantly increased ($P < 0.05$) the atherogenic index compared to controls [Tables 1-3]. Copper (II) dihydrate complex of ofloxacin significantly reduced ($P < 0.05$) serum sodium ion, urea, albumin and total protein concentrations with the atherogenic index, but significantly increased ($P < 0.05$) serum HDL-cholesterol concentration compared to controls. Cuprate (II) hydrate complex of ofloxacin significantly increased ($P < 0.05$) serum glucose concentration while it significantly reduced ($P < 0.05$) serum inorganic phosphate and total cholesterol concentrations compared to controls. Iron (III) complex of ofloxacin had no significant effect ($P > 0.05$) on all the parameters studied compared to controls. Norfloxacin significantly increased ($P < 0.05$) serum glucose concentration while it significantly reduced ($P < 0.05$) serum potassium ion concentration compared to controls [Tables 4-6]. Nickel (II) complex of norfloxacin significantly reduced ($P < 0.05$) serum creatinine and inorganic phosphate concentrations but significantly increased ($P < 0.05$) serum total cholesterol concentration compared to controls.

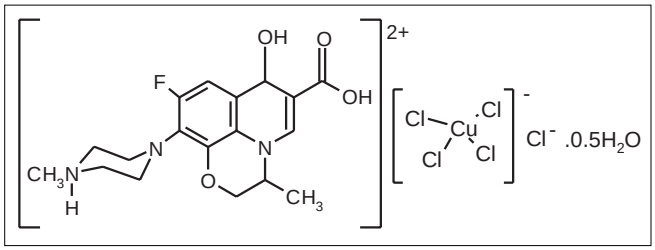

Figure 4: (H_3Ofl)[$CuCl_4$].0.5H_2O-[Ofloxacinium(2+) tetrachlorocuprate(II) hydrate]

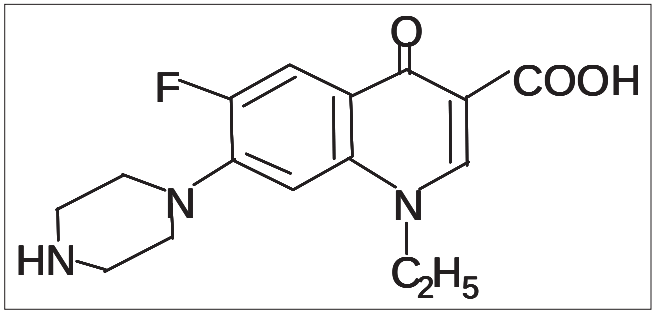

Figure 5: Norfloxacin

DISCUSSION

Serum creatinine and urea concentrations are kidney function indices [32], though creatinine concentration is more specific than urea concentration [33]. An increase in serum urea and creatinine concentrations indicates renal dysfuction [34]. Ofloxacin and its metal complexes did not significantly increase serum urea and creatinine concentrations, suggesting that they may not impair normal kidney function. However, serum urea concentration was significantly reduced by ofloxacin and its copper (II) dihydrate complex, suggesting a dysfunction of the liver rather than that of the kidney. Urea is mainly synthesized in the liver through the concerted efforts of enzymes of the urea cycle. It suggests that the ligand and its complex may be inhibitors of at least one of the urea cycle enzymes. Also, norfloxacin and its nickel (II) complex did not significantly increase serum urea and creatinine concentrations compared to controls, suggesting that they may not adversely affect the function of the kidney. However, nickel (II) complex of norfloxacin reduced serum creatinine concentration, suggesting that the complex adversely affects the creatine metabolism in the muscle [34]. These findings corroborate earlier

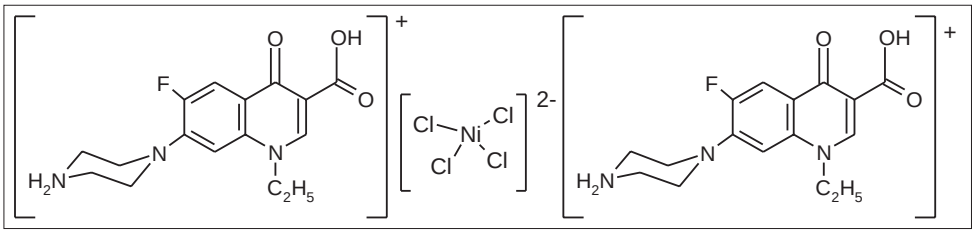

Figure 6: (H_2Nor)$_2$[$NiCl_4$]-[Di(norfloxacinium (1+)) tetrachloronickelate(II)]

Table 1: Effects of metal complexes of ofloxacin on the concentrations of some serum biomolecules in rats

Groups	Concentrations of serum biomolecules				
	Glucose (g/L)	Albumin (g/L)	Total protein (g/L)	Urea (mmol/L)	Creatinine (μmol/L)
Control	2.66±0.14^{a}	32.48±1.81^{a}	70.38±1.81^{a}	5.47±0.47^{a}	65.66±16.98^{a}
Ofloxacin	2.76±0.24^{a,b}	31.13±0.90^{a,b}	64.06±0.45^{b}	3.37±0.31^{b}	54.34±4.87^{a}
Copper (II) dihydrate complex	2.72±0.14^{a,b}	30.23±0.90^{b}	59.55±2.71^{c}	3.74±0.39^{b}	61.70±7.36^{a}
Iron (III) complex	2.90±0.01^{a,b}	31.58±1.35^{a,b}	69.02±1.35^{a,d}	5.67±0.87^{a}	65.67±8.49^{a}
Cuprate (II) hydrate complex	2.96±0.20^{b}	32.48±0.90^{a}	65.87±2.71^{b,d}	4.88±0.16^{a}	66.79±4.53^{a}

Values are means±SD. Values with different letter superscripts in each column are significantly different ($P<0.05$). SD: Standard deviation

reports that nephrotoxicity is an uncommon consequence of fluoroquinolone therapy [13].

The liver performs a major role in protein metabolism. Albumin is synthesized in the liver and is involved in the maintenance of oncotic pressure in extracellular fluids [35,36]. Also, a link has been established between insulin and albumin synthesis. The mRNA concentration available for albumin synthesis on ribosomes is an important factor controlling the rate of albumin synthesis. Insulin, apart from its primary function of enhancing the uptake of glucose by cells, has been reported to be required for adequate albumin synthesis because it affects the amount of mRNA available for albumin synthesis [37]. Only the copper (II) dihydrate complex of ofloxacin reduced serum albumin concentration in this study with no significant increase in serum glucose concentration. It thus suggests that the reduction may not be as a result of a dysfunction in insulin secretion by the pancreas, but as a result of decreased albumin synthesis in the liver resulting from factors other than insulin unavailability.

Table 2: Effects of metal complexes of ofloxacin on the concentrations of some serum electrolytes in rats

Groups	Concentrations of serum electrolytes			
	Na^+ (mmol/L)	K^+ (mmol/L)	Ca^{2+} (mmol/L)	PO_4^{3-} (mmol/L)
Control	145.25±1.75[a]	5.41±0.14[a,b]	2.16±0.03[a]	3.31±0.24[a,b]
Ofloxacin	146.13±1.31[a]	5.54±0.10[b]	2.10±0.05[a]	3.55±0.20[b]
Copper (II) dihydrate complex	141.75±1.75[b]	5.27±0.20[a]	2.10±0.05[a]	3.62±0.32[b]
Iron (III) complex	146.13±1.31[a]	5.30±0.14[a]	2.20±0.05[a]	3.11±0.20[a,c]
Cuprate (II) hydrate complex	146.13±0.35[a]	5.34±0.20[a]	2.16±0.01[a]	2.84±0.20[c]

Values are means±SD. Values with different letter superscripts in each column are significantly different ($P<0.05$). SD: Standard deviation

Table 3: Effects of metal complexes of ofloxacin on some serum lipid parameters in rats

Groups	Total cholesterol concentration (mmol/L)	HDL-cholesterol concentration (mmol/L)	Atherogenic index
Control	4.43±0.27[a]	1.89±0.14[a,c]	2.11±0.03[a]
Ofloxacin	3.85±0.14[b]	1.49±0.24[a]	2.48±0.03[b]
Copper (II) dihydrate complex	4.60±0.34[a]	2.30±0.41[b]	1.92±0.01[c]
Iron (III) complex	4.73±0.41[a]	2.23±0.27[b,c]	2.11±0.02[a]
Cuprate (II) hydrate complex	3.51±0.14[b]	1.82±0.14[a]	1.94±0.02[a,c]

Values are means±SD. Values with different letter superscripts in each column are significantly different ($P<0.05$). SD: Standard deviation, HDL: High-density lipoprotein

Cuprate (II) hydrate complex of ofloxacin on the other hand, increased serum glucose concentration without any effect on serum albumin concentration. The same observation was made for norfloxacin. It thus implies that cuprate (II) hydrate complex of ofloxacin and norfloxacin may interfere with the binding of insulin to its cellular receptors in such a way that prevents glucose uptake rather than inhibiting insulin synthesis and secretion by the pancreas since insulin is also required for albumin synthesis. Ofloxacin and its copper complexes reduced serum total protein concentration compared to control. The fact that ofloxacin and its cuprate (II) hydrate complex reduced protein concentration without reducing albumin concentration in the serum suggests that other fractions of serum protein (predominantly globulins) were adversely affected by these compounds, which may invariably affect antibody-mediated immune response.

Only copper (II) dihydrate complex of ofloxacin reduced the serum Na^+ concentration. Reduction in serum Na^+ concentration may be as a result of alterations in the filtration of the ion in the kidney caused by the compound. It may be that the compound interferes with regulatory functions of hormones, such as aldosterone and other mineralocorticoids, required for the reabsorption of the ion either by inhibiting the synthesis of the hormones or interfering with the stimulation of Na^+/H^+ exchanger by the hormones [21]. The fact that all the metal complexes and their free ligands did not significantly alter the serum calcium ion concentration suggests that they do not adversely affect calcium ion homeostasis. The observed decrease in serum phosphate ion concentration by cuprate (II) hydrate complex of ofloxacin and nickel (II) complex of norfloxacin suggests that the homeostatic mechanism for phosphate ion concentration has been impaired. Despite the alterations in concentrations of various electrolytes studied, all the values obtained were within the normal range, suggesting that the observations are of no clinical importance.

The ratio of the serum total cholesterol concentration to serum HDL-cholesterol concentration is a strong indicator of cardiovascular diseases [38]. The increase in this ratio indicates a greater tendency of developing cardiovascular diseases and vice versa. In the present study, ofloxacin increased the atherogenic index while its copper (II) dihydrate complex reduced it compared to control. Other compounds studied did not significantly affect the atherogenic index. This suggests that the metal complexes of ofloxacin and norfloxacin may not predispose subjects to cardiovascular diseases as the free ligand.

Of the four metal complexes of the two fluoroquinolones studied in comparison with their ligands, the results suggest that Iron (III) complex of ofloxacin may be a better therapeutic option, being less toxic than its parent compound.

Table 4: Effects of nickel complex of norfloxacin on the concentrations of some serum biomolecules in rats

Groups	Concentrations of serum biomolecules				
	Glucose (g/L)	Albumin (g/L)	Total protein (g/L)	Urea (mmol/L)	Creatinine (μmol/L)
Control	2.66±0.14[a]	32.48±1.81[a]	70.38±1.81[a]	5.47±0.47[a,b]	65.66±16.98[a]
Norfloxacin	3.16±0.16[b]	36.19±6.61[a]	64.51±9.02[a]	6.59±1.01[b]	76.42±15.85[a]
Nickel (II) complex	2.76±0.35[a]	29.86±2.72[a]	64.96±2.25[a]	4.85±0.34[a]	39.62±5.66[b]

Values are means±SD. Values with different letter superscripts in each column are significantly different ($P<0.05$). SD: Standard deviation

Table 5: Effects of nickel complex of norfloxacin on the concentrations of some serum electrolytes in rats

Groups	Concentrations of serum biomolecules			
	Na^+ (mmol/L)	K^+ (mmol/L)	Ca^{2+} (mmol/L)	PO_4^{3-} (mmol/L)
Control	145.25±1.75[a]	5.41±0.14[a]	2.16±0.03[a]	3.31±0.24[a]
Norfloxacin	143.66±3.64[a]	4.83±0.39[b]	2.10±0.27[a]	3.38±0.61[a]
Nickel (II) complex	142.80±2.73[a]	5.41±0.41[a]	2.13±0.01[a]	2.30±0.14[b]

Values are means±SD. Values with different letter superscripts in each column are significantly different ($P<0.05$). SD: Standard deviation

Table 6: Effects of nickel complex of norfloxacin on some serum lipid parameters in rats

Groups	Total cholesterol concentration (mmol/L)	HDL-cholesterol concentration (mmol/L)	Atherogenic index
Control	4.43±0.27[a]	1.89±0.14[a]	2.11±0.03[a]
Norfloxacin	4.26±0.25[a]	1.75±0.22[a]	2.07±0.00[a]
Nickel (II) complex	4.93±0.27[b]	1.95±0.07[a]	2.05±0.03[a]

Values are means±SD. Values with different letter superscripts in each column are significantly different ($P<0.05$). SD: Standard deviation, HDL: High-density lipoprotein

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Source of Support: Nil, Conflict of Interest: None declared.