African Journal of Biotechnology Vol. 6 (24), pp. 2826-2832, 17 December, 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Toxicological studies and antimicrobial properties of some Iron(III) complexes of Ciprofloxacin

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Accepted 23 November, 2007

Two iron(III) complexes of Ciprofloxacin were synthesized by reaction of the ligand with iron(III) chloride hexahydrate in different solutions. The nature of bonding of the ligands and the structure of the isolated metal complexes were elucidated on the basis of their physical and spectroscopic studies. The infrared spectra suggest that two classes of compounds were obtained: *molecular complex* in which the ligands were bidentately bonded to the metal through the ring carbonyl oxygen and one of the oxygen of the carboxylate group and the *ionic complex* consisting of a tetrachlorometalate ion which is electrostatically attached to the ligand. The antibacterial activities of the products against various microorganisms were tested and it was established that their activities were comparable with those of their parent drug. Toxicological studies were carried out in which therapeutic doses of the Ciprofloxacin drug and the metal complexes were administered to albino rats and the results showed that the metal complexes are not toxic.

Key words: Quinolone, fluoroquinolone, Ciprofloxacin, metal complexes, toxicological study, antimicrobial study.

INTRODUCTION

According to Chu et al. (1996) there is a worldwide agreement over the present need to develop novel agents to treat bacterial infections that have become increasingly unresponsive to standard antibacterial therapy. Emergence of bacteria resistance to a number of antimicrobial agents is becoming a major health problem.

This has led to studies on the metal complexes of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid hydrochloride ($C_{17}H_{18}FN_3O_3.HCl.H_2O$) (Ciprofloxacin), a fluoroquinolone derivative, in which biological studies were carried out to determine their potency (Wise et al., 1983; Kucers and Mck.Bennet, 1987; Singleton, 1997). Some copper(II) Ciprofloxacin complexes $(cfH_2)(cfH_3)[CuC_4]Cl.H_2O$, $[Cu(cf)(H_2O)_3]SO_4.2H_2O$ and $[Cu(cf)_2]Cl_2.6H_2O$ were tested against the growth of various gram positive and gram negative microorganism (Turel, 2002; Drevenski et al., 2002). The complexes showed comparable antimicrobial activity with the free ligand.

In this report, antimicrobial assay and toxicity studies of Ciprofloxacin (Figure 1) and two synthesized and analyzed iron (III) complexes of Ciprofloxacin were carried out.

MATERIALS AND METHOD

Ciprofloxacin was obtained from Sigma-Aldrich Chemie, Germany. The metal salt used is $FeCl_3.6H_2O$. Reagents and solvents were used without further purification.

Melting point was determined using Gallenkamp melting point apparatus. The conductivity was measured by using the Hanna instrument conductivity meter. The analysis of chemical elements (C, H, and N) was carried out at the laboratory of Desert Analytics, Tucson, Arizona, USA. The metal concentration was determined on an alpha 4 atomic absorption spectrophotometer at the central laboratory, O.A.U, Ile-Ife, Nigeria. The samples were digested in concentrated HCl and diluted with a known volume of water. The I.R. spectra were recorded using KBr pellets with Buck scientific infrared spectrometer M500 at the range of 4000 – 600 cm^{-1}.

Synthesis

Compound 1-$[Fe(Cip)_2Cl_2]Cl.6H_2O$

Twenty millilitres of the aqueous Ciprofloxacin (1.0 mmole) in a 250 ml round bottomed flask was stirred for 1 min. 10.0 ml of aqueous

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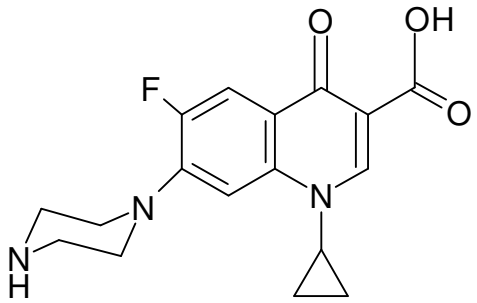

Figure 1. Structure of Ciprofloxacin.

solution of the $FeCl_3.6H_2O$ (0.5 mmole) were added to the ligand solution. The mixture was stirred with a magnetic stirrer for 2 h and left to stand overnight. The orange precipitate formed was filtered and washed with distilled water.

Compound 2 -(H$_3$Cip)[FeCl$_4$]Cl.H$_2$O

1 mmole of the Ciprofloxacin was dissolved in 10 ml of 1M HCl solution. To this was added 20 ml of $FeCl_3.6H_2O$ (1 mmole) dissolved in 1M HCl. The solution was stirred with a magnetic stirrer for 1 h. The colour changed from orange to red. The resulting solution was then concentrated and products collected by filtration.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal concentration (MBC)

To each of a series of sterile stoppered test tubes, a standard volume of medium that will support the growth of the test organism was added; this was followed by the addition of 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.50, 0.80, 1.00, 1.50, 2.00, 3.00, 4.00, 5.00, 6.00 and 7.00 ml of each of the antimicrobial metal complex and ligand solutions representing 10, 15, 20, 25, 30, 35, 40, 45, 50, 80, 100, 150, 200, 300, 400, 500, 600 and 700 µg/ml respectively in final mixture of 10 ml. Standard volume of the inoculum (0.2 ml) of each of the test organism was added to the reacting mixture. Two controls were set up for each of the test, one in which the test sample (antimicrobial) was omitted and the other in which the test organism was omitted. All the tubes were incubated at 35°C for 24 h.

All tubes showing turbidity (evidence of growth) were removed while those showing no turbidity were subcultured into nutrient broth by transferring a loopful of the culture which has been properly shaken into 10 mls of the broth and incubated for 8 h at 35°C. This broth culture was further subcultured on to nutrient agar media by a single stroke streaking and incubated at 35°C for 24 h. The plates were observed for growth after the period of incubation. The minimum concentration plates showing no growth after this period represents the minimum bactericidal concentration (MBC).

Toxicological studies

The drugs were dissolved in distilled water and administered orally to the male albino rats in proportion to their body weight. The control groups were given only distilled water. The test solutions were prepared based on therapeutic dose as follows: Ciprofloxacin - 500 mg, per 70 kg body weight, twice daily for seven days after which they were sacrificed. The homogenates of the liver, kidney, small intestine and heart were prepared in ice-cold 0.25 M sucrose solution to give a final volume of five times the original tissue weight (1:5, w:v).The tissue homogenates were all diluted in ratio 1:20 with saline water before enzyme assay. All enzymes activity was determined using enzyme kits prepared by Randox Laboratories ltd (1997).

RESULTS AND DISCUSSION

Compound 1- [Fe(Cip)$_2$Cl$_2$]Cl.6H$_2$O

Bright orange, amorphous product; 70.2%; m.p > 400°C; Soluble in H_2O, MeOH, EtOH; Mol. cond. (Λ) = 0.62 Scm^2mol^{-1}; IR(KBr) *V*max/cm^{-1}: 3500 - 3000(OH for water molecule) 1621 $(C=O)_p$, 1573, $(OCO)_{as}$, 1475 (C-C +C-N), 1383 $(OCO)_s$; Anal. Calcd. for $C_{34}H_{46}N_6F_2O_{12}FeCl_3$ (M_r= 931.15): C= 43.76, H= 5.18, N= 9.01 and Fe= 5.98%. Found: Fe 5.73%.

Compound 2 - (H$_3$Cip)[FeCl$_4$]Cl.H$_2$O

Deep red crystalline product; 52.2%; m.p 250 - 252 °C; Soluble in H_2O and MeOH. Insoluble in EtOH; Mol. cond. (Λ) = 1.09 Scm^2mol^{-1}; IR(KBr) *V*max/cm^{-1}: 3562 - 3000(OH for water molecule), 1743 $(C=O)_c$ 1641 $(C=O)_p$, 1514 (C-C +C-N), 1407.7$(OCO)_s$, 1251.9 (C-F). Anal.Calcd. for $C_{17}H_{22}N_3FO_4FeCl_5$ (M_r= 583.75): C= 34.92, H= 3.79, N= 7.19 and Fe= 9.55% Found: C 35.41, H 4.02, N 7.24, Fe 9.18%;

From the elemental analysis data, the mole ratio, anion present and water molecules were determined. The $AgNO_3$ solution was used to confirm the presence of chloride ions outside the coordination sphere (The CHN for Compound I was not determined.) Chloride ions from the metal salts appears in **[Fe(Cip)$_2$Cl$_2$]Cl.6H$_2$O** complex either within and outside the coordination spheres as shown in the conductivity measurements. The terminal nitrogen on the 7-piperazinyl group is protonated where simple chloride ions are present to balance the charge on the complex as has been shown in similar studies (Turel, 2002; Turel et al., 2003; Al- Mustafa, 2002; Zupančič et al., 2001). Also the presence of water (lattice or coordinated water) is shown by the broad peak at 3600 – 3000 cm^{-1} of the IR spectra.

Infrared spectra

It has been observed from the previous studies that fluoroquinolones coordinate to metals through the ring carbonyl oxygen and one of the oxygens of the carboxylate group as observed for **[Fe(Cip)$_2$Cl$_2$]Cl.6H$_2$O**. This is supported by the absence of the $\nu(C=O)_c$ frequencies and the presence of $\nu(OCO)_{as}$ and $\nu(OCO)_{as}$ in the infrared spectra of **[Fe(Cip)$_2$Cl$_2$]Cl.6H$_2$O**.

In the IR spectra of **(H$_3$Cip)[FeCl$_4$]Cl.H$_2$O**, the intense band at 1743 cm^{-1} was assigned to $\nu(C=O)_c$. The position of $\nu(C=O)_p$ appears at higher frequency around 1630 cm^{-1}.

Table 1. Minimum inhibitory concentration (ug/ml) and minimum bactericidal concentration (ug/ml) of Ciprofloxacin, $[Fe(Cip)_2Cl_2]Cl.6H_2O$-1 and $(H_3Cip)[FeCl_4]Cl.H_2O$-2.

Bacteria	Minimum inhibitory concentration (ug/ml			Minimum bactericidal concentration (ug/ml)		
	Ciprofloxacin	Compound 1	Compound 2	Ciprofloxacin	Compound 1	Compound 2
S. typhi	60	180	80	80	100	100
Shigella spp	150	150	150	180	180	180
E. coli	450	250	450	500	300	500
Klebsiella spp.	150	500	450	200	550	500
S. aureus	150	400	450	200	450	500
Pseudomonas spp.	150	250	180	180	300	200
N. gonorrhoea	60	20	180	80	30	200

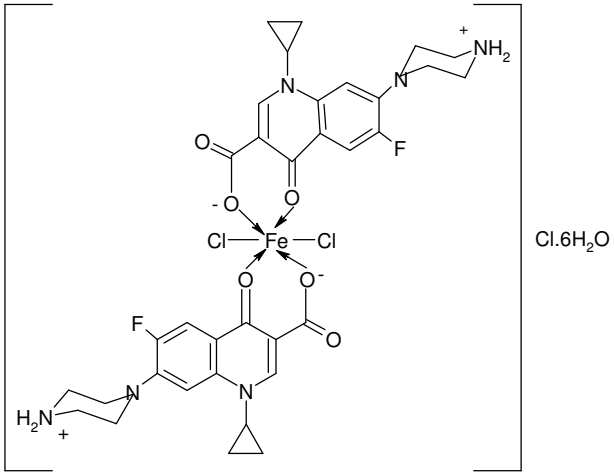

Figure 2. Di(ciprofloxacin)dichloro iron(III) chloride hexahydrate-$[Fe(Cip)_2Cl_2]Cl.6H_2O$-1.

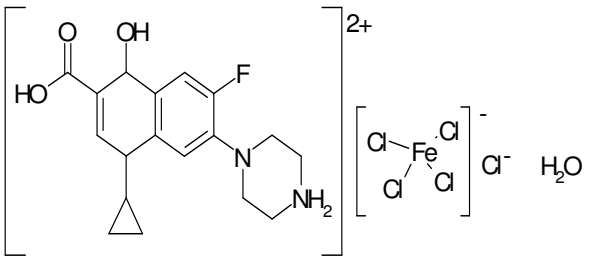

Figure 3. Ciprofloxacinium tetrachloroferrate(III) chloride hydrate-$(H_3Cip)[FeCl_4]Cl.H_2O$-2.

This indicates that the carboxylic group is not deprotonated, therefore the Ciprofloxacin ligand is not directly coordinated to the metal. It is proposed that in this class of compounds, the ligand exist as a singly (+1) or doubly (+2) protonated cation as for **$(H_3Cip)[FeCl_4]Cl.H_2O$** The positive charge on the ciprofloxacium ion is neutralized by the tetrachlorometalate anion and any extra chloride ion. Similar *ionic complexes* consisting of a tetrachlorometalate ion which is electrostatically attached to the ligand have being reported (Zupančič et al., 2001; Turel et al., 1996).

Based on these results, the following structures were proposed for the compounds **$[Fe(Cip)_2Cl_2]Cl.6H_2O$** and **$(H_3Cip)[FeCl_4]Cl.H_2O$** (Figures 2 and 3).

Antimicrobial Studies

The result of the minimum inhibitory concentrations (bacteriostatic) (MIC) and the minimum bactericidal concentrations (MBC) of the ligands and metal complexes determined are presented in Table 1. Generally, ligands and metal complexes showed antimicrobial effect against the tested organism species except against the molds of *Penicillim* and *Aspergillus* as presented. *Neissera gonorrhoea* was the most sensitive organism to the fluoroquinolones and their complexes. Some of the metal complexes showed comparable activity or greater activity against some of the microorganisms in comparison to the parent compounds. The MIC of the samples against the various isolates ranged from 20 to 450 g/ml of the antimicrobial dilutions, while that of the MBC ranged from 30 to 550 g/ml.

Results of toxicological study

Figures 4 – 8 show the effects of Ciprofloxacin and its metal complexes on some enzymes and tissue/ bodyweight ratio in some rat tissues.

Tissue-bodyweight ratio

The observed reduction in liver-body weight ratio caused by Ciprofloxacin and its metal complexes suggests that the drugs may retard the growth of the liver. This may result from the inhibition of some metabolic processes (e.g. the synthesis of DNA), which are needed for normal

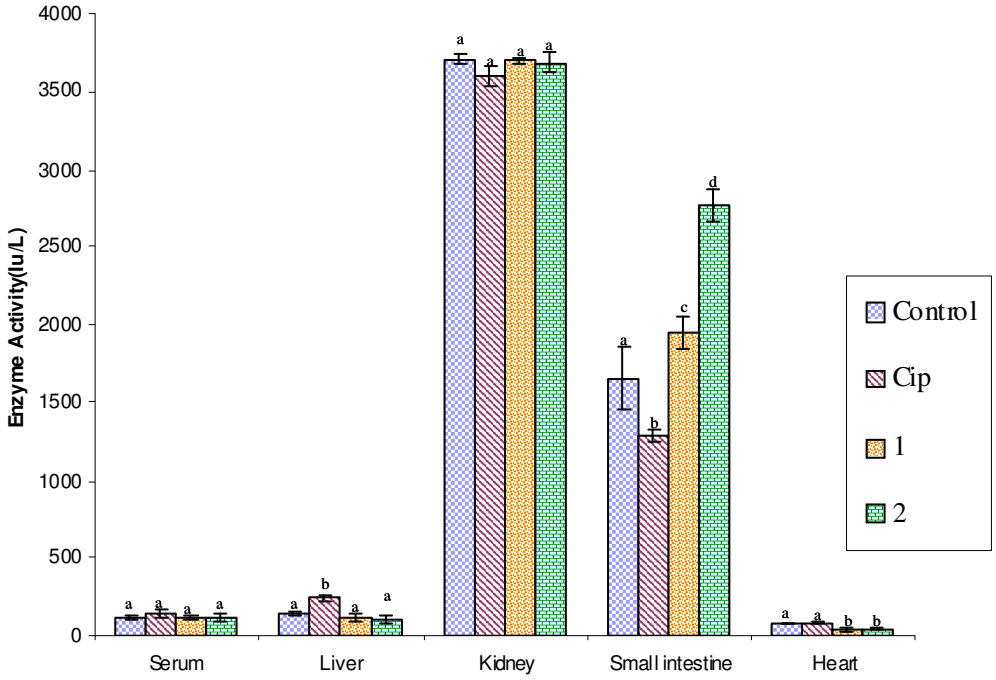

Figure 4. Effects of Ciprofloxacin and its metal complexes on ALP activity in some tissues. [Where Cip = Ciprofloxacin, 1 = $[Fe(Cip)_2Cl_2]Cl.H_2O$ and 2 = $(H_3Cip)[FeCl_4]Cl.H_2O$-]. Values with different superscripts are significantly different at $P < 0.05$.

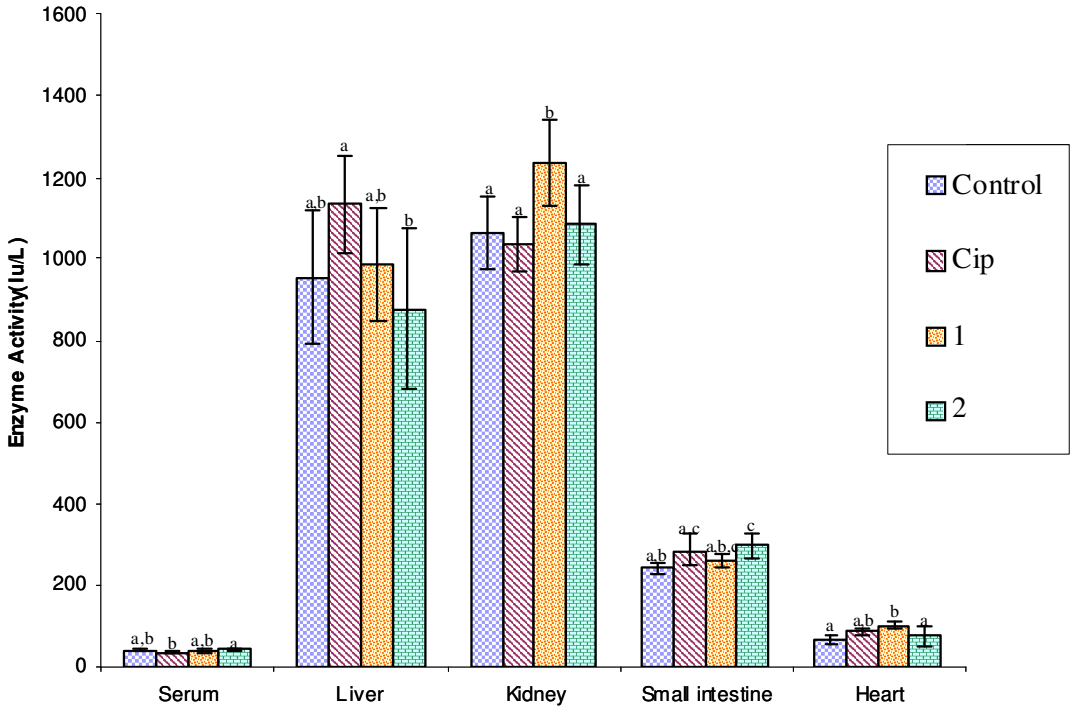

Figure 5. Effects of Ciprofloxacin and its metal complexes on ACP activity in some tissues. [Where Cip = Ciprofloxacin, 1 = $[Fe(Cip)_2Cl_2]Cl.H_2O$ and 2 = $(H_3Cip)[FeCl_4]Cl.H_2O$-]. Values with different superscripts are significantly different at $P < 0.05$.

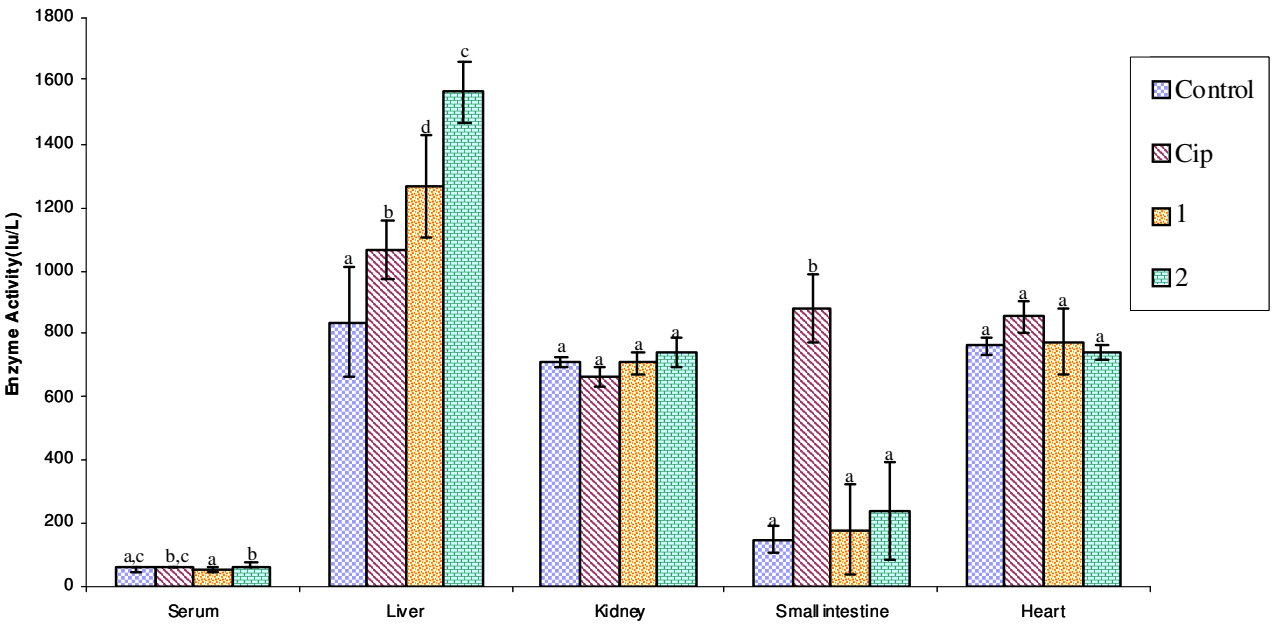

Figure 6. Effects of Ciprofloxacin and its metal complexes on ALT activity in some tissues. [Where Cip = Ciprofloxacin, **1** = $[Fe(Cip)_2Cl_2]Cl.H_2O$ and **2** = $(H_3Cip)[FeCl_4]Cl.H_2O$-]. Values with different superscripts are significantly different at $P < 0.05$.

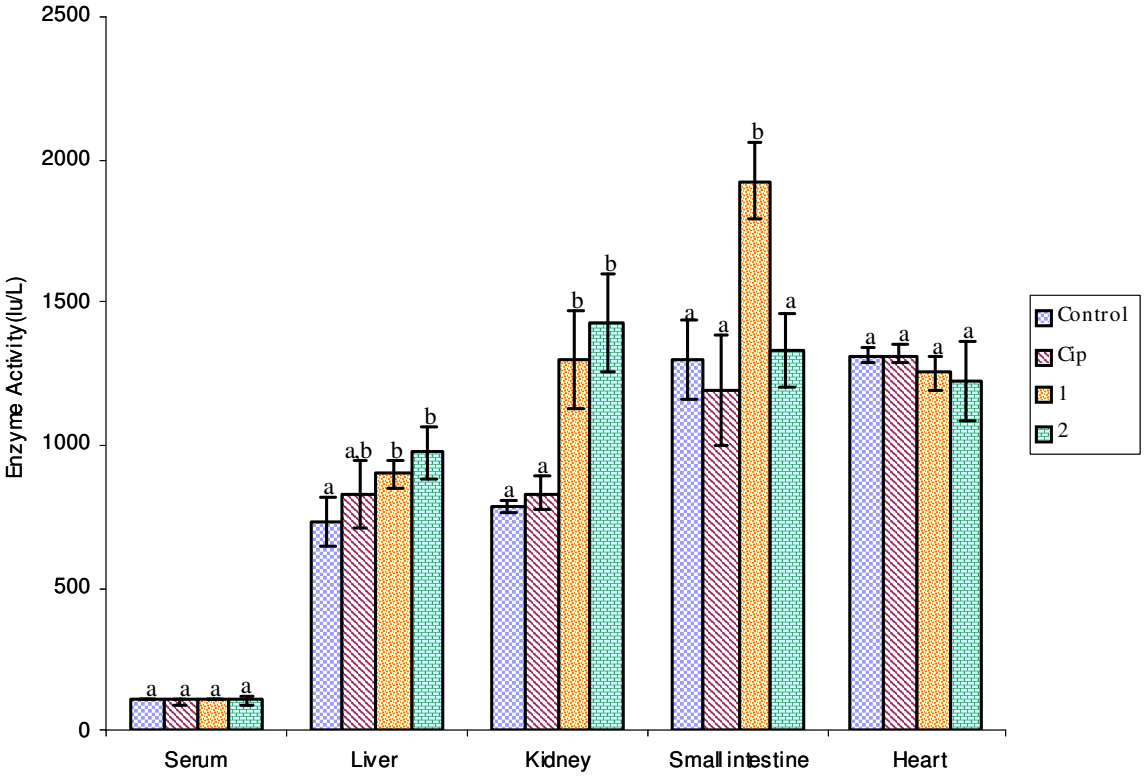

Figure 7. Effects of Ciprofloxacin and its metal complexes on AST activity in some tissues. [Where Cip= Ciprofloxacin, **1** = $[Fe(Cip)_2Cl_2]Cl.H_2O$ and **2** = $(H_3Cip)[FeCl_4]Cl.H_2O$-]. Values with different superscripts are significantly different at $P < 0.05$.

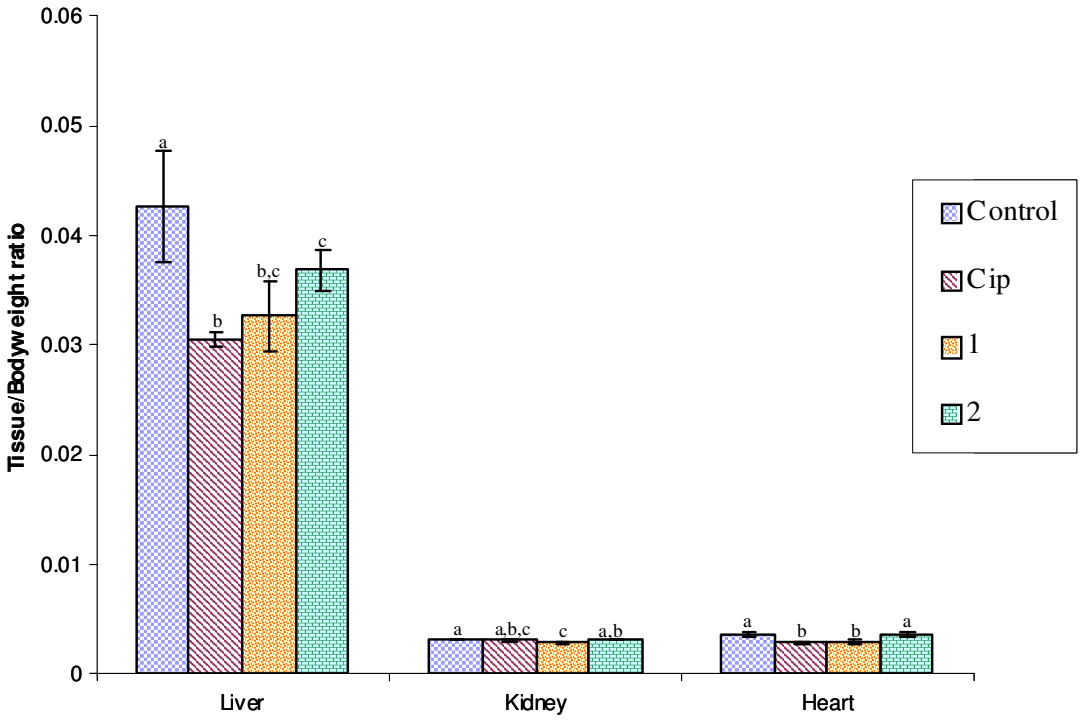

Figure 8. Effects of Ciprofloxacin and its metal complex on tissue-bodyweight ratio in some tissues. [Where Cip = Ciprofloxacin, **1** = $[Fe(Cip)_2Cl_2]Cl.H_2O$ and **2** = $(H_3Cip)[FeCl_4]Cl.H_2O$-]. Values with different superscripts are significantly different at $P < 0.05$.

cell growth and proliferation (Dollery, 1999). However, the reduction caused by **compound 2** is not as severe as that caused by Ciprofloxacin. The same pattern is exhibited with respect to heart- body weight ratio, although **compound 2** did not cause any significant reduction in heart-body weight ratio compared to control. This result suggests that **compound 2** may have a less adverse effect on the liver and heart compared to Ciprofloxacin and **compound 1**. Also prolonged administration of Ciprofloxacin and its complexes should be discouraged.

Alkaline Phosphatase

The fact that there was no significant difference in serum ALP activities of Ciprofloxacin and its metal complexes compared with control suggests that the integrity of the plasma membrane of the cells in the various tissues might not have been adversely affected. This is because ALP is a membrane-bound enzyme often used to assess the integrity of the plasma membrane and endoplasmic reticulum and an increase in ALP activity of the serum implies membrane damage to the tissues (Akanji et al., 1993). Since there was no increase in serum ALP activities of the various groups, the observed decrease in ALP activities in the small intestine (of rats administered with Ciprofloxacin), and heart (of rats administered with **1** and **2**) may imply inactivation of the enzyme *in situ* by the drugs or their metabolites. Moreover, the observed increase in the ALP activities in the liver (of rats administered Ciprofloxacin) and small intestine (of rats administered **1** and **2**) suggests an enhancement of the activities of the existing enzymes by the drugs and their metabolites. The increase may also be as a result of stress imposed on the tissue by the drugs, which may lead to loss of the enzyme molecule through leakage into extracellular fluid, which has not been significantly noticed in the serum. In a bid to offset this stress, the tissue may increase the *de novo* synthesis of the enzyme, thus accounting for the increase in ALP activities in these tissues (Umezawa and Hooper, 1982; Malomo et al., 1993, 1995).

Acid phosphatase

The serum ACP activities also showed a similar trend with serum ALP activities in rats administered Ciprofloxacin and metal complexes suggesting that the integrity of the lysosomal membrane in the cells of the various tissues has not been compromised. This is because ACP is a lysosomal enzyme, which is often used to monitor prostatic cancer (Burtis and Ashwood, 1999). Since there was no increase in serum ACP activities, the observed increase in ACP activities in the kidney (of rats administered **1**), small intestine (of rats administered **2**)

and heart (of rats administered **1**) suggests either an increased production of ACP due to stimulation of the enzyme synthesis or facilitated activities of existing enzymes. However, the observed reduction in ACP activities in the liver (of rats administered **2**) could be due to inactivation of the enzyme synthesis or activity by the drugs and their metabolites.

Alanine aminotransferase

In a similar trend with the ALP and ACP activities, the serum ALT activities in rats administered with Ciprofloxacin and **1** did not show significant increase compared with control, which suggests that the integrity of the cell membranes in the various tissues studied has not been adversely affected. However **2** caused a significant increase in serum ALT activity compared with control, with a corresponding increase in liver ALT activity, but comparable with the parent drug. For the treatment groups that did not show an increase in serum ALT activity, observed increases therefore in ALT activities in the liver and small intestine (of rats administered Ciprofloxacin) suggests stimulation of the enzyme activities by the drugs and their metabolites which could be due to stress imposed on the tissues by the drugs.

Aspartate aminotransferase

Ciprofloxacin and the metal complex groups did not have a significant increase in serum AST activities compared with control as in ALT activities. This suggests that the integrity of the cell membranes of the various tissues (especially the heart and liver) were not adversely affected. However, observed increases in AST activities in the liver (of rats administered **1** and **2**), kidney (of rats administered **1** and **2**) and small intestine (of rats administered **1**) without corresponding increase in serum AST activities for the groups may imply enhancement of enzyme activities as observed for other enzymes.

Conclusion

The stoichiometries and the structure of the complexes reported here are based essentially on the microanalytical data obtained. Based on this, structures are proposed such that steric constraints are eliminated. The Ciprofloxacin ligand formed molecular complex in the zwitterionic form (Cip±) for **$[Fe(Cip)_2Cl_2]Cl.6H_2O$** while it formed ionic complex in the acidic cation (Cip^+) for **$(H_3Cip)[FeCl_4]Cl.H_2O$** as reported in some previous work (Turel, 2002; Al- Mustafa, 2002).

In general, the metal complexes were most active against *N. gonorrhoea*, most especially $[Fe(Cip)_2Cl_2]Cl.6H_2O$-**1.** From the toxicological studies of Ciprofloxacin and metal complexes, the metal complexes $[Fe(Cip)_2Cl_2]Cl.6H_2O$-**1** and $(H_3Cip)[FeCl_4]Cl.H_2O$-**2** had less negative effect on the body tissues studied compared to their parent drug. Therefore the metal complexes are much better than the parent drug.

ACKNOWLEDGEMENT

We would like to thank Dr. Bamidele Omotowa of Fluorine Products Division, International Isotopes Inc., Idaho Falls, USA for results of elemental analyses.

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