ORIGINAL ARTICLE

IN VITRO AVAILABILITY OF OFLOXACIN IN PRESENCE OF METALS ESSENTIAL TO HUMAN BODY

NAJMA SULTANA , M. SAEED ARAYNE* AND NUVAIRA YASMEEN

Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy,
University of Karachi, Karachi-75270, Pakistan
**Department of Chemistry, University of Karachi, Karachi-75270, Pakistan*

ABSTRACT

The significance of interaction between ofloxacin and metals ions was evaluated in this study. The absorption of ofloxacin can be negatively affected by concomitant administration of agents containing metal cations. Current studies examine alterations of ofloxacin availability by metal cations and is limited to conventional metal-containing agents such as antacids and mineral supplements. The *in vitro* availability of ofloxacin was studied in presence of metal ions as magnesium, calcium, chromium, manganese, ferric, ferrous, cobalt, nickel, copper, zinc and cadmium in their salt form in simulated gastric juice, buffers of pH 7.4 and 9 at 37°C by using a modified B.P 2002 dissolution apparatus. UV/VIS (Shimadzu 1601) spectrophotometer was used to analyze the drug by measuring absorbance at 294 nm in simulated gastric juice and at 288 nm in pH 7.4 and 9. The result showed that availability of ofloxacin slightly changed in presence of all metals in all these dissolution mediums.

Keywords: Ofloxacin; essential and trace elements; in vitro availability.

INTRODUCTION

Ofloxacin, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxa-zine-6-carboxalic acid (Susan *et al*. 1991), is a second generation fluorinated quinolone, a pyridone carboxylic acid derivative which exert a broad-spectrum antimicrobial effect in a variety of systemic infections (Monk and Compoli, 1987; Bassaris *et al*., 1995). It blocks bacterial DNA synthesis by inhibiting DNA gyrase and topoisomerase IV. Inhibition of DNA gyrase prevents the relaxation of positively supercoiled DNA that is required for normal transcription and replication (Drlica, 1987 and Gellert, 1981).

Corresponding author: Email: arayne@chemist.com

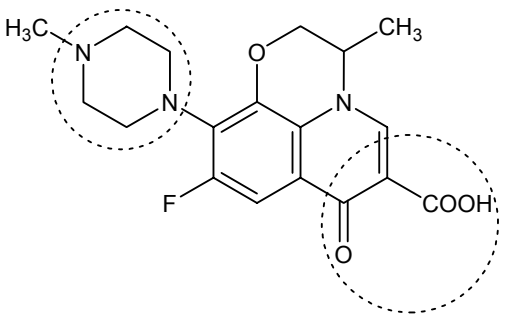

Fig. 1: Ofloxacin showing different pKa centers in the molecule.

Ofloxacin exists as zwitterions at the pH condition in the small intestine (figure 1) as it has both slightly acidic and basic centers having different pKa values. Ofloxacin is considered to be soluble in aqueous solutions with pH between 2 and 5 (Villanova, 1990). It is sparingly to slightly soluble in aqueous solution with pH 7 and freely soluble in aqueous solution with pH above 9. It is soluble in glacial acetic acid, slightly soluble in methylene chloride and in methanol (BP, 2000). The stability of ofloxacin in several transfusion solutions was found compatible with 5% glucose; 0.9% NaCl, and peritoneal dialytic solutions at room temperature and is suitable for use in intravenous infusion and dialysis (Hu and Shao, 1994).

Ofloxacin has a chiral center having methyl group on pyridobenzoxazine nucleus, where the orientation of the substituent can be of critical importance to the compound's antibacterial activity (Vincent, 1998). When ofloxacin was first introduced it was made available as the racemate (1:1 mixtures). Later the optical isomers were prepared and was found that the (S)-enantiomer, DR-3355 (6b), was substantially more active (8-128-fold) than the (R)- isomer against broad range of bacteria (Atarashi and coworkers, 1987; Hayakawa and coworkers, 1987; Imamura *et al*., 1987).

The trace elements comprise of metals in biological fluids at concentrations < 1 μg/g weight. Functions of calcium and magnesium are determined, in part, by their charges, mobilities, and binding constants to biological ligands. Calcium and magnesium form moderately stable complexes with enzymes, nucleic acids, and other ligands. They act as triggers, altering and/or controlling various biological functions (Beedwarl and Bakuguna, 1994).

Metal ions have been reported to significantly alter the absorption of different drugs especially antibiotics probably through chelation or adsorption mechanism. Fluoroquinolones are capable of forming chelate complexes with metal ions, resulting in reduced bioavailablity of the quinolones antibiotic (Lomaestro and Bailie, 1995; 1991). Chelation between 3-carboxy and 4-oxo-functional groups of quinolone molecule and metallic cation (e.g., Al^{+++} or Mg^{++}) result in the formation of insoluble complexes and reduced quinolone absorption (Arayne *et al*., 2005). Concurrent use of ofloxacin and an aluminum, magnesium or calcium containing antacid may result in decreased serum concentration of antibiotic (Flor *et al*., 1991; Monk and Campoli, 1987). The absorption of fluroquinolones is reduced by 50 to 90% in presence of antacids containing magnesium (Gugler and Allgayer, 1990; Hoffken *et al*., 1985; Holt, 1998). Many studies have confirmed that iron compounds can also decrease absorption of fluoroquinolones (Brouwers, 1992). A number of interactions of ofloxacin with metals have been reported by various workers (Flor *et al*., 1990; Shiba *et al*., 1992). Ofloxacin has potential to form stable coordination compounds with many metal ions. This *in vitro* chelation potential has the following formation order: Fe^{+3}> Al^{+3}> Cu^{+2}> Ni^{+2}> Pb^{+2}> Zn^{+2}> Mg^{+2}> Ca^{+2}> Ba^{+2} (Villanova, 1990; Physician's Desk Reference, 1997). Administration of ferrous salts with ofloxacin has been found to significantly reduce the absorption of ofloxacin (Lehto *et al*., 1994). Co administration of zinc and calcium with ofloxacin may reduce the absorption of ofloxacin (Sanchez *et al*., 1994; Lomaestro and Bailie, 1991; Marchbanks, 1993). It is necessary to be aware of these interactions because they may greatly affect the antibiotic availability that it may compromise the patient outcome.

The aim of the present work is to study the *in vitro* availability of ofloxacin in presence of essential and trace elements which may either be present in low concentrations in human body or may be ingested as a result of multiple drug therapy.

EXPERIMENTAL

Materials

Ofloxacin reference standard (99.99% purity) was provided by Lab-9 of the Department of Chemistry, University of Karachi. Ofloxacin tablets were gift from Aventis Pharmaceutical Laboratories Ltd Karachi, Pakistan. The metal chlorides used were of analytical grade.

Methods

Simulated gastric juice containing 0.1 M hydrochloric acid, phosphate buffers of pH 7.4 and 9 were prepared according to B.P. 2003 methods.

A dissolution apparatus as outlined in British Pharmacopoeia (2002) was used with slight modifications, details of which have already been described earlier (Iftikhar *et al*., 2005). UV/VIS spectrophotometer (Shimadzu, Japan Model 1601), which was serially coupled with a PIII PC loaded with a UVPC-ver. 3.9 software was used to monitor the drug.

A. *Reference standard of ofloxacin*

Solutions of ofloxacin reference standard prepared in simulated gastric juice, buffers of pH 7 and 9 were scanned in the region 200–650 nm, absorption maxima recorded and the absorbance was measured at the particular wave length. Epsilon values were calculated in different mediums, which were used to calculate the drug contents in later studies.

B. *Availability of ofloxacin*

The *in vitro* availability of ofloxacin in simulated gastric juice, buffer pH 7 and 9 at 37°C was found by using the dissolution apparatus as described earlier. The absorbance

Table 1: Availability of ofloxacin (%) in presence of metals at different time intervals in stimulated gastric juice.

Sample	Time [min]											
	15	30	45	60	75	90	105	120	135	150	165	180
Ofloxacin	46.7	95.3	97.8	100.1	100.2	100.3	103.7	100.7	101.1	101.4	101.5	102.6
Ofloxacin + magnesium	65.9	27.0	78.3	95.8	97.22	97.3	98.2	98.3	99.0	101.1	101.6	101.7
Ofloxacin + calcium	78.6	26.8	81.5	99.5	103.3	103.4	103.7	103.8	103.8	103.9	101.9	100.7
Ofloxacin + chromium	35.2	87.5	98.3	99.8	100.5	100.8	101.4	101.6	102.4	102.3	101.2	99.8
Ofloxacin + manganese	29	77.6	94.6	98.7	101.2	101.3	101.9	101.9	101.8	101.7	101.7	100.2
Ofloxacin + ferrous	48	94.5	101	101.6	102	102.8	103	103.1	103.2	103.3	103.4	102
Ofloxacin + ferric	34	92	99	99.8	101	102.8	102.9	102.3	101.9	101.6	101.2	99
Ofloxacin + cobalt	60	96	99	100	101.3	101.8	102.1	102.1	102.4	101.9	101.7	101
Ofloxacin + nickel	40	92	98	98.7	98.9	99.5	100	100.3	100.4	102.3	101.2	99
Ofloxacin + copper	16	77	100	102	102	102.8	103	102.8	102.6	101	99	94
Ofloxacin + zinc	44	93	98	102	103	103.5	103.7	102.5	102	102	99	97
Ofloxacin + cadmium	21	60	89	98	99	101.5	101.7	102.5	102.5	103.4	103.5	101

Table 2: Availability of ofloxacin (%) in presence of metals at different time intervals in buffer of pH 7.4.

Sample	Time [min]											
	15	30	45	60	75	90	105	120	135	150	165	180
Ofloxacin	42	55	72	76	81	90	91	93	93	93.6	94	98
Ofloxacin + magnesium	39	59	62	68	70	72	78	81	82	83	85	86
Ofloxacin + calcium	33	49	62	66	70	71	74	74	75	76	77	77
Ofloxacin + chromium	22	32	48	58	65	69	72	76	78	80	80.5	84
Ofloxacin + manganese	48	67	80	90	91	91.6	93.7	93.9	94	94.3	94.7	96
Ofloxacin + ferrous	56	75	95	100	101	103	102.6	102	101.6	101	101	94
Ofloxacin + ferric	32	41	48	54	64	70	79	83	87	89	89	88
Ofloxacin + cobalt	61	92	95	97	99	100	98.7	98.6	98.5	98.5	98.5	98
Ofloxacin + nickel	16	45	63	90	96.8	96	95.8	95.4	95	94	93	93
Ofloxacin + copper	18	24	31	35	42	46	49	54	57	60	63	67
Ofloxacin + zinc	21	30	35	39	42	46	53	60	65	69	71	76
Ofloxacin + cadmium	38	58	69	79	86	87	88	88	89	89.7	91	92

was measured at the given wave length and % drug available was calculated.

C. *Interaction of ofloxacin with essential and trace element*

Ofloxacin was reacted with hydrated salts of various essential and trace elements in simulated gastric juice, in buffers of pH 7.4 and 9 at 37°C using dissolution apparatus. In individual experiments metal salt and drug were added in the ratio of 1:2 at the start of experiment to the dissolution medium maintained at 37°C. Aliquots were withdrawn periodically at fifteen minutes time intervals for 180 minutes and assayed for drug content. The volume of the dissolution medium was maintained by adding the volume of the dissolution fluid withdrawn, which was maintained at the same temperature in the same bath.

RESULT AND DISCUSSION

Quinolones affect trace metal metabolism being potent inhibitors of some copper or zinc dependent enzymes. Present studies were carried out with the metal chlorides of magnesium, calcium, chromium, manganese, ferric, cobalt, nickel, copper, zinc and cadmium in the form of their hexahydrate salts, except zinc and cadmium, which were dihydrates. Ferrous was used in the form of ferrous sulfate heptahydrate. Interactions of ofloxacin were carried out with these essential and trace elements in simulated gastric juice and buffers of pH 7.4 and 9; the later two simulating blood and intestinal pH.

To study *in vitro* interactions of ofloxacin and elements essential to human body in dissolution test apparatus has a number of advantages as it provides the simulation of peristaltic movements of the stomach and the human body temperature. There are many designs of the dissolution test apparatus described in the literature. In present studies, the apparatus described in B.P. 2002 with slight modifications to eliminate air entrapment was used in these studies (Iftikhar *et al*., 2005).

The decline observed in the availability of ofloxacin in presence of all essential and trace elements in all dissolution mediums is evident in the data given in tables 1-3 and plotted in figures 2-4. The intrinsic dissolution rate constants and dissolution time T50% and T90% of ofloxacin in presence of essential and trace elements are given in table 4. Ofloxacin followed first order dissolution rates in presence of all these metals, the dissolution time T50% and T90% was substantially increased in presence of all metals in simulated gastric juice. Similarly, the

Table 3: Availability of ofloxacin (%) in presence of metals at different time intervals in buffer of pH 9.

Sample	Time [min]											
	15	30	45	60	75	90	105	120	135	150	165	180
Ofloxacin	41	59	73	83	89	95	97	100	102	103	103	103
Ofloxacin + magnesium	14	18	23	27	31	34	37	40	43	46	48	48
Ofloxacin + calcium	40	62	74	83	90	94	99	102	102	102	102	102
Ofloxacin + chromium	40	57	71	79	85	90	93	96	98	100	101	100
Ofloxacin + manganese	49	62	73	81	86	91	93	98	100	101	86	83
Ofloxacin + ferrous	17	24	30	36	41	45	50	54	57	60	64	63
Ofloxacin + ferric	31	42	50	56	60	66	70	74	77	80	82	84
Ofloxacin +cobalt	88	88.3	92	94	96	97	99	100	100	100	100	100
Ofloxacin + nickel	54	75	90	95	98	99	99	99	99.3	98.7	98	97
Ofloxacin + copper	34	43	52	60	65	69	75	78	80	83	103	103
Ofloxacin + zinc	40	57	69	80	89	93	96	102	102	102	102	87
Ofloxacin + cadmium	56	73	78	80	83	85	87	87.9	89	91	91.9	90

Table 4: First order dissolution rate constants of ofloxacin in presence of metals.

	Simulated gastric juice pH 7.4			pH 9					
	$T_{50\%}$	$T_{90\%}$	K_{294nm}	$T_{50\%}$	$T_{90\%}$	K_{288nm}	$T_{50\%}$	$T_{90\%}$	K_{288nm}
Ofloxacin	18.4	56.7	0.09	31.8	105	0.02	20.0	66.0	0.03
Ofloxacin + magnesium	16.4	54.7	0.04	6.23	207	0.01	188	627	0.003
Ofloxacin + calcium	5.8	19.3	0.11	47.0	156.3	0.01	15.2	50.5	0.04
Ofloxacin + chromium	6.3	21.2	0.10	66.8	222	0.01	21.3	70.9	0.03
Ofloxacin + manganese	9.5	31.7	0.07	62.3	207	0.01	20.7	69.0	0.03
Ofloxacin + ferrous	7.1	23.7	0.05	44.1	146	0.01	124	412	0.05
Ofloxacin + ferric	6.3	21.2	0.10	56.8	189	0.01	67.7	225	0.01
Ofloxacin + cobalt	5.9	19.8	0.11	31.0	103	0.02	14.3	47.5	0.04
Ofloxacin + nickel	11.6	38.6	0.05	44.4	147	0.01	34.9	116	0.01
Ofloxacin + copper	13.9	46.1	6.05	109	363	0.01	57.1	189	0.01
Ofloxacin + zinc	7.5	25.0	0.09	85.7	284	0.01	21.2	70.6	0.03
Ofloxacin + cadmium	0.05	3.77	5.37	0.07	3.32	0.075	53.8	178	0.01

dissolution time T50% and T90% was also substantially increased in buffer of pH 7.4 in presence of magnesium, chromium, manganese, copper and zinc. Likewise, the dissolution time T50% and T90% was also increased to a large extent in buffer of pH 9 in presence of magnesium, ferrous, ferric, copper and cadmium.

In simulated gastric juice, in presence of cobalt and chromium 102% of drug was available at 135 minutes and 101 & 99% were available in presence of cobalt and chromium at 180 minutes. In presence of zinc 103% of drug was available at 105 minutes, which decreased to 97% at 180 minutes. Similarly, in presence of calcium 103% of drug was available at 150 minutes which decreased to 100% at 180 minutes. In presence of copper 103% of drug was available at 105 minutes but availability decreased to 99% at 180 minutes. In presence of nickel 102% of drug was available at 150 minutes, which decreased to 99% at 180 minutes. In presence of ferrous 103% of drug was available at 165 minutes but availability decreased to 102% at 180 minutes. In presence of cadmium 103% of drug was available at 150 minutes but availability decreased to 101% at 180 minutes. In presence of ferric 102% of drug was available at 105 minutes but availability decreased to 99% at 180 minutes. In presence of magnesium 101% of drug was available at 180 minutes. In presence of manganese 101% of drug was available at 120 minutes but availability decreased to 100% at 180 minutes.

In case of buffer pH 7.4, in presence of ferric ions 89% of drug was available at 150 minutes, which decreased to 88% at 180 minutes. In presence of calcium 77% of drug was available at 180 minutes. In presence of cobalt 100% of drug was available at 90 minutes which decreased to 98% at 180 minutes. Similarly, in presence of nickel 96% of drug was available at 75 minutes was decreased to 93% at 180 minutes. In presence of chromium 84% of drug was available at 180 minutes. In presence of copper 67% of drug was available at 180 minutes. In presence of magnesium, manganese and zinc 86%, 96% and 76% of drug was available at 180 minutes respectively.

In case of buffer pH 9, similar pattern was followed in presence of all essential and trace elements. In most of these cases the availability of drug achieved at 120 minutes persisted till the end of the experiment. However, in presence of manganese and zinc 101% and 102% of drug available at 150 minutes was decreased to 83% and 87% at 180 minutes respectively.

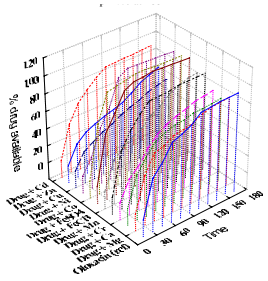

Fig. 2: Availability of ofloxacin (%) in presence of metals essential to human body in simulated gastric juice at 37ºC.

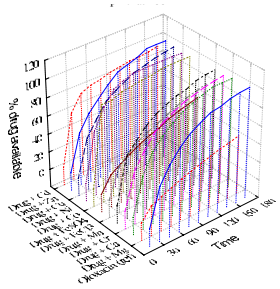

Fig. 3: Availability of ofloxacin (%) in presence of metals essential to human body in pH 7.4 at 37ºC.

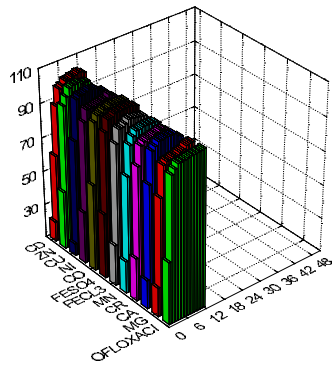

Fig. 4: Availability of ofloxacin (%) in presence of metals essential to human body in pH 9 at 37 ºC.

To facilitate our studies first-order dissolution constants (K), $T_{50\%}$ and $T_{90\%}$, of ofloxacin in presence of various metals in simulated gastric and in buffer pH 7.4 and intestinal juices were also calculated as given in (table 4).

CONCLUSION

From these drug-metal interaction studies we have concluded that concurrent use of ofloxacin and drugs containing metal ions may result in decreased absorption, decreased serum concentration and decreased bioavailability of ofloxacin, leading to loss of therapeutic efficacy. Therefore metal supplements or metal containing products should be administered two hours before or two hours after the administration of ofloxacin.

REFERENCES

Abid I, Arayne MS and Sultana N (2005). *In vitro* release of tetracyclines in presence of H_2-receptor antagonists. *Pak. J. Pharm. Sci.,* **18**(2): 55-60.

Arayne MS, Sultana N and Hussain F (2005). Interaction between ciprofloxacin and antacids. adsorption and dissolution studies. *Drug Met. & Drug Inter*. **21**(2): 117-129.

Atarashi S and co-workers (1987). *Chem. Pharm. Bull*., **35:** 1896.

Bassaris H, Akalin E and Calangu S (1995). A randomised, multinational study with sequential therapy comparing ciprofloxacin twice daily and ofloxacin once daily. *Infection*, **23**: 39-45.

Beedwarl RS and Bakuguna A (1994). Zinc, copper and selenium in reproduction. *Experentia*, **50**: 626.

British Pharmacopoeia (2000). Published by the stationary office, **1**: 1121-1122.

Brouwers JR (1992). Drug interactions with quinolone antibacterials. *Drug Saf*., **7**(4): 268-281.

Drlica K (1984). Biology of bacterial deoxyribonucleic acid topoisomerases. *Microbiol. Rev.,* **48**: 273-289.

Flor S, Guay DRP and Opsahi JA (1990). Effects of magnesium-almunium hydroxide and calcium carbonate antacids on bioavailability of ofloxacin. *Antimicrob. Agents Chemother*., **34**: 2436-2438.

Flor S, Guay DRP and Opsahl JA (1991). Effect of magnesium- aluminum hydroxide and calcium carbonate antacids on the bioavailability of ofloxacin. *Antimicrob. Agents Chemother*., **34**: 2436-2438.

Gellert M (1981). DNA topoisomerases. *Ann. Rev. Biochem*., **50**: 879-910.

Gugler R and Allgayer H (1990). Effects of antacids on the clinical pharmacokinetics of drugs. An update. *Clin. Pharmacokine*., **118**(3): 210-219.

Hayakawa I and co-workers (1987). *Antimicrob. Agents Chemother*., **29**: 163.

Hoffken G, Borner K, Glatzel PD, Koeppe P and Lode H (1985). Reduced absorption of ciprofloxacin in the presence of antacids. *Eur. J. Clin. Microbiol.,* **4**(3): 334.

Holt GA (1998). Food and Drug Interactions. Chicago: Precept Press.

Hu D and Shao Z (1994). Stability of ofloxacin in several transfusion solutions. *Zhongguo Yiyuan Yaoxue Zazhi.* **14**(4): 168-9.

Imamura M, Shibamura S, Hayakawa I and Osada Y (1987). *Antimicrobial. Agents. Chemother*., **31**: 325.

Johnson MA (1990). Iron: Nutrition monitoring status assessment. *J. Nutr*., **120**: 1486-1491.

Lehto P and Kivisto KT and Neuvonen PJ (1994). The effect of ferrous sulphate on the absorption of ofloxacin. *Br. J. Clin. Pharmacol.,* **37**: 82-85.

Lomaestro BM and Bailie GR (1991). Quinolone-cation interactions: a review. *DICP*. **25**: 1249-58.

Lomaestro BM and Bailie GR (1991). Quinolone-cation interactions: a review. *DICP Ann. Pharmacother*., **25**: 1249-1258.

Lomaestro BM and Bailie GR (1995). Absorption interactions with fluoroquinolones. *Drug Safety*, **12**: 314-33.

Marchbanks CR (1993). Drug-drug interactions with fluoroquinolones. *Pharmacotherapy*, **13**: 23-28.

Monk JP and Campoli RDM (1987). Ofloxacin: A review of its antibacterial activity, pharmacokinetics properties and therapeutic use. *Drugs*, **33**: 346-391.

Monk JP and Campoli RDM (1987). Ofloxacin: a review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drug*, **33**: 346-528.

Physicians' Desk Reference, (1997). 5th ed., Published by Medical Economics Company, 1577.

Sanchez NA, Martinez CM and Dominguez GHA (1994). Comparative study of the influence of Ca on absorption parameters of ofloxacin. *J. Antimicrob. Chemother*., **34**(1): 119-25.

Shiba K, Sakai O and Shimada J (1992). Effects of antacids, ferrous sulphate, and ranitidine on absorption of DR-3355 in humans. *Antimicrob. Agents Chemother.,* **36**: 2270-2274.

Susan B, Maryadele JON, Ann S and Patricia EH (1991). The Merck Index, 11th ed., Published by Merck and Co., 1071.

Villanova PA (1990). National Committee for Clinical Laboratory Standards, *Performance Standards for Antimicrobial Disk Susceptibility Tests*, 4th ed., **10**: 7.

Villanova PA (1990). National Committee for Clinical Laboratory Standards, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 2nd ed., **10**: 8.

Vincent TA (1998). The Quinolones, 2nd ed., Academic Press, 51.

Received: 21-08-2006 – Accepted: 06-11-2006