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Clinical and chemical interactions between iron preparations and ciprofloxacin

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1 The effect of ferrous sulphate (300 mg), ferrous gluconate (600 mg), and a combination tablet of iron (10 mg), magnesium (100 mg), zinc (15 mg), calcium (162 mg), copper (2 mg), and manganese (5 mg) (Centrum Forte) co-administration on ciprofloxacin bioavailability was tested in eight healthy subjects.

2 Peak serum ciprofloxacin concentrations and area under the curve (AUC) were significantly reduced when ciprofloxacin was administered with 300 mg ferrous sulphate (3.0 *vs* 2.0 mg l^{-1}, $P < 0.05$ and 12.3 *vs* 6.7 mg l^{-1} h, $P < 0.01$, respectively). Reductions in peak ciprofloxacin concentrations and AUC also occurred when ciprofloxacin was ingested with 600 mg ferrous gluconate (1.3 mg l^{-1}, $P < 0.01$ and 4.1 mg l^{-1} h, $P < 0.01$, respectively) and a Centrum Forte tablet (1.4 mg l^{-1}, $P < 0.01$ and 5.4 mg l^{-1} h, $P < 0.01$, respectively).

3 When ferrous ion was mixed with ciprofloxacin, rapid spectral changes occurred ($t_{1/2} = 1.9$ min). Additional studies were consistent with oxidation of the ferrous form of iron to its ferric form, which is followed by rapid formation of a Fe^{3+}-ciprofloxacin complex. Ciprofloxacin seems to bind to ferric ion in a ratio of 3:1 by interacting with the 4-keto and 3-carboxyl groups on ciprofloxacin.

4 The formation of a ferric ion-ciprofloxacin complex is probably the cause of the reduction in ciprofloxacin bioavailability in the presence of iron.

Keywords ciprofloxacin iron complex formation chelation drug interaction

Introduction

Ciprofloxacin is a second generation quinolone antimicrobial with a broad spectrum of activity against common aerobic gram negative and gram positive bacterial pathogens (Walker & Wright, 1987). Although ciprofloxacin is readily absorbed, a variety of medications containing metal ions have been shown to interfere with its bioavailability (Davies & Maesen, 1989; Frost *et al.*, 1989; La Pennec *et al.*, 1990; Nix *et al.*, 1989; Polk, 1989; Polk *et al.*, 1989). The decreased bioavailability is presumably the result of formation of poorly absorbed metal ion-ciprofloxacin complexes (Davies & Maesen, 1989; Frost *et al.*, 1989; Nix *et al.*, 1989; Polk, 1989; Polk *et al.*, 1989). Ciprofloxacin and iron supplements are among the most commonly prescribed drugs (La Piana Simonsen, 1990) and iron is also taken in over-the-counter preparations. We have examined some aspects of the formation of iron-ciprofloxacin complexes, confirmed the clinical interaction between ferrous sulphate and ciprofloxacin and have examined the effect of ferrous gluconate and a commonly used multi-vitamin and mineral preparation (Centrum Forte) on ciprofloxacin bioavailability.

Methods

Seven adult males (average age 28.3, range 21 to 42 years) and one female (age 20 years) participated in the study. None of the subjects was ill or taking any medication at the time of the study. All subjects fasted from midnight on the day of the study. Indwelling intravenous catheters were inserted in an antecubital vein of each subject for blood sampling. Blood was drawn for routine renal, hepatic and haematological function tests (sodium, creatinine, serum aspartate aminotransferase, alkaline phosphatase, total bilirubin and an automated blood cell count). The results of the blood tests were normal for

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all subjects. The subjects were randomly assigned to take a single 500 mg tablet of ciprofloxacin (Miles Canada Inc., Eticbicoke, Ont. Canada) with or without a 300 mg tablet of ferrous sulphate (60 mg 'elemental iron') (Wampole Inc., Perth, Ont, Canada). The alternative treatment was taken 7 days later. After a further 1 week interval the subjects were randomly designated to take ciprofloxacin 500 mg with two 300 mg ferrous gluconate tablets (69.6 mg 'elemental iron') (Wampole Inc., Perth, Ont, Canada) or one tablet of Centrum Forte (Lederle-dyanamid Canada Inc., Markland, Ont, Canada). The alternative drugs were taken 7 days later. Each Centrum Forte tablet contains the following minerals: iron 10 mg, magnesium 100 mg, zinc 15 mg, calcium 162 mg, copper 2 mg, and manganese 5 mg. Blood samples were drawn into clot tubes before drug ingestion and at 0.5, 1, 1.5, 2, 3, 4, 5, 7 and 9 h afterwards. The tablets were taken with about 250 ml of tap water. The subjects continued to fast for 3 h after drug ingestion. These studies were approved by the Human Investigation Committee of Memorial University.

Ciprofloxacin was extracted from serum for subsequent measurement by h.p.l.c. with u.v. detection using a modification of the methods of Nix and of Vallee within a day of blood collection (Nix *et al.*, 1989; Vallee *et al.*, 1986). The interday coefficient of variation of the assay was 1.7% and 3.8% at 4.2 mg l^{-1} and 0.52 mg l^{-1} ciprofloxacin, respectively. The limit of assay was 0.05 mg l^{-1} ciprofloxacin (signal : noise 5 : 1).

In vitro experiments were performed to assess the stability of ciprofloxacin in the presence of ferrous sulphate. Ciprofloxacin was incubated with ferrous sulphate at various intervals up to 90 min after which the iron was removed by EDTA and the quantity of ciprofloxacin measured by h.p.l.c.

A number of ciprofloxacin-iron binding experiments were performed. Optical absorbance experiments were carried out in 1 cm cells on a Shimadzu UV-260 double beam recording spectrophotometer with the cell compartment maintained at 25° C. Oxygen uptake experiments to determine if the ferrous form of iron was being aerobically oxidized to the ferric form were carried out at 25° C using a Yellow Springs Instruments micro-oxygen electrode system (Yeollow Springs, Ohio), calibrated with air saturated solutions of known oxygen solubility. Both the spectrophotometric and oxygen uptake experiments were conducted in 50 mM Bis-Tris/HCl buffer (pH 6.0) at 25° C. Ferrous sulphate solutions were made immediately prior to use.

AUC values were measured by the linear trapezoidal method up to 9 h. Unless otherwise stated the AUC (0, 9 h) value was used in all calculations. To determine the total AUC the AUC after 360 min was estimated using the log linear terminal portion of the log serum ciprofloxacin-time curve. The kinetic data are shown as means ± s.d., unless indicated otherwise. The data were subjected to an analysis of variance (ANOVA) with a repeated measures design followed by one tailed Dunnett's *t*-tests.

Results

The pharmacokinetic parameters of ciprofloxacin following each treatment are shown in Table 1. Co-administration of ferrous sulphate resulted in a 46% decrease in ciprofloxacin AUC ($P < 0.01$) and a 37% decrease in peak serum drug concentration ($P < 0.05$). The decreases in AUC were 67% ($P < 0.01$) and 56% ($P < 0.01$) and the decreases in peak drug concentrations were 57% ($P < 0.01$) and 53% ($P < 0.01$) with ferrous gluconate and Centrum Forte, respectively. There was no significant difference in the time to peak ciprofloxacin concentrations when the antibiotic was given alone and when given with the different iron preparations or Centrum Forte. The average serum ciprofloxacin concentrations resulting from the different treatments are shown in Figure 1.

The addition of ferrous ammonium sulphate to ciprofloxacin in pH 6.0 buffer resulted in the spectral changes shown in Figure 2. To test if the final spectrum was due to formation of a ferric-ciprofloxacin complex, the ferric ion-ciprofloxacin complex was formed directly by adding ferric chloride to ciprofloxacin. This resulted in the formation of an orange complex. The addition of this complex to pH 6.0 buffer produced a spectrum that was nearly identical to the spectrum shown in Figure 2, indicating that the ferric ion-ciprofloxacin complex had formed following addition of ferrous ammonium sulphate to ciprofloxacin.

In experiments to confirm the formation of ferric ion : ciprofloxacin complexes, rapid consumption of oxygen from the ferrous sulphate solutions indicated that aerobic oxidation of Fe^{2+} to Fe^{3+} was occurring (Figure 3a) (Campbell *et al.* (1989, 1990)). The oxygen consumption was faster in the presence of ciprofloxacin (Figure 3a). Removal of iron following incubation of ciprofloxacin with ferrous sulphate for up to 90 min resulted in a 97–104% recovery of ciprofloxacin

Table 1 Mean (± s.d.) pharmacokinetic parameters of ciprofloxacin (500 mg) alone and with ferrous sulphate, ferrous gluconate, or Centrum Forte

	AUC (0, 9 h) *(mg l^{-1} h)*	*AUC (0, ∞)* *(mg l^{-1} h)*	t_{max} *(h)*	C_{max} *(mg l^{-1})*	$t_{1/2}$ *(h)*
Ciprofloxacin	12.3 (3.3)	16.2 (4.6)	1.2(0.5)	3.0(1.0)	4.5(0.8)
Ciprofloxacin + ferrous sulphate	6.7 (2.0)**	9.4 (3.1)**	0.9(0.2)	2.0(0.7)*	5.1(1.0)
Ciprofloxacin + ferrous gluconate	4.1 (2.8)**	5.8 (3.7)**	1.1(0.5)	1.3(1.5)**	4.8(1.2)
Ciprofloxacin + Centrum Forte	5.4 (1.0)**	7.7 (2.1)**	0.9(0.4)	1.4(0.4)**	5.2(1.6)

* $P < 0.05$ significantly less compared with ciprofloxacin alone.
** $P < 0.01$ significantly less compared with ciprofloxacin alone.

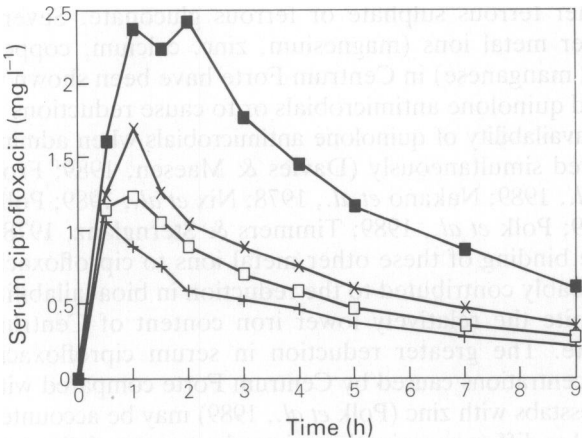

Figure 1 Mean serum ciprofloxacin concentrations after ingestion of 500 mg ciprofloxacin alone (■), with 300 mg ferrous sulphate (×), with 600 mg ferrous gluconate (+) and with Centrum Forte (□) (n = 8).

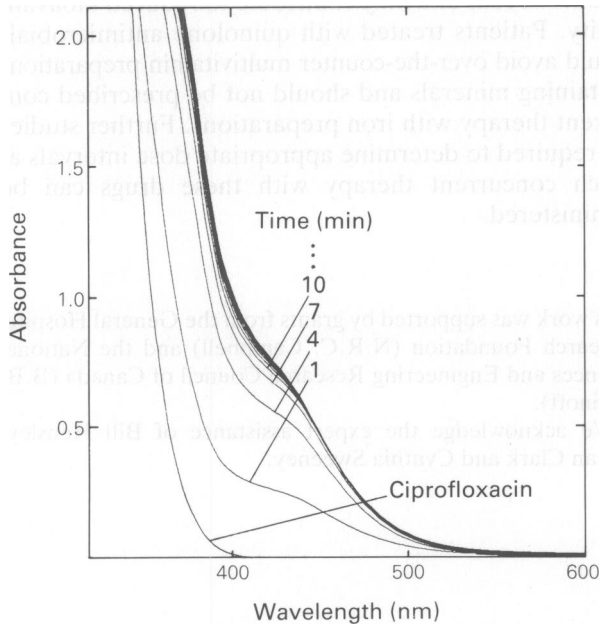

Figure 2 Spectral changes following addition of ferrous ammonium sulphate (0.50 mM) to ciprofloxacin (0.43 mM) in pH 6.0 Bis-Tris buffer (50 mM) at 25° C. The spectrum was scanned every 3 min after the addition of ferrous ammonium sulphate. The shoulder that appeared with increased absorbance at approximately 435 nm is due to the formation of an Fe^{3+}-ciprofloxacin complex.

indicating that no irreversible alteration of ciprofloxacin had occurred. This suggests that ciprofloxacin increases the rate of Fe^{2+} oxidation as ciprofloxacin is stable in the presence of ferrous sulphate. The absorbance changes that occur when ferrous sulphate is added to ciprofloxacin (Figure 3b) indicate that upon oxidation of Fe^{2+}, ciprofloxacin binds rapidly to the Fe^{3+} that is produced. The half-time for the absorbance changes on adding ferrous sulphate to ciprofloxacin was 1.9 min (Figure 3b).

To assess the stoichiometry of the complex formed between the ferric ion and ciprofloxacin, a spectrophotometric titration was carried out at 450 nm. Increasing concentrations of ciprofloxacin were added to ferric

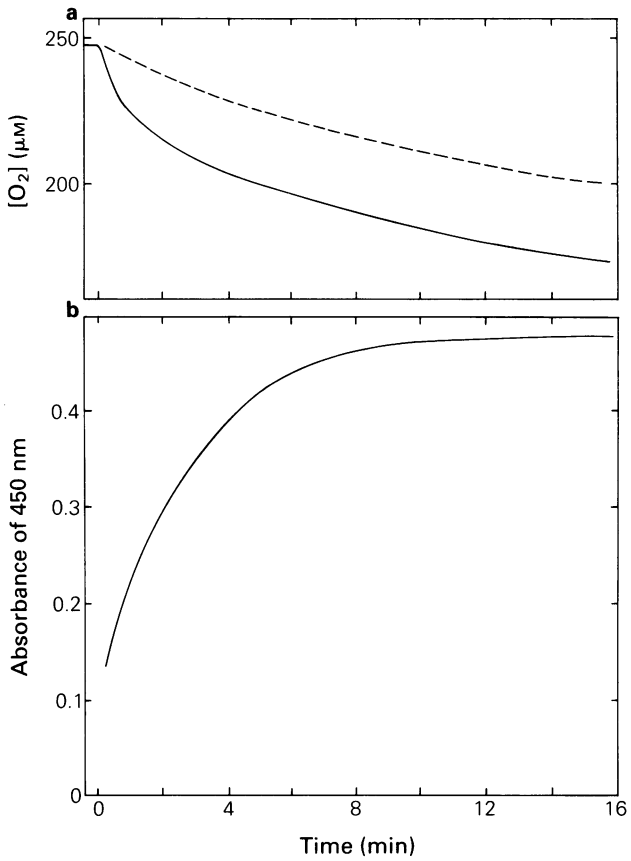

Figure 3 a) Oxygen consumption following addition of ferrous sulphate (0.5 mmol l^{-1}) alone (– – –) and with ciprofloxacin (0.5 mmol l^{-1}) (——) to an air saturated pH 6.0 Bis-Tris buffer. b) Absorbance change occurring at 450 nm when ferrous sulphate (0.5 mmol l^{-1}) is added to ciprofloxacin (0.5 mmol l^{-1}).

chloride in 50 mM Bis Tris buffer (pH 6.0). The titration yielded a plot with a break point close to a value of 3, which is consistent with the formation of a 3:1 (ciprofloxacin:ferric ion) complex.

Discussion

Quinolone antimicrobials are known to bind several metal ions (Davies & Maesen, 1989; Frost *et al.*, 1989; Nakano *et al.*, 1978; Nix *et al.*, 1989; Polk, 1989; Polk *et al.*, 1989; Timmers & Sternglanz, 1978). Comparing previous reports (Polk *et al.*, 1989), we have shown a large reduction in peak serum ciprofloxacin concentrations and in AUC when ciprofloxacin was ingested with ferrous sulphate. We have also shown that ferrous gluconate and Centrum Forte cause large reductions in ciprofloxacin AUC and peak serum concentrations. This is probably the result of a lowering of ciprofloxacin absorption secondary to the formation of iron-ciprofloxacin complexes. The ferrous form of iron is rapidly oxidized to the ferric form *in vitro* in pH conditions similar to those found in the small bowel (Campbell *et al.*, 1989, 1990), and the ferric ion binds rapidly to ciprofloxacin forming a complex. At pH 6.0 a 3:1 (ciprofloxacin:Fe^{3+}) complex is formed. The close

proximity of the carboxyl and keto groups on the ciprofloxacin molecule would account for its good chelating properties. Bidentate chelators typically form 3:1 complexes with the normally six coordinate ferric ion. Thus, the chemistry of the ciprofloxacin-iron interaction is consistent with the following overall reaction:

$$Fe^{2+} + 3 \text{ ciprofloxacin} + 1/4\ O_2 + H^+ \rightarrow Fe^{3+}(\text{ciprofloxacin})_3 + 1/2\ H_2O$$

Iron ions can alter drugs irreversibly by catalyzing oxidation and reduction reactions (Campbell *et al.*, 1990). However, ciprofloxacin is chemically stable in the presence of ferrous sulphate for up to 90 min. Thus, chemical instability of ciprofloxacin in the presence of iron is unlikely to contribute to the ciprofloxacin iron interaction. Strong binding of ciprofloxacin by the ferric ion is the likely explanation for the interaction of ciprofloxacin with ferrous sulphate and ferrous gluconate.

Significant differences between the effects of ferrous gluconate and sulphate salts were not seen in this study. However, effects of ferrous gluconate and ferrous sulphate co-administration were found to be different in an earlier study of tetracycline bioavailability (Neuvonen & Turakka, 1974). This discrepancy may be explained by differences in the dissolution rates of ferrous sulphate and ferrous gluconate tablets between the studies. However, the slightly higher iron content of the ferrous gluconate tablets used in our study may also be responsible. Slower dissolution of the ferrous sulphate tablets in this study may explain the smaller decrease in ciprofloxacin AUC and peak serum concentrations compared with that observed by Polk *et al.* (1989).

Tablets with a lower iron content may be expected to have less effect on ciprofloxacin bioavailability, yet Centrum Forte co-administration was as effective as either ferrous sulphate or ferrous gluconate. Several other metal ions (magnesium, zinc, calcium, copper, and manganese) in Centrum Forte have been shown to bind quinolone antimicrobials or to cause reductions in bioavailability of quinolone antimicrobials when administered simultaneously (Davies & Maesen, 1989; Frost *et al.*, 1989; Nakano *et al.*, 1978; Nix *et al.*, 1989; Polk, 1989; Polk *et al.*, 1989; Timmers & Sternglanz, 1978). The binding of these other metal ions to ciprofloxacin probably contributed to the reduction in bioavailability despite the relatively lower iron content of Centrum Forte. The greater reduction in serum ciprofloxacin concentrations caused by Centrum Forte compared with Stresstabs with zinc (Polk *et al.*, 1989) may be accounted for by differences in the mineral content of the two multivitamin and mineral preparations.

Two other quinolone antimicrobials, nalidixic acid and norfloxacin are known to bind iron (Issopoulos, 1989; Sulkowska & Staroscik, 1978). The carboxyl and keto groups common to these molecules are likely to bind ferric ions and may contribute to reduced bioavailability. Patients treated with quinolone antimicrobials should avoid over-the-counter multivitamin preparations containing minerals and should not be prescribed concurrent therapy with iron preparations. Further studies are required to determine appropriate dose intervals at which concurrent therapy with these drugs can be administered.

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