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Effect of Oral Administration of Fennel (*Foeniculum vulgare*) on Ciprofloxacin Absorption and Disposition in the Rat

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Abstract

The aim of this study was to investigate the possibility of a drug–drug interaction between ciprofloxacin and fennel (*Foeniculum vulgare*) in a rat model.

Pharmacokinetic assessment of ciprofloxacin was performed in two groups of male Sprague–Dawley rats. One group (n = 5) received 20 mg kg^{-1} antibiotic orally with concomitant oral dosing of the aqueous fennel extract (2 g herb kg^{-1}) whereas the controls (n = 5) received 20 mg kg^{-1} oral ciprofloxacin. Blood and urine samples were collected over 6 and 24 h, respectively, for quantitation of ciprofloxacin by HPLC. A non-compartmental model was employed for pharmacokinetic analysis. Major ingredients and metal cations in the fennel extract were determined. Compared with the control, maximum plasma concentration, area under the curve and urinary recovery of ciprofloxacin were significantly ($P < 0.05$) lower, by 83, 48 and 43%, respectively, in rats receiving concomitant dosing of the two agents. The relative bioavailability of ciprofloxacin, under the influence of fennel, was estimated to be 0·52. In addition, its apparent volume of distribution and terminal elimination half-life were significantly ($P < 0.05$) increased, from 30·8 ± 11·1 (L kg^{-1}) and 2·0 ± 0·4 (h) to 143·8 ± 31·6 (L kg^{-1}) and 5·2 ± 2·0 (h), respectively. Although none of the organic components of fennel seemed to cause this interaction, the total amount of ten metal cations measured was found to be 13 mg g^{-1}.

Significant interaction between ciprofloxacin and fennel was observed in this study. Absorption, distribution and elimination of ciprofloxacin were all affected. These changes might be because of the formation of a more lipophilic ciprofloxacin chelate in the presence of relatively large amounts of metal cations. If, therefore, the two therapeutic agents are used concurrently, an adequate dosing interval is needed to ensure the efficacy of ciprofloxacin.

Oral absorption of quinolone antibiotics is known to be adversely affected by metal cations (Yukinori et al 1996). For instance, the bioavailability of ciprofloxacin has been shown to be significantly reduced by concomitant administration of agents containing Ca, Al, Fe or Mg cations (Frost et al 1992; Lehto et al 1994; Li et al 1994). However, these interaction studies have been mostly confined to conventional therapeutic agents—the influence of herbal remedies on the pharmacokinetics of quinolones has not been well studied despite widespread parallel use of antibiotics and alternative medicines in many Eastern countries.

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This aim of this study was to explore the possibility of interaction between ciprofloxacin and an antibacterial and anti-inflammatory herbal medicine, *Foeniculum vulgare* Mill (Fam. Umbelliferae, so-called fennel). In some European countries, fennel seeds are used to enhance milk secretion in nursing mothers and to treat visual problems (Weiner & Weiner 1994). The plant can also serve as an aromatic, appetite stimulant and as a carminative, especially for prevention of colic in infants and to soothe gastrointestinal upsets (Weiner & Weiner 1994). The antibacterial activity of fennel has been demonstrated against pathogens including *Pseudomonas aeruginosa*, *Bacillus typhi*, *Proteus vulgaris*, *Salmonella paratyphi*, *Staphylococcus aureus* and *Shigella dysenteriae* (Li 1992). The use of this herb as a natural antimicrobial against different infections is common in the Orient.

Chemical investigation of fennel fruit has shown that it contains mainly volatile oils, with small amounts of flavonoids and furanocoumarins (Bruneton 1995).

Because of the high likelihood of the concurrent use of the two agents, the pharmacokinetics of ciprofloxacin have been evaluated with and without concomitant administration of fennel extract. To define more accurately the potential interaction, the chemical composition and content of ten metal cations in fennel were examined in more detail by gas chromatography–mass spectrometry (GC–MS), thin-layer chromatography (TLC) and plasma emission spectrometry.

Materials and Methods

Plant materials

Fennel, the fruits of *Foeniculum vulgare*, was purchased from a herbal shop in Hong Kong. It was authenticated by macroscopic examination and microscopic identification in the Pharmacognosy Laboratory, Department of Pharmacy, The Chinese University of Hong Kong. To prepare the fennel extract used in animal studies, the powdered herb (5 g) was treated with boiling distilled and deionized water (50 mL) for 1 h and the extract solution was evaporated to a concentration equivalent to 0·5 g herb mL^{-1}.

Chemical reagents

Ciprofloxacin hydrochloride was kindly provided as a gift by Bayer (Leverkusen, Germany). Enoxacin, the internal standard (IS) employed for quantitation of ciprofloxacin, was purchased from Sigma (St Louis, MO). Acetonitrile (HPLC grade, Mallinckrodt–Baker, USA), triethylamine (Riedel-de Haën, Germany) and other chemical reagents were acquired commercially.

Measurement of metal cation content of fennel extract

Fennel powder was digested with 9 : 1 HNO_3–$HClO_4$. A plant-free acid control was also prepared for comparison. The Zn, Fe, Cu, Ca, Mn, Mg, Sr, Cr, Pb and Ni content of the digest was determined by means of a Shimadzu ICPQ-1012 inductive plasma emission spectrometer.

Identification of major chemical ingredients in fennel

The aqueous fennel extract was partitioned with chloroform and the resulting chloroform extract was concentrated and analysed by GC–MS with a Finnigan (USA) GCQ instrument. The remaining water fraction was freeze–dried and reconstituted in 70% methanol for analysis by thin-layer chromatography (TLC).

GC–MS separation and identification of the organic constituents of fennel in the chloroform extract was achieved by means of a 30 m × 0·25 mm (i.d.) capillary column coated with a 0·25-μm film of PTE-5 QTM (Supelco, USA). The injection volume was 3 μL and the injector temperature was 280°C. The column temperature was maintained at 60°C for 1·5 min after injection and then programmed at 8° min^{-1} to 300°C which was maintained for another 12·5 min. The temperature of the GC–MS interface was 300°C. Mass spectra were generated by both electron impact ionization at an electron energy of 70 eV and by chemical ionization. Spectra were acquired between 35 and 500 atomic mass units at 2·2 scans s^{-1}.

The ingredients of water fraction were examined by TLC on silica gel 60 F_{254} plates (Merck) and identified by comparison with standards. The sample was separated with the solvent systems toluene–ethyl acetate, 97 : 3 (for non-polar components), chloroform–methanol, 8 : 2 (for components of medium polarity) and chloroform–methanol–water, 6 : 4 : 0·5 (for polar components). TLC plates were subsequently treated with Dragendorff reagent, vanillin–sulphuric acid reagent, or concentrated ammonia solution or aluminium chloride reagent for the detection of alkaloids, terpenoids and saccharides, and flavonoids and coumarins, respectively.

Pharmacokinetic studies

Male Sprague–Dawley rats, 210–230 g, were housed under controlled conditions (23–25°C, 55% relative humidity, 12 h light–dark cycle) and were allowed free access to food and water before experiments. The study animals were fasted overnight before the oral administration of ciprofloxacin and were cannulated through the right jugular vein one day before blood sampling. In the test group (n = 5), rats were dosed orally with 1 mL aqueous fennel extract (equivalent to 2 g herb kg^{-1}) immediately followed by a single oral dose of ciprofloxacin (0·5 mL, 20 mg kg^{-1}). Control rats (n = 5) received ciprofloxacin (0·5 mL, 20 mg kg^{-1}) orally after pretreatment with deionized water (1 mL). Blood (0·3 mL) was withdrawn via the cannula immediately before dosing with ciprofloxacin (t = 0) and then 5, 30, 60, 90, 120, 150, 180, 240,

300 and 360 min after dosing. Plasma was immediately separated by centrifugation at 10 000 *g* for 5 min. Urine samples were collected between 0 and 2 h, between 2 and 4 h, between 4 and 6 h and between 6 and 24 h, the total volume within each interval was recorded. Plasma and urine samples were stored at −80°C until assayed (license for approval of the pharmacokinetic study: 98/024/ERG).

Chromatographic assay of ciprofloxacin

High-performance liquid chromatography (HPLC) was performed with a Hewlett–Packard 1050 instrument, including UV-detector and autosampler. Compounds were separated on a 250 mm × 4·6 mm i.d., 5 μm particle, Phenomenex reversed-phase ODS column protected by a guard column (Novapak C_{18}, Waters). The mobile phase was 16% acetonitrile, 1% methanol and 83% aqueous buffer (pH 3·0), which comprised sodium dihydrogen phosphate monohydrate (0·1 M), glacial acetic acid (1% v/v) and triethylamine (0·5% v/v). The flow rate was 1·1 mL min^{-1} and detection was performed at 278 nm. The assay used was that developed by Nix et al (1985), with minor modifications. For quantitation of ciprofloxacin in plasma, acetonitrile (200 μL) was added to the plasma sample (0·3 mL), to precipitate protein, followed by the addition of enoxacin (internal standard, final concentration 1·0 ng μL^{-1}). The mixture was centrifuged at 10 000 *g* for 5 min and 200 μL of the supernatant was transferred to a microcentrifuge tube and concentrated by means of a Concentrivap Concentrator (Labconco, USA) at 35°C. The residue was reconstituted in mobile phase (70 μL) and 50 μL of the sample was injected. For urine analysis samples were diluted with deionized distilled water containing 10 ng mL^{-1} internal standard. The dilution factor was 1 : 50 for samples collected over the 0–2, 2–4 and 4–6-h intervals and 1 : 10 for the sample collected over the 6–24-h interval. The diluted samples were centrifuged at 10 000 *g* for 5 min and the supernatant (50 μL) was injected.

Ciprofloxacin and enoxacin were separated completely; their retention times were 9·0 and 7·0 min, respectively. The calibration curves were linear over the ranges 0 to 3 μg mL^{-1} and 1·0 to 10 μg mL^{-1} for plasma and urine assays, respectively, with correlation coefficients $> 0{\cdot}999$ for both matrices. The detection limit for ciprofloxacin in both assays was 25 ng mL^{-1}. The adequacy of this analytical methodology was supported by the 9·3% coefficient of variation for the inter-day assay variability. Sample stability was found to be > 4 months without significant degradation under the specified storage condition.

Analysis of the pharmacokinetic parameters of ciprofloxacin

Plasma concentration–time data for ciprofloxacin were obtained by non-compartmental analysis. The maximum plasma concentration (C_{max}) and the time corresponding to this (T_{max}) were directly observed from data obtained from individual animals. Least-square regression analysis was employed on the terminal elimination phase to estimate the elimination rate constant (λ_z). The elimination half-life ($t_{½,\lambda_z}$) of ciprofloxacin was computed as $0{\cdot}693/\lambda_z$ and the area under the curve from time zero to infinity ($AUC_{0\to\infty}$) was estimated by trapezoidal integration as:

$$AUC_{0\to\infty} = AUC_{0\to t} + C_t/\lambda_z \qquad (1)$$

where $AUC_{0\to t}$ is the AUC from time zero to time t and C_t is the plasma ciprofloxacin concentration at time t. Other parameters including total clearance (CL/F), renal clearance (CL_r) and apparent volume of distribution ($V_{d,\lambda_z}/F$) were estimated by standard procedures. The relative bioavailability of ciprofloxacin was estimated as the ratio of the mean $AUC_{0\to\infty}$ value for animals receiving both ciprofloxacin and fennel to that for animals receiving ciprofloxacin alone. The statistical significance of differences between the derived pharmacokinetic parameter estimates for the groups was assessed by means of the unpaired Student *t*-test with the level of statistical significance (*P*) set at 0·05.

Results

The chemical components of fennel

GC–MS analysis of the fennel extract indicated the presence of anethole (70%), α-fenchone (10%), anisalcohol (7%), anisaldehyde (4%), estragole (3%) and minor amounts of anisalcohol acetate, 1-methyl-4-(1-methylethyl)benzene, and 4,8-dienementha, etc. The chemical structures of major components of the fennel extract are shown in Figure 1. As with the TLC examination, flavonoids and coumarins including quercetin, quercetin glycosides, kaemaptin, imperatorin, dergapten and xanthotoxol were also detected in the fennel extract.

The determination of the metal cation content of fennel revealed that the amounts of Ca, Mg, Fe, Sr, Zn, Mn, Cr, Cu, Ni and Pb present were 6754, 6092, 260, 51, 49, 32, 14, 11, 3·9 and 1·0 μg g^{-1}, respectively.

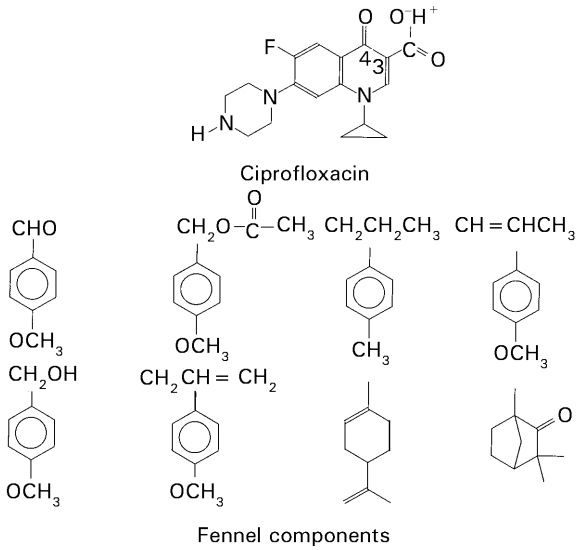

Figure 1. The chemical structures of ciprofloxacin and the major components of the fennel extract.

Pharmacokinetics of ciprofloxacin in the rat

After oral administration of ciprofloxacin ($20\,mg\,kg^{-1}$) to control rats, the drug was rapidly absorbed in the gastrointestinal tract and the maximum plasma concentration (C_{max}) was $1{\cdot}31 \pm 0{\cdot}49\,\mu g\,mL^{-1}$ at time (T_{max}) $0{\cdot}42 \pm 0{\cdot}17$ h. The apparent volume of distribution ($V_{d,\lambda_z}/F$) of ciprofloxacin was $30{\cdot}8 \pm 11{\cdot}1\,L\,kg^{-1}$, approximately 50 times the total body-water content, indicating significant tissue penetration and uptake. The drug was eliminated from the systemic circulation with a terminal elimination half-life ($t_{½\lambda_z}$) of $1{\cdot}96 \pm 0{\cdot}43$ h. Estimates of respective total clearance (CL/F) and renal clearance (CL_r) were $10{\cdot}8 \pm 2{\cdot}71\,L\,h^{-1}\,kg^{-1}$ and $2{\cdot}36 \pm 0{\cdot}45\,L\,h^{-1}kg^{-1}$, suggesting the domination of non-renal clearance in the elimination process. Similarly, urinary recovery of ciprofloxacin over a 24-h period accounted for 22·9% of the dose administered. All pertinent pharmacokinetic parameter estimates of ciprofloxacin for the control animals are given in Table 1.

Pharmacokinetic alternations of ciprofloxacin with co-administration of fennel

The mean plasma concentration–time profiles of ciprofloxacin for control rats and those with concomitant oral administration of fennel are shown in Figure 2. Significant changes in the pharmacokinetic estimates of ciprofloxacin were observed when fennel was administered concurrently (Table 1). In particular, the C_{max} of ciprofloxacin ($0{\cdot}23 \pm 0{\cdot}07\,\mu g\,mL^{-1}$) decreased by 83% ($P < 0{\cdot}01$) with T_{max} remaining similar. In the presence of fennel the relative bioavailability of ciprofloxacin was estimated to be 0·52 because $AUC_{0\to\infty}$ was significantly ($P < 0{\cdot}01$) reduced from $1{\cdot}97 \pm 0{\cdot}51$ to $1{\cdot}02 \pm 0{\cdot}21\,\mu g\,h\,mL^{-1}$. A 4·7-fold increase of $V_{d,\lambda_z}/F$ for ciprofloxacin was also detected and $t_{½\lambda_z}$ was significantly prolonged 2·7-fold after concurrent dosing of fennel. In concert with the

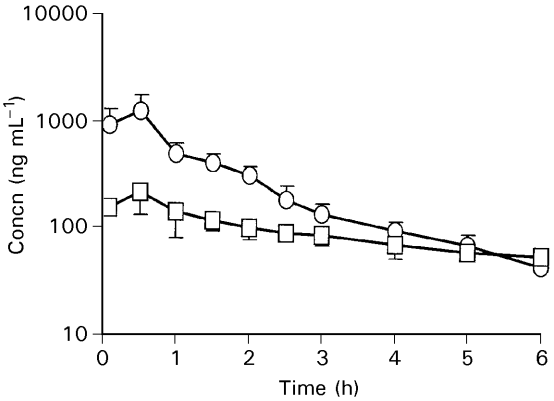

Figure 2. Mean ± s.d. plasma concentration–time profiles of ciprofloxacin after a single $20\,mg\,kg^{-1}$ oral dose with (□) or without (○) concomitant administration of fennel extract ($2\,g\,herb\,kg^{-1}$). Each treatment group comprised five animals.

Table 1. Pharmacokinetic parameters of ciprofloxacin in rats after oral administration ($20\,mg\,kg^{-1}$) with or without concomitant oral administration of aqueous fennel extract ($2\,g\,herb\,kg^{-1}$).

Parameter	Ciprofloxacin (control)	Ciprofloxacin + fennel
Maximum concentration (C_{max}; $mg\,L^{-1}$)	$1{\cdot}31 \pm 0{\cdot}49$	$0{\cdot}23 \pm 0{\cdot}07$**
Time to maximum concentration (T_{max}; h)	$0{\cdot}42 \pm 0{\cdot}17$	$0{\cdot}42 \pm 0{\cdot}17$
Volume of distribution ($V_{d,\lambda_z}/F$; $L\,kg^{-1}$)	$30{\cdot}8 \pm 11{\cdot}1$	$143{\cdot}78 \pm 31{\cdot}6$**
Elimination half-life ($t_{½\lambda_z}$; h)	$1{\cdot}96 \pm 0{\cdot}43$	$5{\cdot}23 \pm 2{\cdot}04$**
Elimination rate constant (λ_z; h^{-1})	$0{\cdot}37 \pm 0{\cdot}07$	$0{\cdot}15 \pm 0{\cdot}05$**
Total clearance (CL/F; $L\,h^{-1}\,kg^{-1}$)	$10{\cdot}8 \pm 2{\cdot}71$	$20{\cdot}39 \pm 4{\cdot}21$**
$AUC_{0\to\infty}$ ($mg\,L^{-1}$ h)	$1{\cdot}97 \pm 0{\cdot}51$	$1{\cdot}02 \pm 0{\cdot}21$**
Urinary recovery (mg)	$1{\cdot}02 \pm 0{\cdot}25$	$0{\cdot}58 \pm 0{\cdot}09$**
Renal clearance (CL_r; $L\,h^{-1}\,kg^{-1}$)	$2{\cdot}36 \pm 0{\cdot}45$	$2{\cdot}59 \pm 0{\cdot}17$

$AUC_{0\to\infty}$ is the area under the plasma concentration–time curve from time zero to infinity. Values are means ± s.d. (n = 5). **$P < 0{\cdot}01$ compared with control.

decrease in $AUC_{0\rightarrow\infty}$, urinary recovery was reduced by 44%. Despite this CL_r did not change significantly; CL/F was twice as high as the control value (Table 1).

Discussion

The dramatic increase in the consumption of herbal remedies world-wide raises the possibility of drug–drug interactions between conventional medications and natural products. Indeed, current data suggest that co-administration of fennel with ciprofloxacin significantly reduces plasma concentrations of the antibiotic (Figure 2). The most prominent pharmacokinetic perturbation was the impairment of absorption of ciprofloxacin after oral administration, as evidenced by the 83% reduction in C_{max}, 48% reduction in $AUC_{0\rightarrow\infty}$ and 43% reduction in urinary recovery. The rate of absorption of ciprofloxacin was not, apparently, affected, because T_{max} remained unchanged after co-administration of fennel.

Among other possibilities, the lower absorption of ciprofloxacin after oral administration might result from precipitation, from chelation or from chemical reaction with fennel that can lead to poor drug permeation through the gut wall or change of drug structure. Nevertheless, it has been convincingly demonstrated that reduction, by metal cations, of the bioavailability of ciprofloxacin after oral administration is the result of metal chelate formation with the 3-carboxyl and 4-oxo groups of the ciprofloxacin molecule (Figure 1). A positively charged ligand of suitable molecular size is therefore essential for chelation with the quinolones (Frost et al 1992).

Analysis of the structural characteristics of the phenolic and terpene components present in the fennel extract indicates that these natural substances are neutral or slightly acidic. It is, therefore, unlikely that these plant ingredients are sufficiently reactive to cause the pharmacokinetic changes observed for ciprofloxacin. By the principle of exclusion, the metal cations contained in the herb are the remaining probable cause of the behaviour observed.

The total content of ten metal ions in fennel was $13\,mg\,g^{-1}$, with Ca, Mg, Fe, Zn, Mn and Cu accounting for 99·5% of the total. Because the respective doses of cations and ciprofloxacin administered to the rats were 26 and $20\,mg\,kg^{-1}$, and because each metal cation can potentially complex with as many as three fluoroquinolone molecules (Kuhlmann et al 1998), the presence of these cations should be sufficient to trigger the negative effect on ciprofloxacin absorption. In addition, due to the increased molecular size formation of the metal–ciprofloxacin chelates may reduce drug permeability through the gut wall.

For that portion of the antibiotic entering the systemic circulation after escaping interaction in the gastrointestinal tract, drug elimination would then be subject to the excretory processes of the liver and kidneys. As shown in Table 1, concomitant dosing with the fennel extract caused a twofold increase in CL/F for ciprofloxacin. Because of the identical renal clearance in both groups, such an increase in CL/F must be related to an increase in hepatic clearance or a reduction in F, or both. Interestingly, the magnitude of the increase in CL/F mirrored that of the decrease in urinary drug recovery, indicating a decrease in F is the more feasible explanation. Reduced absorption after oral administration should, in principle, lead to changes in both renal and faecal excretion of the drug. Although not measured, higher faecal recovery of ciprofloxacin should be expected.

Besides the reduction in absorption, co-administration of fennel also caused a 4·7-fold increase in $V_{d,\lambda_z}/F$ and a 2·7-fold increase in $t_{½\lambda_z}$ of the antibiotic. These changes in distribution and elimination were, at least in part, induced by the same mechanism, i.e. formation of the ciprofloxacin cation complex. An increase in molecule size and hence lipophilicity should facilitate tissue uptake and reduce elimination by reducing the size of the drug pool available in the central compartment. Therefore, this factor affecting ciprofloxacin absorption might play a role in altering tissue distribution and elimination.

In conclusion, the drug–drug interaction observed between ciprofloxacin and fennel is well characterized by the reduction in absorption of the antibiotic after oral administration. This interaction also affected the overall pharmacokinetic profile of ciprofloxacin, by increasing its tissue distribution and impeding its terminal elimination. The findings of this study suggest that if ciprofloxacin must be employed concurrently with herbal remedies with a high mineral content, such as fennel, sufficient time should be allowed between the two to reduce the possibility of unwanted interaction.

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