Effect of Licorice Extract on the Pharmacokinetics of Ciprofloxacin in Rabbits after Oral Administration Using an Improved High-performance Liquid Chromatography Assay

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ABSTRACT

This study was undertaken to evaluate the significance of pharmacokinetic interaction between ciprofloxacin and licorice (*Glycerhyza glabra*). The study was designed as a comparative, randomized, two-period, two-treatment, two-sequence, single dose, crossover study in order to investigate the effect of licorice extract on ciprofloxacin in 12 rabbits (1.8-3.2kg). Rabbits were administered single oral doses of 40 mg/kg ciprofloxacin either with licorice extract or water. A simple and sensitive high performance liquid chromatography method for the detection and quantification of ciprofloxacin in rabbit plasma was developed specifically for this study. The resulting concentrations versus time curves were analyzed using non–compartmental pharmacokinetic analysis. Study results showed that licorice extract slightly reduced the rate and extent of ciprofloxacin absorption to around 80% [maximum plasma concentration (Cmax); from 1714 ng/ml to 1241 ng/ml, with a p-value of 0.25 and a 90% confidence interval (90% CI) 43.930–119.3 and the area under the plasma concentration time curve from zero to infinity (AUC∞); from 6964 ng.hr/ml to 5777 ng.hr/ml, with a p-value of 0.33 and a 90% CI 58.8–117.4 ng.hr/ml]. This interaction is speculated to be due to the interaction between the metals in the licorice extract and ciprofloxacin.

In conclusion, the non-statistically significant pharmacokinetic interaction between ciprofloxacin and licorice that was observed in this study is not expected to have significant clinical consequences.

Keywords: ciprofloxacin, HPLC, pharmacokinetics, rabbit.

INTRODUCTION

The fluoroquinolones are broad-spectrum antimicrobials with bactericidal activity against most gram-negative organisms. They also have good activity against gram-positive bacteria.[1-4] These antimicrobials are used clinically by oral and parenteral administration to treat infections.

Ciprofloxacin (figure 1) is a second-generation fluoroquinolone widely used and extensively studied in humans. The mechanism of action of ciprofloxacin is the inhibition of DNA gyrase, an enzyme that is critical to bacterial chromosome replication.[5]

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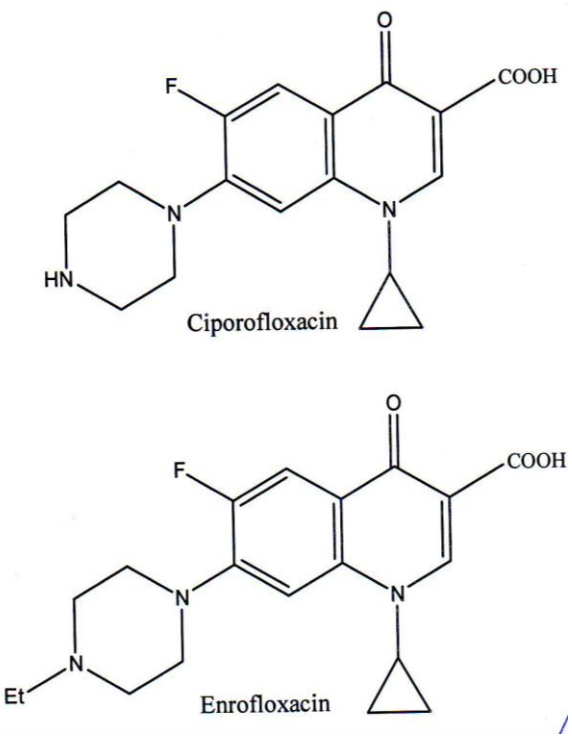


Figure 1: Chemical structure of ciprofloxacin and enrofloxacin

Licorice is used extensively as a demulcent, mild expectorant and anti-inflammatory agent. It is indicated for sore throats with hoarseness of voice and cough, peptic and duodenal ulcers, allergic conditions, rheumatoid arthritis, adrenal insufficiency and liver toxicity.[6]

The main constituents are glycyrrhizin, the potassium and calcium salts of glycyrrhitinic acid. Flavonoid rich fractions include liquirtin, isoliquertin, liquiritigenin and rhamnoliquirilin. Glycerhizine is a pentacyclic saponin compound comprised of a triterpenoid aglycone, which constitutes 10-25% of licorice root extract and is considered the primary active ingredient of water extract. [7]Ciprofloxacin is indicated to be used twice daily (immediate release products) or once-daily (long-acting products). This makes it an appropriate agent in fasting periods (Ramadan) where also licorice extract is extensively used in islamic countries as a drink that prevents thirst in Ramadan (the month of fasting).

The present study was undertaken to determine the effect of licorice extract on plasma concentration and on pharmacokinetics of ciprofloxacin after a single oral administration to 12 rabbits at a dose of 40 mg/kg. We have also utilized a simple and sensitive method for quantification of ciprofloxacin in rabbit plasma after protein precipitation with orthophosphoric acid and perchloric acid utilizing internal standardization quantitative analysis that uses enrofloxacin (figure 1) as an internal standard. This method has several advantages over other published ciprofloxacin assays (a shorter procedure with a lower limit of quantification of 6 ng/ml).

MATERIALS AND METHODS

Animals

Twelve rabbits (local strains), 6 males and 6 females weighing between 1.8 and 3.2 kg were included in the study. Animals had not been previously exposed to antibiotics. All the animals were subjected to clinical examination prior to the start of the experiment to rule

out the possibility of any disease. Animals were handled with care and consideration in line with the rules of the institution (Animal Housing, Faculty of Medicine, University of Jordan).

Drugs

Ciprofloxacin hydrochloride was provided by the Jordan Sweden Medical and Sterilization Company (JOSWE). The enrofloxacin base was provided by the Arab Veterinary Industrial Company (AVICO) and was employed as an internal standard in the high performance liquid chromatography (HPLC) assay. Crude licorice roots were purchased from the local market and were authenticated by macroscopic examination and microscopic identification at the pharmacognosy laboratory at the Faculty of Pharmacy, University of Jordan.

Experimental Design

Animals were individually weighed just before drug administration to establish administration of a precise dose. All rabbits fasted overnight before the first drug administration. A single oral dose of ciprofloxacin (40mg/kg of body weight) was administered in the form of a solution of ciprofloxacin hydrochloride (10 mg/ml) to the 12 rabbits, 6 males and 6 females, via a nasogastric tube. The tube was then washed with 20 ml of either licorice extract or water (control). About 1ml of blood was withdrawn from the rabbit's ear into a K3-EDTA tube. Blood samples were immediately centrifuged at 1500g for 10 minutes and were then stored at -20ºC until analysis. A 0.2 ml sample of plasma was used in the analysis. Sampling of rabbits was performed in the Animal Housing, Faculty of Medicine, University of Jordan. Blood samples were withdrawn at 0 (pre-dose), 5, 15, 25, 40, 60, 90, 120, 180, 240, 360, 480 and 570 minutes. The total volume of blood collected in the two periods from each animal was less than 15 % of the total blood volume.[8]

High-performance Liquid Chromatographic Assay

A simple and sensitive reversed-phase high-performance liquid chromatography was specifically developed and validated for the determination of ciprofloxacin in rabbit plasma. The HPLC system was a Shimadzu Class-VP System consisting of a SIL-10ADVP autosampler, a LC-10ADVP pump, a DGE-14A degasser, a SCL-10ADVP controller and a RF-10AXL fluorescence detector. Chromatographic separation was achieved in 22 minutes using a reversed phase C_{18} column (MetaChem polaris 5 µ C18-A 250 x 4.6mm) with a flow rate of 1.5 ml/minute. For fluorescence detection, the excitation wavelength was set at 280 nm and the emission wavelength was 442 nm. The pressure did not exceed 2500 psi. An isocratic elution procedure was used throughout. The mobile phase consisted of acetonitrile/phosphate buffer pH 3.0 (13.5:86.5). The buffer was prepared by dissolving 5.0 gm of sodium dihydrogen phosphate monohydrate into 865 ml double-distilled water, then the pH was adjusted to 3.0 using ortho-phosphoric acid 85%, and then 135 ml of HPLC grade acetonitrile were added. The solution was then filtered through a 0.45 µm membrane filter and sonicated in an ultrasonic bath for 5 minutes prior to use. The pH of the final solution was 3.4.

Sodium dihydrogen phosphate monohydrate was obtained from Merck (Germany). Water used in all preparations was bi-distilled, filtered and degassed prior to use. Acetonitrile (HPLC grade) was obtained from Scharlau (Spain). Perchloric acid (70%) was obtained from Riedel-de Haen (Germany). Ortho-phosphoric acid (GPR) was obtained from BDH chemicals (England). Blank plasma was obtained from rabbits.

Stock standard solutions were prepared through dissolving 20 mg of ciprofloxacin hydrochloride in 200 ml of bi-distilled water to produce a stock solution of 100 µg/ml concentration. Different sets of working standards at different concentrations were prepared by appropriate serial dilution of the stock solution. For internal standard stocks, 20 mg of the enrofloxacin base were dissolved into 200 ml of water and a few drops of concentrated phosphoric acid were added in order to improve the solubility. Further dilution was performed in order to achieve a stock solution of an internal standard with a concentration of 5 µg/ml.

Sample Preparation

Quality control (QC) and calibration curve samples were prepared by the addition of 50 µl of an internal standard stock to 500 µl of rabbit plasma spiked with ciprofloxacin concentrations ranging between 6 and 4000 ng/ml. The mixture was vortexed for 30 seconds. Protein precipitation was then achieved by introducing 100 µl of ortho-phosphoric acid (17%) and 25 µl perchloric acid (70%). The mixture was vortexed for 30 seconds and centrifuged at 1500g for 3 minutes at room temperature. A 20 µl of the supernatant was injected directly into the HPLC system.

Pharmacokinetic and Statistical Calculations

The drug concentration time data in plasma for each animal were analysed by non-compartmental techniques using WinNonlin® software (Cary, North Carolina, version 5.0). For peak concentration in plasma (Cmax) and time to peak concentration in plasma (tmax), observed values were taken. The apparent terminal rate constant, λ_z was determined by linear regression of the terminal linear phase of the logarithmic plasma concentration vs. time curve. The terminal half-life, $t_{1/2\lambda}$ was calculated as ln $2/\lambda_z$. The area under the plasma concentration–time curve (AUC) from time zero to time (t) at which the final measurable concentration was obtained (AUC_{0-t}) was calculated by the linear trapezoidal rule. The AUCt–∞ from the final time point to time infinity was estimated as the ratio of the final observed concentration/λ_z. The total area under the concentration–time curve ($AUC_{0-\infty}$) was calculated by addition of AUC_{0-t} and AUCt–∞.

Statistical comparisons of the estimated pharmacokinetic values were performed using the analysis of variance (ANOVA) with factors for treatment, period, sequence and for subjects nested within sequence.

The 90% confidence limits were calculated on the basis of the least-square means and the mean square error obtained from the ANOVA. ANOVA and 90 % confidence intervals were obtained using WinNonlin (Version 5.2.1).

The calculations for testing the interaction were carried out using ln-transformed values. An interaction was accepted as absent if the 90% confidence interval around the point estimates of the ratios of true means were entirely within the 80-125% interval. This is the US Food and Drug Administration (FDA) recommended approach for interaction testing which is based on the bioequivalence principle.

RESULTS

HPLC Method Validation

Selectivity, Precision, Accuracy and Recovery

To show selectivity, plasma blank samples were prepared without the addition of ciprofloxacin and enrofloxacin. Plasma blank chromatogram is shown in Figure 2-A. Five concentrations were used as quality-control samples. Each one of the QC samples was injected six times in one day to examine the day repeatability. Also each of these concentrations was injected over five successive days in order to check the between-day precision. The obtained results with their coefficients of variation (CV %) for the five concentrations are summarized in Table 1. The accuracy was expressed as a percent deviation of the observed concentration from the theoretical concentration. The obtained results for accuracy of three concentration levels are listed in Table 2.

The average recovery for both the analyte (ciprofloxacin) and the internal standard (enrofloxacin) was 88.1 ± 7.75%, and 93.8 ± 5.45%, respectively. Recovery was examined by comparing the detector response obtained from an amount of the analyte added to and extracted from the plasma and the detector response obtained for the true concentration.

Table 1: Intraday and interday coefficient of variation for measurement of five concentrations of ciprofloxacin

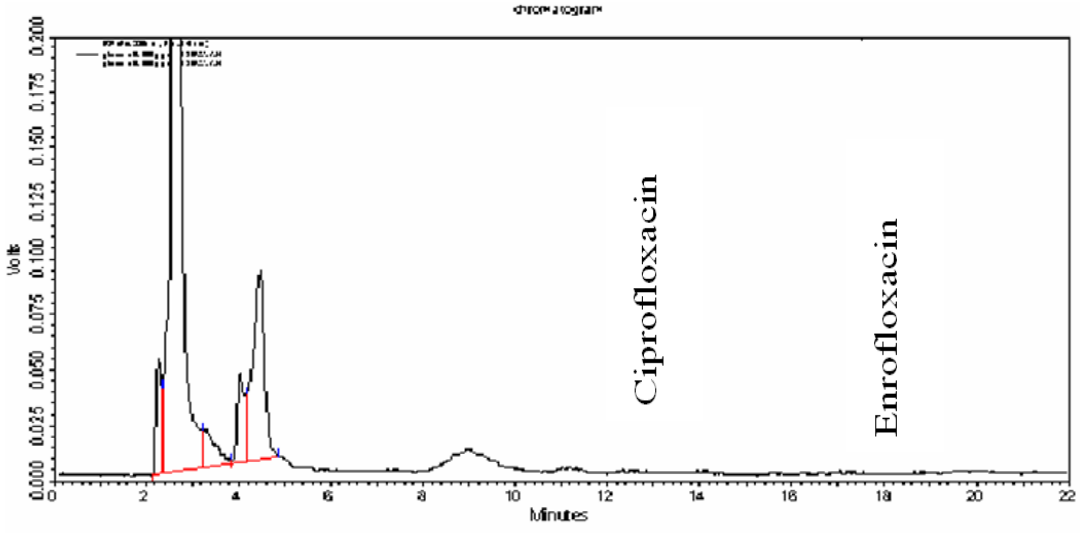
Ciprofloxacin concentration (ng/ml)	Within day CV %	Between-days CV %
10	4.23	4.84
400	0.87	0.89
1200	0.65	0.99
1600	0.93	1.32
4000	0.27	2.92

Table 2: Accuracy of ciprofloxacin at three concentration levels (low, intermediate and high)

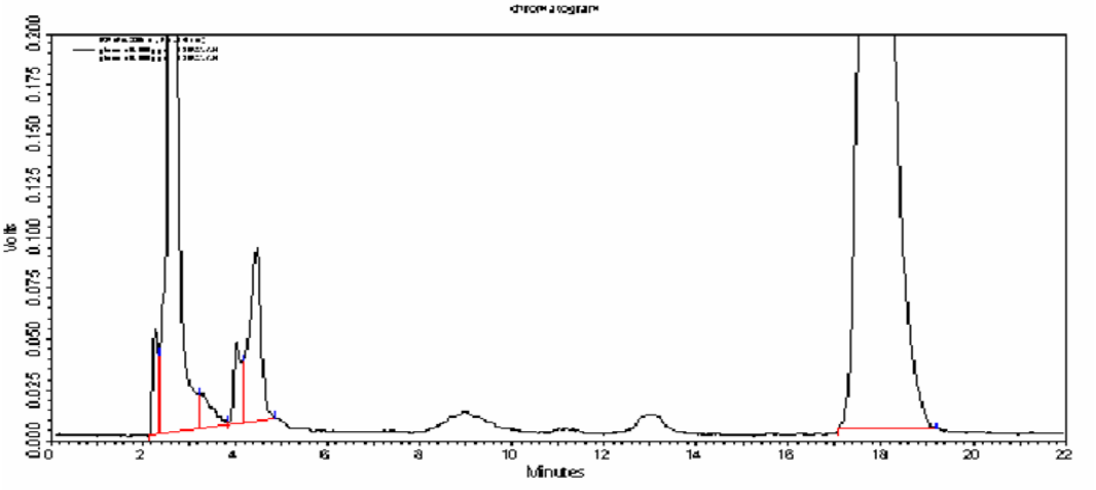
Concentration level	Accuracy %
Low concentration (24 ng/ml)	115.5
Medium concentration (400 ng/ml)	97.5
High concentration (4000 ng/ml)	97.3

Limits of Detection and Quantitation

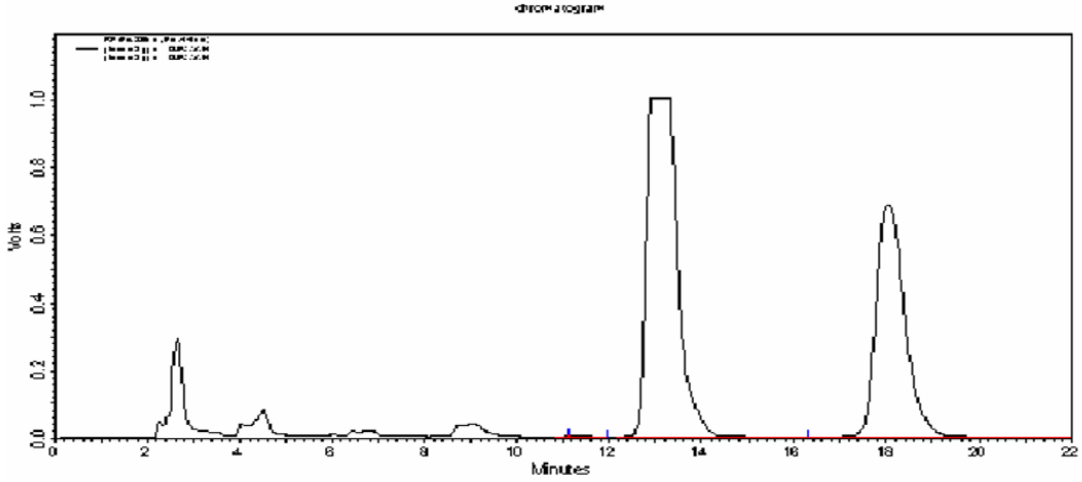
The limit of detection (LOD) was 2 ng/ml of ciprofloxacin, taken as the concentration that gives rise to a signal that is three times the noise of the method. While the limit of quantitation (LOQ) was 6 ng/ml taken as the concentration that produces a signal that is ten times the noise. Two representative chromatograms at The LOQ and at a higher concentration are shown in Figure 2-B and 2-C.



A) Blank Plasma



B) 6 ng/ml



C

Figure 2: Representative chromatograms. A; Blank plasma, B; 6 ng/ml C; 1500 ng/ml ciprofloxacin and enrofloxacin

Linearity

Calibration curves were constructed by plotting the area ratio of ciprofloxacin and enrofloxacin versus the ciprofloxacin concentration. A linear relationship between the area ratio and the concentration of ciprofloxacin was obtained over the examined concentration range 6-4000 ng/ml. Four calibration curves were constructed in four different days, each of them had good linearity within this range which was verified using least-squares linear regression (correlation coefficient r). The results showed that each calibration curve had an R^2 value of at least 0.999. Multiple linear regression analysis showed no significant differences in either the slope or the intercept. Accordingly, the average of the four calibration curves was used in calculating the plasma concentration of ciprofloxacin in the samples collected. The average regression equation was $y = 1.037x + 0.0084$ and R^2 was found to be 0.9998 ($r = 0.9999$). The average calibration curve is presented in Figure 3.

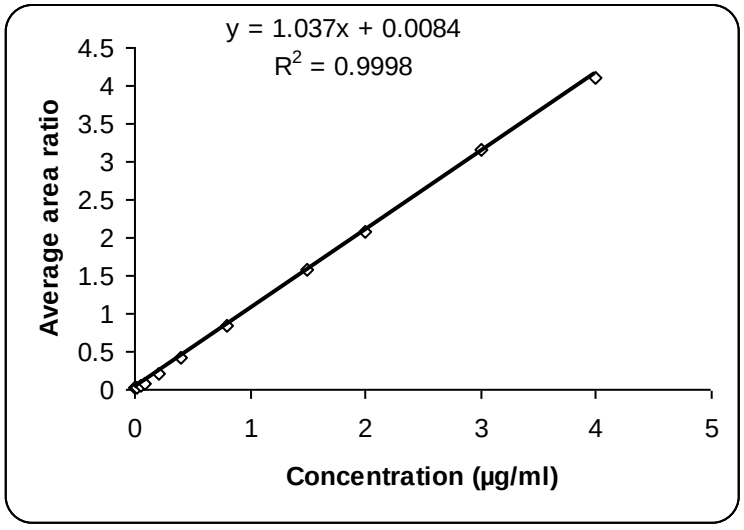


Figure 3: Average calibration curve of ciprofloxacin concentration

Pharmacokinetic and Statistical Results

All 12 rabbits completed the study. The mean plasma concentration-time curves of ciprofloxacin after oral administration of 40 mg/kg oral solution to 12 rabbits either with or without licorice are shown in Figure 4. The pharmacokinetic parameters are listed in Table 3 along with the statistical significance values. The 90% confidence intervals for Cmax, AUCt and AUC∞ after log-transformation are shown in Table 4.

Table 3: Pharmacokinetic parameters after the two treatments

	Treatment		**Significance**
	Licorice	**Water**	**P-value**
Half-life (hr)	2.56±1.85º	2.87± 1.75º	0.69ε
Tmax (hr)	0.50	1.00	0.55•
Cmax (ng/ml)	1240.6687(79.9)*	1713.74(53.85)*	0.25ε
AUCt(ng,hr/ml)	4862.70(62.8)*	5864.82 (25.8)*	0.36ε
AUC∞(ng,hr/ml)	5777.33(55.91)*	6964.15(27.5)*	0.33ε

º: Harmonic mean ± SD

*: Geometric mean (% CV)

ε: Paired two sample t-test after logarithmic transformation

•: Wilcoxon signed rank test

Table 4: 90% confidence intervals for the equality of the two means (log transformed)

Parameter	Ratio * (licorice/water)	Lower limit of the 90 % confidence interval	Upper limit of the 90 % confidence interval
Cmax	72.40 %	43.93	119.30
AUCall	82.91 %	57.17	120.25
AUCINF	82.96 %	58.63	117.39

* The point estimate of the ratio of μ licorice period / μ water period

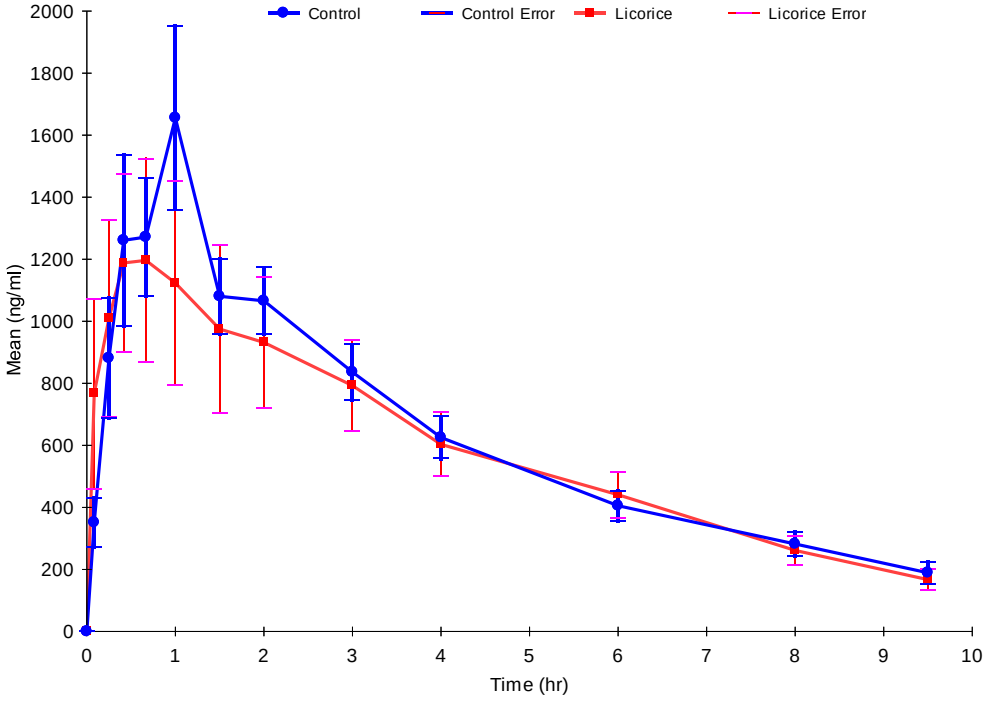


Figure 4: Mean rabbit plasma concentrations (12 rabbits)

DISCUSSION

The HPLC assay was specifically developed and validated for use in the present study to allow the accurate detection of ciprofloxacin. This method has several advantages over other published ciprofloxacin assays.[9-11] First, the presented method avoids multiple time-consuming extraction steps used in previous studies by utilizing the protein precipitation of the plasma samples. Second, the method was developed to allow for the quantitation of very low ciprofloxacin concentrations from rabbit plasma that was necessary for pharmacokinetic analysis.

The results of the present study provided sufficient evidence that the developed HPLC method is highly sensitive, accurate and reproducible for the determination of ciprofloxacin in rabbit plasma.

In the present study, ciprofloxacin was found to be rapidly and well absorbed in rabbits after oral administration. Pharmacokinetic parameters of ciprofloxacin in this study are in agreement with previous studies.[12] Statistical analysis using paired t-tests showed no significant differences for Cmax, AUC, and the half-life between the two treatments as shown in Table 3.

Furthermore, the results of the statistical analyses using the standard two period, two sequence, crossover design indicate that licorice did not reduce the bioavailability of ciprofloxacin. It should be noted that all the 90% confidence intervals of the pharmacokinetic parameters included unity supports that the difference between the two treatments is not statistically significant. Furthermore, the point estimates were within the limits of the bioequivalence acceptance zone (80-125%). As a function of the sample size and the variability, the confidence intervals were quite broad.

There are few previous studies that investigated the interaction between ciprofloxacin and other medicinal plants including Singuisorba [13] and Taraxacum in rats [11]. In those studies, both plants were found to reduce bioavailability of ciprofloxacin due to their high content of metals. Lin *et al* investigated the effect of licorice on pharmacokinetics of methotrexate in rabbits, and they found that both the AUC and the mean residence time were increased.[14] In a study investigating the effect of licorice on exposure to verapamil [15], the researchers observed a significant decrease in the AUC and Cmax of verapamil. In another study, licorice increased the elimination of lidocaine in rats.[16]

However, in this study there was no significant effect of licorice on ciprofloxacin pharmacokinetics, which might be due to its two contradictory effects: the content of both saponins and metals. The saponins in licorice are known to have surface activity and can increase the solubility of several drugs. However, ciprofloxacin is also affected by metals where its solubility is reduced. It is suggested that these two contradictory effects are responsible for the results of this study.

In conclusion, ciprofloxacin is rapidly absorbed and therapeutically effective concentrations of ciprofloxacin are achieved in the plasma of rabbits after a single oral administration at a dosage of 40 $mg.kg^{-1}$. The developed HPLC method is valid and accurate and can be used for pharmacokinetic analysis. The non-statistically significant pharmacokinetic interaction between ciprofloxacin and licorice that was observed in this study is not expected to have significant clinical consequences.

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REFERENCES

(1) Wise R., Andrews J. M. and Edwards L.J. In vitro activity of Bay 09867, a new quinoline derivative, compared with those of other antimicrobial agents. Antimicrob Agents Chemother. 1983, 23, 559–564.

(2) Roy C., Foz A., Segusa C., Tirado M. and Tesvel D. Activity of ciprofloxacin (BAYo 9867) against Pseudomonas aeruginosa and ampicillin-resistant Enterobacteriaceae. Infection. 1983, 11, 326–328.

(3) Chin N. X. and Neu H.C. Ciprofloxacin, a quinolone carboxylic acid compound active against aerobic and anaerobic bacteria. Antimicrob Agents Chemother. 1984, 25, 319–326.

(4) Smith M. J., Hodson M. E. and Batten J.C. Ciprofloxacin in cystic fibrosis, Lancet. 1986, 10, 1103.

(5) LeBel M.Ciprofloxacin: chemistry, mechanism of action, resistance, antimicrobial spectrum, pharmacokinetics, clinical trials, and adverse events. Pharmacotherapy, 1988, 8, 3-33.

(6) Isbrucker R. A. and Burdock G.A. Risk and safety assessment on the consumption of Licorice root (Glycyrrhiza sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. Regul Toxicol Pharmacol. 2006, 46,167-92.

(7) Prescott J. and Yielding K. H. In vitro susceptibility of selected veterinary bacterial pathogens to ciprofloxacin, enrofloxacin and norfloxacin. Can J Vet Res. 54, 1990, 195-197.

(8) Diehl K., Hull R., Morton D., Pfister F., Rabemampianina Y., Smith D., Jean-Marc Vidal J. and Vorstenbosch C. A Good practice guide to the administration of substances and removal of blood, including routes and volumes. *J. Appl. Toxicol.* 2001, 21, 15–23.

(9) Scheer M. Studies on the antimicrobial activity of Baytril. *Veterinary Medicine Reviews*, 1987, 2, 90–9.

(10) Manceau J., Gicquel M., Laurentie M. and Sanders P. Simultaneous determination of enrofloxacin and ciprofloxacin in animal biological fluids by high-performance liquid chromatography. Application in pharmacokinetic studies in pig and rabbit. Journal of Chromatography B., 1999, 726, 175-184.

(11) Zhu M., Wong P.Y. and Li R.C. Effects of taraxacum mongolicum on the bioavailability and disposition of ciprofloxacin in rats. J Pharm Sci. 1999, 88, 632-634.

(12) Hanan M.S., Riad E.M. and el-Khouly N.A. Antibacterial efficacy and pharmacokinetic studies of ciprofloxacin on Pasteurella multocida infected rabbits. *Dtsch Tierarztl Wochenschr*. 2000, 107, 151-5.

(13) Zhu M., Wong P.Y. and Li R.C. Influence of Sanguisorba officinalis, a mineral-rich plant drug, on the pharmacokinetics of ciprofloxacin in the rat. J Antimicrob Chemother. 1999, 44, 125-8.

(14) Lin S.P., Tsai S.Y., Hou Y.C. and Chao P. D. Glycyrrhizin and licorice significantly affect the pharmacokinetics of methotrexate in rats. J Agric Food Chem. 2009, 57, 1854-9.

(15) Al-Deeb I. D., Arafat T.A. and Irshaid Y.M. The effect of licorice drink on the systemic exposure of verapamil in rabbits. Drug Metab Lett. 2010, 4, 173-9.

(16) Tang J., Song X., Zhu M. and Zhang J. Study on the pharmacokinetics drug-drug interaction potential of Glycyrrhiza uralensis, a traditional Chinese medicine, with lidocaine in rats. Phytother Res. 2009, 23, 603-7.

دراسة تأثير مستخلص عرق السوس على حرائك السيبروفلوكساسين

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ملخص

تم إجراء هذه الدراسة لتقييم تأثير مستخلص عرق السوس على حرائك السيبروفلوكساسين، وتم تصميم هذه الدراسة المقارنة بشكل عشوائي على فترتين، ولمعالجتين وتسلسلين بجرعة واحدة وبتبادل متعاكس من أجل دراسة تأثير مستخلص عرق السوس على السيبروفلوكساسين في اثني عشر ارنبا (1.8–3.2 كغم). وتم إعطاء جرعة فموية واحدة تبلغ 40 ملغم/ كغم مرة مع الماء، ومرة مع مستخلص عرق السوس لكل واحد من الأرانب.
تم تحليل تراكيز السيبروفلوكساسين في بلازما الدم عن طريق جهاز التشريب السائل عالي الإنجاز خصيصا لهذه الدراسة.
اظهرت نتائج الدراسة أن مستخلص عرق السوس قد قلل سرعة امتصاص السيبروفلوكساسين ومقداره إلى ما يقرب من 80% على النحو الآتي: تركيز القمة من (1714 ng/ml) إلى (1241 ng/m) وكانت قيمة مستوى المعنوية-p) value=0.25) والمساحة تحت المنحنى من (6964ng.hr/ml) الى (5777 ng.hr/ml) وكانت قيمة مستوى المعنوية (p-value=0.33).
ويعتقد بأن هذا التداخل ناتج عن التفاعل ما بين المعادن الموجودة في مستخلص عرق السوس والسيروفلوكساسين.
وفي الختام، لا يتوقع أن يكون التداخل بين السيبروفلوكساسين ومستخلص عرق السوس ذا تأثير على اسخدام السيبروفلوكساسين والمستخلص في نفس الوقت.

الكلمات الدالة: مستخلص عرق السوس، حرائك السيبروفلوكساسين.

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