EFFECT OF CEPHALEXIN ON THE PHARMACOKINETICS OF METFORMIN IN HEALTHY HUMAN VOLUNTEERS

G. Jayasagar, M. Krishna Kumar, K. Chandrasekhar, C. Madhusudan Rao and Y. Madhusudan Rao*

University College of Pharmaceutical Sciences, Kakatiya University, Warangal - 506 009 (A.P.), India

SUMMARY

The purpose of this study was to assess the effect of a single dose of cephalexin on the pharmacokinetics of metformin in healthy human volunteers. A 2 x 2 double blind randomized crossover study was conducted in 12 healthy human volunteers. Each subject received orally either 500 mg of metformin with a placebo or a combination of 500 mg of metformin and 500 mg of cephalexin. Serum and urine levels of metformin were estimated by a validated HPLC method. The systemic disposition of metformin was altered by the co-administration of cephalexin. Cephalexin increased C_{max} and AUC by an average of 34% and 24%, respectively, and reduced renal clearance to 14%. The renal clearance of metformin was reduced in a time-dependent manner in the presence of cephalexin. Hence, it is concluded that cephalexin inhibits the renal tubular secretion of metformin resulting in higher circulating serum concentrations.

KEY WORDS

metformin, cephalexin, drug interaction, pharmacokinetics, renal clearance

* Author for correspondence
Professor Y. Madhusudan Rao
University College of Pharmaceutical Sciences
Kakatiya University
Warangal - 506 009 (A.P.), India
e-mail: ymrao123@yahoo.com

INTRODUCTION

Metformin hydrochloride has been successfully used for many years in the treatment of type 2 diabetes mellitus. Absolute bioavailability of a dose of 500 mg metformin is approximately 50% and the bioavailability decreases as the dose increases /1/. The bioavailability of metformin decreases in the presence of food with 20% lower area under the serum concentration-time curve (AUC) and 35% lower peak serum concentrations (C_{max}) /2/. After intravenous administration, metformin undergoes near complete excretion in urine and probably does not undergo metabolism in humans. Renal clearance is much higher than the glomerular filtration rate, indicating secretion by the proximal tubules /1/. Metformin has been indicated to produce adverse effects, especially lactic acidosis, which may be related to high circulating concentrations of the drug /3/.

Cephalexin is primarily indicated in respiratory tract infections, otitis, skin and skin structure infections. It is well absorbed from the gastro-intestinal tract, and absorption may be delayed by food, but the amount absorbed is not affected /4/. Cephalexin undergoes complete excretion in urine and it does not undergo metabolism /5,6/.

The purpose of this study was to determine whether cephalexin, a zwitterionic drug, excreted by both organic cationic and anionic transporters, alters the renal excretion of metformin, which is excreted by organic cationic transporters in the proximal tubules.

MATERIALS AND METHODS

Chemicals

Metformin hydrochloride 500 mg (Gluformin®; Nicholas Piramal India Ltd., Mahad, India), cephalexin 500 mg (Phexin®; Glaxo India Ltd., Mumbai, India), phenformin (generous gift from Wallace Pharmaceuticals Ltd., Goa, India), acetonitrile (Ranbaxy Chemicals Ltd., India); all other chemicals used were of AR grade.

Study design

The study was conducted with a double blind, randomized and crossover design. The treatments administered were 500 mg metformin with placebo and 500 mg metformin with 500 mg of

cephalexin. Serial serum and urine samples were collected up to 24 hours post dose.

Subjects

Twelve healthy male volunteers who met all eligibility requirements and successfully passed the exclusion criteria were admitted to the study after signing informed consent.

Protocol

After an overnight fast, volunteers received one of the two treatments mentioned above. No food or drink was permitted for 3 hours after drug administration. Collection of 3 ml blood samples was made at the following times after drug administration: 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours. Blood was allowed to clot, centrifuged and serum was separated and stored at -20°C until analysis. Serial urine samples were collected at 4, 8, 12 and 24 hours after drug administration. There was a 7-day washout period between consecutive treatments. The clinical protocol for the study was approved by the institutional ethics committee.

Sample assays

Serum samples were analyzed for metformin by HPLC using the modified method reported by Huupponen *et al.* /7/. The serum samples (0.5 ml) were added to phenformin (20 µl; 100 µg/ml) as an internal standard. To this, 0.1 ml of 2 M sodium hydroxide solution was added to make it alkaline, so that the metformin would be in its non-ionized form, and 750 mg of sodium chloride was added to reduce the solubility of metformin in serum. The mixture was then vortexed for 1 min. Acetonitrile (6 ml) was then added and the mixture vortexed for 5 min. The obtained emulsion was centrifuged at 7,500 rpm for 15 min, the supernatant was separated and evaporated, and the residue reconstituted in 100 µl of mobile phase.

Urine samples (100 µl) were diluted 5 times with water, centrifuged, and the supernatant was injected onto the HPLC column.

Separation was achieved at room temperature on a Wakosil 5 PH (phenyl) column (5µ, 25 cm x 0.46 cm) using a mobile phase of 25% acetonitrile and 75% 0.01 M phosphate buffer; pH was adjusted to 7

with diethylamine. The fraction of interest was then filtered with a 0.45-μm Millipore filter and degassed by sonication.

The HPLC system (Shimadzu, Japan) consisted of an LC-10 AT solvent delivery module, SPD-10A UV-VIS spectrophotometric detector with LC10 software. Flow rate was 1.35 ml/min and the eluate was monitored at 236 nm. Sensitivity was set at 0.05 AUFS. The retention times of internal standard and metformin were 8.5 min and 6.2 min, respectively.

Treatment of bioavailability data

Each serum concentration-time profile was analyzed by non-compartmental methods /8/. C_{max} and T_{max} were determined as the highest observed concentrations and the time to reach the maximum concentrations, respectively. Elimination half life ($t_{½}$), area under the serum concentration curve (AUC), volume of distribution (Vd/f) and clearance (Cls/f) were calculated using a noncompartmental model. Total percentage urinary recovery (%UR) was calculated from urinary data.

Statistical analysis

The pharmacokinetic parameters obtained from the two treatments were compared using Student's paired t-test. Differences in the samples means were considered significant at $p < 0.05$.

RESULTS

Mean serum concentrations of metformin in the presence or absence of cephalexin are shown in Figure 1. The mean pharmacokinetic parameters of metformin when given alone and upon co-administration with cephalexin are shown in Table 1.

All subjects tolerated both treatments well, with no side effects. However, significant changes in AUC, C_{max} and clearance were found in the presence of cephalexin.

There was no difference in the time to achieve the maximum serum metformin concentration, but the concentration itself was increased by cephalexin by an average of 34%. There was no significant difference in elimination half life between the treatments. However, cephalexin

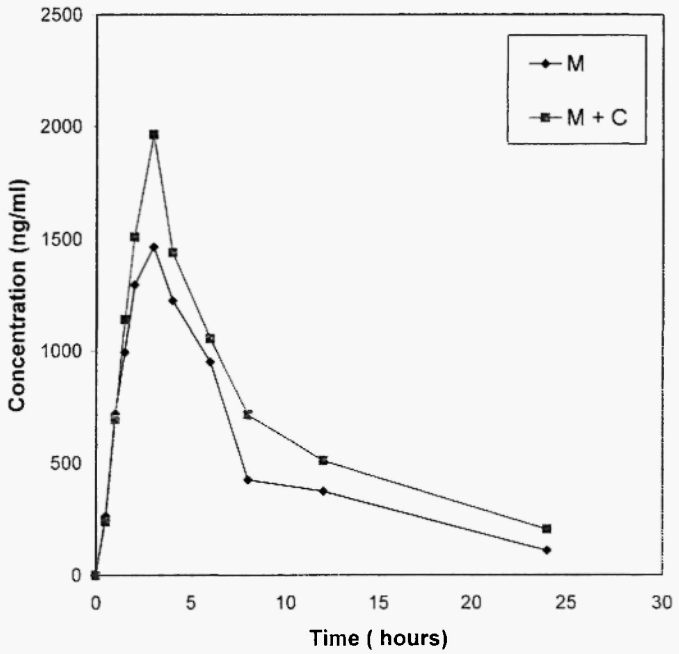

Fig. 1: Mean plasma metformin concentration vs time profiles after metformin alone (M) and metformin with cephalexin (M + C).

TABLE 1

Mean (SD) serum pharmacokinetic parameters of metformin alone and in combination with cephalexin (n = 12)

	Metformin	**Metformin + cephalexin**
C_{max} (ng/ml)	1465 (404)	1964 (498)*
T_{max} (h)	3.0 (2.0, 5.0)	3.0 (2.0, 5.0)
$AUC_{(0, \infty)}$ (ng-h/ml)	8645 (2344)	10712 (2953)*
$t_{½}$ (h)	2.82 (0.44)	3.24 (0.92)
CLr (ml/min)	433 (96)	374 (92) *
% UR	47.5 (7.36)	48.3 (10.55)

* $p < 0.05$.

TABLE 2

Effect of cephalexin on the renal disposition of metformin (n = 12)

Time (h)	**Metformin renal clearance** (ml/min)	
	Metformin	**Metformin + cephalexin**
0-4	558 ± 210	244 ± 120 *
4-8	692 ± 306	408 ± 160 *
8-12	494 ± 210	386 ± 184
12-24	264 ± 144	218 ± 94

* $p < 0.05$.

increased the AUC of metformin by an average of 24%. Metformin renal clearance was reduced significantly by an average of 14%. This effect was more prominent during the first 8 hours, as shown in Table 2.

DISCUSSION

From the present study, it appears that a 500 mg oral dose of cephalexin affects the pharmacokinetics of metformin. Cephalexin increased the serum concentrations of metformin in all subjects. This was not due to the increase in the absorption of metformin, as the total urinary recovery was same with both treatments. It is known that the excretion of metformin is via the organic cationic transporter system of the proximal tubules /1/. The amount of metformin recovered was ~50% which is consistent with earlier reports /9-11/. Cephalexin reduced the overall renal clearance of metformin by 57 ml/min (Table 1), indicating that the interaction involved renal tubular secretion. Cephalexin is a zwitterionic drug and is reported to be excreted by both the organic anionic transporter and cationic

transporter systems of the proximal tubules /12/. The mechanism of this interaction is considered to be competitive in nature by the organic cationic transport system of proximal tubular secretion.

The inhibitory effect of cephalexin on metformin renal clearance was time dependent and significant up to 8 hours after cephalexin administration (Table 2). This observation strengthens the hypothesis that the interaction is competitive in nature rather than non-competitive.

Previous reports /3/ suggested that high doses of metformin in patients with declining renal function were a risk factor for metformin-associated lactic acidosis. Some cationic drugs, such as cimetidine, digoxin, quinidine, vancomycin, procainamide, etc., also reduce the excretion of metformin through the organic cationic transporter system of the renal proximal tubules /13-15/.

We report here an interaction between metformin (a cationic drug) and cephalexin (a zwitterionic drug) which results in the elevation of serum concentrations of metformin by reducing tubular secretion. Hence, the co-administration of cephalexin with metformin is considered to be a risk factor, especially during chronic administration. It is recommended that patients who are prescribed these two drugs concomitantly should have serum metformin levels monitored or an alternative to cephalexin should be considered.

In conclusion, cephalexin reduces the renal clearance of metformin by inhibiting tubular secretion via the organic cationic system. This results in elevated blood metformin concentrations which in turn may lead to adverse effects upon repeated administration.

REFERENCES

1. Tucker GT, Casey F, Phillips PJ, Conner H, Ward JD. Metformin kinetics in healthy subjects and in patients with diabetes mellitus. Br J Clin Pharmacol 1981; 12: 235-246.
2. Scheen AJ. Clinical pharmacokinetics of metformin. Clin Pharmacokinet 1996; 30: 359-371.
3. Phillips PJ, Scicchitano R, Clarkson AR, Gilmore HR. Metformin associated lactic acidosis. Aust N Z J Med 1978; 8: 281-284.
4. Kastrup EK, Parker VJ, Hebel SK, Olin BR, Rivard R, Threlkeld DS, et al. Drug Facts and Comparisons: Anti-infectives, Cephalosporins. Missouri: Kluwer, 1998; 2209-2220.

5. Finkelstein E, Quintiliani R, Lee R, Bracci A, Nightingale CH. Pharmacokinetics of oral cephalosporins: cephradine and cephalexin. J Pharm Sci 1978; 67: 1447-1450.
6. Spyker DA, Thomas BL, Sande MA, Bolton WK. Pharmacokinetics of cefaclor and cephalexin: dosage nomograms for impaired renal function. Antimicrob Agents Chem Ther 1978; 14: 172-177.
7. Huupponen R, Ojala-Karisson P, Rouru J, Koulu M. Determination of metformin in plasma by high performance liquid chromatography. J Chromatogr 1992; 583: 270-273.
8. Gibaldi M, Perrier D. Pharmacokinetics, 2nd Ed. New York: Marcel Dekker, 1982.
9. Sirtori CR, Baldwin DS, Villarreal H. Disposition of metformin (*N,N*-dimethyl biguanide) in man. Clin Pharmacol Ther 1978; 24: 683-693.
10. Pentikainen PJ, Neuvonen PJ, Penttila A. Pharmacokinetics of metformin after intravenous and oral administration to man. Eur J Clin Pharmacol 1979; 16: 195-202.
11. Karttunen P, Uusitupa M, Lamminsivu U. The pharmacokinetics of metformin: a comparison of the properties of a rapid-release and a sustained-release preparation. Int J Clin Pharmacol Ther Toxicol 1983; 21: 31-36.
12. Van Crugten J, Bochner F, Keal J, Somogyi A. Selectivity of the cimetidine-induced alterations in the renal handling of organic substrates in humans. Studies with anionic, cationic and zwitterionic drugs. J Pharmacol Exp Ther 1986; 236: 481-487.
13. Somogyi A, Stockley C, Keal J, Rolan P, Bochner F. Reduction of metformin renal tubular secretion by cimetidine in man. Br J Clin Pharmacol 1985; 23: 545-551.
14. Kastrup EK, Parker VJ, Hebel SK, Olin BR, Rivard R, Threlkeld DS, et al. Drug Facts and Comparisons: Anti-diabetic Agents, Biguanides. Missouri: Kluwer, 1998; 620-627.
15. Somagyi, A, McLean A, Heinzow B. Cimetidine-procainamide pharmacokinetic interaction in man. Evidence of competition for tubular secretion of basic drugs. Eur J Clin Pharmacol 1983; 25: 339-345.