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Short Communication

Determination of metformin in plasma by high-performance liquid chromatography

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ABSTRACT

A high-performance liquid chromatographic method for the determination of metformin, an oral antidiabetic agent, in plasma is described. Plasma samples containing the internal standard, phenformin, are eluted through Amprep extraction columns before injection into the chromatographic column, packed with μBondapak phenyl. The eluent is monitored at 236 nm. At a mobile phase flow-rate of 1.35 ml/min, the retention times of metformin and phenformin are 2.8 and 5.6 min, respectively. The intra-day coefficients of variation are 1.5 and 4.3% at metformin concentrations of 0.05 and 1 mg/l, respectively.

INTRODUCTION

Metformin is an antidiabetic agent used in the treatment of type 2 diabetes mellitus. Owing to its weight-decreasing and serum lipid-normalizing effects, it has been especially recommended for obese patients [1]. The kidneys have an important role in the elimination of metformin; its renal clearance exceeds its creatinine clearance four-fold [2]. The administration of radioactive labelled metformin indicated that no metabolism of the drug occurs in either humans [3] or animals [4]. However, the fate of *ca.* 20% of orally administered metformin was not determined in a study using gas chromatography for drug detection [2]. The elimination half-life of intravenous metformin was found to be between 2 and 5 h [2,3]; on the basis of urinary data, longer elimination half-lives have been suggested [2]. For pharmacokinetic studies, a method that allows an accurate measurement of low concentrations of metformin in plasma is needed.

Besides gas chromatographic methods [4], high-performance liquid chromatography (HPLC) has been used for the quantitation of metformin in biological fluids [5,6].

This paper describes a rapid HPLC method, which enables the determination of metformin

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with great accuracy, even at low drug concentrations in plasma, without deproteinization of the sample. We also demonstrate the applicability of this method for pharmacokinetic studies in humans and rats.

EXPERIMENTAL

Materials

Amprep extraction columns with 100 mg of C_8-bonded phase were from Amersham International (Amersham, UK). Acetonitrile, KH_2PO_4, methanol, diethyl ether and diethylamine (analytical grade) were purchased from E. Merck (Darmstadt, Germany). Metformin hydrochloride was a generous gift from Leiras Pharmaceuticals (Turku, Finland), and phenformin hydrochloride was purchased from Sigma (St. Louis, MO, USA). Type HV filters (0.45 μm) were from Millipore (Yonezawa, Japan). Distilled water was first deionized using a Millipore Q apparatus (Millipore, Bedford, MA, USA).

Instrumentation

A vacuum-operated sample processing station designed for use with solid-phase extraction columns (VacElut SPS 24, Analytichem International, Harbor City, CA, USA) was used. The HPLC system consisted of an LKB Model 2150 HPLC pump (LKB, Bromma, Sweden), a Guard-pak precolumn, a C_{18}-bonded cartridge (Millipore) and a μBondapak phenyl column (30 cm × 3.9 mm I.D., particle size 10 μm, Millipore). The column effluent was monitored with a programmable UV detector (Model 783A, Applied Biosystems, Foster City, CA, USA) set at 236 nm. The peaks were recorded with a Pharmacia recorder (Bromma, Sweden) set at a chart speed of 5 mm/min. The injector was a syringe-loading sample injector with a 50-μl loop (Model 7125, Rheodyne, Berkeley, CA, USA).

Mobile phase preparation

The mobile phase was acetonitrile–0.01 *M* KH_2PO_4 (40:60, v/v) the pH of which was adjusted to 7 with a few drops of diethylamine. The mixture was filtered with a 0.45-μm Millipore HV filter and degassed by sonification. The flow-rate was 1.35 ml/min (pressure 77 bar). The mobile phase was not allowed to recirculate during the analysis.

Sample preparation

Blood samples were collected into prechilled K_2EDTA tubes and centrifuged at 3000 *g* for 10 min at 4°C. The plasma was stored at −70°C until analysis. Before analysis, aliquots of 500 μl were supplemented with the internal standard phenformin to give a final concentration of 2 mg/l (1 mg/l in some analyses).

Amprep extraction columns were activated in the VacElut manifold by 2 ml of methanol followed by 1 ml of water. The columns were not allowed to run completely dry during this procedure. With the vacuum off, samples containing the internal standard were loaded on the columns. After passage of the samples through the columns under reduced pressure, they were washed with 2 ml of diethyl ether. Metformin (and the internal standard phenformin) were then eluted from the columns with 500 μl of 0.01 *M* KH_2PO_4 (pH 3.5) in 70% acetonitrile. The eluates were then filtered with Millipore HV filters, or centrifuged in a Beckman Microfuge II for 5 min, and injected into the chromatograph. Standard solutions from 0 to 2 mg/l metformin hydrochloride were prepared in blank plasma from an aqueous stock solution, spiked with phenformin, and extracted as above.

Pharmacokinetic applicability

A healthy male volunteer, who had fasted, received 1 g of metformin hydrochloride (two tablets of Diformin, Leiras Pharmaceuticals). Blood samples were drawn into K_2EDTA tubes at the times given in Fig. 1.

In an animal experiment, 32 rats received either 320 mg/kg metformin hydrochloride dissolved in drinking water or water alone for two weeks. After decapitation, trunk blood was collected into K_2EDTA tubes and centrifuged, and the plasma was analysed for metformin content.

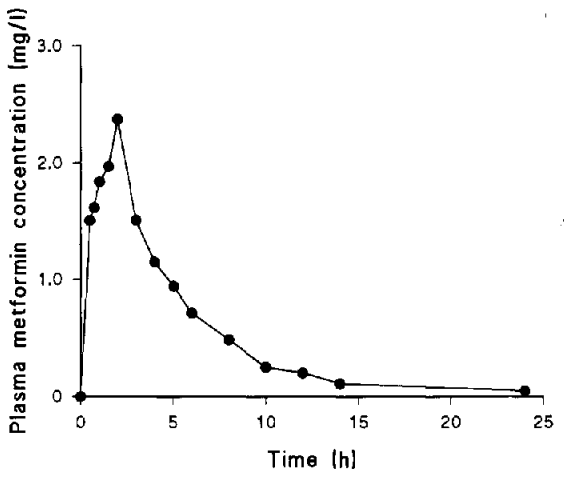

Fig. 1. Metformin concentrations in plasma after administration of 1 g of metformin hydrochloride to a healthy volunteer.

RESULTS AND DISCUSSION

The retention times of metformin and phenformin were 2.8 and 5.6 min, respectively. Both peaks were well resolved from each other, and there was no interference from endogenous plasma (Fig. 2).

Calibration graphs were constructed in blank plasma for metformin concentrations ranging from 0.05 to 2 mg/l. At an internal standard concentration of 2 mg/l and a detector range of 0.05 a.u.f.s., the graphs were straight lines passing through the origin with slopes near unity (*e.g.*, $y = 0.98x - 0.001$, $r = 0.999$). The intra-day coefficient of variation (C.V.), based on peak-height ratios, was 1.5% at a metformin concentration of 0.05 mg/l ($n = 11$) and 4.3% at 1 mg/l ($n = 10$). The inter-day C.V. was estimated by measuring the drug concentration repeatedly over several days in a sample spiked to contain 0.10 or 1 mg/l metformin. The mean (± S.D.) metformin concentration in the samples were 0.11 ± 0.01 ($n = 5$) and 1.02 ± 0.09 mg/l ($n = 12$) corresponding to C.V.s of 7.8 and 8.4%, respectively.

The recovery of metformin from plasma was 92% when estimated by comparing the peak-height ratios due to the same amount of metformin (1 mg/l) added to buffer and blank plasma.

Metformin concentrations in excess of 2 mg/l were analysed either after dilution of the sample, or after constructing a calibration graph up to 10 mg/l with a greater detector attenuation. The lowest metformin concentration that could be detected under the conditions described was 50 ng/ml. If necessary, the sensitivity of the method can be improved by reducing the elution volume from the cartridge. Thus, when the elution volume was reduced to 250 μl, metformin concentrations at least down to 30 ng/ml could be detected while still preserving the signal-to-noise ratio well above 3.

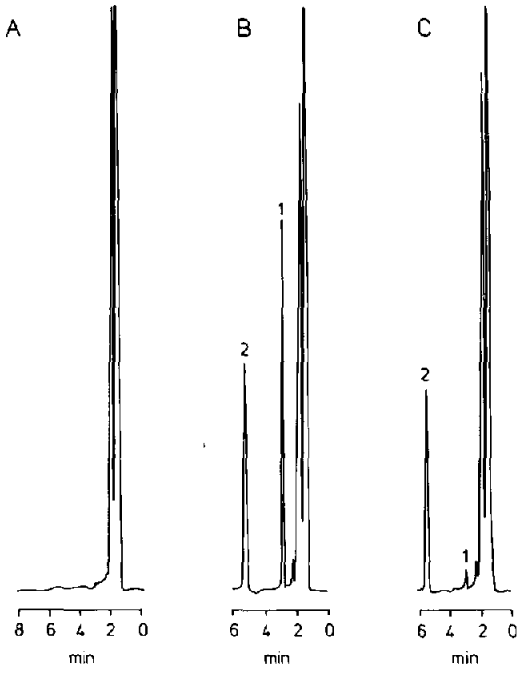

Fig. 2. Chromatograms of blank plasma (A), and plasma from a volunteer containing 1.51 mg/l (B) or 0.1 mg/l (C) metformin. Peaks: 1 = metformin; 2 = phenformin (internal standard). The range was set to 0.05 a.u.f.s.

The applicability of the method for pharmacokinetic studies is demonstrated in Fig. 1. In the volunteer, the peak drug concentration after administration of 1 g of metformin (2.4 mg/l) was reached after 2 h. After 24 h, the concentration had fallen to 0.05 mg/l.

No interference from endogenous substances was noted in rat plasma either. The mean (± S.E.M., $n = 15$) plasma drug concentration after two weeks treatment with 320 mg/kg metformin

hydrochloride in drinking water was 5.6 ± 1.9 mg/l. No metformin was found in the plasma of the rats that received water only.

CONCLUSION

The present method allows an accurate determination of plasma metformin concentrations in humans and rats. The method is flexible and gives reproducible results even at low plasma concentrations of the drug, making it suitable for pharmacokinetic studies.

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REFERENCES

1 R. Vigneri and I. D. Goldfine, *Diabetes Care*, 10 (1987) 118.
2 G. T. Tucker, C. Casey, P. J. Phillips, H. Connor, J. D. Ward and H. F. Woods, *Br. J. Clin. Pharmacol.*, 12 (1981) 235.
3 P. J. Pentikäinen, P. J. Neuvonen and A. Penttilä, *Eur. J. Clin. Pharmacol.*, 16 (1979) 195.
4 R. Beckmann, *Diabetologia*, 5 (1969) 318.
5 M. S. Lennard, C. Casey, G. T. Tucker and H. F. Woods, *Br. J. Clin. Pharmacol.*, 6 (1978) 183.
6 B. G. Charles, N. W. Jacobsen and P. J. Ravenscroft, *Clin. Chem.*, 27 (1981) 434.