CHROMBIO. 379

Note

Rapid determination of diazoxide in plasma and urine of man by means of high-performance liquid chromatography

T.B. VREE and B. LENSELINK

Department of Clinical Pharmacy, Sint Radboud Hospital, University of Nijmegen, Nijmegen (The Netherlands)

F.T.M. HUYSMANS

Department of Internal Medicine, Division of Nephrology, Sint Radboud Hospital, University of Nijmegen, Nijmegen (The Netherlands)

H.L.J. FLEUREN

Department of Pharmacology, Sint Radboud Hospital, University of Nijmegen, Nijmegen (The Netherlands)

and

Th.A. THIEN

Department of Internal Medicine, Division of Nephrology, Sint Radboud Hospital, University of Nijmegen, Nijmegen (The Netherlands)

(Received March 12th, 1979)

The non-diuretic benzothiazine, diazoxide, is used as an antihypertensive drug, especially for the intravenous treatment of hypertensive crises. It is recommended to administer the drug as a bolus injection, 300 mg within 10—30 sec [1], but recently it was shown that also an infusion during 20—30 min was effective [2] and could avoid hypotension and consequent myocardial ischaemia. Orally administered diazoxide is seldom used for chronic treatment of severe hypertension.

No clear relationship has been established between plasma concentration, half-life ($T_{1/2}$) of elimination and vascular activity [1]. In hypertensive patients, the $T_{1/2}$ of elimination varies markedly between 21 and 50 h [1, 3,4]. The large

variation in pharmacokinetics observed may be as much related to analytical problems as to inherent variations in the patients. Currently analysis is carried out by a simple UV method [1, 3, 5], which is of low specificity since possible metabolites may have the same absorption characteristics. Sadee et al. [6] developed an excellent gas chromatographic—mass spectrometric (GC—MS) method for measuring diazoxide plasma concentrations but access to such equipment and the availability of the deuterated internal standard makes the assay difficult for routine applications. Grevink and Fleuren [7] developed a gas—liquid chromatographic method, which required derivatization of diazoxide as the methyl derivative. Both methods are quite laborious and the large series of samples that are required in kinetic studies or with therapy compliance control pose considerable methodological problems.

High-performance liquid chromatography (HPLC) combines simple UV detection with chromatographic specificity. The method is somewhat less specific than GC—MS but its simplicity, speed and the possibility of analysing large series of blood samples are advantages. An HPLC method for the analysis of diazoxide was developed for the purpose of studying pharmacokinetics of diazoxide in healthy human volunteers, hypertensive patients and control of therapy compliance, and some results of the pharmacokinetics of diazoxide in healthy volunteers are reported.

MATERIALS AND METHODS

Apparatus

A Spectra Physics 3500B high-performance liquid chromatograph was used, equipped with a spectrophotometric detector (Model 770). The detector was connected to a 1-mV recorder (BD 7, Kipp en Zonen, Emmen, The Netherlands). A stainless-steel column (10 cm × 4.6 mm I.D.) was packed with LiChrosorb RP-8, particle size 5 μm, obtained from Chrompack (Middelburg, The Netherlands). An injection loop of 100 μl was used. Detection of diazoxide was effected at 270 nm. The detection limit of diazoxide is 0.1 μg/ml.

Solvents

For diazoxide as a simple drug the solvent was a degassed mixture of 300 ml water and 200 ml methanol. The solvent flow-rate was 1 ml/min, at a pressure of 125 atm. (k' diazoxide = 4.4.)

For diazoxide with comedication of chlorthalidon, the solvent was a degassed mixture of 325 ml of 0.005 M sodium acetate and 175 ml of methanol. (k' diazoxide = 5.75; k' chlorthalidon = 3.25.)

Sample preparation

Plasma. Plasma (100 μl) is mixed with 400 μl of perchloric acid (0.33 N) on a Vortex mixer. Deproteinization is completed after standing for 5 min. The mixture is centrifuged for 5 min at 4000 rpm (2600 g) in a Heraus Christ centrifuge; 100 μl of the supernatant are injected onto the column.

Urine. Urine (10 μl) is mixed with 500 μl of perchloric acid (0.33 N) on a Vortex mixer; 100 μl of the mixture are injected onto the column.

Drugs

Diazoxide was obtained from Schering (Essex Nederland B.V., Amstelveen, The Netherlands).

Subjects and patients

Two healthy Caucasian subjects, both employees of the Department of Nephrology, volunteered for this study. Blood samples for routine control or pharmacokinetics studies in hypertensive patients were obtained from patients in the Department of Nephrology. Doses of 25, 100, 100 and 200 mg diazoxide were administered intravenously (i.v.) to the volunteers. Blood samples of 1 ml were taken at regular time intervals by venipuncture.

Spontaneously voided urine was collected for 60 h. One volunteer excreted the drug under acidic urinary conditions achieved by the daily intake of 8 g of ammonium chloride. The other volunteer excreted diazoxide under alkaline urinary conditions maintained by the intake of 10 g of sodium bicarbonate per day.

Recovery

The recovery of diazoxide from plasma was 83.7 ± 1.2% (S.D.) at a concentration of 10 μg/ml and 83.1 ± 2.8% (S.D.) at 5 μg/ml; from urine it was 98.1 ± 1.2% (S.D.).

The calibration curves were obtained by adding known amounts of diazoxide to human plasma and urine samples. They were linear for the concentration range 0.10 –50 μg (r = 0.998). The sensitivity limit was 0.1 μg/ml.

RESULTS

Fig. 1 shows a chromatogram of two different human plasma samples containing diazoxide. The second peak (×) is an endogenous compound as it appeared in the plasma of both volunteers and patients, and is sometimes present in markedly varying concentrations in plasma of patients and volunteers who did not receive diazoxide.

Fig. 2 shows the plasma concentration, salivary concentration and renal excretion rate—time profiles in a volunteer after an intravenous dose of 100 mg of drug under alkaline urinary conditions. The half-life of elimination in the volunteers varies from 15 to 20 h. The ratio between the concentration of diazoxide in plasma and saliva is found to be constant in each volunteer, but varies between 1.5 and 4.8.

The two human volunteers excreted about 20% of the drug unchanged after all doses (Table I). The relationship of renal excretion rate (μg/min) to the plasma concentration (μg/ml) appeared to be linear, showing an average renal clearance constant of 5.36 ml/min, as shown in Fig. 3. The renal clearance of diazoxide was calculated from each urine sample and corresponding plasma sample and plotted against urine flow or urinary pH.

When the urinary pH is not modified by the intake of ammonium chloride or sodium bicarbonate the renal clearance—urinary pH relationship shows a relatively high correlation coefficient (Table I). In both cases the renal clearance is also dependent on the urine flow (Fig. 4). The average renal

clearance over the whole time course of the elimination process of diazoxide is low (2—6 ml/min). The calculated pharmacokinetic parameters of diazoxide are summarized in Table I.
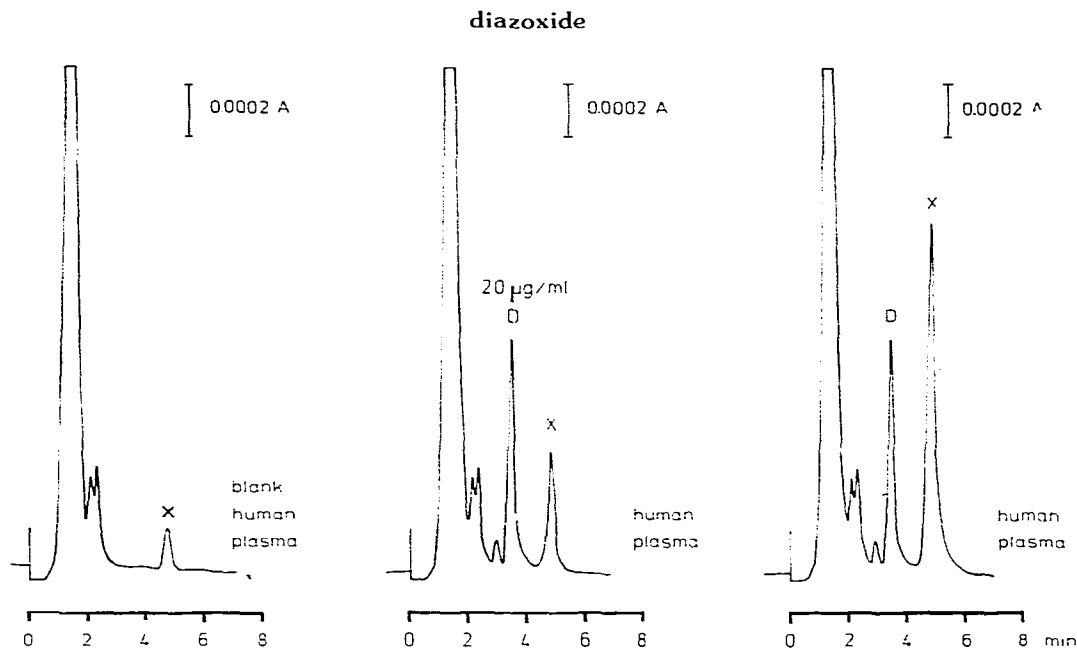
diazoxide

Fig. 1. Chromatogram of diazoxide (D) as single medication in a human plasma sample. Compound x is an endogenous compound present in markedly varying concentrations. Solvent: 300 ml water—200 ml methanol. Solvent flow-rate: 1 ml/min. Column: LiChrosorb RP-8, 5 μm particle size.

DISCUSSION

The HPLC method is found to be reliable and extremely simple and it exhibits a good sensitivity limit of 0.1 μg/ml. This value is between the sensitivity limit of the UV method of Symchowicz et al. [4] and that of the GC—MS method of Sadee et al. [6] (10 ng/ml). Even with a subclinical dose of 25 mg i.v., the plasma concentration elimination curve is followed for 60 h, which is long enough for estimation of $T_{1/2}$ values.

The low renal clearance of the unchanged drug implies high protein binding, strong tubular reabsorption and therefore dependency on urinary pH and urine flow. Because of the low renal clearance, the main route of elimination therefore must be oxidation to a carboxylic acid, followed by glucuronidation and renal excretion.
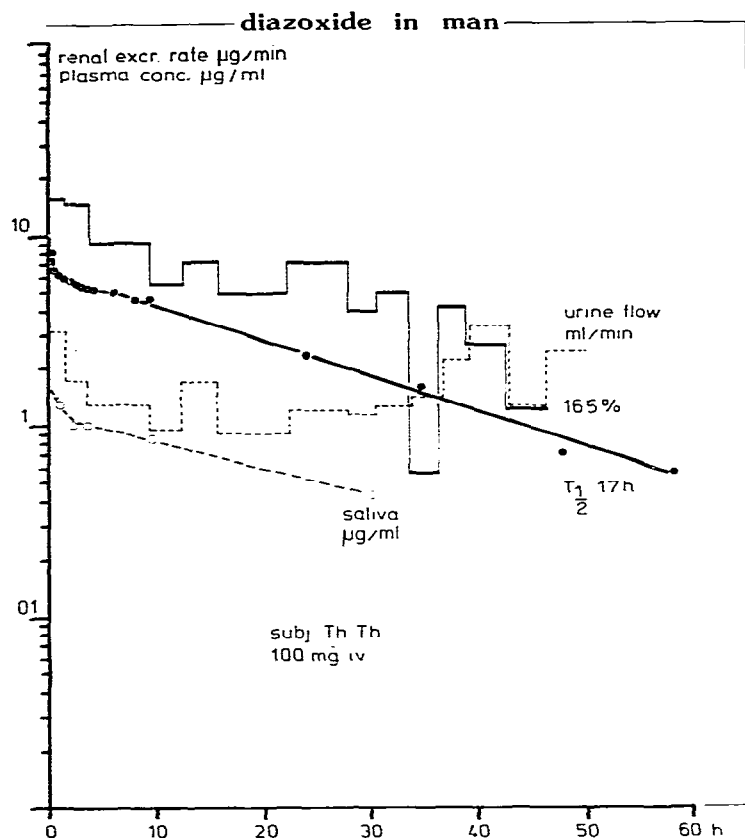
Fig. 2. Plasma and saliva concentrations and renal excretion rate—time profiles of diazoxide after an i.v. dose of 100 mg in a volunteer. There is a constant ratio (4.8) between the plasma and saliva concentration. The urinary pH in this experiment has been kept alkaline [pH 7.70 ± 0.49 (S.D.)].

TABLE I

SOME PHARMACOKINETIC PARAMETERS OF DIAZOXIDE IN MAN

Subject	Dose (mg, i.v.)	Urine flow (ml/min ± S.D.)	Urine pH (± S.D.)	$T_{1/2}$ (h)	Percentage excreted[*]	Renal clearance (ml/min ± S.D.)
T.Th.	25	0.99 ± 0.30	6.19 ± 0.28	17	27.8	6.70 ± 1.79
T.Th.	100	1.69 ± 0.76	7.70 ± 0.49	15	16.5	2.05 ± 0.79
F.H.	200	1.45 ± 0.89	6.42 ± 0.59	20	20.4	3.50 ± 1.76
F.H.	100	2.61 ± 1.81	5.34 ± 0.17	16	14.7	3.25 ± 1.96

[*]Percentage of the dose excreted in the urine unchanged.
[**]Correlation coefficient relationship between urinary pH and renal clearance.
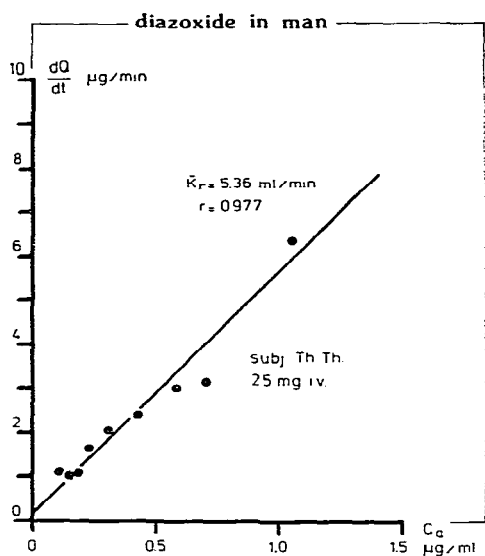[***]Correlation coefficient relationship between urine flow and renal clearance.

Fig. 3. Linear relationship between the renal excretion rate dQ/dt (μg/min) and plasma concentration C_a (μg/ml) of diazoxide in man with uncontrolled urinary pH. The average renal clearance (K_r) over the whole excretion period is 5.36 ml/min.
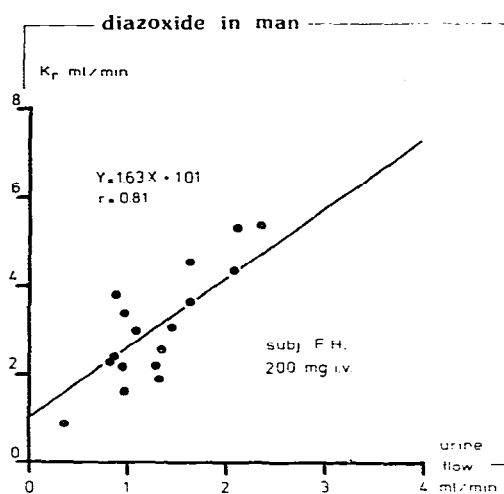
Fig. 4. Relationship between the renal clearance of diazoxide (K_r) and the urine flow in a volunteer receiving 200 mg iv. The urinary pH is 6.42 ± 0.59 (S.D.).

The half-life of elimination in the two healthy volunteers did not vary much. The $T_{1/2}$ may be dependent on the renal function, since with impaired kidney function the $T_{1/2}$ is prolonged to 40—50 h [8], but it also depends on the degree of hypertension [6].

The availability of a simple, reliable and fast analytical method for the measurement of diazoxide in human plasma samples may reveal the relationships between dose, speed of injection, plasma concentration and blood pressure lowering effects.

Ratio of plasma/ saliva concentration	r	
	pH—K_r**	flow—K_r***
—	0.46	0.72
4.8	0.22	0.38
1.5	0.62	0.81
3.5	0.020	0.22

REFERENCES

1 E.M. Sellers and J. Koch-Weser, New Engl. J. Med., 281 (1969) 1141.
2 Th.A. Thien, F.T.M. Huysmans, P.G.G. Gerlag, R.A.P. Koene and P.G.A.B. Wijdeveld, Clin. Pharmacol. Ther., in press.
3 A.W. Pruit, P.G. Dayton and J.H. Patterson, Clin. Pharmacol. Ther., 14 (1973) 73.
4 S. Symchowicz, L. Winston, J. Black, M. Smith, B. Calesnick and I.I.A. Tabachnick, J. Pharm. Sci., 56 (1967) 912.
5 B. Calesnick, B. Katchen and J. Black, J. Pharm. Sci., 56 (1965) 1279.
6 W. Sadee, J. Segal and C. Finn, J. Pharmacokin. Biopharm., 1 (1973) 295.
7 R. Grevink and H.L.J. Fleuren, Report Diazoxide Study, Department of Pharmacology, University of Nijmegen, 1977.
8 A.K. Riethling, G. Rostock, G. Teichmann, K. Schröder and H. Rohmann, Zentralbl. Pharm., 116 (1977) 615.