Hepatic First-Pass Effect of Thiazinamium Methylsulfate (*N*-Methylpromethazine Methylsulfate) in Dogs

JAN H. G. JONKMAN *‡x, **HERMAN G. M. WESTENBERG** *§, and **EPPO VAN DER KLEYN** ¶

Received July 16, 1980, from the *Department of Pharmaceutical and Analytical Chemistry, State University of Groningen, Antonius Deusinglaan 2, 9713 AW Groningen, The Netherlands, the ‡Laboratory for Drug Analysis, Assen, The Netherlands, the §Department of Psychiatry, Academic Hospital, Utrecht, The Netherlands, and the ¶Department of Clinical Pharmacy, St. Radboud Hospital, University of Nijmegen, Nijmegen, The Netherlands. Accepted for publication January 26, 1981.

Abstract □ Plasma concentration–time curves are described for the intravenous infusion and portal vein infusion of the quaternary ammonium compound thiazinamium methylsulfate to dogs. Comparison of areas under the curve indicate that hepatic first-pass elimination occurred on the order of 25–50%.

Keyphrases □ Thiazinamium methylsulfate—hepatic first-pass effect in dogs, plasma concentration–time curves □ *N*-Methylpromethazine methylsulfate—hepatic first-pass effect in dogs, plasma concentration–time curves □ First-pass effect—thiazinamium methylsulfate, dogs, plasma concentration–time curves

Numerous drugs representing several therapeutic groups and chemical classes have been reported to be subject to either intestinal first-pass effect or hepatic first-pass effect (1–4). However, no evidence could be obtained from the literature concerning this phenomenon for quaternary ammonium compounds.

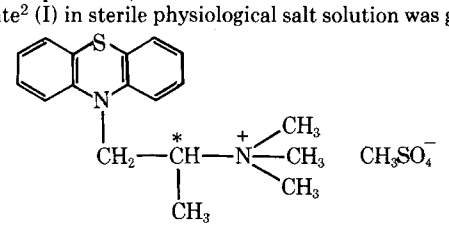
Recently, clinical pharmacokinetic studies on thiazinamium methylsulfate (*N*-methylpromethazine methylsulfate, I), a phenothiazine derivative containing a quaternary ammonium group, were reported (5–7). The drug has anticholinergic and antihistaminic properties and is used as a bronchodilator in patients suffering from generalized chronic obstructive lung diseases.

Absorption of the drug after oral administration was low (~10% of the dose), and considerable inter- and intrasubject variations in bioavailability were found. Such variations are often explained by the hepatic first-pass effect (8). The extent of a hepatic first-pass effect is often related to hepatic clearance (9). Since I is cleared largely by the liver (10), it was decided to investigate whether the hepatic first-pass effect also contributes to its incomplete bioavailability in the dog.

EXPERIMENTAL

Dogs (Table I) were anesthetized by intravenous injection of pentobarbital sodium (30 mg/kg). Anesthesia was maintained by inhalation of oxygen, nitrous oxide, and halothane[1]. Each dog was used in two experiments with an interval of ~2 weeks.

In the first experiment, a slow continuous infusion of thiazinamium methylsulfate[2] (I) in sterile physiological salt solution was given in the vena cephalica antebrachii sinister for 100 min by pump (20 ml/hr). The dose rate was 1 mg/kg/hr. During and after the infusion, blood samples (~6 ml) were taken from the vena saphena parva dexter by a permanent cannula[3] at the time intervals indicated in Fig. 1.



I

In the second experiment, the same procedure was repeated but the infusion was given in the vena porta (portal vein). The abdomen was opened, and a cannula was inserted *via* a side branch.

Plasma concentrations were determined by ion-pair extraction, followed by GLC separation and alkali flame-ionization detection (11).

The area under the plasma concentration–time curve (*AUC*) was measured by cutting and weighing a standard high-quality paper. The absolute bioavailability was calculated by dividing the *AUC* after portal vein infusion by the *AUC* after the intravenous infusion and was expressed as a percentage.

The pharmacokinetic parameters were calculated using the NONLIN program (12). A three-compartment body model was used, with zero-order input in the central compartment and final elimination from this compartment. The initial estimates of the parameters were determined graphically. Examination of the plot of the weighted residuals against time indicated that a weighting factor of $W = 1/\sqrt{y_i}$ resulted in the best fit.

Table I—Data for the Four Dogs Used in the Experiment with Linear Infusion of I in the Portal Vein

Parameter	Dog 268	Dog 343	Dog 367	Dog 515
Breed	Labrador	Dalmatian	Labrador	Labrador
Sex	Female	Male	Female	Male
Weight, kg	22	26	19	24
Dose, mg/kg/hr	1	1	1	1
F_{abs}[a], %	72.0	78.0	51.0	51.7

[a] Absolute bioavailability is expressed as a percentage of the dose.

RESULTS AND DISCUSSION

The hepatic elimination of thiazinamium methylsulfate (I) during the first passage through the liver was investigated by determining the absolute bioavailability obtained after infusion of a drug-containing solution in the portal vein. In this way, the intestinal first-pass effect and the observed irregular and incomplete absorption were avoided. After intravenous infusion, plasma concentrations of ~600 ng/ml were obtained. After the infusion was stopped, plasma concentrations fell steeply (Fig. 1). The $(t_{1/2})_\alpha$ was estimated to be ~1 min, and the $(t_{1/2})_\beta$ was ~5–10 min; $(t_{1/2})_\gamma$, the elimination half-life, was ~6–7 hr. These figures should be considered as estimations because of the very steep decrease in plasma concentrations immediately after the infusion was stopped and the very low concentrations during the final elimination phase.

Studies in rats (10) indicated that the extremely rapid disappearance of the drug from the plasma was mainly due to both fast biliary elimination of the unchanged drug (3.8% of the dose) and rapid biotransformation to thiazinamium sulfoxide, which also can be excreted rapidly in the bile (16.1% of the dose). Two other metabolites also were excreted in bile (13.1 and 2.7% of the dose).

A substantial part of the dose in humans also was excreted in the bile [16.6% unchanged and 6.9% as the sulfoxide (13)].

After infusion in the portal vein, the plasma concentration–time curve rose somewhat slower than in the intravenous infusion. In Dogs 343 and 367, the plasma concentration–time curves reached a plateau level of

[1] Fluothane.
[2] Specia, Rhône Poulenc, Paris.
[3] Braunule, B. Braun, Melsungen, West Germany.

0022-3549/81/0800-0947$01.00/0

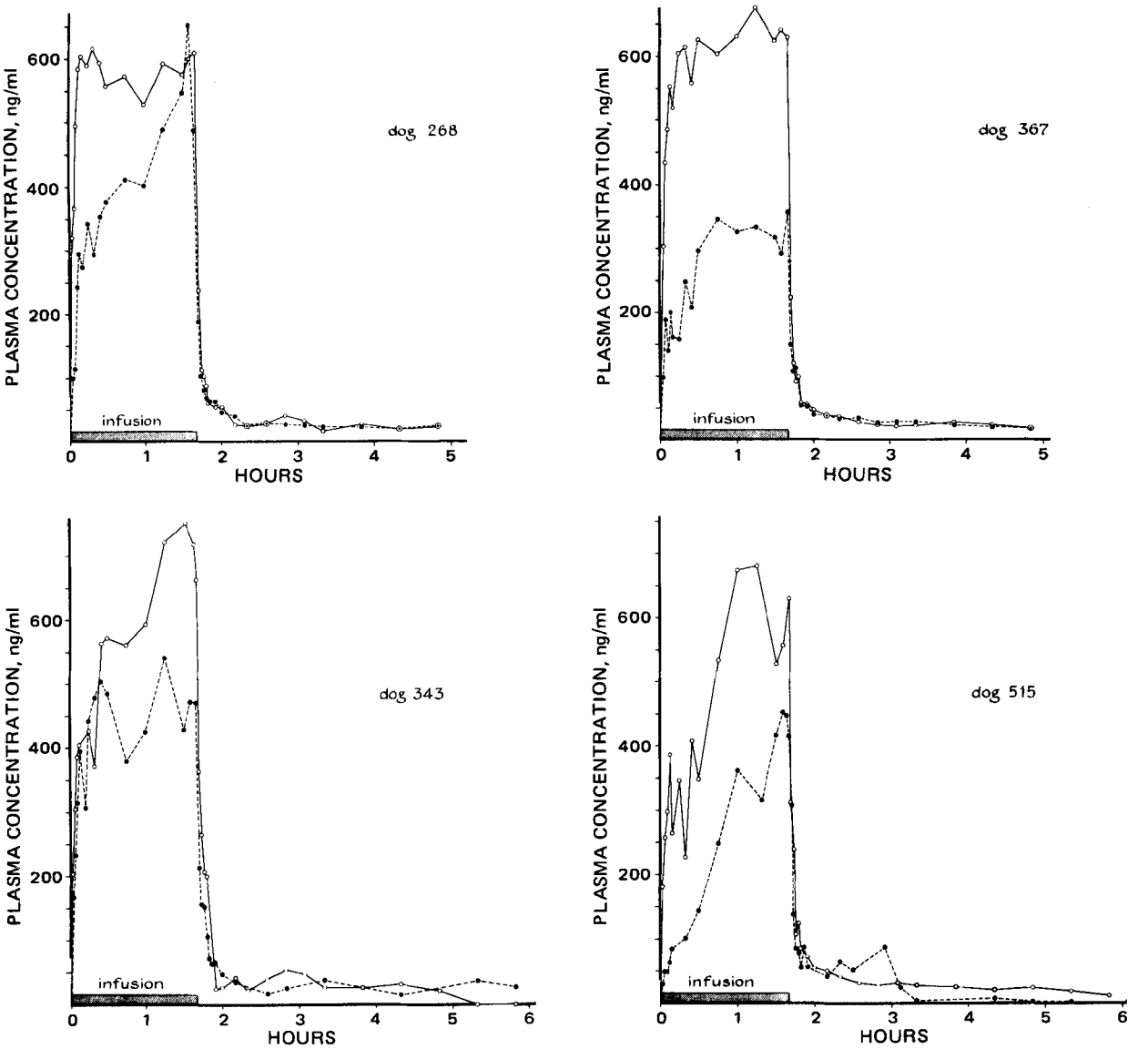


Figure 1—*Plasma concentrations in dogs during and after the infusion (1 mg/kg/hr) of I. Key: o—, intravenous infusion; and ●– –, infusion in portal vein.*

~300–400 ng/ml. The phenomenon can be explained by assuming that a substantial part of the drug offered to the liver by portal blood flow is extracted by this organ before the drug can enter the general circulation.

For Dogs 268 and 515, the curve was still going up at the end of the infusion time, suggesting that the biotransformation and/or elimination processes in the liver became saturated and were no longer able to remove the drug from the portal vein to the same extent as before.

The calculated absolute bioavailability (F_{abs}) after infusion in the portal vein varied from 51.0 to 78.0% (mean 63.2 ± 13.9 *SD*%), indicating a hepatic first-pass effect that removed 22.0–49.0% (mean 37.8 ± 14.9 *SD*%) of the dose before entering the general circulation.

Oguma *et al.* (14) found that, when valethamate bromide or scopolamine-*N*-butyl bromide (both quaternary ammonium compounds) was infused in the portal vein in the rat, biliary excretion was more pronounced than when infusion was given in a femoral vein. This result is an indirect indication of a hepatic first-pass effect. Gibaldi *et al.* (15) studied the metabolism of *N,N*-bis(phenylcarbamoylmethyl)dimethylammonium chloride, a quaternized lidocaine derivative, after intravenous and intraperitoneal administration and found that, during passage of drug through the liver after intraperitoneal administration, a fraction of the dose was irreversibly lost by metabolic conversion and subsequent biliary excretion (hepatic first-pass effect).

The results of this study on I are comparable with the observations of Taylor *et al.* (16) on the tertiary analog promethazine in rabbits. They reported a systemic availability of 59 ± 19% after promethazine injection into the portal vein. Although a different species is involved, the indication is that both the tertiary and the quaternary compound can be subjected extensively to a hepatic first-pass effect.

The results of the present study indicate that the low and variable bioavailability of an oral dose of I can be explained in part by an extensive hepatic first-pass effect.

REFERENCES

(1) C. Jacquot, J. Cariou, J. R. Rapin, and Y. Cohen, in "Formulations and Preparations of Dosage Forms," J. Polderman, Ed., Elsevier, Amsterdam, The Netherlands, 1977, pp. 153–169.

(2) J. Caldwell and R. L. Smith, in *ibid.*, pp. 169–181.

(3) A. Bieder and J. Gaillot, in *ibid.*, pp. 181–195.

(4) M. Mayersohn, in "Principles and Perspectives in Drug Bioavailability," J. Blanchard, R. J. Sawchuk, and B. B. Brodie, Eds., S. Karger, Basel, Switzerland, 1979, pp. 248–255.

(5) J. H. G. Jonkman, L. E. van Bork, J. Wijsbeek, R. A. de Zeeuw, and N. G. M. Orie, *Clin. Pharmacol. Ther.*, **21**, 457 (1977).

(6) J. H. G. Jonkman, L. E. van Bork, J. Wijsbeek, A. S. Bolhuis-de Vries, R. A. de Zeeuw, N. G. M. Orie, and H. L. M. Cox, *J. Pharm. Sci.*, **68**, 69 (1979).

(7) J. H. G. Jonkman, L. E. van Bork, J. Wijsbeek, A. S. Bolhuis-de Vries, R. A. de Zeeuw, N. G. M. Orie, and H. L. M. Cox, *Int. J. Pharm.*, **3**, 55 (1979).

(8) J. T. Wilson, G. T. Atwood, and D. G. Shand, *Clin. Pharmacol.*

Ther., **19**, 264 (1976).
(9) M. Rowland, *J. Pharm. Sci.*, **61**, 70 (1972).
(10) K. Neef, J. H. G. Jonkman, and D. K. F. Meyer, *ibid.*, **67**, 1147 (1978).
(11) J. H. G. Jonkman, J. Wijsbeek, S. Hollenbeek Brouwer-de Boer, R. A. de Zeeuw, L. E. van Bork, and N. G. M. Orie, *J. Pharm. Pharmacol.*, **27**, 849 (1975).
(12) C. M. Metzler, G. L. Elfring, and A. J. McEwen in "A Users Manual for NONLIN and Associated Programs," Upjohn Co., Kalamazoo, Mich., 1974.
(13) J. H. G. Jonkman, Ph.D. thesis, State University of Groningen, Groningen, The Netherlands, 1977.
(14) T. Oguma, T. Muramatsu, T. Iga, T. Fuwa, S. Awazu, and M. Hanano, *Chem. Pharm. Bull.*, **21**, 1554 (1973).
(15) M. Gibaldi, J. E. Axelson, and W. D. Conway, *J. Pharm. Sci.*, **64**, 359 (1975).
(16) G. Taylor and J. B. Houston, *J. Pharm. Pharmacol., Suppl.*, **31**, 40P (1979).

ACKNOWLEDGMENTS

The authors are indebted to T. H. M. Arts, A. J. Peters, and P. G. H. Philips (Central Animal Laboratory, St. Radboud Hospital, University of Nijmegen) for technical assistance, and they gratefully acknowledge J. Konings (University of Nijmegen) for preparation of the illustrations.

High-Performance Liquid Chromatographic Analysis of Triamterene and *p*-Hydroxytriamterene in Plasma

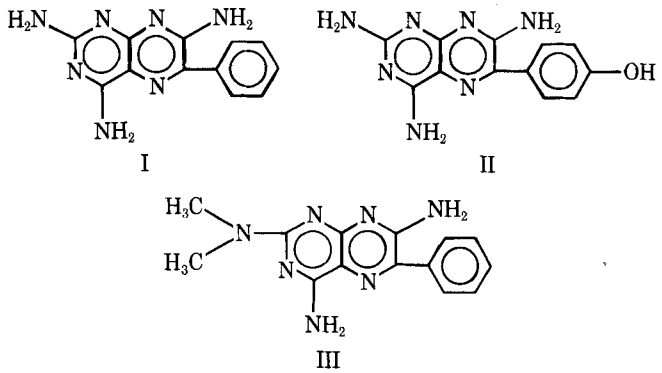
GERALD J. YAKATAN *x and JOSEF E. CRUZ

Received September 24, 1980, from the *Drug Dynamics Institute, College of Pharmacy, University of Texas at Austin, Austin, TX 78712.* Accepted for publication January 23, 1981. *Present address: Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48105.

Abstract □ A rapid and sensitive high-performance liquid chromatographic assay was developed for triamterene and 6-*p*-hydroxytriamterene in plasma. Plasma (0.5 ml), after addition of the internal standard, was extracted with 10 ml of ether–isopropanol (95:5). After thorough mixing and separation of phases, the organic layer was evaporated to dryness under nitrogen. The residue was reconstituted with 500 μl of mobile phase [acetonitrile–water–acetic acid (60:39.5:0.5)], and 100 μl was injected into the chromatograph. Chromatographic separation was carried out on a C_{18} μBondapak column at a flow rate of 1 ml/min. Detection of compounds in the column eluent was by UV absorption at 365 nm. The retention times for 6-*p*-hydroxytriamterene, triamterene, and the internal standard were 7.5, 9.0, and 12.0 min, respectively. The lower limit of detection for each compound was 20 ng/ml. Recoveries of triamterene and 6-*p*-hydroxytriamterene were 91–99 and 82–95%, respectively, over a 40–240-ng/ml range.

Keyphrases □ Triamterene—high-performance liquid chromatographic analysis, plasma □ *p*-Hydroxytriamterene—high-performance liquid chromatographic analysis, plasma □ Diuretics—high-performance liquid chromatographic analysis of triamterene and *p*-hydroxytriamterene, plasma □ High-performance liquid chromatographic analysis—triamterene and *p*-hydroxytriamterene, plasma

Triamterene, 2,4,7-triamino-6-phenylpteridine (I), is a natriuretic and a potassium-sparing diuretic. It is used mainly in combination with hydrochlorothiazide in the treatment of edema associated with congestive heart failure, cirrhosis of the liver, and the nephrotic syndrome.



I

II

III

BACKGROUND

Studies of plasma and urine concentrations of triamterene and its major metabolite, 2,4,7-triamino-6-*p*-hydroxyphenylpteridine (II), in humans and animals have been performed either with nonspecific fluorescence methods or more specific methods that require time-consuming and involved separation procedures (1–5). The separation techniques utilized have included paper chromatography (4), TLC (5), and liquid–liquid extraction (2, 6). One method (5) detected nanogram levels of the compounds, but the other methods lacked the sensitivity to measure nanogram concentrations of triamterene in plasma. A sensitive and specific assay is particularly desirable for study of triamterene pharmacokinetics since concentrations of the metabolite may be 10-fold those of the parent compound 30 min following oral administration (2).

Recently, two high-performance liquid chromatographic (HPLC) assays for triamterene in plasma were reported. One method (7) involved extraction of triamterene as its perchlorate ion-pair and subsequent normal-phase HPLC utilizing a fluorescence detector. Triamterene concentrations as low as 2 ng/ml could be detected, although the investigators did not employ an internal standard. The other method (8) involved extraction with ethyl acetate and subsequent reversed-phase HPLC with fluorescence detection; it had a sensitivity to 1 ng/ml. Neither research group attempted to quantitate metabolites.

This article describes a rapid, sensitive, and selective assay for triamterene and its major metabolite in plasma. The method involves a simple extraction of plasma with organic solvent and reversed-phase HPLC of the extract using UV detection.

EXPERIMENTAL

Reagents—Acetonitrile[1] and methanol[1] were chromatography grade. Anhydrous ether[2], acetic acid[2], and isopropanol[2] were spectranalytical grade.

Equipment—A high-performance liquid chromatograph[3] with a syringe-loading sample injector[4], a recorder[5], and a variable-wavelength

[1] MCB Manufacturing Chemists Inc., Cincinnati, Ohio.
[2] Fisher Scientific Co., Fair Lawn, N.J.
[3] Tracor 950 chromatographic pump, Tracor Instruments, Austin, Tex.
[4] Model 7120, Rheodyne, Berkeley, Calif.
[5] OmniScribe, Houston Instruments, Austin, Tex.

0022-3549/81/0800-0949$01.00/0