Pharmacokinetic Studies on Ditazole, a Novel Inhibitor of Platelet Aggregation

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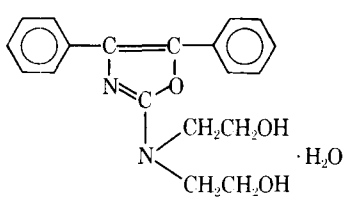
Abstract □ The distribution of ditazole in blood and tissues of rats was determined by a simple GLC technique. Ditazole, after intravenous injection in rats (20 mg/kg), entered preferentially into the brain, the liver, and the heart in decreasing order. In the epididymal adipose tissue, the drug was present only in small amounts. Ditazole disappeared from the rat organs 4 hr after the treatment. The apparent ditazole half-life in rat blood was 41 min, the volume of distribution was 2.068 liters/kg, and the body clearance was 0.0345 liter/kg/min.

Keyphrases □ Ditazole—GLC analysis, pharmacokinetic study of blood and tissue distribution in rats □ GLC—analysis, ditazole in rat blood and tissues □ Pharmacokinetics—ditazole in rats, GLC analysis □ Distribution, tissue—ditazole in rats, GLC analysis □ Platelet aggregation inhibitors—ditazole, GLC analysis, pharmacokinetic study of blood and tissue distribution in rats

Ditazole (I) is a new nonsteroidal drug selected from a series of new oxazole derivatives (1). It possesses anti-inflammatory and antipyretic peripheral analgesia activities (2) and markedly inhibits platelet aggregation (3, 4).



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Some data illustrating ditazole metabolism in rats, rabbits, and humans after oral administration, using radioisotope techniques, were reported (5). Although these techniques are very sensitive, they cannot always detect whether the radioactivity found is wholly due to the administered labeled drug or to its metabolites. To overcome this limitation, a simpler and more rapid procedure for the analysis of blood and tissue ditazole levels was developed.

The GLC method described in this report was utilized for a pharmacokinetic investigation of ditazole in rats.

EXPERIMENTAL

Male Sprague–Dawley rats, 150 ± 5 g, were used.

Ditazole (20 mg/kg) in dimethyl sulfoxide–absolute ethanol–polysorbate 80[1]–water (0.2:1:2:7) was administered by intravenous injection.

Whole blood (1 ml), 1 *M* pH 7.4 phosphate buffer (2 ml), water (1 ml), and ether (10 ml) were placed in glass-stoppered centrifuge tubes. The tubes were shaken on a reciprocating shaker for 10 min and centrifuged at 4000×*g* for 10 min. The water phase was reextracted with 10 ml of ether; the combined organic phases were dried under vacuum and redissolved in a suitable volume of a solution containing the internal standard (diazepam) in acetonitrile (10 μg/ml). A 1-μl aliquot of the acetonitrile solution was mixed with 1 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide[2] in the same syringe and then injected into a chromatographic column.

The ditazole derivatization increased its GLC sensitivity by about 100-fold.

The animals were sacrificed by decapitation at various times after ditazole injection. The brain, heart, liver, and epididymal adipose tissue were rapidly removed and homogenized in ice-cold absolute ethanol (1:4 w/v). The homogenates were centrifuged at 4000×*g* for 20 min. The supernate was evaporated to dryness under vacuum, and the residue was dissolved in 5 ml of 6 *N* HCl and washed twice with 10 ml of ether. After shaking for 10 min, ethereal phases were discarded. The acidic phase was adjusted to neutral pH (7–7.5) and then reextracted twice with 10 ml of ether. The procedure with the ether extracts was identical with that described for blood extraction.

Recovery of ditazole from the tissues studied was around 80%, ranging from 75 ± 1 to 85 ± 3%.

The gas chromatograph[3] was equipped with a hydrogen flame-ionization detector. The stationary phase was 3% OV-17 on 100-120-mesh Gas Chrom Q packed into a 1-m glass column (internal diameter 4 mm, external diameter 6 mm).

The flow rates of hydrogen and air were 15 and 300 ml/min, respectively. The carrier gas, nitrogen, was used at a flow rate of 33 ml/min. The injection port temperature was 300°, the detector temperature was 300°, and the oven temperature was 270°.

Derivatization was performed on-column by mixing 1 μl of ditazole solution in acetonitrile with 1 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide in a syringe.

For ditazole identification and determination, the internal standard technique was used. Diazepam was employed as the internal standard because of its suitable retention time (1 min and 45 sec in comparison to 2 min for ditazole).

The peak area was determined by measuring, in convenient units, the height and the width of the peak at half-height. Ditazole can be quantitated by GLC when the relative peak area is used as a concentration index since a linear relationship exists between relative peak areas and drug concentration in the 10–40-μg range.

A typical gas chromatogram showing the separation of the ditazole derivative and the internal standard is shown in Fig. 1.

The chemical structure of the ditazole silyl derivative was confirmed by GLC–mass spectrometry. A mass spectrometer[4], fitted with a 2-m × 2-mm i.d. spiral glass column packed with 3% OV-17 on 100–120-mesh Gas Chrom Q, was employed. Helium was used as the carrier gas, and all mass spectra were obtained at 70 ev. Other parameters were: injection site temperature, 300°; molecular separator temperature, 260°; ion source temperature, 290°; accelerating voltage, 3 kv; and ionizing current, 20 μamp.

For the determination of the change of spectrum produced by the binding of ditazole to hepatic cytochrome P-450, the technique described by Omura and Sato (6, 7) was used.

RESULTS AND DISCUSSION

Table I shows the disposition pattern of ditazole in blood, brain, heart, liver, and adipose tissue of rats after intravenous administration of ditazole at 20 mg/kg. The ditazole concentration reached peak values at 5 min in all tissues except adipose tissue, for which peak values were attained at 30 min. The highest values of ditazole in the brain were reached 5 min after administration. The brain level of ditazole was reduced to an unmeasurable value by 4 hr after treatment.

[1] Tween 80.

[2] Pierce Chemical Co., Rockford, Ill.

[3] Model GI, Carlo Erba, Milan, Italy.

[4] LKB model 9000.

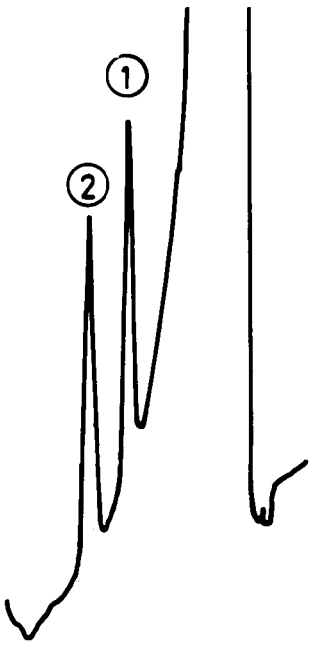

Figure 1—*Separation of the ditazole derivative (2) from the internal standard, diazepam (1).*

Ditazole also was found in high concentration in the liver 5 min after treatment; ditazole levels in the liver decreased with a slope similar to that seen in the brain. The heart levels, with a maximum at 5 min, decreased rapidly so that only small amounts were detected 1 hr after treatment.

Small amounts of ditazole were found in the adipose tissue, with a maximum of 5 μg at 30 min after administration. No measurable amounts were present after 1 hr.

The pharmacokinetic parameters of ditazole in rat blood following a 20-mg/kg iv administration were calculated using the NONLIN program (8) on a digital computer[5]. The apparent ditazole half-life in the rat blood was 41 min, its distribution volume was about 2 liters/kg, and its clearance was about 0.03 liter/kg/min. Areas under the blood ditazole concentration–time curves at time 0 and ∞ were 490 and 578 μg/ml × min, respectively.

[5] UNIVAC 1106.

Table I—Ditazole Levels[a] in the Blood, Brain, Liver, and Adipose Tissue of Rats after Administration of 20 mg/kg iv

Minutes after Ditazole Administration	Blood, μg/ml ± *SE*	Brain, μg/g ± *SE*	Heart, μg/g ± *SE*	Liver, μg/g ± *SE*	Adipose Tissue, μg/g ± *SE*
5	8.7 ± 1.0	79.7 ± 4.0	18 ± 0.2	29.6 ± 1.0	3.4 ± 0.1
30	4.9 ± 0.7	15.4 ± 1.0	10.2 ± 0.5	24.7 ± 0.7	5.0 ± 0.5
60	3.6 ± 0.7	7.6 ± 0.3	1.7 ± 0.2	7.0 ± 0.7	1.1 ± 0.1
120	1.5 ± 0.4	2.6 ± 0.1	<0.4	2.5 ± 0.3	<0.4
240	<0.4	<0.4	<0.4	<0.4	<0.4

[a] Each figure is the average of at least four determinations.

Studies on ditazole metabolism in rats after intravenous administration are lacking. Marchetti *et al.* (5) reported that ditazole elimination occurred slowly through urine and feces after oral administration; most of the drug was excreted unchanged. Among its metabolites, 4,5-diphenyl-4-oxazolin-2-one, 4,5-diphenyl-2-(2-oxyethyl)aminoxazole, and benzil were identified. Preliminary *in vitro* studies on ditazole metabolism by rat liver microsomal enzymes indicate that this drug is metabolized into two or three compounds not yet identified.

It is well known that drugs and other foreign compounds combine with hepatic cytochrome P-450 to produce difference spectra of two general types, I and II (9). With hepatic cytochrome P-450, ditazole gave a type II spectrum with λ_{max} and λ_{min} falling within the usual range.

REFERENCES

(1) E. Marchetti, G. Mattalia, and V. Rosnati, *J. Med. Chem.*, **11**, 1092 (1968).

(2) L. Caprino, F. Borrelli, and R. Falchetti, *Arzneim.-Forsch.*, **23**, 1972 (1973).

(3) *Ibid.*, **23**, 1277 (1973).

(4) G. de Gaetano, M. C. Tonolli, M. P. Bertoni, and M. C. Roncaglioni, *Haemostasis*, **6**, 127 (1977).

(5) E. Marchetti, G. Mattalia, and G. Bergesi, *Arzneim.-Forsch.*, **23**, 1291 (1973).

(6) T. Omura and R. Sato, *J. Biol. Chem.*, **239**, 2370 (1964).

(7) *Ibid.*, **239**, 2379 (1964).

(8) C. M. Metzler, "A User's Manual for NONLIN," Tech. Rep. 7292/69/7292/005, The Upjohn Co., Kalamazoo, Mich., Nov. 25, 1969.

(9) B. N. La Du, M. C. Mandel, and E. L. Way, "Fundamentals of Drug Metabolism and Drug Disposition," Williams & Wilkins, Baltimore, Md., 1971, p. 206.

Electronic Structure–Activity Relationships of Antibacterial Acridines

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Abstract □ The antibacterial activity of a series of amino- and fluorinated acridines was studied in the framework of their electronic structures. To calculate the electronic structure, a simple Hückel molecular orbital theory was used. A statistical regression analysis revealed linear correlations between the activity and the electronic indexes, particularly the electron density at the ring nitrogen.

Keyphrases □ Acridines, various—antibacterial activity related to electronic structure □ Antibacterial activity—various acridines, related to electronic structure □ Electronic structure—various acridines, related to antibacterial activity □ Structure–activity relationships—various acridines, antibacterial activity related to electronic structure

The antibacterial activity of acridines has been found to be proportional to the fraction ionized as the cation (1–3). The simplest interpretation of the mode of action of acridine cations is that they compete with hydrogen ions for a vitally important anionic group on the bacterium (4). The vital activity of the vulnerable anionic group (A^-) of the bacterium is supposed to be reduced by the formation of a feebly dissociated complex (ABH) with the cation