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Note

Quantitative high-performance liquid chromatographic determination of antispasmodic trimebutine in human plasma

Pharmacokinetic studies after intravenous administration in humans

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Trimebutine maleate, 2-dimethylamino-2-phenylbutyl-3,4,5-trimethoxybenzoate maleate (Debridat®) (Fig. 1), is an antispasmodic compound used in France in various gastrointestinal diseases and in radiological examinations [1].

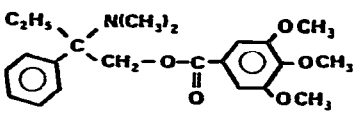

Fig. 1. Structural formula of trimebutine maleate.

Side-effects have been described [2, 3] after high doses. The pharmacokinetic behaviour of this drug in patients has been investigated using radiolabelled drug [4], but little is known about the characteristics of its absorption and elimination. The main reason for this paucity of information appears to be due to the lack of analytical methods applicable to human studies.

A simple, rapid, routine and non-radioactive assay of the drug in plasma was required for further investigations of its pharmacokinetics. This type of assay using reversed-phase high-performance liquid chromatography with C_{18} bonded-phase, is the subject of this report.

EXPERIMENTAL

Reagents

Methanol was obtained from Carlo Erba (Milan, Italy) and filtered (0.22 μm

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Fluoropore; Millipore, Molsheim, France) before use. Hexane and 2-pentanol (analytical grade) were purchased from Merck (Darmstadt, G.F.R.). The Pic-A reagent was obtained from Waters Assoc. (Milford, MA, U.S.A.). Trimebutine was generously supplied by Jouveinal Laboratories (Fresnes, France), procaine hydrochloride (internal standard) was obtained from the Pharmacie Centrale des Hôpitaux (Paris, France). All other solvents and reagents were of analytical quality.

Chromatography

A liquid chromatograph (Altex 380-Chromatem, Touzart et Matignon, Paris, France) equipped with a variable-wavelength detector (Pye Unicam, Cambridge, Great Britain) was used in a reversed-phase system with Partisil ODS-2 as the stationary phase (250 × 4.6 mm I.D.; particle size 10 μm) (Touzart et Matignon) and methanol—aqueous Pic-A (85:15, v/v; Pic-A = 0.005 *M*) as the mobile phase. The effluent stream was monitored at 265 nm and the volume of sample injected was 20 μl (Rheodyne, Berkeley, CA, U.S.A.).

The mobile phase was degassed by a helium stream during the determination and pumped at a constant flow-rate of 2.00 ± 0.02 ml/min. A chart speed of 5 cm/min was employed.

Standard curve and sample preparation

A 1-ml sample of patient plasma and 0.1 ml of a procaine hydrochloride solution (20 μg/ml; freshly prepared daily from a stock solution of 1 mg/ml in water) were added to a 20-ml culture tube with PTFE-lined caps (Prolabo, Paris, France).

The plasma was made alkaline (pH adjusted to 11) by adding 2 ml of 2.9% ammonia solution and was extracted with 3 ml of hexane containing 2-pentanol (0.1%) in a rotary shaker (Cenco, Breda, The Netherlands) for 15 min. The organic (upper) phase was separated by centrifugation at 600 *g* for 5 min and transferred to a clean evaporating tube.

The hexane was evaporated at 30°C under vacuum (Rotavapor, Buchi, Switzerland). The residue was taken up with 50 μl of methanol. Aliquots of 20 μl were injected into the chromatograph.

The concentration of trimebutine in the plasma was determined from a calibration curve of peak height ratio (drug/internal standard) versus drug concentration in spiked plasma (0.05—1 μg/ml) carried through the procedure.

In vivo study

Three in-patients with normal renal and hepatic functions participated voluntarily in the study. Trimebutine maleate (150 mg) was administered intravenously (right hand vein) in a rapid injection (2 min). Venous blood samples were collected via a Teflon catheter inserted in an antecubital vein of the contralateral arm in heparinized vials at various times and were centrifuged within 10 min at 1200 *g* (+ 4°C) to obtain plasma fractions (stored at −30°C until analysis).

RESULTS AND DISCUSSION

Method

A chromatogram obtained from a patient plasma sample containing trimebutine and procaine, extracted and assayed as described, is shown in Fig. 2. The retention times for procaine and trimebutine were 3.8 and 8 min, respectively.

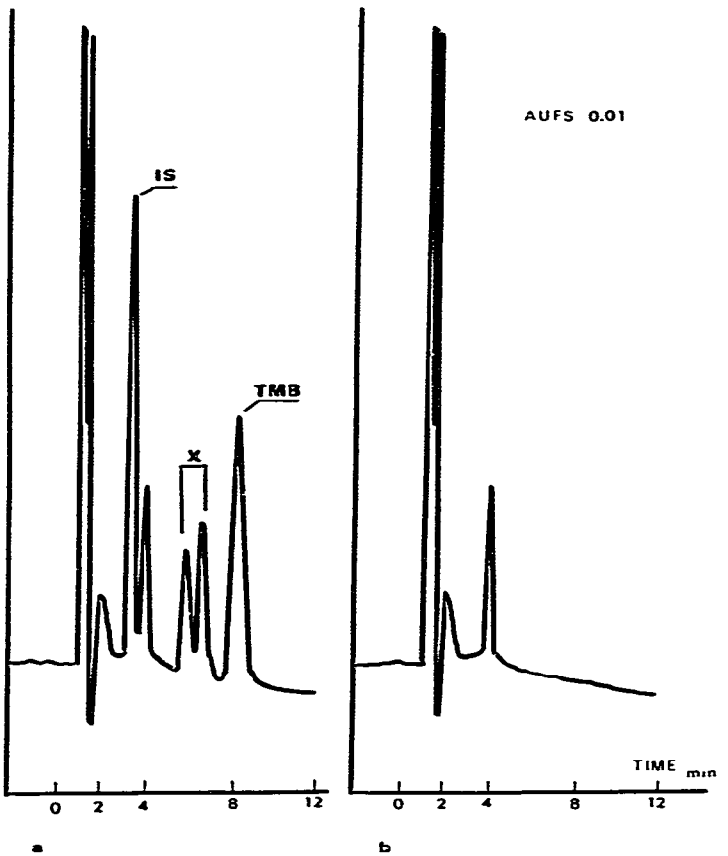

Fig. 2. Chromatograms of (a) a patient plasma sample (IS = procaine, 2 μg/ml; TMB = trimebutine, 250 ng/ml; X = unidentified peaks), 90 min after a single intravenous dose of 150 mg of trimebutine maleate, (b) a patient plasma before drug injection.

The trimebutine peak was well separated from all extraneous plasma peaks. The peaks marked "X" were unidentified compounds, probably biotransformation products of trimebutine. The calibration curves were linear over the concentration range studied. The least-squares regression line, which represents the mean of ten determinations for each point, has a slope of 1.21 and a y-intercept (y = peak height ratio of drug to internal standard) of 0.005 (R = 0.9979).

The precision of the method was determined by repeated analyses of spiked plasma samples containing low (0.5 μg/ml) and high (1 μg/ml) concentrations of trimebutine. The mean standard deviation and coefficient of variation for intra- and inter-day analyses are shown in Table I and were satisfactory. The

TABLE I

INTRA- AND INTER-DAY REPRODUCIBILITY OF TRIMEBUTINE ASSAYS IN PLASMA

		Amount added to plasma (μg/ml)	
		0.5	1.0
Intra-day ($n = 10$)	R^* (mean ± S.D.)	0.35 ± 0.015	0.80 ± 0.02
	Coefficient of variation (%)	4	2.5
Inter-day ($n = 10$)	R^* (mean ± S.D.)	0.38 ± 0.02	0.79 ± 0.03
	Coefficient of variation (%)	5	3.8

*R = (peak height of trimebutine)/(peak height of procaine).

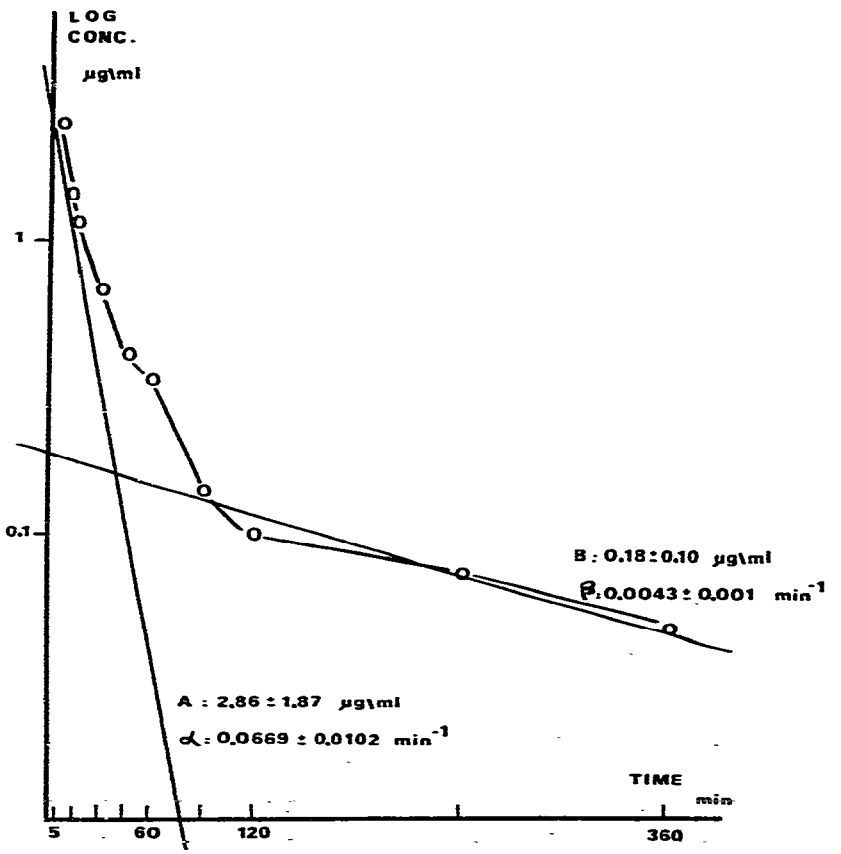

Fig. 3. Mean plasma concentrations of trimebutine after a single intravenous dose of 150 mg of trimebutine maleate.

TABLE II

PHARMACOKINETIC PARAMETERS* IN THREE VOLUNTEERS AFTER A SINGLE 150-mg INTRAVENOUS DOSE OF TRIMEBUTINE

Initials	A (μg/ml)	α(min^{-1})	$t_{1/2}\,\alpha$(min)	AUC_0^∞ (α) (μg per ml per min)	B (μg/ml)	β (min^{-1})	$t_{1/2}\,\beta$ (min)	AUC (β) (μg per ml per min)	AUC ($\alpha + \beta$) (μg per ml per min)
A.	1.43	0.0670	10.3	21.2	0.119	0.0048	144.3	24.8	46.0
L.G.	2.17	0.0565	12.3	38.5	0.308	0.0047	151.6	67.4	105.9
Y.	4.97	0.0769	9.0	64.7	0.133	0.0035	197.4	37.9	102.6
Mean	2.86	0.0669	10.5	41.5	0.187	0.0043	164.4	43.3	84.8
± S.D.	± 1.87	± 0.0102	± 1.6	± 21.9	± 0.105	± 0.0007	± 28.8	± 21.8	± 33.6

*Clearance (1800 ml/min) = $D \times \frac{\alpha\beta}{A + B}$, where α = constant of distribution phase, β = constant of elimination phase, D = dose administered, AUC = area under the plasma level curve, $t_{1/2}\,\alpha$ = distribution half-life, $t_{1/2}\,\beta$ = elimination half-life.

analytical recovery of trimebutine from plasma was determined by comparing the ratio of the peak heights of trimebutine to internal standard in spiked plasma specimens (trimebutine 0.5 μg/ml, internal standard 2 μg/ml), to the ratio in spiked plasma with only 2 μg/ml of internal standard (trimebutine added just before injection to chromatograph). Recovery (mean ± S.D.) was 91 ± 4% (n = 5).

The sensitivity of this method was estimated to be 0.04 μg/ml. Greater sensitivity may be possible by increasing the plasma volume or increasing the injection volume (50 μl).

In vivo study

No adverse effects were observed in any of the volunteers after the administration of trimebutine.

The plasma concentration kinetics of trimebutine (mean of three volunteers) are shown in Fig. 3. The various pharmacokinetic parameters were calculated with a non-linear least-squares regression program using a "feathering method" [5] written for a microcalculator (Hewlett-Packard HP 97). A summary of the pharmacokinetic parameters is shown in Table II. The AUC_0^∞ values were measured using the trapezoidal rule.

The data show that trimebutine kinetics follow a biexponential pattern, with an initial rapid phase (distribution) lasting for up to 10.5 ± 1.6 min after an intravenous infusion. This is followed by a slower (elimination) phase with a half-life of 164.4 ± 28.8 min. Maximum concentration (mean 2.6 ± 1.8 μg/ml) was obtained in the first sample taken 5 min after the end of the 2-min intravenous infusion.

The typical compartmental scheme corresponding to this model might be written as shown in Fig. 4, where k_{12} and k_{21} are first-order microconstants for transfer and k_e is the rate constant of elimination [5—7].

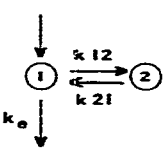

Fig. 4. Compartmental scheme (trimebutine, intravenous).

CONCLUSION

This assay provides an efficient and accurate method for the analysis of trimebutine in human plasma. We are currently using the method for pharmacokinetic studies on trimebutine following intravenous administration in humans. The method has been applied successfully in a study of the pharmacokinetics of trimebutine in liver dysfunction and these results will be presented elsewhere.

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