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Note

High-performance liquid chromatography of propanidid in rat plasma

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Propanidid [3-methoxy-*p*-(N,N-diethylcarbamoylmethoxy)phenylacetic acid *n*-propyl ester] is used for intravenous anaesthesia in man [1—3]. The extremely short duration of action of propanidid is a result of rapid hydrolysis of the ester linkage by tissue esterases [3—5]. The metabolites have been shown to be anaesthetically inactive [6]. Serum concentrations of propanidid have previously been determined by photometric [3, 7] and gas chromatographic [8] methods. The photometric method lacks specificity and sensitivity, and the gas chromatographic technique is relatively time-consuming. Moreover, these methods require several millilitres of blood and cannot be applied for determination of propanidid in microvolumes of plasma.

In the present report we describe a new and rapid high-performance liquid chromatographic (HPLC) method for the determination of propanidid in plasma. The method can quantify propanidid in 0.1 ml of plasma and is applicable for studies such as the experimental pharmacokinetics of propanidid.

MATERIALS AND METHODS

Chemicals

Propanidid in injectable (Propantan®, 50 mg/ml) and in pure (B.P. 1980) form was obtained from Leiras (Turku, Finland), and it was used without

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further dilution. The internal standard α-naphthyl propionate was purchased from Sigma (St. Louis, MO, U.S.A.). Acetonitrile (Orion, Espoo, Finland) and water obtained from Milli-Q system (Millipore, Bedford, MA, U.S.A.) were of HPLC grade, and they were degassed under vacuum immediately before use. Other solvents and chemicals were of reagent grade.

Liquid chromatography and mass spectrometry

A liquid chromatograph, Altex Model 420, equipped with Altex Model 110A pumps, a Rheodyne 7125 injector (20 μl), a Kratos UV-spectroflow monitor SF 770 and a monochromator GM 770 was used. Separations were achieved on an Ultrasphere ODS (Beckman, Irvine, CA, U.S.A.) column, 4.6 × 250 mm (particle size 5 μm) and a mobile phase consisting of water—acetonitrile (30:70, v/v) at a flow-rate of 1.0 ml/min. Propanidid and internal standard were detected at ambient room temperature at 280 nm (see Fig. 3). The propanidid peaks were identified using a Shimadzu PR-1 spectrophotometer and a JEOL JMS-D 300 mass spectrometer equipped with a JMA 2000 mass data analysis system. The conditions for the mass spectrometer were ionization current 300 μA and ionization potential 70 eV, and the instrument was calibrated using perfluorokerocine.

Extraction procedure

Plasma was separated by centrifuging the blood at 800 *g* for 15 min. For both assay calibration and in vivo studies plasma samples (0.1 ml) were transferred to 90 mg of sodium chloride, 0.05 ml of α-naphthyl propionate (10 μg/ml in acetonitrile) and 0.3 ml of acetonitrile, and they were shaken horizontally (Desaga, Heidelberg, F.R.G.) for 30 min. Samples containing 1.0 ml of plasma, 900 mg of sodium chloride, 0.05 ml of α-naphthyl propionate (100 μg/ml in acetonitrile) and 3.0 ml of acetonitrile were prepared in a similar manner. After centrifugation at 2000 *g* for 15 min the acetonitrile layer was collected and used for the determination of propanidid.

Standard curve and assay calibration

A standard curve was prepared by adding known amounts of propanidid and internal standard to acetonitrile and determining the peak ratios of propanidid to internal standard. The recovery of propanidid from rat plasma was determined by comparing the amounts resulting from spiked plasma samples (1.0 ml and 0.1 ml) to those obtained from standard measurements. Inter-assay and intra-assay standardizations were performed with 1.0 ml and 0.1 ml of rat plasma using samples spiked with 5, 10 and 20 μg/ml propanidid. The inter-assay precision was carried out over a two-month period.

Animal study

During light ether anaesthesia the tail vein and the portal vein of male Wistar rats (weight 300 ± 35 g) were cannulated. Propanidid (25 mg/kg) was injected into the tail vein during 10 sec. Blood samples (0.20—0.25 ml) were collected from the portal vein 15, 30, 45, 60, 90, 120 and 180 sec after the injection, in Eppendorf microcentrifuge tubes containing 20% sodium citrate and 0.01 ml of 0.1 *M* diisopropylfluorophosphate (DFP). The tubes were carefully revolved

several times and stored at ambient room temperature for further extraction.

RESULTS AND DISCUSSION

Fig. 1 shows the separation and quantitation of propanidid (peak I) in rat plasma using α-naphthyl propionate (peak II) as an internal standard. In the chromatogram obtained after extraction of 1.0 ml of blank plasma, no additional peaks are seen which could interfere with the determination of propanidid and α-naphthyl propionate (Fig. 1A). Fig. 1B represents the quantitation of propanidid from plasma spiked with propanidid and α-napththyl propionate. Fig. 1C is a chromatogram obtained after extracting 0.1 ml of plasma from a rat 30 sec after the intravenous injection of propanidid (25 mg/kg). Propanidid and the internal standard were well separated with retention times of 4.4 and 6.9 min, respectively.

The peak eluting at 4.4 min in HPLC was identified by mass spectrometry. The structure and the fragmentation of propanidid in the mass spectrometer are presented in Fig. 2. The most prominent ions were the fragments *m/e* 114 (F_1), 100 (F_2) and 72 (F_3), accompanied by the molecular ion *m/e* 337. The mass spectra of pure propanidid and the plasma eluate were similar with respect to the dominant ions. Identification was also made by comparing ultraviolet spectra of the peak (t_R = 4.4 min) and propanidid standard (Fig. 3), which gave identical results. These studies proved that the peak eluting 4.4 min is due to propanidid with no interference from its metabolites.

The calibration curves were obtained using rat plasma spiked with 2—40 μg/ml propanidid and 5 μg/ml α-naphthyl propionate. There was a good correlation between the amount of propanidid added to rat plasma and the

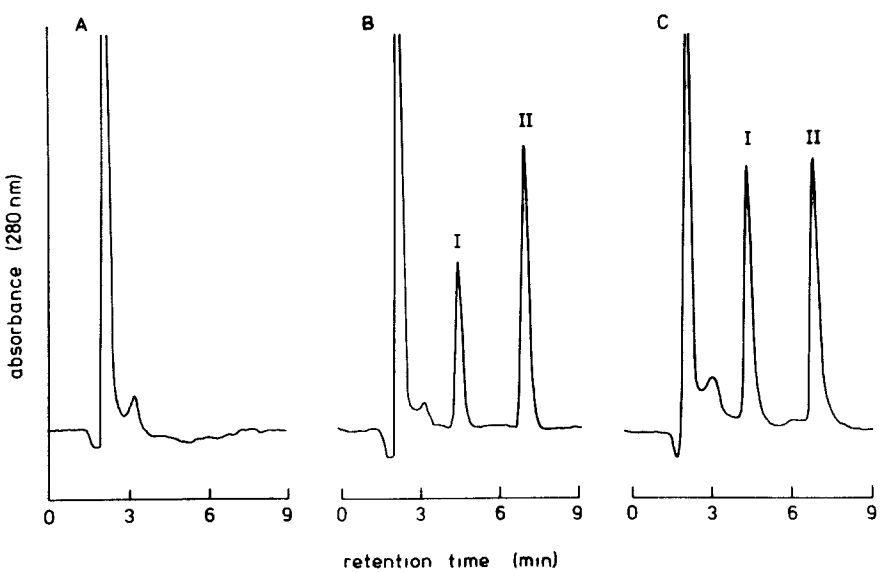


Fig. 1. Chromatograms of rat plasma: (A) blank plasma; (B) plasma spiked with propanidid (10 μg/ml) and α-napththyl propionate (5 μg/ml) as an internal standard; (C) plasma obtained from a rat 30 sec after the injection of propanidid (25 mg/kg, intravenously) and spiked with internal standard. Peaks: I = propanidid; II = α-naphthyl propionate.

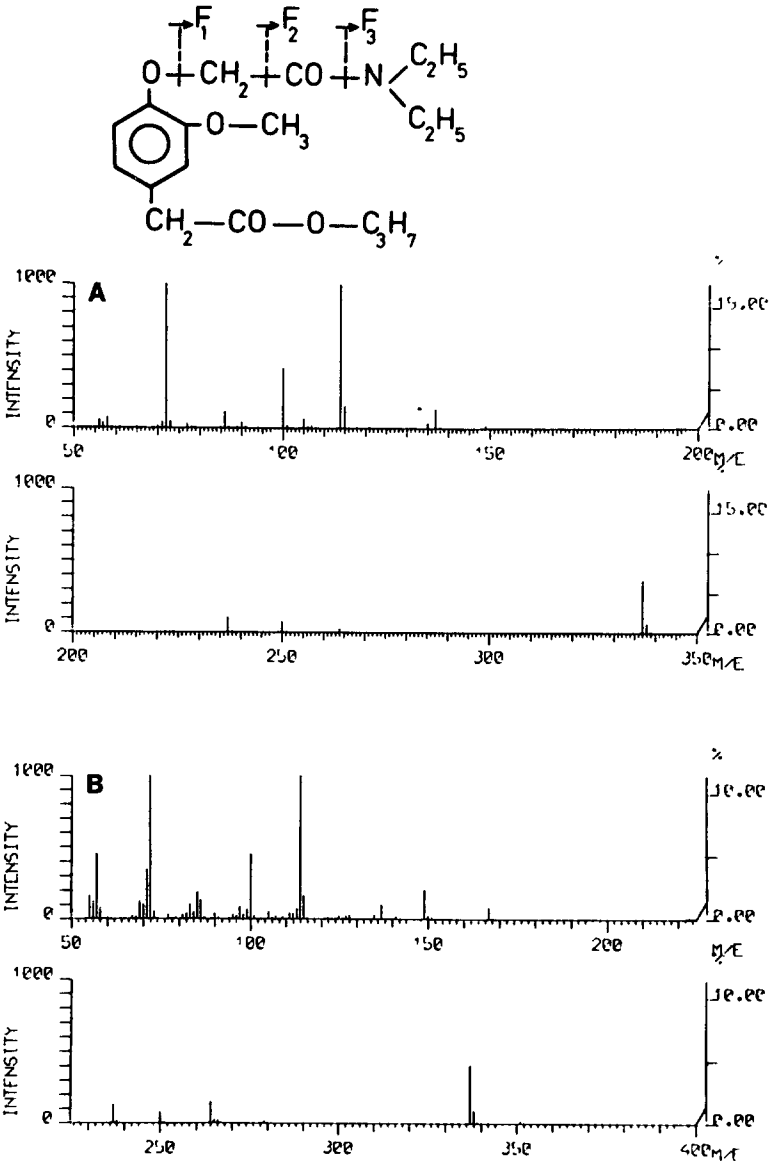
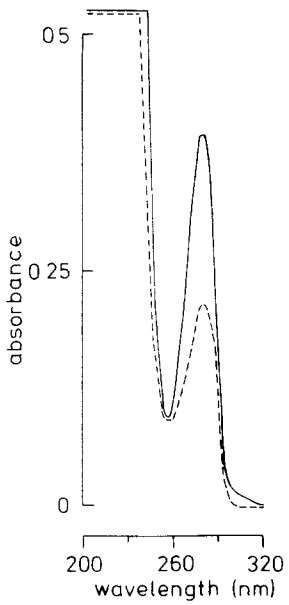


Fig. 2. Mass spectra of the pure propanidid (A) and the eluate (B) at 70 eV.

amount detected in the samples of both 1.0 ml and 0.1 ml plasma. The regression equations of these data are $Y = 17.280X - 0.04$ ($r^2 = 0.9999$) and $Y = 17.909X - 0.05$ ($r^2 = 0.9998$), respectively. The curves passed through the origin and were linear from 1 to 50 μg/ml. The present method could quantify up to 1 ng of propanidid with reasonable accuracy at 100 ng/ml. The intra-assay variations determined using 1.0 ml and 0.1 ml of spiked rat plasma were 1.2 and 2.0% ($n = 10$), and the inter-assay variations 3.0 and 5.9% ($n = 10$), respectively.

The plasma concentration—time curve for propanidid in rats (25 mg/kg,



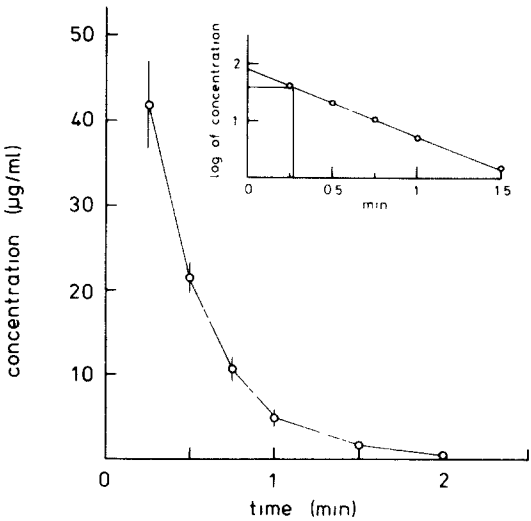


Fig. 3. Ultraviolet spectra of pure propanidid (50 µg/ml) (——) and the eluate (25 µg/ml) (– – –)

Fig. 4. Plasma concentration—time curve (mean ± S.E.) in rats (n = 15) following a parenteral administration of propanidid (25 mg/kg, intravenously)

intravenously) is shown in Fig. 4. The half-life (mean ± S.D.) of propanidid was 0.27 ± 0.08 min (n = 15). As has been assumed previously [3—5], the esterases in blood play an important role in the degradation of propanidid. The rapid hydrolysis of propanidid must be taken into account when collecting the blood samples. To avoid spontaneous hydrolysis of propanidid, we used DFP as an esterase inhibitor because it inhibits the activities of all B-esterases [9]. We noticed that plasma cholinesterase (butyrylthiocholine iodide as substrate) and carboxylesterase (*p*-nitrophenylacetate as substrate) were inhibited 80—90% and 40—50%, respectively, after treatment of the blood with DFP. The stability of propanidid and the internal standard in plasma samples were tested under the experimental conditions described above. No significant decrease in concentration of these two substances was observed during a follow-up period of 60 min.

The applicability of heptane [3] as the extraction medium was also tested. The half-life of propanidid in vitro was determined by incubating hepatic microsomes (0.25 mg protein per ml in a final volume of 2.0 ml of Tris—HCl buffer pH 8.2) in the presence of propanidid (5 µg/ml). The extraction of propanidid with 6.0 ml of acetonitrile or heptane showed similar half-lives of 7.0 and 7.8 min, respectively. However, heptane was not suitable for extraction of propanidid in small plasma volumes because of the poor linearity and the low recovery of different concentrations of the drug. Plasma also tended to precipitate during the extraction with this method, which caused difficulties in collecting representative samples for the analysis. These studies suggest that acetonitrile is superior to heptane for the extraction of propanidid in micro-volumes.

The present method is more selective than the photometric method [3, 7] and more sensitive than the gas chromatographic technique [8], and only microvolumes of plasma are required for reliable determinations. No pre-purification or concentration of samples before injection into the HPLC system were necessary. HPLC can be applied for the analysis of propanidid in other biological samples and hydrolysis of propanidid is easily detectable also in vitro conditions. Thus, this HPLC method offers a new way to study the function of tissue esterases both in vivo and in vitro.

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