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Note

High-performance liquid chromatographic analysis of pentazocine in blood and plasma

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Pentazocine [1,2,3,4,5,6-hexahydro-*cis*-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol] is a narcotic analgesic, structurally related to morphine, which has been widely used in man. Various analytical techniques including fluorimetry [1], gas chromatography [2,3], radioimmunoassay [4] and thin-layer chromatography [5], have been developed for pentazocine, although to date no high-performance liquid chromatographic (HPLC) method has been reported.

In the present investigation, an HPLC method for pentazocine has therefore been developed to allow rapid quantification of the drug in biological fluids.

Pentazocine has a relatively low molar extinction coefficient of 1910 (λ_{max} = 278 nm), and per se is not readily detected by its UV absorption. To overcome this sensitivity limitation, a derivative of pentazocine with 2-*p*-chlorosulphophenyl-3-phenylindone (DIS-CL), previously used by Vinson et al. [6] in the thin-layer chromatographic analysis of narcotic analgesics has been used. DIS-CL reacts with the phenolic hydroxyl group of pentazocine to produce a derivative with a λ_{max} at 275 and has a considerably greater UV absorption than pentazocine itself.

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MATERIALS AND METHODS

Extraction of pentazocine

One milliliter of whole blood or whole blood β-glucuronidase—sulphatase hydrolysate was alkalinised by the addition of 0.2 ml 1 *M* sodium hydroxide, 1 ml of levallorphan (internal standard, 0.2 mg/ml in water) was added, and the mixture was extracted by shaking with 2 × 5 ml dichloromethane for 10 min. After centrifugation, the organic phase was removed, combined and evaporated to dryness at 45°C under a stream of dry nitrogen.

The residue was redissolved in 100 μl acetonitrile and 5-μl aliquots of DIS-CL (1 mg/ml in acetonitrile), and 2 *M* sodium carbonate were added. Derivatization was complete after heating at 45°C for 10 min. The reaction mixture was evaporated under a stream of dry nitrogen and the residue redissolved in 100 μl of the HPLC mobile phase.

High-performance liquid chromatography

Analyses were performed using a Waters Assoc. (Milford, MA, U.S.A.) HPLC system Model M6000A pump, Model 440 detector set at 280 nm, U6K injector and C_{18} μBondapak column (30 cm × 4 mm I.D.; 10 μm particle size). A mobile phase of acetonitrile—0.7% ammonium chloride (adjusted to pH 8 with ammonium hydroxide) (80:20) was pumped at a flow-rate of 2 ml/min.

In situ isolated rabbit intestinal loops

The technique used was essentially that described by George et al. [7]. Briefly, using unanaesthetized rabbits, a 10—15 cm section of jejunum was isolated between two catheters and pentazocine solution (10.4 m*M*) was introduced into the gut lumen. Venous blood from the isolated loop was collected in fractions (2—4 min periods for 46 min) by means of a 21-gauge scalp vein catheter placed in the mesenteric vein draining the isolated loop. Aliquots of blood (0.2—0.4 ml), 1 ml of acetate buffer pH 5.0, and 1 ml of β-glucuronidase (E.C. 3.2.1.31)—sulphatase (E.C. 3.1.6.1) mixture (1000 U/ml) were incubated at 37°C for 48 h to achieve total hydrolysis of conjugated pentazocine.

Materials and reagents

Dichloromethane (nanograde, Mallinckrodt, St. Louis, MO, U.S.A.), acetonitrile (HPLC grade, Waters Assoc.), levallorphan·HCl (Roche Products, Sydney, Australia), pentazocine (Winthrop Labs., Sydney, Australia), 2-*p*-chlorosulphophenyl-3-phenylindone (DIS-CL; Polysciences, Warrington, PA, U.S.A.). β-Glucuronidase—sulphatase was obtained from Sigma (St. Louis, MO, U.S.A.). All other chemicals were of analytical reagent grade.

RESULTS AND DISCUSSION

Under the conditions used for the HPLC separation, pentazocine and levallorphan have retention times of approximately 5.7 min and 7.8 min respectively (Fig. 1).

Other structurally similar narcotics had very similar retention times (codeine 5.5 min, morphine 5.9 min, ethylmorphine 6.2 min, and methadone 5.8 min).

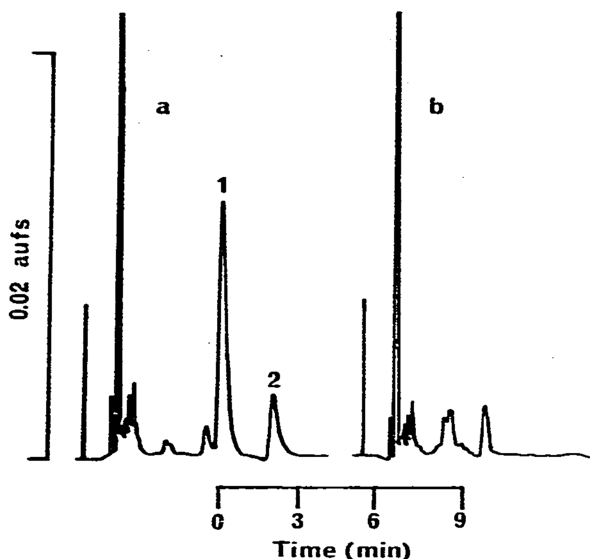

Fig. 1. Chromatograms of an extract of whole blood (pentazocine added) showing (a) separation of pentazocine (1) 5 μg/ml and the internal standard levallorphan (2) 2 μg/ml, and (b) an extract of drug-free blood.

TABLE I

COMPARISON OF ANALYTICAL METHODS FOR PENTAZOCINE

Method	Sensitivity	Comments	Reference
Thin-layer chromatography	1 μg/ml		Reid and Gerbeck [5]
Fluorimetry	50 ng/ml	Selectivity?	Berkowitz et al. [9]
Gas chromatography (flame ionisation detector)	not stated	Large volume of plasma required	Vaughn and Beckett [2]
Gas chromatography (electron-capture detector)	100 ng/ml	Extensive clean-up needed in sample preparation	Pittman and Davison [10]
Gas chromatography (electron-capture detector)	1 ng/ml		Brötell et al. [11]
Gas chromatography (electron-capture detector)	1 ng/ml		Swezey et al. [3]
Radioimmunoassay	1 ng/ml		Peterson et al. [4]

Results were quantified from a plot of peak height ratio pentazocine to internal standard versus pentazocine concentration, which was found to be linear over the range 20 ng—10 μg pentazocine base per ml blood.

Recovery of pentazocine through the extraction procedure, assessed by measuring peak height of the pentazocine derivative prepared from a blood extract (1 μg/ml) with that from a non-extracted standard, was found to be 78%. The relative standard deviations of the method at 200 ng, 1 μg and 10 μg pentazocine per ml blood were 4% (n = 6), 3.8% (n = 4) and 3.5% (n = 4), respectively. Blood to plasma ratios (rabbit blood) of 2.05 ± 0.2 (mean ± S.D.; n = 6) were obtained over the concentration range 1—10 μg/ml. Ehrnebo et al. [8] obtained a mean value of 1.06 for human blood at 200 ng pentazocine per ml.

This difference may be a reflection of the drug concentration range used, or of inherent differences in blood chemistry between the two species.

The HPLC method described above can be compared to previously reported methods by reference to Table I. It has a detection limit of approximately 10 ng/ml, which makes it 5—10 times more sensitive than fluorimetric or some gas chromatographic methods, but is some 10 times less sensitive than electron-capture gas chromatography or radioimmunoassay. A major advantage of the HPLC method is that it requires minimal sample preparation by comparison to the extensive clean-up procedures needed for electron-capture gas chromatography.

Fig. 2 illustrates the application of the HPLC method to the measurement of pentazocine in venous blood from an isolated in situ rabbit intestinal loop preparation. Both free (unmetabolised) and total (after hydrolysis with β-glucuronidase—sulphatase) blood pentazocine were measured. The difference between total and free drug gives a measure of the extent of conjugation of pentazocine. The results indicate that peak concentration of pentazocine occurs at about 4 min, and that there is substantial metabolism of the drug during intestinal absorption.

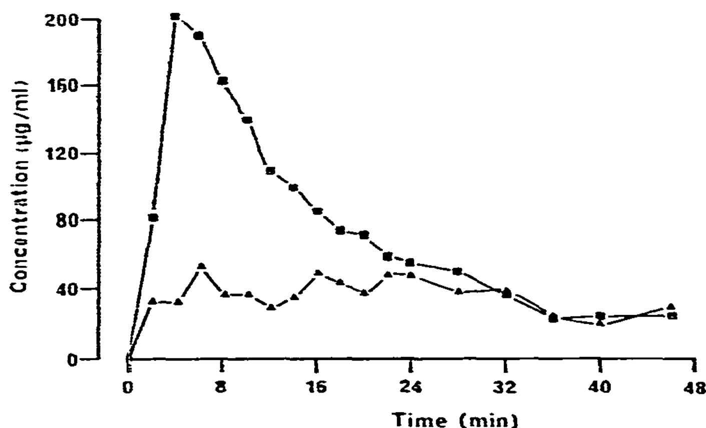

Fig. 2. Blood concentration—time curve for unmetabolized pentazocine (■) and conjugated pentazocine (▲) in mesenteric venous blood from an isolated in situ rabbit intestinal loop containing 10.4 mM pentazocine solution.

In summary, we have developed a rapid, simple, sensitive analytical technique for pentazocine in blood and have demonstrated its application in studying the intestinal absorption of this drug. Use of a fluorimetric detector would further increase sensitivity. Since steady-state concentrations of pentazocine in blood following administration of the usual analgesic dose to man are in the range 10—50 ng/ml [2] this method should also be applicable to the monitoring of pentazocine in the clinical situation.

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