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Note

Determination of tauromustine, a nitrosourea-based antitumour agent, in plasma by high-performance liquid chromatography

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Tauromustine, 1-(2-chloroethyl)-3-[2-(dimethylaminosulphonyl)ethyl]-1-nitrosourea (TCNU) (Fig. 1), is a novel antitumour nitrosourea derived from taurine, an endogenous aminoethanesulphonic acid. Tauromustine is a neutral and semi-polar compound with a partition coefficient of log $P=0.6$ (n-octanol–water). It is active against a wide variety of experimental tumours [1] and produces a higher therapeutic index than 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU) against Walker 256 carcinosarcoma and L 1210 leukaemia when administrated orally. On the basis of these results, tauromustine was selected for clinical evaluation in cancer patients.

For pharmacokinetic studies in animals and humans a method for the quantitative determination of tauromustine in plasma was required. A selective and sensitive method is described in this paper.

EXPERIMENTAL

Chemicals

Tauromustine and its demethylated analogue were synthesized at Leo Labs. [1]. 4-Hydroxybenzoic acid isopropyl ester (isopropagin), used as an internal standard, was purchased from INC Pharmaceutical (Plainview, NY, U.S.A.), chloroform and dichloromethane (BDH, Poole, U.K.) were all-glass distilled prior

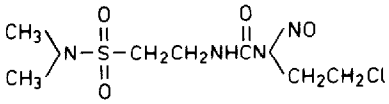

Fig. 1. Structure of tauromustine.

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to use, and acetonitrile (Merck, Darmstadt, F.R.G.) was used as supplied. Water was deionized by a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.). All other chemicals were purchased from standard sources and used without further purification.

Procedure

A frozen plasma sample was acidified with 2 *M* hydrochloric acid (ca. 10 μl per ml of plasma) and thawed under occasional shaking in a water-bath at room temperature. Then 1.0 ml of plasma was transferred to a test-tube containing 2.0 ml of 0.1 *M* phosphate buffer (pH 6) and 4.0 ml of internal standard solution (isopropagin, 200 ng/ml in dichloromethane–chloroform, 1:1), shaken for 10 min and centrifuged at 1000 *g* for 10 min. The aqueous phase was re-extracted with 2.0 ml of chloroform–dichloromethane (1:1). The organic phases were combined, washed with 1.0 ml of 0.1 *M* phosphate buffer (pH 8), filtered through dry sodium sulphate and evaporated under a gentle stream of nitrogen. The residue was reconstituted in 0.2 ml of methanol–acetic acid (99:1), and transferred to autosampler vials. Aliquots of 25 μl were injected and chromatographed in duplicate.

Apparatus

The chromatographic system consisted of a Waters (Milford, MA, U.S.A.) WISP 710 B autoinjector, a Model 6000 A solvent delivery system, coupled with a Model 441 UV absorbance detector equipped with a cadmium lamp for detection at 229 nm and a Spectra-Physics (Santa Clara, CA, U.S.A.) SP 4270 integrator. The column was an Ultrasphere ODS (250 mm × 4.6 mm I.D., 5 μm particle size; Altex, Berkeley, CA, U.S.A.) preceded by a Guard-Pak™ C_{18} pre-column module (Waters).

Mobile phase

The mobile phase was prepared by mixing 630 ml of 0.005 *M* potassium dihydrogenphosphate with 370 ml of acetonitrile, adjusting the pH to 4.7 by the addition of 0.1 *M* phosphoric acid and vacuum-filtering through a FHUP-047 Millipore filter. The flow-rate was 1.3 ml/min.

Calibration

At least six different plasma standards covering the expected concentration range were processed as described above. The calibration graph was established by the least-squares linear regression method, and the regression equation was used to calculate concentrations of tauromustine in unknown plasma samples.

Pharmacokinetic application

The pharmacokinetics of tauromustine was studied in a patient participating in a phase I clinical trial [2]. The patient is denoted C 131 in the Copenhagen phase I study. The drug (130 mg/m^2) was administrated orally, and 10-ml blood samples were collected in heparinized tubes through an infusion catheter prior to drug administration and subsequently 5, 15, 30, 60, 90 and 120 min, and 4, 6, 8

and 10 h after administration. The samples were immediately cooled in ice, and plasma was separated by centrifugation at 1300 *g* and 0°C for 6 min. The centrifugation was completed within 15 min after sampling, and plasma was at once frozen and stored below −20°C until analysis.

The area under the plasma concentration–time curve (AUC) was calculated using the linear trapezoidal rule. Exponential equations were fitted to plasma concentration–time data by the extended least-squares non-linear regression program ELSFIT [3] run on a VAX 11/750 computer.

RESULTS AND DISCUSSION

Extraction procedure

Preliminary stability studies of tauromustine in aqueous media and plasma in vitro at various pHs have indicated that tauromustine, like other nitrosoureas [4], shows optimum stability at lower pH. Frozen plasma was therefore acidified before thawing. However, it was beneficial to raise the pH of the plasma by addition of buffer (pH 6) before extraction as well as washing the organic phase with another buffer (pH 8). This procedure minimized interference from peaks originating from endogenous plasma substances. The extraction efficiency was determined using human plasma spiked with 470 ng/ml tauromustine. Samples were extracted according to the procedure described above, except that only two thirds of the collected organic layer was evaporated. Reference solutions were prepared from mixtures of tauromustine and internal standard by evaporation and reconstitution. The recovery rate was 94.5%, with 95% confidence interval (92.3, 96.7).

Selectivity

The method was tested for the absence of interfering peaks originating from various sources. Different blank plasmas from humans were tested for endogenous components. One known metabolite [1], desmethyl tauromustine and metoclopramide, which is used as an antiemetic during treatment of patients with tauromustine, have also been tested. The metabolite is well separated and metoclopramide is not detected. The chromatograms obtained with plasma from a cancer patient before treatment and 30 min and 2 h after oral administration of tauromustine are shown in Fig. 2. The chromatograms reveal no interference.

Linearity

Plasma standards were prepared from human plasma spiked with tauromustine in the range 40–8000 ng/ml. In a typical experiment a linear relationship between the peak-height ratios of tauromustine and the internal standard versus the concentrations of tauromustine added to the plasma was described by the equation $y = 0.001649x + 0.0278$ ($r = 0.9992$). The lowest r value from sixteen calibration graphs was 0.9964.

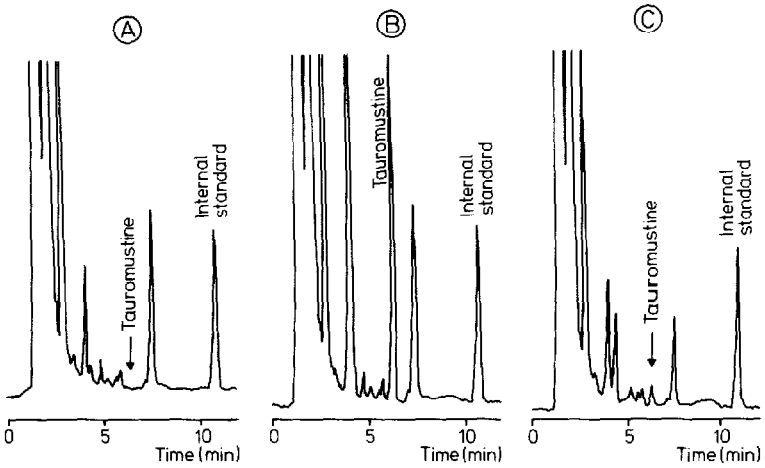

Fig. 2. Representative chromatograms obtained for plasma from a cancer patient before treatment (A) and 30 min (B) and 2 h (C) after oral administration of tauromustine (130 mg/m^2).

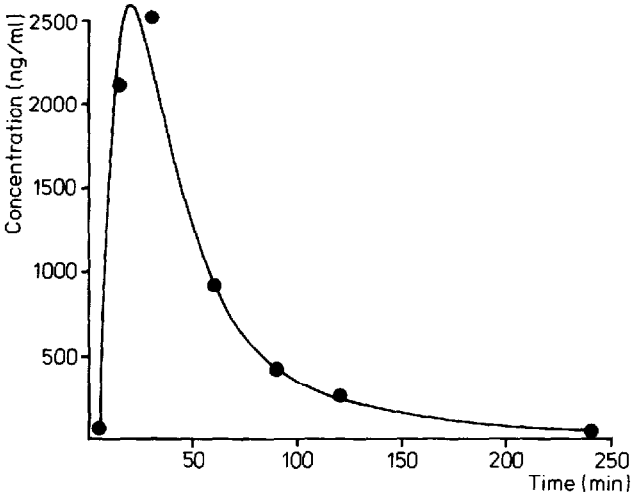

Fig. 3. Plasma concentration of tauromustine after oral administration of tauromustine (130 mg/m^2) to a patient.

Precision and detection limit

Human plasma was spiked with tauromustine at concentrations 1000 and 100 ng/ml, and eight replicates were analysed. Coefficients of variation were 1.25 and 4.1%, respectively, and the detection limit was ng/ml at a signal-to-noise ratio of 3:1.

Pharmacokinetic applications

The present method has been used to study the pharmacokinetics of tauromustine in dogs [1] and humans [5]. In dogs, tauromustine could be detected in plasma after oral (1.5 mg/kg) as well as intravenous (0.5 mg/kg) administration. The half-life was ca. 20 min. The oral absorption was rapid and the bio-

availability was ca. 26%. In cancer patients the plasma concentrations of parent tauromustine were even higher than in dogs after oral administration [5].

The data from a representative patient were further evaluated by compartmental analysis (Fig. 3). A two-compartment model with first-order absorption was best-fitted to the experimental data. The maximum concentration (C_{max}) and the time to reach C_{max} (t_{max}) were calculated as 2.6 μg/ml and 20 min, respectively. The half-life of the absorption phase was 8 min and of the elimination phases 12 and 60 min, respectively. Thus the sensitive and selective assay presented here has been successfully used in the characterization of tauromustine pharmacokinetics both in animals and humans.

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