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Note

High-performance liquid chromatographic determination of bromural in serum upon hemoperfusion

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Patients suffering from acute intoxication caused by bromural (BU) have usually been treated by symptomatic therapy. More positive therapy has been needed medically, and the application of hemoperfusion has been tried. There are few detailed reports on the effectiveness of hemoperfusion in intoxication [1—6], and none describing a comprehensive study of BU in serum upon hemoperfusion. This paper reports a rapid, precise and simple high-performance liquid chromatography (HPLC) method for the routine determination of BU in serum upon hemoperfusion.

EXPERIMENTAL

Reagents

Acetonitrile (HPLC grade), methanol (analytical grade), ethanol (analytical grade) and 3-nitro-*p*-anisidine (technical grade, 3NPA) were obtained from Wako (Osaka, Japan), and used without further purification. BU was purchased from Iwaki (Tokyo, Japan) and recrystallized from ethanol (m.p. 153—155°C).

Stock solutions

Bromural stock solution (10 mg per 100 ml) was prepared by dissolving BU in acetone. Internal standard stock solution (13 mg per 100 ml) was prepared by dissolving 3NPA in ethanol. Ten-fold and fifty-fold dilutions of these two solutions, respectively, were made before use.

Apparatus

An Hitachi 635 T liquid chromatograph equipped with a variable-wavelength detector, and also a liquid chromatograph consisting of a Kyowa Seimitsu Mini Micro Pump Type KHU 16 and a Kyowa Seimitsu variable-wavelength detector Type KLC-200, were used in this work. A Kyowa Seimitsu Universal Injector Type KHP-130 was used to introduce samples into the chromatographic system.

Column preparation

LiChrosorb RP-18 (Merck, Darmstadt, G.F.R.; particle size 5 μm) was packed into a stainless-steel column (125 mm × 4 mm I.D.) using a balance density method via a 10-ml stainless-steel packer at the rate of 800 kg/cm^2 using a Kyowa Seimitsu Ultra High Pressure Pump Type KHW-20. The efficiency of the columns was tested with a standard mixture using sodium nitrate as an unretained marker. The solvent system was methanol—water (80:20, v/v). Under these conditions, the column efficiency was 22,000 theoretical plates for benzene and 20,000 for anthracene.

Hemoperfusion

A Shaldon catheter was inserted into the femoral vein of adult dogs. The perfusion circuit had a priming volume of about 200 ml of 0.9% saline containing 10 units of heparin per ml. The charcoal columns were designed and supplied by Asahi Medical Co. and contained, on average, 200 g of bead-type activated charcoal from petroleum pitch with a 0.5-μm thick collodion coating. BU was administered orally via a stomach tube. The blood flow-rate was maintained at 60 ml/min and perfusion time was 3 h (Fig. 1).

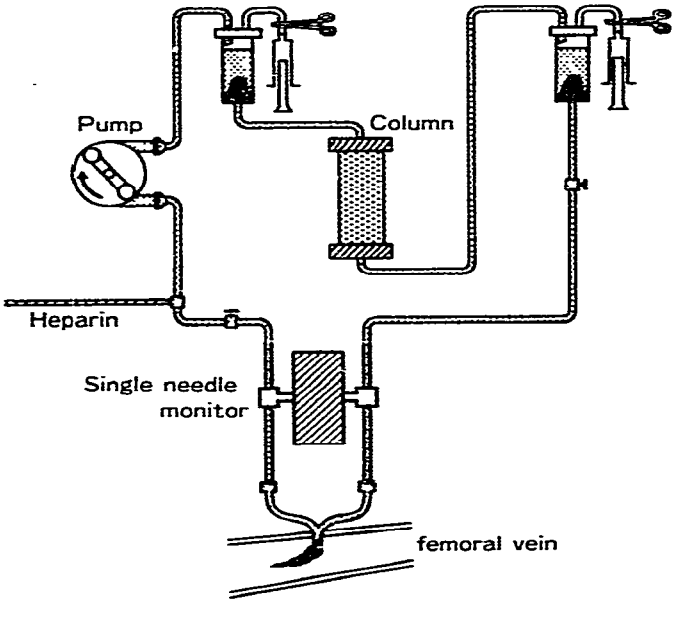

Fig. 1. The hemoperfusion circuit.

Procedure

Serum (0.1—0.5 ml) was transferred to a 10-ml glass-stoppered centrifuge tube and diluted with two volumes of ethanol containing the internal standard 3NPA (2.6 μg/ml) [7]. The serum was mixed with ethanol by stirring on a vortex mixer (60 sec) and ultrasonics (45 W, 38 kHz, 5 min). After centrifugation (1500 *g*, 5 min), a 50-μl aliquot of the upper phase was injected into the HPLC system.

A standard graph was prepared by adding BU to "blank" (no drug) dog serum so that the final concentration of BU in serum was 10—500 μg/ml.

RESULTS AND DISCUSSION

To determine the optimum extracting solvent for BU in serum, the chromatographic profile and the peak height ratio of BU/3NPA were measured on 5-μm LiChrosorb RP-18 columns with methanol, ethanol or acetonitrile. The peak height ratios for these solvents were 3.62, 3.78 and 2.42, respectively. Consequently, ethanol was chosen as the optimum extracting solvent for BU in serum.

A liquid chromatogram from dog serum 4 h after oral administration of BU is shown in Fig. 2, which corresponds to the experiment with 150 mg/kg BU in Fig. 3. BU and 3NPA eluted from the columns gave symmetrical peaks

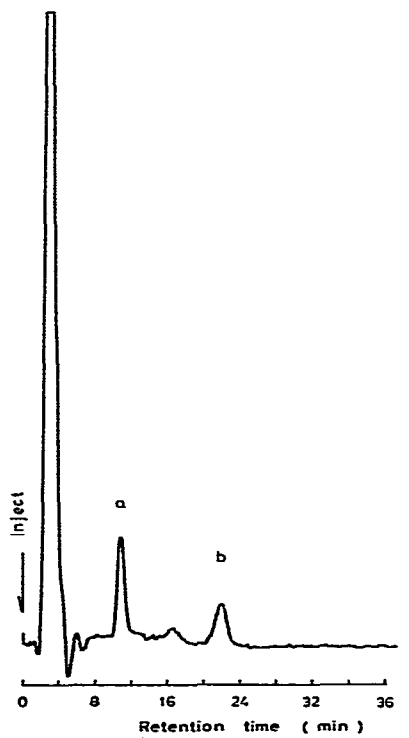

Fig. 2. Liquid chromatogram of bromural (BU) extracted from dog serum. Operating conditions: packing, LiChrosorb RP-18 (5 μm); column, 125 mm × 4 mm I.D.; mobile phase, acetonitrile—water (1:3, v/v); column temperature, ambient; flow-rate, 0.38 ml/min (80 kg/cm²); detection, 210 nm; sensitivity, 0.04 a.u.f.s.; injection volume, 50 μl. Peaks: a = BU; b = 3-nitro-*p*-anisidine (I.S.).

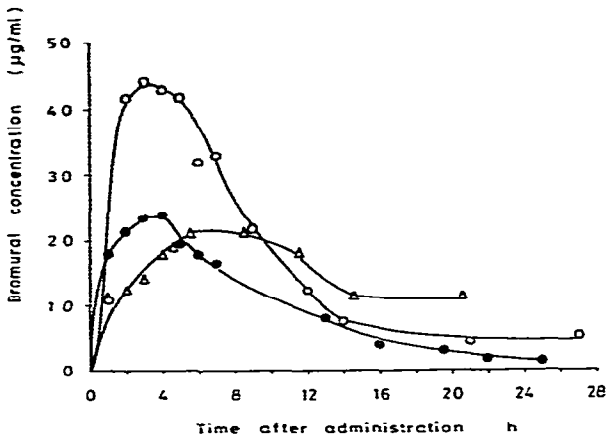

Fig. 3. Time courses of bromural (BU) concentration in serum after oral administration of BU to dog. o——o, BU 220 mg/kg; △——△, BU 220 mg/kg; •——•, BU 150 mg/kg.

with retention times of 11 and 22.5 min, respectively. Blanks were prepared from serum of BU-free subjects, and no interfering peaks were observed at the retention times of the compounds of interest.

The calibration curve of detector response vs. BU in serum was linear over the range 0.5—25 μg. The extraction of BU from serum was substantially complete within the 10—500 μg/ml range, and the reproducibility of the assay was good down to a concentration of 10 μg/ml of serum. The mean recovery from nine samples containing 10—500 μg of BU per ml was 98.3% (C.V. 1.85%).

In the experimental case without hemoperfusion, the serum level of BU in dog reached a maximum 4 h after oral administration and decreased for 24 h, except in the case of one dog administered 220 mg/kg (△ in Fig. 3), which died 21 h after the oral administration of BU.

In the experimental case with hemoperfusion, the serum level of Bu in dog was decreased dramatically during hemoperfusion (Fig. 4). A serum level rebound was observed after the end of the hemoperfusion. This suggests that the phenomenon is primarily a delayed gastrointestinal absorption.

Fig. 5 shows comparative chromatograms of BU extracted from a female patient suffering from acute BU intoxication.

CONCLUSION

The HPLC method described permits the rapid determination of BU in serum down to 10 μl/ml. The preparation of serum samples prior to chromatography is simple, only requiring a single-step extraction. No derivatization procedure is necessary. The precision of the method is good and no interfering peaks are seen with serum. Only a small sample size is needed for analysis, and this method is therefore suitable for the routine clinical monitoring of serum levels of BU in patients and for use in research studies involving pharmacokinetics and bioavailability.

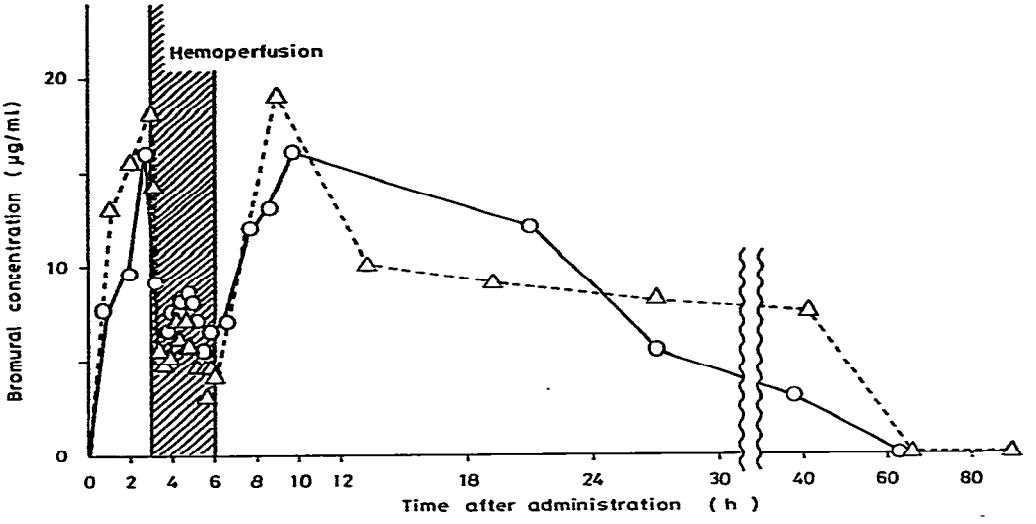

Fig. 4. Time courses of bromural (BU) concentration in serum upon hemoperfusion. △———△, BU 200 mg/kg; ○———○, BU 220 mg/kg.

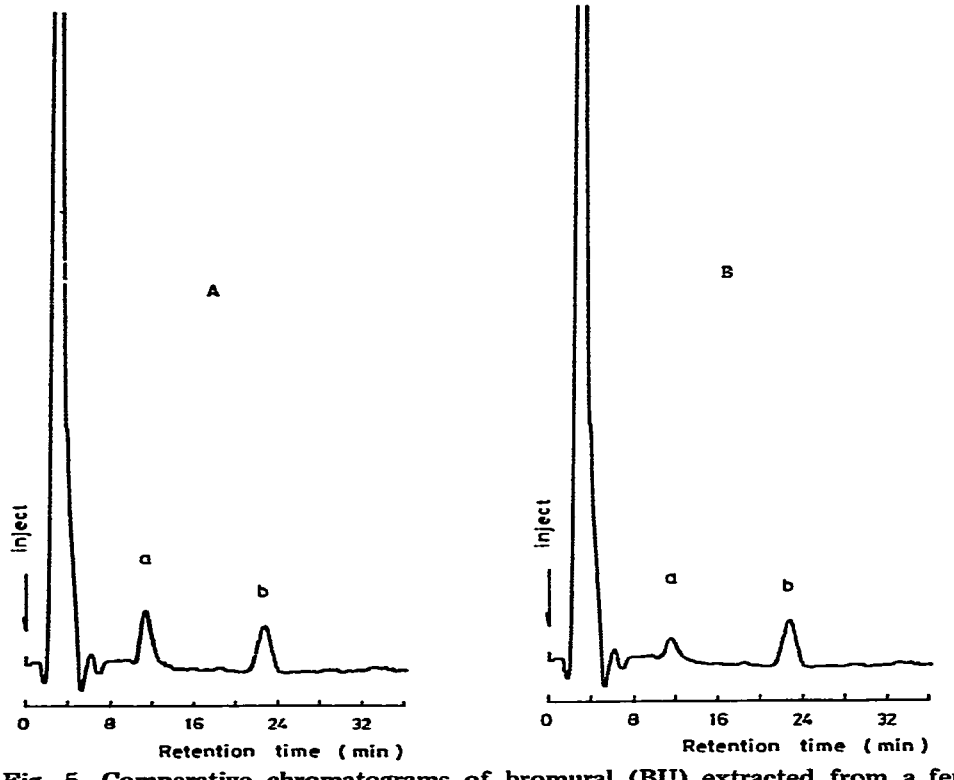

Fig. 5. Comparative chromatograms of bromural (BU) extracted from a female patient suffering acute intoxication. (A) during hemoperfusion, (B) after hemoperfusion. Peaks: a = BU, b = 3-nitro-*p*-anisidine (I.S.). Chromatographic conditions as shown in Fig. 2.

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