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Simultaneous determination of berberine in rat blood, liver and bile using microdialysis coupled to high-performance liquid chromatography

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Abstract

High-performance liquid chromatography coupled to microdialysis was used for the simultaneous determination of unbound berberine in rat blood, liver and bile for a pharmacokinetic study. Microdialysis probes were simultaneously inserted into the jugular vein toward the right atrium, the median lobe of the liver, and the bile duct of male Sprague–Dawley rats for biological fluid sampling after administration of berberine (10 mg/kg) through the femoral vein. Berberine and dialysates were separated using a Zorbax SB-phenyl column and a mobile phase comprised of acetonitrile–methanol–20 m*M* monosodium phosphate (pH 3.0) (35:20:45, v/v) together with 0.1 m*M* 1-octanesulfonic acid. The detection limit for berberine was 10 ng/ml. The concentration–response relationship was linear (r^2>0.995) over the concentration range 0.05–50 μg/ml; intra-assay and inter-assay precision and accuracy for berberine fell within predefined limits. The disposition of berberine in the blood, liver and bile fluid suggests that berberine might be metabolized in the liver and undergo hepatobiliary excretion.

Keywords: Microdialysis; Berberine; Alkaloids

1. Introduction

Berberine (Fig. 1), a well-known alkaloid found in medicinal herbals such as *Coptis chinensis* and *Hydrastis canadensis*, has commonly been used in Oriental medicine as an intestinal antiseptic [1] via oral and parenteral administration. Currently, besides its significant antimicrobial activity [2], other varied pharmacological effects have been reported such as anticancer [3], immunosuppression [4], anti-inflammatory [5], as well as vasorelaxant and antiproliferative effects [6]. Several methods have been reported for the qualitative and quantitative analysis of berberine, including thin-layer chromatography (TLC) [7,8], capillary zone electrophoresis (CE) [9,10], high-performance liquid chromatography (HPLC)

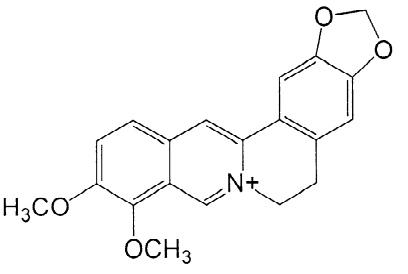

Fig. 1. Chemical structure of berberine.

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[11–14] and capillary electrophoresis–electrospray ion trap mass spectrometry (CE–ESI-MS) [15,16]. In addition, several assay methods have been used to examine the disposition of berberine or quantify its plasma concentration from different dosage regimens. For example, berberine absorption and distribution in rats [17] and rabbits [18] by non-specific UV spectrophotometric and fluorimetric assays, berberine absorption in rats [19] and human subjects [20] using tritium-labeled berberine or by gas chromatography–chemical ionization mass spectrometry, and determination of berberine in rats [21], rabbits [22] and human subjects [23] by HPLC have been reported. However, few reports have presented detailed pharmacokinetic information about berberine.

The aim of this investigation was to study the pharmacokinetics, liver distribution and hepatobiliary excretion of berberine after single i.v. bolus administration in rats using microdialysis. To achieve this, three microdialysis probes were inserted into the blood, liver and bile of a rat for simultaneous biological fluid sampling and analysis by HPLC.

2. Experimental

2.1. Animals

Male, specific pathogen-free Sprague–Dawley rats were obtained from the Laboratory Animal Center of the National Yang-Ming University, Taipei. The animals had free access to food (Laboratory Rodent Diet No. 5P14, PMI Feeds, Richmond, IN, USA) and water until 18 h prior to being used in experiments, at which time food was removed. Six Sprague–Dawley rats (280–320 g) were initially anesthetized with urethane 1 g/ml and α-chloralose 0.1 g/ml (1 ml/kg, i.p.), and remained anesthetized throughout the experimental period. The femoral vein was exposed for further drug administration. The rat's body temperature was maintained at 37 °C with a heating pad.

2.2. Chromatography

HPLC-grade solvents and reagents were obtained from E. Merck (Darmstadt, Germany). Triply deionized water (Millipore, Bedford, MA, USA) was used for all preparations. The HPLC system consisted of a chromatographic pump (BAS PM-80, West Lafayette, IN, USA), an injector (CMA 160, Stockholm, Sweden) equipped with a 20 μl sample loop, and an ultraviolet detector (Varian, Walnut Creek, CA, USA). Berberine and dialysates were separated using a Zorbax SB-phenyl column (150×4.6 mm I.D.; particle size 5 μm) maintained at ambient temperature. The mobile phase was comprised of acetonitrile–methanol–20 m*M* monosodium phosphate (pH 3.0) (35:20:45, v/v) and 0.1 m*M* 1-octanesulfonic acid, and the flow-rate of the mobile phase was 1 ml/min. The buffer was filtered through a Millipore 0.45 μm filter and degassed prior to use. The UV detector was set at 346 nm and connected to an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

2.3. Method validation

All calibration curves were required to have a correlation coefficient of at least 0.995. The intra-assay and inter-assay variabilities were determined by quantitating six replicates on the same day and six consecutive days, respectively [24] (Table 1). The

Table 1
Intra-assay and inter-assay precision (RSD) and accuracy (bias) of the HPLC method for the determination of berberine

Nominal conc. (μg/ml)	Observed conc.[a] (μg/ml)	RSD (%)	Bias (%)
Intra-assay			
0.05	0.055±0.003	5.5	10.0
0.10	0.105±0.005	4.8	5.0
0.50	0.522±0.017	3.2	4.4
1.00	1.02±0.03	2.9	2.0
5.00	5.06±0.12	2.4	1.2
10.00	10.12±0.21	2.1	1.2
50.00	50.31±0.64	1.3	0.6
Inter-assay			
0.05	0.048±0.005	10.4	−4.0
0.10	0.104±0.006	5.8	4.0
0.50	0.514±0.019	3.7	2.8
1.00	0.99±0.03	3.0	−1.0
5.00	5.02±0.04	0.8	0.4
10.00	9.97±0.09	0.9	−0.3
50.00	50.01±0.02	0.04	0.02

[a] Data expressed as mean±SEM ($n=6$). SEM, standard error of the mean.

accuracy (bias) was calculated from the nominal concentration (C_{nom}) and the mean value of observed concentrations (C_{obs}) as follows:

$$\text{bias}\ (\%) = [(C_{nom} - C_{obs})/C_{nom}] \cdot 100$$

The relative standard deviation (RSD) was calculated from the observed concentrations as follows:

$$\text{RSD}\ (\%) = [\text{standard deviation (SD)}/C_{obs}] \cdot 100$$

2.4. Microdialysis experiment

Blood, liver and bile microdialysis systems consisted of a CMA/100 microinjection pump (CMA, Stockholm, Sweden), microdialysis probes and a CMA/140 fraction collector. The dialysis probes for blood and liver (10 mm in length) and bile (7 cm in length) were made of silica capillary using a concentric design with their tips covered by dialysis membrane (Spectrum, 200 μm inner diameter with a nominal molecular mass cut-off of 13 000, Laguna Hills, CA, USA) [25–28]. The blood and liver microdialysis probes were positioned within the jugular vein toward the right atrium and the median lobe near the center of the liver, respectively. The microdialysis systems were perfused with anticoagulant citrate dextrose, ACD solution (citric acid, 3.5 m*M*; sodium citrate, 7.5 m*M*; dextrose, 13.6 m*M*) at a flow-rate of 3 μl/min.

The bile duct microdialysis probe was constructed in our laboratory [28] based on the design originally described by Scott and Lunte [29] and Hadwiger et al. [30]. A 7-cm dialysis membrane was inserted into polyethylene tubing (PE-60; 0.76 mm I.D.×1.22 mm O.D., Clay-Adams, NJ, USA). The ends of the dialysis membrane and PE-60 were inserted into silica tubing (40 μm I.D.×140 μm O.D., SGE, Australia) and PE-10 (0.28 mm I.D.×0.61 mm O.D.), respectively. Both ends of the tubing and the union were cemented with epoxy, which was allowed to dry for at least 24 h. For post-bile duct cannulation, the microdialysis probe was then perfused with Ringer's solution at a flow-rate of 3 μl/min.

2.5. Drug administration

After a 2 h post-surgical stabilization period subsequent to the implantation of probes, berberine (10 mg/kg, i.v.) was administered via the femoral vein in an injection volume of 1 ml/kg. The dialysates from the blood, liver and bile were connected into a fraction collector. The sampling interval was 10 min for each probe. Blood, liver and bile dialysates were immediately measured by a validated HPLC system.

2.6. Recovery of microdialysate

ACD solution or Ringer's solution containing berberine was passed through the microdialysis probe at a constant flow-rate (3 μl/min) and the perfusate (C_{perf}) and dialysate (C_{dial}) concentrations of berberine were determined by HPLC. The in vivo relative recovery (R_{dial}) of berberine across the microdialysis probe in rat blood, liver and bile duct was calculated from the following equation [31]:

$$R_{dial} = (C_{perf} - C_{dial})/C_{perf}$$

Berberine microdialysate concentrations (C_m) were converted into unbound concentrations (C_u) as follows [31]:

$$C_u = C_m/R_{dial}$$

Pharmacokinetic curves of berberine in rat blood, liver and bile were constructed by individual sets of data using a logarithmic scale. The elimination half-life ($t_{1/2}$) of berberine in blood, liver and bile was calculated from the slope (k) of this semilog graph as $t_{1/2} = 0.693/k$. The areas under the concentration curves (AUCs) were calculated by the linear trapezoid method as

$$\text{AUC} = \text{AUC}_{last} + C_{last}/\lambda_z$$

where C_{last} and λ_z are the last observed concentration and slope.

3. Results and discussion

Typical chromatograms of berberine are shown in Fig. 2. The retention time of berberine was 3.1 min. In order to improve the symmetry of the berberine peak, 0.1 m*M* 1-octanesulfonic acid was added to the mobile phase. Fig. 2A shows a standard injection of

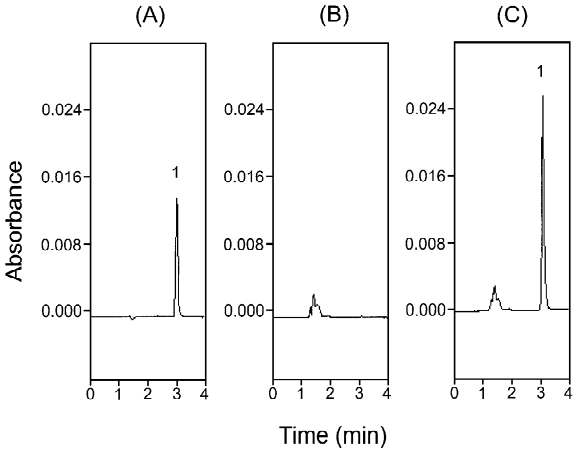

Fig. 2. Typical chromatograms of (A) standard berberine (1 μg/ml), (B) blank blood dialysate from the microdialysis probe before drug administration, and (C) blood dialysate sample containing berberine (1.81 μg/ml) collected 10 min after berberine administration. 1 = berberine.

berberine (1.0 μg/ml), and Fig. 2B shows the chromatogram of a blank blood dialysate. None of the observed peaks interfered with the analyte. Fig. 2C shows the chromatogram of a blood dialysate sample containing berberine (1.81 μg/ml) collected 10 min after berberine administration.

Fig. 3A shows a standard injection of berberine (0.1 μg/ml), and Fig. 3B shows the chromatogram of a blank liver dialysate. Fig. 3C shows the chromatogram of a liver dialysate sample containing berberine (0.34 μg/ml) collected 20 min after berberine administration. Similarly, none of the observed peaks interfered with the analyte in the chromatogram of the liver sample.

Fig. 4A shows a standard injection of berberine (10.0 μg/ml), and Fig. 4B shows the chromatogram of a blank bile dialysate. None of the observed peaks interfered with the analyte. Fig. 4C shows the chromatogram of the bile dialysate sample containing berberine (19.21 μg/ml) collected 20 min after berberine administration. In addition, an unidentified peak was found in the rat liver and bile dialysates; it may correspond to the metabolite of berberine.

The calibration curve of berberine was obtained prior to HPLC analysis of the dialysates over the concentration range 0.05–50 μg/ml. The concentration of berberine was linearly related to the peak areas in the chromatogram ($r^2 > 0.995$). The detection limit of berberine was 10 ng/ml at a signal-to-noise ratio of 3.

As shown in Table 1, the overall RSD ranged from 0.04 to 10.4%. Analytical accuracy, expressed

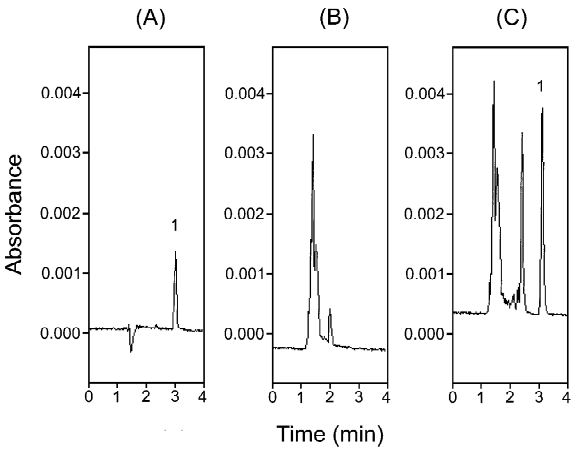

Fig. 3. Typical chromatograms of (A) standard berberine (0.1 μg/ml), (B) blank liver dialysate from the microdialysis probe before drug administration, and (C) liver dialysate sample containing berberine (0.34 μg/ml) collected 20 min after berberine administration. 1 = berberine.

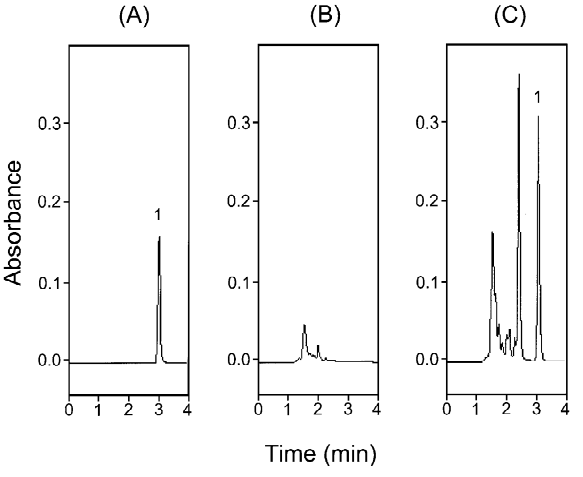

Fig. 4. Typical chromatograms of (A) a standard berberine (10 μg/ml), (B) a blank bile dialysate from the flow-through microdialysis probe before drug administration, and (C) a bile dialysate sample containing berberine (19.21 μg/ml) collected 20 min after berberine administration. 1 = berberine.

as the bias, varied from −4.0 to 10.0%. Thus, the intra-assay and inter-assay accuracy and precision were found to be acceptable for the analysis of a dialysis sample in support of pharmacokinetic studies. From Table 2, the average in-vivo recovery ($n=6$) of berberine in blood (0.5, 1 and 5 μg/ml), liver (0.5, 1 and 5 μg/ml), and bile (1, 5 and 10 μg/ml) was 33.5±2.5, 22.5±2.0 and 65.9±2.1%, respectively. Hence, the actual concentration of berberine in rat blood, liver and bile could be corrected by the respective recoveries.

The concentration versus time curves of berberine in rat blood, liver and bile are shown in Fig. 5, indicating that the disposition of berberine in rat bile has a slower and longer elimination phase. In addition, both the bile and liver graphs have a peak concentration at 20 min after berberine administration. On the whole, the concentration of berberine in rat bile was significantly higher than that in blood and liver.

With regard to the pharmacokinetic parameters, the elimination half-life ($t_{1/2}$) of berberine in blood, liver and bile was 12.5±1.6, 29.4±5.7, and 160.0±16.5 min, respectively. Furthermore, the blood-to-bile distribution ratio (AUC_{bile}/AUC_{blood}) of berberine was 7.2. These results suggest that the hepatobiliary excretion of berberine may undergo active transportation against a concentration gradient. In addition, it is suspected that liver metabolism causes the AUC of berberine in liver (70±14 min μg/ml) to be less than that in blood (291±59 min μg/ml). Both of the above results are consistent with the previous report indicating that biliary excretion of berberine may be one of the major elimination pathways after parenteral administration [22]. But the amount of berberine eliminated or in plasma is a small part of that administered, and it is doubtful that the remaining berberine is metabolized [22,23]. Further investigation on this question is currently underway.

Table 2
In vivo microdialysate recoveries of berberine from rat blood, liver and bile

Concentration (μg/ml)	Recovery (%)
In rat blood	
0.5	33.6±1.5
1	33.5±3.0
5	32.5±2.8
In rat liver	
0.5	22.3±1.1
1	23.4±2.2
5	21.8±2.5
In rat bile	
1	65.1±1.5
5	66.0±2.6
10	66.8±2.1

Data expressed as mean±SD ($n=6$).

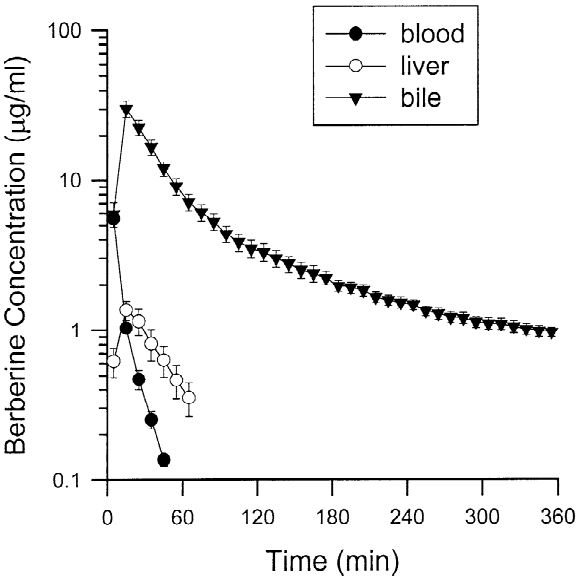

Fig. 5. Mean unbound levels of berberine in rat blood, liver and bile after berberine administration.

In conclusion, we have developed a specific, sensitive, endogenous interference-free, and economical microdialysis sampling method for the determination of protein-unbound berberine in rat blood, liver and bile. The data obtained suggest that berberine may be excreted in the bile.

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