Determination of Dibucaine and Its Metabolites in Human Urine by High-Performance Liquid Chromatography with Fluorescence Detector

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A high-performance liquid chromatographic (HPLC) method using a fluorescence detector is described for the simultaneous determination of dibucaine and its metabolites (M-4, M-8 and M-10) in human urine. Urine samples from obstetric patients were chromatographed in a reversed-phase system. When a ultraviolet detector (320 nm) was used, some interfering peaks appeared on the chromatogram, but this interference could be overcome by employing a fluorescence detector (Ex 330 nm and Em 440 nm) instead. The calibration curves were linear in the range of 0.05—5.0 µg/ml for all compounds and the detection limits of dibucaine and its metabolites were about 5 ng/ml in urine. The urinary excretion of dibucaine and its metabolites by obstetric patients infused with Percamine S® in the spinal cord were determined. The mean cumulative amounts of dibucaine, M-4, M-8 and M-10 excreted during 10 h after administration were 1.1, 10.5, 3.5 and 1.1% of the dose, respectively. The total urinary excretion was 16.2% of the dose. This method is sufficiently sensitive and specific to permit the determination of dibucaine and its metabolites in biological fluids.

Keywords—dibucaine; metabolite; human urine; spinal anesthesia; HPLC; fluorescence detector; Percamine S®; urinary excretion; metabolism

Dibucaine is a potent local anesthetic used for relief of pain or for spinal anesthesia. This drug has occasionally caused unsuspected death because it is highly toxic. From the viewpoint of clinical toxicology it would be useful to determine dibucaine concentration in the biological fluids and to elucidate the metabolism of dibucaine in order to minimize the risks.

The analysis of dibucaine in the biological fluids is complicated by the low dose levels employed. A specific and sensitive method for the analysis of dibucaine is therefore needed. Several methods for the micro-determination of dibucaine alone in the biological fluids by gas chromatography (GC) or gas chromatography–mass spectrometry (GC-MS) have been reported,1–4) and the analysis of dibucaine and its metabolites by high performance liquid chromatography (HPLC) is also possible.5,6)

In the previous study,7) we showed that in humans dibucaine is metabolized to six basic metabolites, of which the 2-hydroxyethoxy (M-8), O-debutyl (M-4) and α-1 hydroxy (M-10) compounds are the major ones (Fig. 1). The present work describes a sensitive and specific assay for the determination of dibucaine and its metabolites in urine by HPLC with a fluorescence detector, and its application to the analysis of urine from obstetric patients infused with Percamine S® in the spinal cord.

Experimental

Chemicals and Reagents—Dibucaine hydrochloride was purchased from Teikoku Chemical Industry Co., Ltd. (Osaka, Japan). Metabolites of dibucaine, O-debutyl (M-4), 2-hydroxyethoxy (M-8) and α-1 hydroxy (M-10)
compounds, and the internal standard (I.S.), 2-butoxy-N-(2-dimethylaminoethyl)cinchoninamide were prepared as described previously (Fig. 1). All other solvents and reagents used were of analytical-reagent grade.

**Apparatus and Chromatographic Conditions**—A Shimadzu LC-3A high-performance liquid chromatograph was used. The ultraviolet (UV) detector (Shimadzu SPD-2A) was set at 320 nm. The excitation (Ex) and the emission (Em) wavelengths of the fluorescence spectrometer (Shimadzu RF-530) were 330 and 440 nm, respectively. The column used was a Cosmosil® 5 C18 (15 cm x 4.6 mm i.d., Nakarai Chemical Co., Japan). The mobile phase was consisted of methanol–water (60:40, v/v) containing 30 mm triethylamine, the pH being adjusted to 7.5 with acetic acid. The mobile phase was passed through the column at a flow-rate of 1 ml/min.

**Extraction Procedure**—A urine sample (4 ml) was adjusted to pH 11 with conc. NH₄OH, and then 0.2 ml of the I.S. solution (20 µg/ml) and 5 ml of ethyl acetate were added. The mixture was vigorously shaken for 1 min and centrifuged. After phase separation, the organic layer was back-extracted by shaking with 2 ml of 0.5 N HCl. The aqueous layer was made alkaline with conc. NH₄OH and reextracted with 5 ml of dichloromethane. The organic layer was evaporated to dryness, and the residue was dissolved in 0.1 ml of the mobile phase. A 10 µl sample was injected into the HPLC column.

**Clinical Study**—Urine samples were taken from seven obstetric patients (25—33 years old) infused with 2 ml of Percamine S® (containing 0.3% dibucaine) in the spinal cord for the surgical treatment; urine was collected every 2 h up to 10 h after dosing. The urine samples were stored at -20°C until analysis.

**Results and Discussion**

Typical chromatograms of urine samples from obstetric patients infused with Percamine S® in the spinal cord are shown in Fig. 2. Although they differed from sample to sample, some unidentified peaks were observed on the chromatogram when the UV detector (320 nm) was used and interfered with the simultaneous determination of dibucaine and its metabolites (Fig. 2A). These interfering peaks were not seen in the urine of healthy volunteers or patients before surgical treatment. Therefore, they may have arisen from administered drug(s) other than dibucaine. In order to overcome this problem, we carried out the HPLC analysis with a fluorescence detector. The HPLC analysis of dibucaine using a fluorescence detector has been reported by Takeoka et al. However, the method was directed to the analysis of dibucaine in injectable ampoules. We used the fluorescence detector for the simultaneous analysis of dibucaine and its metabolites. From the viewpoint of sensitivity and specificity of analysis, the detection wavelengths were set at Ex 330 nm and Em 440 nm. As shown in Fig. 2B, we were able to minimize the interference by exogenous substances in the urine samples under clinical conditions.

The peak height ratios of authentic samples of dibucaine and its metabolites with respect to the I.S. were linearly related (r > 0.995) with the concentrations in urine in the range from 0.05 µg/ml to 5.0 µg/ml. The calibration curves showed little day-to-day variability in slopes and intercepts (CVs below 5%). The minimum detectable amounts of dibucaine and its metabolites in urine were calculated to be about 5 ng/ml with a signal-to-noise ratio of 5:1. As compared with the UV detector, the use of the fluorescence detector offered a considerable sensitivity enhancement in the detection of dibucaine and its metabolites (10 times for M-4, 6 times for M-8, 4 times for M-10 and dibucaine).

The recoveries of known concentrations (0.25 and 2.5 µg/ml) of dibucaine and its
metabolites from blank human urine were determined by comparing the peak heights of the extracted compounds to those obtained from aqueous standard solutions injected directly. The mean recoveries of dibucaine, M-4, M-8 and M-10 were approximately 98%, 76%, 90% and 94%, respectively (n=5). The CV values were below about 5%. The recovery of M-4 was relatively poor, probably due to higher polarity.

The reproducibility of the method was also assessed by the repeated analysis of urine samples from obstetric patients infused with Percamine S® in the spinal cord. As shown in Table I, the CV values were satisfactory for all compounds (below 7%). Thus, the method was proved to be reproducible.

Using this method, we determined the urinary excretion of dibucaine and its metabolites by obstetric patients infused with Percamine S® in the spinal cord. In Table II the urinary excretion of dibucaine and its metabolites during 10 h after administration is presented. In the early period a small amount of dibucaine was observed (about 0.02 mg), and thereafter the amount was very small. On the other hand, metabolites were excreted at an approximately constant rate during 10 h after dosing. Ranges of cumulative excreted M-4 and M-8 in seven patients were 5.2—19.9% and 2.2—5.6% of the dose, respectively. Dibucaine and M-10 were excreted in the ranges of 0.3—3.2% and 0.6—1.7%, respectively. The range of total urinary excretion was 9.1—28.3%, with an average of 16.2% of the dose. These values are reasonably consistent with the findings in male healthy volunteers receiving dibucaine orally (10 mg); total excretion during 9 h after dosing, averaged 12.6% of the dose.6) Fukui has also reported that only 1—2% of the dose was excreted as unchanged drug in the 0—12 h urine of patients infused with Percamine L® (containing 0.5% dibucaine) in the spinal cord.9) From these results, we consider that dibucaine is rapidly and extensively metabolized in humans.

In conclusion, we have developed a sensitive and specific method to determine dibucaine and its metabolites in urine under clinical conditions.
TABLE II. Urinary Excretion of Dibucaine and Its Metabolites in Seven Obstetric Patients Infused with Percamine S® in Spinal Cord

<table>
<thead>
<tr>
<th>Collection time (h)</th>
<th>M-4</th>
<th>M-8</th>
<th>M-10</th>
<th>Dibucaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0— 2</td>
<td>0.041</td>
<td>0.011</td>
<td>0.007</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>(0.011—0.104)</td>
<td>(0.006—0.025)</td>
<td>(0.002—0.017)</td>
<td>(0.004—0.037)</td>
</tr>
<tr>
<td>2— 4</td>
<td>0.116</td>
<td>0.036</td>
<td>0.019</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>(0.042—0.361)</td>
<td>(0.015—0.114)</td>
<td>(0.009—0.051)</td>
<td>(0.004—0.070)</td>
</tr>
<tr>
<td>4— 6</td>
<td>0.109</td>
<td>0.042</td>
<td>0.015</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>(0.020—0.186)</td>
<td>(0.008—0.092)</td>
<td>(0.004—0.032)</td>
<td>(0.002—0.065)</td>
</tr>
<tr>
<td>6— 8</td>
<td>0.174</td>
<td>0.069</td>
<td>0.018</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>(0.047—0.460)</td>
<td>(0.022—0.072)</td>
<td>(0.007—0.033)</td>
<td>(0.001—0.025)</td>
</tr>
<tr>
<td>8—10</td>
<td>0.090</td>
<td>0.043</td>
<td>0.010</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>(0.016—0.180)</td>
<td>(0.007—0.075)</td>
<td>(0.001—0.021)</td>
<td>(0.001—0.027)</td>
</tr>
<tr>
<td>0—10</td>
<td>0.528</td>
<td>0.201</td>
<td>0.068</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>(0.260—0.997)</td>
<td>(0.125—0.321)</td>
<td>(0.035—0.104)</td>
<td>(0.015—0.197)</td>
</tr>
</tbody>
</table>

Mean values are given with ranges in parentheses (n = 7).

References

6) K. Igarashi, F. Kasuya, and M. Fukui, submitted for publication in J. Pharm. Sci.,