Determining Zinc Coproporphyrin in Maternal Plasma—a New Method for Diagnosing Amniotic Fluid Embolism

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We measured the concentration of zinc coproporphyrin I (ZnCP-I), a characteristic component of meconium, in maternal plasma by fluorometry after HPLC. We obtained plasma samples from 89 women: 35 at weeks 10–40 of normal pregnancy, 41 shortly after normal delivery, 4 from patients with amniotic fluid embolism (AFE), and 9 from non-AFE patients with intra- or postpartum shock caused by genital bleeding. The plasma ZnCP-I concentration was 97 (SD 83, range 38–240) nmol/L in the AFE patients, 11 (SD 9.2) nmol/L in the non-AFE patients, 12 (SD 7.9) nmol/L during normal pregnancy, and 26 (SD 10) nmol/L shortly after normal delivery. We suggest that measuring ZnCP-I in maternal plasma by fluorometry on HPLC is a rapid, noninvasive, and sensitive method for diagnosing AFE and propose 35 nmol/L as the cutoff value for the ZnCP-I concentration in maternal plasma for the diagnosis of AFE.

Additional Keyphrases: pregnancy • fluorometry • meconium • maternal health

Amniotic fluid embolism (AFE) is a rare cause of sudden maternal death during or shortly after delivery (1–3). Entry of amniotic fluid into the maternal bloodstream may induce AFE, and several studies have sought the substance in amniotic fluid responsible for AFE (4–7). Therefore, a simple, rapid, and noninvasive method for diagnosing AFE would be desirable.

Recently, we reported (8) that coproporphyrins in meconium are complexed with various metals and that their complexed with Zn2+ are fluorescent. Moreover, the fluorescence of zinc coproporphyrins (ZnCPs), especially zinc coproporphyrin I (ZnCP-I), is a characteristic of meconium (8, 9). Therefore, using fluorometry after HPLC, we investigated ZnCP-I concentrations in amniotic fluid and maternal plasma.

Materials and Methods

Specimens

We obtained plasma samples from 89 women: 35 samples at weeks 10–40 of normal pregnancy; 41 samples shortly after normal delivery (in the second stage of labor); 9 samples from non-AFE patients with intra- or postpartum shock caused by genital bleeding (3 with rupture of uterus, 2 with cervical laceration, and 1 each with abruptio placentae, placenta previa, abortive bleeding, or unknown reason); 3 samples from patients with AFE diagnosed at autopsy, and 1 sample from a patient showing AFE-like symptoms.

Using an intra-uterine catheter, we obtained specimens of clear amniotic fluid (n = 12) and meconium-stained amniotic fluid (n = 6) during normal deliveries. We could not obtain amniotic fluid from AFE patients because they were asymptomatic until after delivery.

AFE Patients

The four plasma samples from AFE patients were obtained from three university hospitals and a medical center in Japan between January 1988 and June 1991. (We are in contact with 10 university hospitals and the medical center to obtain plasma from patients with AFE-like symptoms as part of our government’s project, Reduction of Maternal Mortality.) Therefore, we believe that we have samples from the AFE cases from more than 10^6 deliveries during this period.

All the patients experienced sudden intrapartum hypoxia, hypotension, and cardiovascular collapse. Subsequent investigation excluded the possibility of confounding cardiovascular-, septic-, or anesthesia-related conditions. Characteristics of the four patients are shown in Table 1. In three cases AFE was confirmed by the postmortem finding of fetal squamous cells in the lung. The one case in which cure was achieved by supportive care is reported below.

Case History. A 26-year-old woman, gravida 1, was admitted to hospital in the third trimester because of preeclampsia (gestosis index = 9). She was treated by diet therapy and bed rest, but her blood pressure increased to 190/90 mmHg. A nonstress test showed late deceleration with loss of variability. From ultrasound examination, abruptio placentae was suspected, and a

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**Table 1. Data on Patients with Amniotic Fluid Embolism**

<table>
<thead>
<tr>
<th>Age</th>
<th>Delivery and weeks of gestation</th>
<th>Symptoms</th>
<th>Time of blood sampling, h</th>
<th>ZnCP-I in plasma, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>y</td>
<td>Para</td>
<td>Symptom</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>Normal, 39</td>
<td>Dyspnea, shock</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>CS, 37</td>
<td>Chest pain, shock</td>
<td>6</td>
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<tr>
<td>3</td>
<td>34</td>
<td>Normal, 40</td>
<td>Dyspnea, shock</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>CS, 33</td>
<td>Bleeding, shock</td>
<td>15</td>
</tr>
</tbody>
</table>

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CS, cesarean section.

* Hours after onset of shock.

* Dead.

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cesarean section was promptly performed. Grade I premature separation was recognized. One hour after the operation, she suddenly suffered dyspnea and massive genital bleeding and became unresponsive. Her blood pressure was 40/20 mmHg and pulse rate was 140/min. She was resuscitated with oxygen and dopamine and referred to our clinic. We performed intubation, blood transfusion, and Swan–Ganz catheterization. Simultaneously, we obtained plasma and pulmonary blood samples and detected fetal squamous cells in these samples. Disseminated intravascular coagulation, pulmonary edema, left heart failure, liver dysfunction, and renal failure developed. We controlled the circulation and respiration, began hemodialysis, and began treatment with antithrombin III for disseminated intravascular coagulation; gradually, her condition improved. After a 28-day hospital stay, her renal function had recovered appreciably and she was discharged.

Reagents

CP-I dihydrochloride was purchased from Porphyrin Products (Logan, UT 84321). HPLC-grade acetonitrile, reagent-grade acetic acid, and other reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan).

Procedures

Extraction of ZnCP-I from meconium. Dissolve 1 g of fresh meconium in 1 mL of dimethyl sulfoxide and stir vigorously in a vortex-type mixer for 1 min with 5 mL of 20 mmol/L phosphate buffer, pH 7.4, containing NaCl, 9 g/L (phosphate-buffered saline, PBS). Centrifuge the mixture at 10 000 × g for 20 min and filter the supernate through a membrane filter (0.45-μm pore size).

High-performance liquid chromatography of ZnCP-I. The HPLC system consisted of a Model Tri Rotor-V pump (Jasco, Tokyo, Japan), a Model 7225 injector (Rhododyne, Berkeley, CA 94710) equipped with a 100-μL sample loop, a spectrophotometer (Model FP-210; Jasco), and a data processor (Model DP-L230; Jasco). For ZnCP-I analysis we used an 8 mm × 10 cm column prepacked with Radial-Pak C18 (10-μm particle size; Waters Associates, Inc., Milford, MA 01757), and a mobile phase of acetonitrile/potassium phosphate, 50 mmol/L (1/5 by vol), pH 6.8. The flow rate was 2.0 mL/min at room temperature. The excitation and emission wavelengths were 405 and 580 nm, respectively.

Synthesis of ZnCP-I. CP-I dihydrochloride (4.9 mg; 6.7 μmol) was dissolved in 2.0 mL of dimethyl sulfoxide and mixed with 0.3 mL of aqueous zinc acetate (1.5 mg; 6.8 μmol). A mixture of equal volumes of 10 mmol/L acetic acid and n-butanol was added, and ZnCP-I was extracted into the organic layer. Removal of the solvent under reduced pressure yielded ~3.8 mg of ZnCP-I.

Atomic absorption spectrophotometry. We determined atomic absorption spectra with a Model 581 atomic absorption spectrophotometer (Hitachi, Tokyo, Japan) that had a hollow cathode lamp for zinc analysis with an absorption line at 213.8 nm.

Assay of ZnCP-I content of plasma and amniotic fluid.

We thawed samples of plasma or amniotic fluid that had been stored at -80 °C and centrifuged them at 1000 × g for 15 min. The supernate was filtered through a Minisart syringe filter (0.45-μm pore size; Sartorius GmbH, Göttingen, F.R.G.). ZnCP-I concentration in the filtrate (100 μL per filtrate) was assayed by HPLC with synthetic ZnCP-I as the standard. The concentration of ZnCP-I in a standard solution was determined from the concentration of zinc, measured by atomic absorption spectrophotometry. The minimum detectable amount of ZnCP-I was ~0.6 pmol (signal-to-noise ratio = 2) per 100 μL of sample injected under our experimental conditions.

Results

Recently we showed that the sharp fluorescence peak at 580 nm (excitation at 405 nm) in an extract of meconium in PBS is due to ZnCP-I (Figure 1A) and that this is a characteristic fluorescence component of meconium (8, 9). Figure 1B shows a typical fluorometric pattern on HPLC of a solution of CP-I in PBS (absorbance 10.1 at 392 nm) mixed with an equal volume of 100 μmol/L zinc nitrate in PBS and monitored by emission at 580 nm or at 620 nm (excitation at 405 nm). In humans, ZnCP-I has been reported only in urine (10), so we tested for it in human plasma. A typical fluorometric pattern on HPLC of plasma obtained from a nonpregnant woman is shown in Figure 2A. We did not detect ZnCP-I in any samples unrelated to pregnancy, but we did detect a small ZnCP-I peak in plasma of women during normal pregnancy and in plasma of women shortly after normal delivery (Figure 2B). We also observed a clear ZnCP-I peak in plasma of the AFE patients (Figure 2C).

The concentrations of ZnCP-I in plasma during normal pregnancy, shortly after normal delivery, and in patients with or without AFE are shown in Figure 3. The mean value in plasma samples unrelated to pregnancy was <6 nmol/L (below the detection limit; n > 30);
the mean values in samples during normal pregnancy and shortly after normal delivery were 12 (SD 7.9) and 26 (SD 10) nmol/L, respectively. The mean value in plasma was 97 (SD 83, range 38–240) nmol/L for the AFE patients and 11 (SD 9.2) nmol/L for the non-AFE patients. The values for the individual AFE patients are shown in Table 1.

ZnCP-I was detected in amniotic fluids with and without meconium, but its mean concentration in meconium-stained amniotic fluid was 928 (SD 397) nmol/L, about fourfold that in clear amniotic fluid: 212 (SD 135) nmol/L (Figure 4).

Discussion

We show here that ZnCP-I is present in the plasma of normal women during pregnancy and shortly after delivery, in patients with or without AFE, and in amniotic fluids with or without contaminating meconium. No zinc porphyrin was previously reported in human plasma except for zinc protoporphyrin IX in lysates of blood from patients with lead poisoning [11, 12]. Recently, we [8] and Gourley et al. [9] showed that ZnCP-I is a characteristic fluorescence component of meconium. Here we show that ZnCP-I is present in maternal plasma during and shortly after normal pregnancy or in maternal plasma of non-AFE patients with intra- or postpartum shock caused by genital bleeding at a concentration twofold greater than the lower limit for its detection. The ZnCP-I in the amniotic fluid was probably derived from meconium and then transferred through the placenta via the fetal plasma to the maternal plasma.

The difference in the plasma concentrations of ZnCP-I during pregnancy and shortly after normal delivery was not significant, but tended to be slightly higher shortly after normal delivery. This tendency suggests that a small amount of amniotic fluid entered the maternal circulation during delivery, but was insufficient to cause any symptoms.

We examined only four samples of plasma from patients diagnosed as having AFE and, therefore, do not have enough data for statistical analysis. However, we also have data on three other plasma samples from patients showing AFE-like symptoms shortly after delivery. Fetal squamous cells could not be measured in these patients’ lungs, and supportive care resulted in their cure. We obtained these samples from the patients within 6 h after the onset of symptoms, and the mean ZnCP-I value was 315 (SD 92, range 245–445) nmol/L. From these results, we conclude that the concentration of ZnCP-I in the plasma of AFE patients increases and that amniotic fluid with ZnCP-I enters the maternal bloodstream directly by an unknown mechanism.

If 10 mL of amniotic fluid enters the maternal bloodstream, its ZnCP-I must be diluted >500-fold with ~5 L of maternal blood. Therefore, judging from our data on the concentration of ZnCP-I in the plasma of AFE
patients and in amniotic fluid with or without contaminating meconium, the entry of a larger volume of amniotic fluid or the entry of amniotic fluid with a greater concentration of ZnCP-I into the maternal bloodstream of women during delivery may induce AFE.

For diagnosis of AFE, the demonstration of squamous cells, lanugo hairs, or mucinous material by pulmonary catheterization is required—methods thought to be too complex to be practical. Squamous cells in the maternal pulmonary arterial circulation are also a common finding in AFE (13, 14). Our method for detecting ZnCP-I in the plasma of patients showing AFE-like symptoms directly demonstrates the influx of amniotic fluid into the maternal bloodstream during delivery.

When the cutoff value of ZnCP-I was defined as >35 nmol/L, only 1 of 41 plasma samples obtained shortly after normal delivery exceeded the cutoff (36 nmol/L). As calculated from the data for the 41 samples obtained after normal delivery and for the 4 samples from the AFE patients, the positive and negative predictive values and the specificity and sensitivity of prediction of AFE are 80%, 100%, 98%, and 100%, respectively. Therefore, if a ZnCP-I concentration greater than the cutoff value is found in plasma of a patient showing AFE-like symptoms, we recommend supportive care such as circulation and respiration control and treatment for disseminated intravascular coagulation with antithrombin III, as used in the case reported here.

In conclusion, we suggest that the assay of ZnCP-I is a simple and reliable way to diagnose AFE, even though we could study plasma samples from only four patients.

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References