Introduction

Premature delivery is defined as giving birth between weeks 22 and 37 of gestation. Threatened premature delivery is a condition in which women are at high risk of premature delivery and the symptoms include lower abdominal pain (more than one contraction every 10 min), vaginal bleeding and ruptured membrane with regular uterine contractions determined by external tocography, and cervical dilation or effacement occurring between weeks 22 and 27. In Japan, long-term tocolysis is used in perinatal treatment to prolong the in utero period and improve the maturity of the fetus to avoid disorders caused by low birth weight and immaturity due to premature delivery. The β2 selective agonist ritodrine has been used clinically to inhibit uterine contractions for extended periods from mid-term pregnancy until delivery.

Maternal physiological functions vary considerably during pregnancy and change the pharmacokinetics of drugs. Furthermore, because of the differences in maternal circulating blood and amniotic fluid volumes, drug clearance may differ between women who are pregnant with singletons and those with twins. Because reduced ritodrine clearance will result in increased serum concentrations in pregnant women receiving intravenous infusion, caution should be exercised when complications develop.

Several studies have examined the effects of the number of fetuses on drug pharmacokinetics. Ballabh et al. reported that the half-life of betamethasone is significantly longer in singleton than in twin pregnancies (9.0 ± 2.7 vs. 7.2 ± 2.4 h). Callesen et al. further...
reported that the weekly increase in daily dose of insulin between weeks 14 and 27 is two times higher in twin pregnancies compared with singleton.

We previously reported that ritodrine clearance decreases as twin pregnancies progress. However, the effects of ritodrine pharmacokinetics and number of fetuses on delivery outcomes have not been reported. We measured serum ritodrine concentrations under steady state conditions in women pregnant with singletons or twins treated with continuous infusion of ritodrine, and examined the relationship between dynamic parameters in vivo and delivery outcomes.

Patients and Methods

Patients

One hundred and five women (singleton pregnancy, n = 67; twin pregnancy, n = 38) received continuous infusion of ritodrine to prevent threatened premature delivery at Tenshi Hospital between November 2004 and March 2010 (Table 1), and 213 and 182 blood samples, respectively, were collected. We previously reported a progressive increase in systemic clearance of ritodrine toward the end of pregnancy, based on the data obtained from the initial 14 pregnant women. To study whether there is a difference in ritodrine clearance between pregnant women with singleton and those with twins, we newly recruited 24 pregnant women with twins and 67 with singletons. A steady state of ritodrine was considered to be reached at 24 h after receiving an identical dose. All laboratory parameters including liver and kidney function were within normal ranges in all participants.

Ethics

The Ethics Committee at Tenshi Hospital approved this study and all the women provided written consent to participate.

Measurement of serum ritodrine concentrations

Serum ritodrine levels were determined by HPLC using a fluorescence detector. Blood samples were separated by centrifugation at 3000 rpm for 5 min and serum was separated and stored at −20°C. Serum ritodrine concentrations were measured within one week of sampling. In brief, 1 mL of 0.1 M sodium carbonate buffer (pH 9.75) was added to 1 mL of serum, then 5 mL of ethyl acetate was added and the mixture was agitated for 15 min. The mixture was separated by centrifugation and the supernatant (4 mL) was evaporated to dryness at 40°C for one hour. The residue was dissolved in 100 μL of mobile phase comprising 0.04 M phosphoric acid : acetonitrile (90:10). Fifty μL was injected into a Lichrospher RP-18 (Kanto Chemical Co., Tokyo) HPLC column using an L-2130 pump (Hitachi, Tokyo, Japan), and ritodrine was detected with an L-2480 fluorescence detector (Hitachi). Serum ritodrine concentration was determined using an absolute calibration method at excitation and emission wavelengths of 280 and 305 nm, respectively. The column temperature was 35°C and the flow rate was 1.0 mL/min. Ritodrine was eluted at approximately 12 min, without evident interfering substances. Quantitative reproducibility expressed as intra- and inter-day coefficients of variation (CV) were within 5.0% and 10.0%, respectively.

Calculation of pharmacokinetic parameters of ritodrine

Ritodrine clearance (CLtot) was calculated as the dosage divided by the serum concentration of
ritodrine, as shown in Equation 1:

\[
CL_{\text{tot}} (L/h/kg) = \frac{\text{Continuous infusion dosage} (\mu g/\text{min}/kg)}{\text{Serum ritodrine concentration} (\text{ng/mL})}
\]  

(Eq. 1)

**Delivery outcomes**

Delivery outcomes were determined from birth and medical records, and then associations with serum ritodrine concentrations were analyzed.

**Statistical analysis**

Serum ritodrine concentrations, ritodrine clearance and week of birth (means and standard deviation) were analyzed using t-tests. The regression lines derived from ritodrine dose and serum concentration were analyzed using covariance analysis. The p value for statistical significance was established at 5%.

**Results**

None of the newborns had congenital abnormalities or significant problems, although some pregnant women developed mild palpitation and hand tremor during ritodrine therapy. The ritodrine infusion rates [mean ± SD (range)] were 2.1±1.4 (0.4-7.1) and 2.3±1.1 (0.7-4.7) \(\mu g/\text{min}/kg\), in pregnant women with singletons and those with twins, respectively, and correlated positively with serum ritodrine concentrations \(y=30.1x+9.34, r=0.930\) and \(y=39.2x-0.79, r=0.907\), respectively. Figure 1 shows a linear relationship between the dosage and serum concentration of ritodrine determined from all data. However, serum concentrations at given infusion rates were significantly higher in twin pregnancies \((p<0.01)\) (Fig. 1).

Ritodrine clearance was significantly lower in pregnant women with twins than in those with singletons \([1.59±0.30 (0.83-2.47) \text{ vs. } 1.75±0.43 (0.69-3.65) L/h/kg; p<0.001]\) (Fig. 2).

Figure 3 shows the relationship between gestation week and serum ritodrine concentrations in singleton and twin pregnancies. Serum ritodrine concentration tended to increase as gestation week progressed in twin pregnancies, but did not change in singleton pregnancies.

Of 105 pregnant women in this study, 20 with singletons and 25 with twins delivered prematurely, with no difference in the week of delivery \([33.5±3.60 (26.4-36.9) \text{ vs. } 35.1±2.35 (26.9-36.9) \text{ weeks; } p=\text{NS}]\). Serum ritodrine concentrations in 49 samples from singleton pregnancies and 117 samples from twin pregnancies that delivered prematurely also did not differ significantly \([97.5±61.1 (29.2-220) \text{ vs. } 89.6±50.4 (28.4-290) \text{ ng/mL; } p=\text{NS}]\). Among pregnant women in this study, 47 with singletons and 13
with twins delivered at term, with twin mothers delivering significantly earlier [38.1 ± 1.17 (37.0–40.7) vs. 37.0 ± 0.04 (37.0–37.1) weeks; p < 0.001]. Serum ritodrine concentrations were higher in 65 samples from twin pregnancies than in 164 samples from singleton pregnancies [85.8 ± 39.7 (30.4–208) vs. 65.7 ± 38.7 (18.4–187) ng/mL; p < 0.001] (Table 2).

Ritodrine clearance did not differ significantly between mothers who delivered singletons prematurely and those who delivered singletons at term [1.77 ± 0.45 (0.69–2.84) vs. 1.75 ± 0.43 (0.82–3.65) L/h/kg; p = NS], but was significantly higher in mothers who delivered twins at term than those who delivered twins prematurely [1.69 ± 0.31 (1.14–2.47) vs. 1.53 ± 0.28 (0.83–2.34) L/h/kg; p < 0.001] (Table 2).

**Discussion**

In Japan, prolonging gestation with continuous ritodrine infusion is the basic treatment to prevent perinatal death and delivery of low birth weight infants. In the USA and Canada, the Canadian Preterm Labor Investigators Group recommends tocolytic therapy to prolong gestation and provides the 48-h window required for steroid administration to prevent neonatal respiratory distress syndrome. Few reports have described long-term ritodrine to prevent threatened premature delivery.

Ritodrine is a $\beta_2$ selective adrenergic agonist with weaker $\beta_1$ selective adrenergic activity that may lead to the development of tachycardia, hypoglycemia and intestinal paralysis in singleton neonates. Serum ritodrine concentrations correlates with the frequency of uterine contractions ($y = 4.4x – 19.6$, $r = 0.84$, $p < 0.001$, $n = 17$), and the reported serum ritodrine concentration required to inhibit uterine contractions is 15–45 ng/mL.

Even though infusion rates did not differ significantly between singleton pregnancies and twin pregnancies, serum ritodrine concentrations at given infusion rates were significantly higher in twin pregnancies in the present study. Gabriel et al. reported that the frequency of maternal adverse effects increases...
dramatically with prolonged intravenous ritodrine administration in mothers with multiple pregnancies compared with singletons. Furthermore, ritodrine passes through the placenta, where it may cause the development of tachycardia, hypoglycemia and intestinal paralysis in neonates \(^{10,11}\). We previously found essentially equal maternal-to-fetal ritodrine transfer in 14 women with twin pregnancies who were treated with continuous intravenous ritodrine \(^{13}\). Therefore, fetal development and adverse effects should be considered cautiously when increasing the dose of ritodrine.

Ritodrine clearance was significantly lower in twin pregnancies than in singleton pregnancies, and serum concentrations tended to increase as gestation progressed in twin pregnancies. As 32 to 38% of serum ritodrine is bound to plasma proteins, we consider that a change in the bound fraction has little effect on ritodrine clearance \(^{14,15}\). The birth weights did not differ significantly in prematurely delivered singletons and twins. However, the birth weight was significantly lower in twins than in singletons when born at term. According to the 2006 Maternal and Child Health Statistics in Japan, the average birth weights for singleton and multiple births were 3.01 and 2.21 kg, respectively \(^{16}\), which were very similar to the present findings. The gestation week of birth at term was shorter for twins than for singletons. The fact that most pregnant women with twins underwent Caesarean section at week 37 at the latest in the Department of Obstetrics of our hospital may account for the difference.

Serum ritodrine concentrations did not differ significantly between pregnant women with singletons and those with twins who delivered prematurely, but were higher in pregnant women with twins who delivered at term. Serum ritodrine concentrations were higher in pregnant women with singletons who delivered prematurely, compared with those who delivered at term. Ritodrine clearance was lower in pregnant women with prematurely delivered twins. This might be due to the higher serum concentration of ritodrine required to maintain pregnancy in women with twins. The above findings indicate that pregnancy may be maintained in women carrying multiple fetuses by adjusting the serum ritodrine concentration.

The present study identified some differences in pharmacokinetics of intravenous infusion of ritodrine between mothers of singletons and mothers of twins. Thus, maternal physiology including the capability of drug metabolism in twin pregnancies should be clarified. Ritodrine is generally metabolized through sulfate (45%) and glucuronate (38%) conjugation mainly by the sulfotransferases; SULT1A3 in the intestine and SULT1A1 in the kidney \(^{17}\). The potency ratio of SULT1A3 and SULT1A1 for ritodrine metabolism is approximately 1:5 to 1:4, with large individual differences in liver SULT1A1 activity \(^{17,18}\). Fetal ritodrine is metabolized through glucuronate

### Table 2 Delivery outcomes of the pregnant women studied

<table>
<thead>
<tr>
<th></th>
<th>Singletons</th>
<th>Twins</th>
<th>P (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Means ± SD (range)</td>
<td>Mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td>Premature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>20</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Week of birth</td>
<td>33.5 ± 3.60 (26.4-36.9)</td>
<td>35.1 ± 2.35 (26.9-36.9)</td>
<td>n. s.</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2093 ± 845 (524-3320)</td>
<td>First 2306 ± 442 (1538-2936)</td>
<td>n. s.</td>
</tr>
<tr>
<td>Serum concentration (ng/mL)</td>
<td>First 97.5 ± 61.1 (29.2-220)</td>
<td>89.6 ± 50.4 (28.4-290)</td>
<td>n. s.</td>
</tr>
<tr>
<td>Clearance (L/h/kg)</td>
<td>1.77 ± 0.45 (0.69-2.84)</td>
<td>1.53 ± 0.28 (0.83-2.34)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Term</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>47</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Week of birth</td>
<td>38.1 ± 1.17 (37.0-40.7)</td>
<td>37.0 ± 0.4 (37.0-37.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2932 ± 440 (1524-4004)</td>
<td>First 2627 ± 323 (2106-3200)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serum concentration (ng/mL)</td>
<td>First 65.7 ± 38.7 (18.4-187)</td>
<td>85.8 ± 39.7 (30.4-208)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clearance (L/h/kg)</td>
<td>1.75 ± 0.43 (0.82-3.65)</td>
<td>1.69 ± 0.31 (1.14-2.47)</td>
<td>n. s.</td>
</tr>
</tbody>
</table>

*P<0.01 vs. singleton term deliveries; †P<0.05 vs. twin term deliveries.
(23%) and sulfate (66%) conjugation\(^9\). Ritodrine clearance is reportedly reduced in neonates with low birth weight due to underdeveloped liver and kidney functions\(^9\). Thus, the metabolic capabilities of fetuses may vary according to the stage of development.

Ritodrine pharmacokinetics differ between singleton and twin pregnancies. Analyzing umbilical cord and venous ritodrine concentrations in newborns would be helpful to determine ritodrine pharmacokinetics in newborns to ensure appropriate ritodrine administration to pregnant women at high risk of premature delivery.

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Conflict of Interest
All authors have no potential conflict of interest relevant to this article.

References