Stereoselective Analysis of Ritodrine Diastereomers in Human Serum Using HPLC

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Abstract

A sensitive and selective method has been developed for the determination of ritodrine diastereomers in human serum using high-performance liquid chromatography with a chiral stationary phase column and a fluorescence detector. No interfering peaks from endogenous substances were observed. The method showed good reproducibility and accuracy, and the standard curve was linear up to 100 ng/mL with a correlation coefficient of 0.999. Limit of detection (signal-to-noise = 3) and quantitation (signal-to-noise = 10) were found to be 2 and 5 ng/mL, respectively. This method is suitable for chiral pharmacological and pharmacokinetic studies as well as the therapeutic drug monitoring of ritodrine diastereomers for which no information currently exists.

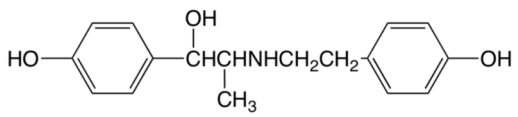
Introduction

Ritodrine itself contains two asymmetric carbons. For clinical use, one of its asymmetric centers is fixed in one configuration, thus, there are two diastereomers of (1RS, 2SR)-erythro-1-(4-hydroxyphenyl)-2-[2-(4-hydroxyphenyl)ethylamino]propanol-1-ol) and for which the racemate is administered as a hydrochloride salt treatment in pregnancies that are threatened by abortion (Figure 1). The effectiveness correlation and pharmacodynamics for the treatment of threatened abortion and the measured serum concentration of racemic ritodrine has been previously demonstrated (1,2). Linear pharmacokinetic behavior and decreased clearance of retodrine diastereomers that are commercially available have been found in the late gestation period in twin pregnancy patients (3).

Among the numerous pharmaceuticals that have chiral centers, it has been reported that the different enantiomers and/or diastereomers may have different in vivo pharmacokinetics and clinical effects (4–6). Separation of the reference standards of ritodrine diastereomers have been reported employing capillary electrophoresis, but their sensitivity was not enough to measure the concentrations of ritodrine diastereomers in biological fluids including serum or plasma, and no data of chiral ritodrines has been demonstrated after extraction from biological fluids (7,8).

Yamasaki et al. have a U.S. patent for therapeutic compositions and use and method of preparation of (–)-ritodrine (9). They have established to resolve ritodrine diastereomers by the selective crystallization method as well as by chromatography, employing optical active columns including Chiralcel OJ and AJ (Daicel Chemical, Tokyo, Japan) at normal phase mode, but detailed procedures are not presented in their patents.They have also demonstrated that suppressing 50% of the contraction of an isolated myometrium caused by oxytocin (IC_{50}) of (–)-ritodrine had an intensity of about 2.6-times that of racemic ritodrine in rats whereas that of (+)-ritodrine had 1/15 activity of racemic ritodrine (9). On comparing the relaxation effects on the isolated tracheal muscle via IC_{50}, (–)-ritodrine had the highest activity, of which intensity was about 2.3-times that of racemic ritodrine. (+)-Ritodrine has the lowest activity in the tracheal muscle and the intensity of about 1/40 that of racemic ritodrine, although the range of the acute toxicity is almost of the same order. (9)

There have been no reports on the clinical effects of the ritodrine diastereomers in relation to their serum concentrations. With the goal of establishing the clinical effects in detail and a safe and effective administration of racemic ritodrine, which is commercially available and used in the treatment of threatened abortion, we have developed a method for quantitative determination of the ritodrine diastereomers when administered as a racemate.



(1*RS*,2*SR*)-1-(4-Hydroxyphenyl)-2-{[2-(4-hydroxyphenyl)ethyl]amino}propan-1-ol

Figure 1. Structure of commercially available ritodrine.

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Experimental

Materials

Ritodrine racemate for clinical use was kindly supplied by Kissei Pharmaceutical (Matsumoto, Japan). Stock solutions were prepared using distilled water and stored at –4°C. All reagents used in this study were of reagent- and high-performance liquid chromatography (HPLC)-grade and purchased from Wako Pure Chemical Industries (Osaka, Japan).

Extraction procedure

Blood samples, collected without any anticoagulant, were centrifuged at 3000 rpm for 5 min. The serum was separated and then stored at –20°C until analysis according to the previous report (10). To 1 mL of serum, 1 mL of a 0.1 M sodium carbonate buffer (pH 9.8) was added, followed by the addition of 5 mL of ethyl acetate and agitation for 15 min by a vortex mixer. The sample was centrifuged for 10 minutes at 2000 g. Subsequently, 4 mL of the organic layer was obtained and allowed to evaporate to dryness at 40°C for approximately 1 h. The residue was dissolved in a 70 µL mobile phase, and then 50 µL aliquots of the resultant solution were injected into the HPLC manually.

Instruments

The HPLC instrument consisted of a CCPM-II pump unit (Tosoh, Tokyo, Japan) and a 0.46 cm × 15 cm OD-RH column (particle size 5 µm, Daicel Chemical). The elution orders of ritodrine diastereomers were identified using an optical rotation detector (Advanced Laser Polarimeter, laser wavelength, 670 nm; PDR-Chiral, Lake Park, FL). A Hitachi F-1150 fluorescence detector (Tokyo, Japan) was used in the determination of the ritodrine diastereomers in the serum at wavelengths of 280 nm for excitation and 305 nm for emission. The mobile phase was 0.1 M KPF_6–acetonitrile (80:20) with a column temperature of 35°C and a flow rate of 0.5 mL/min. The pH value of the mobile phase was found to be 5.5 without any adjustment. Area integration of the elution peaks was calculated using a Hitachi D-2500 Chromato-Integrator (Tokyo, Japan).

Table I. Doses and Pharmacokinetics Parameters of Two Pregnancy Patients

Patient 1						
Gestation (week)	29.0	30.0	31.0	32.7	34.7	
Racemic ritodrine Dose (µg/min/kg)	1.04	1.50	2.00	3.00	2.94	
(–)-ritodrine conc.*	30.1	37.4	51.0	87.6	90.0	
(+)-ritodrine conc.*	28.1	35.4	48.9	84.4	84.5	
(–)-ritodrine clearance†	0.55	0.64	0.61	0.53	0.52	
(+)-ritodrine clearance	0.52	0.60	0.59	0.51	0.49	
Patient 2						
Gestation (week)	25.6	25.7	26.4	27.4	30.4	31.4
Racemic ritodrine Dose (µg/min/kg)	1.21	1.21	1.82	1.78	2.18	2.57
(–)-ritodrine conc.*	32.1	31.2	45.6	46.8	63.8	74.1
(+)-ritodrine conc.	23.9	21.5	34.7	32.6	49.2	62.6
(–)-ritodrine clearance†	0.74	0.82	0.77	0.80	0.65	0.61
(+)-ritodrine clearance†	0.55	0.56	0.59	0.55	0.50	0.52

* (ng/mL). † (L/h/kg).

Standard curves

Known amounts of racemic ritodrine from 5 to 100 ng/mL were added to drug-free serum. The concentrations of ritodrine diastereomers were estimated by the peak height of each of the diastereomers by using the absolute calibration method. Concentration on the calibration curve was determined by six samples of standard racemic ritodrine.

Recovery study and validation of the method

Recovery study was performed at 10, 50, and 100 ng/mL for both diastereomers. Reproducibility was considered by within-day (nine samples at each concentrations) and between-day (nine different experimental days) variation of adjusted samples produced by adding standard racemic ritodrine to serum to get concentrations of 5, 10, 50, and 100 ng/mL, employing the FDA method with lower limit of quantitation (LOQ) values (11).

Clinical study

Subjects of this study were two twin pregnancy patients with threatened premature delivery who underwent treatment in the Department of Obstetrics at Tenshi Hospital. This study received approval from the Ethics Committee of Tenshi Hospital, and a written informed consent was obtained from the subjects regarding the measurement of serum ritodrine concentration. Doses of ritodrine in two patients have decided along with their clinical conditions (Table I). The patients had not taken any medications except ritodrine. To measure the serum ritodrine concentration, 3 mL blood samples were drawn once per week at 6:00 a.m. from a vein in the subject's arm. Patient No. 1 was a 31-year-old woman with a twin pregnancy. Infusion of ritodrine was started at gestation week 28.8 and was continued until the day before delivery (gestation week 34.7). Serum ritodrine concentration measurements started at gestation week 29 with a total of five samples collected. Patient No. 2 was a 26-year-old woman with a twin pregnancy. Infusion of ritodrine was started at gestation week 25.3 and continued until the day before delivery (gestation week 32.1). Measurements of serum concentration started at week 25.6 with a total of six samples collected.

Results

Resolution of ritodrine

In a typical HPLC chromatogram with an optical rotation detector, the early elution peak was (–)-ritodrine while the latter elution peak was (+)-ritodrine. Numerous direct optical resolution columns employing polysaccharides as chiral stationary

Table II. Recovery Values of (–)- and (+)-Ritodrine

Diastereomer	Conc.(ng/mL)	Recovery (mean ± SD, *n* = 9) (%)
(–)-Ritodrine	10	73.8 ± 8.0
	50	91.5 ± 5.2
	100	93.2 ± 5.0
(+)-Ritodrine	10	78.9 ± 8.0
	50	93.9 ± 6.3
	100	95.9 ± 7.1

phases were included: Chiralcel OD-RH, Chiralcel OJ-RH, Chiralpak AD-RH, and Chiralpak AS-RH (Daicel Chemical). Elution conditions also included KPF_6–acetonitrile and H_3BO_3 $Na_2B_4O_7$–acetonitrile system. The optimal conditions were when elution of the (–)- and (+)-ritodrine occurred at 12 and 14 min, respectively (Table II). Under these optimal conditions, the separation factor alpha was calculated to be 1.2, although a complete separation of the two optical isomers was not possible. However, it was possible to measure the concentration of the separate optical isomers by the amplitudes of their peak heights. Chromatograms measured by fluorescence detection showed no interfering substances using patient serum (Figure 2).

Calibration curves

The calibration curve for both the (–)- and (+)-ritodrine within the range of 5–100 ng/mL passed linearly ($y = 435x + 317$ for (–)-ritodrine, $y = 386x + 249$ for (+)-ritodrine) with each exhibiting a correlation coefficient (r) of 0.999. This measurement method was sensitive enough to be able to determine concentration ranges of 15–45 ng/mL, which is the serum concentration of the racemic ritodrine that has been previously reported in clinical applications (12).

Recovery study and validation of the method

The recovery values are shown in Table II. The within-day and between-day reproducibility of each of the four concentrations are shown in Tables III–IV, respectively. The recovery values in this study were found to be more than 73.8% at 10–100 ng/mL for both diastereomers of ritodrine. The coefficients of variations for within-day and inter-day reproducibility for serum concentrations in the range of 5–100 ng/mL were 0.8–13.8% and 1.2–19.8% for (–)- ritodrine and 0.5–12.5% and 1.3–18.6% for (+)-ritodrine, respectively (Tables II–III). Limit of detection (LOD) (signal-to-noise, S/N = 3) and quantitation (LOQ) values (S/N = 10) of ritodrine diastereomers were found to be 2 and 5 ng/mL in this study, respectively.

Table III. Within-day Coefficient of Variation Determination of (–)- and (+)-Ritodrine

	Conc. (ng/mL)		
Diastereomer	Added	Found (mean ± SD, n = 9)	CV (%)
(–)-Ritodrine	5	5.2 ± 0.7	13.8
	10	8.6 ± 1.1	12.9
	50	52.1 ± 1.8	3.4
	100	99.0 ± 0.8	0.8
(+)-Ritodrine	5	5.8 ± 0.7	12.5
	10	9.2 ± 1.0	11.1
	50	49.7 ± 1.3	2.5
	100	100.1 ± 0.5	0.5

Table IV. Between-day Coefficient of Variation Determination of (–)- and (+)-Ritodrine

	Concentration (ng/mL)		
Diastereomer	Added	Found (mean ± SD, *n* = 9)	CV (%)
(–)-Ritodrine	5	5.0 ± 1.0	19.8
	10	9.4 ± 0.8	8.3
	50	50.9 ± 2.4	4.5
	100	99.6 ± 1.2	1.2
(+)-Ritodrine	5	4.8 ± 0.7	14.6
	10	9.7 ± 0.9	9.5
	50	51.2 ± 2.4	4.7
	100	99.4 ± 1.3	1.3

Clinical study

Clinically, both patients avoided aborting the fetuses and successfully delivered. No interfering peaks were observed for the analysis of serum from the two patients enrolled. The relationship between the dosage and serum concentration levels is shown in Figure 3. The serum concentrations of (–)- and (+)-ritodrine were similar in Patient No. 1. However, results for Patient No. 2 showed that (–)-ritodrine had a concentration 1.3-times higher than that of (+)-ritodrine. Their doses and pharmacokinetic data are listed in Table I.

Discussion

There are several methods that are used to measure the serum concentration of ritodrine racemate (13–16). However, a measurement method that can be used to separate and quantify ritodrine diastereomers after intravenous administration of commercially available racemic ritodrine has not been reported, although insensitive separation method of reference standard of ritodrine diastereomers have been demonstrated using capillary electrophoresis (7,8). In this study, separation of the (–)- and (+)-ritodrine was successively executed through the use of a direct chiral separation column, an OD-RH column with elution

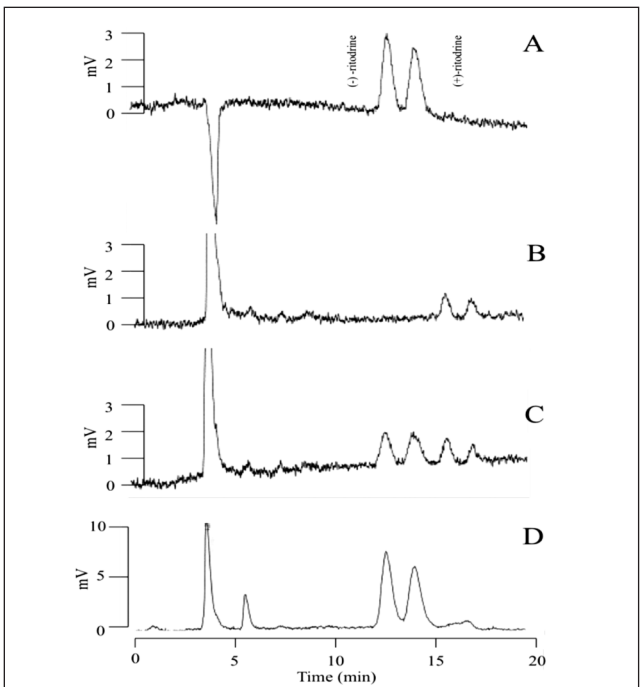

Figure 2. Chromatograms of a blank serum extract (A), an extract of spiked serum with (–)- and (+)-ritodrine (7 ng as racemate) (B), and patient serum containing 73 ng/mL of (–)-ritodrine and 65 ng/mL of (+)-ritodrine (C).

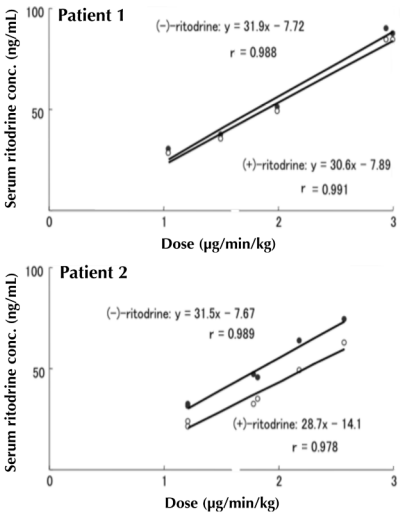

Figure 3. Relationship between dose and serum ritodrine concentration in two patients. Closed circle: (–)-ritodrine; open circle: (+)-ritodrine.

medium containing KPF_6. There is no information on how to interact chiral stationary phase, KPF_6, and ritodrine diastareomers, but several chiral separation studies have enhanced chiral separation of racemic drugs using KPF_6 in mobile phase with chiral stationary phases, which consisted of polysaccharides (17–19). KPF_6 also showed sufficient separation efficiency of ritodrine diastereomers in this study. Further study is needed for the mechanism to resolve ritodrine diastereomes in this condition.

Unidirectional chiral inversions are already demonstrated for non-steroidal anti-inflammatory agents (20–21), and both directional inversions are also reported for thalidomide enantiomers (22–23). There is no information on the chiral inversion of ritodrine diastereomers, and no further experiments were performed in this study because we do not have access to the reference standard of (–)- and (+)-ritodrine.

The method has adequate precision and sensitivity to measure the serum concentration of the ritodrine diastereomers within its clinical dosage range. The measurements of serum from pregnant women undergoing ritodrine treatment show the possibility that there are inter-individual differences in the disposition of ritodrine diastereomers. This method makes it possible to perform a detailed investigation into the development of a safe and effective ritodrine treatment by using serum concentrations of chiral ritodrines as an index.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Manuscript received January 11, 2008;
revision received September 10, 2008.