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Pharmacokinetics of Spiramycin in the Rhesus Monkey: Transplacental Passage and Distribution in Tissue in the Fetus

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Transplacental transfer of spiramycin was investigated in a rhesus monkey model to study whether the antibiotic reaches therapeutic levels in the fetus. Spiramycin concentrations were measured by bioassay and high-performance liquid chromatography. Pharmacokinetic parameters were determined for bioactive spiramycin as measured by the bioassay. Pharmacokinetic pilot studies showed that spiramycin distribution follows a two-compartment model in rhesus monkeys. Following a single intravenous dose of 50 or 250 mg, dose-dependent kinetics were observed. At a dose of 50 mg, 10% of the dose was excreted unchanged in the urine. At the higher dose of 250 mg, an oliguric effect was observed. Spiramycin concentrations in fetal serum were measured over time while the maternal concentration was maintained at a constant level. During a 5-h experiment, a maximum fetal-maternal serum ratio of 0.27 was found. In three fetuses, concentrations in serum and tissue were measured following intravenous administration of 50 mg of spiramycin twice daily to the mother for at least 7 days. The fetal-maternal serum ratios were found to be 0.4 to 0.58 after intravenous administration of the final dose of 50 mg to the mother. It appeared that spiramycin accumulated in the soft tissues, especially in the liver and spleen, of both the mother and the fetus. The concentration in placental tissue appeared to be 10 to 20 times that of the concentration in fetal serum. The concentration of spiramycin in amniotic fluid was about five times higher than the concentration in fetal serum. Another important observation was that absolutely no spiramycin was found in the brain.

Spiramycin is a macrolide antibiotic produced by *Streptomyces ambofaciens* (31) that consists of a complex of three components: spiramycins I, II, and III (43). Spiramycin is mainly bacteriostatic, affecting a broad range of bacteria, while the parasite *Toxoplasma gondii* is also susceptible to this antibiotic (22, 43). The mechanism of action of spiramycin is not precisely known. It is believed that spiramycin inhibits bacterial protein synthesis by stimulating the dissociation of peptidyl-tRNA from ribosomes during translocation (6).

Spiramycin is effective in vivo for a variety of infections, despite the fact that its levels in serum are often below the MIC for the infecting organisms (39). This can be explained in part by the ability of spiramycin to achieve concentrations in tissues and cells that are a factor 10—or even more—higher than the corresponding concentration in serum. High concentrations of spiramycin have been found in female pelvic tissues (1); respiratory tract (3), heart, liver, lung, spleen, and kidney (28, 30) tissues; and macrophages (19, 32, 44). The phenomenon of marked tissue penetration has also been described for the macrolide antibiotic azithromycin (10, 29, 38). The high intracellular concentrations of these macrolides may explain their good antibiotic activity against susceptible intracellular organisms.

Toxoplasma gondii is such a susceptible obligatory intracellular organism. This parasite can cause severe disorders, such as hydrocephalus, chorioretinitis, and mental retardation in congenitally infected fetuses (35). Effective treatment is mandatory to reduce the risk of severe disorders.

Data concerning transplacental transfer of spiramycin have been scarce. In 1968, Garin et al. (15) published the results of assays of levels in human umbilical cord and maternal blood after treatment with spiramycin (3 g/day). The levels in umbilical cord blood were half of those in maternal blood. In 1987, Forestier et al. (13) reinvestigated the transplacental passage of spiramycin in humans. At birth, they found a fetal-maternal serum ratio of 0.74. In addition, they examined samples obtained between the 21st and 24th weeks of pregnancy and found a fetal-maternal serum ratio of 0.47. By using an in vitro perfusion model of isolated placental cotyledons, Quentin et al. (33) found a mass transfer during a 1-h experiment of approximately 9% of the maternal circulating concentrations.

The concentrations measured in fetal serum do not reach therapeutic levels, i.e., levels that equal the MIC found in vitro for *T. gondii* (41). It is possible that effective intracellular concentrations and concentrations in tissue are reached in the fetus, but such data have not been reported. Therefore, a rhesus monkey (*Macaca mulatta*) model was used to study the transplacental transfer and tissue distribution of spiramycin in the fetus. The consequences of the findings for the treatment of congenital *T. gondii* infections will be discussed.

MATERIALS AND METHODS

Animals. In total, 12 rhesus monkeys were used in the experiments. Female 4- to 5-year-old rhesus monkeys weighing about 5 kg were obtained from the Laboratory Animals Breeding Experimental Farm of Shunde Guangdong, Beijing, People's Republic of China. Breeding, housing, and feeding of the monkeys occurred exactly as described previously (37).

Anesthesia. All experiments were carried out under general anesthesia. After a 24-h fasting period, anesthesia was induced with 10 mg of ketamine (Nimatek; Ad Usem Veterinary, Cuyk, The Netherlands) per kg and 0.25 mg of atropine sulfate

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TABLE 1. Design of experiments with rhesus monkeys

Monkey[a]	Wt (kg)	I.v. spiramycin regimen
A1	3.80	Single 50-mg dose
A2[b]	4.50	Single 50-mg dose
A3[b]	4.30	Single 50-mg dose
A4	3.70	Single 250-mg dose
A5[b]	4.95	Single 250-mg dose
A6[b]	5.15	Single 250-mg dose
A7	5.45	Loading dose (1.75 mg/kg) followed by continuous infusion (0.1 mg/kg/min)
B1	4.45	Loading dose (3.25 mg/kg) followed by continuous infusion (0.1 mg/kg/min)
B2	5.80	Loading dose (3.25 mg/kg) followed by continuous infusion (0.1 mg/kg/min)
B3	6.30	100 mg/day in 2 intermittent doses for 7 days, then loading dose (3.25 mg/kg) followed by continuous infusion (0.1 mg/kg/min)
B4	6.00	100 mg/day in 2 intermittent doses for 24 days, then single 50-mg dose on day 25
B5	6.10	100 mg/day in 2 intermittent doses for 24 days, then single 50-mg dose on day 25

[a] A, nonpregnant; B, pregnant.
[b] Urine production was stimulated with mannitol (3 g/h).

(Pharmachemie B.V., Haarlem, The Netherlands) given intramuscularly. The trachea was intubated with a 5.5- or 6-mm-inside-diameter trachea tube without a cuff (Rüsch, Rommelshausen, Germany). General anesthesia was maintained by using a mixture of nitrous oxide and oxygen (2:1) and 1 to 2% enflurane (Ethrane; Abbott, Amstelveen, The Netherlands). The animals were artificially ventilated at a rate of 20 breaths per min with an Amsterdam Infant Ventilation apparatus.

Design of the experiments. The design of the 12 experiments is shown in Table 1.

Pharmacokinetic studies with seven adult, nonpregnant monkeys. Spiramycin (Rovamycine; Rhône-Poulenc, Paris, France) was administered intravenously (i.v.) because coadministration with food can reduce bioavailability by 50% (14). Dose finding experiments were performed with seven monkeys, A1 to A7. Monkeys A1, A2, and A3 were given a single i.v. dose of 50 mg. The concentrations in serum dropped below the detection limit 6 h after administration of the 50-mg dose. The dose for monkeys A4, A5, and A6 was increased to 250 mg to enable measurement of detectable concentrations in serum for a longer time. Monkey A7 was given a loading dose of 1.75 mg of spiramycin per kg, followed by continuous infusion of 0.1 mg/kg/min to achieve a constant level in serum of approximately 3.0 to 3.5 μg/ml. A level of 3.0 to 3.5 μg/ml in serum was chosen because this concentration is normally achieved in humans given spiramycin to treat *T. gondii* infections (22, 43).

The loading dose was calculated with the formula $C_{ss} = D/V_1$ (C_{ss} is the steady-state concentration, and V_1 is the volume of distribution in the central compartment), with C_{ss} = 3.0 to 3.5 μg/ml and a volume of distribution in the central compartment of 0.55 liter/kg, and was 1.75 mg/kg. The maintenance dose was subsequently calculated with the formula $C_{ss} = D/(T \times CL)$ (T is time, and CL is clearance), with C_{ss} = 3.0 to 3.5 μg/ml and CL = 37.6 ml/min/kg, and was 0.1 mg/min/kg. The volume of distribution in the central compartment and CL were derived from the single-dose studies with 50 mg of spiramycin.

To obtain a sufficiently high and constant urine flow, urine production was stimulated in monkeys A2, A3, A5, and A6 by i.v. administration of 3 g of mannitol per h; 30 ml of a 0.1-g/ml solution was administered via the vena saphena with an infusion pump. Urine samples were collected with a catheter (0.6 by 40 cm; Argyle; Sherwood Medical).

Pharmacokinetic studies with fetuses. Transplacental transfer of spiramycin was measured in five monkeys (B1 to B5) under various conditions. The duration of pregnancy in rhesus monkeys is about 170 days. The transfer experiments were performed at day 140 of pregnancy, which is comparable to the third trimester of organogenetic development in humans. Concentrations in fetal serum were measured in monkeys B1 and B2 in a 1-day experiment while the maternal antibiotic concentration was maintained at a constant level by means of a loading dose of 3.25 mg/kg, followed by continuous infusion of 0.1 mg/kg/min for 3 and 5 h, respectively. As a result of pregnancy, the distribution volume of the central compartment is increased considerably in the third trimester of gestation. It was therefore necessary to increase the loading dose in these monkeys. Transplacental transfer of spiramycin at a steady state in the mother was also studied in monkey B3. This monkey was given 50 mg by venous puncture twice a day for 1 week before starting the experiment to achieve maximum concentrations in tissue (24). A loading dose of 3.25 mg/kg was administered, since the trough level dropped nearly to zero. Maximum concentrations in tissue were also achieved in monkeys B4 and B5 by administration of 50 mg i.v. twice a day for 3 weeks. Transplacental transfer was studied in these monkeys after administration of a final i.v. dose of 50 mg.

Collection of samples. Spiramycin was administered in glucose isotonic solution via the vena saphena, and blood samples were taken from the vena jugularis. The blood vessel was cannulated with a heparinized catheter and connected to a three-way stopcock (Viggo, Helsingborg, Sweden), and samples of 2 to 3 ml of blood were collected. The fluid balance was maintained by replacing the blood with a physiological salt solution.

Samples of 0.5 ml of fetal blood were collected from a blood vessel that interconnects the two placental lobes that are present physiologically in this monkey species, exactly as previously described (37). In brief, an interplacental blood vessel was visualized by illumination of the uterus with two sterile lamps. After careful incision of the uterus wall and the decidua, the interplacental blood vessel, which is of fetal origin, was exposed. The vessel was cannulated with a catheter, and the samples were collected. The blood was replaced with a physiological salt solution to maintain the fluid balance.

During the transplacental transfer experiment, blood from the mother, blood from the fetus, and amniotic fluid samples were collected at serial time intervals (see Table 2). From monkey A1, urine samples were collected over 0 to 4, 4 to 8, and 8 to 24 h. At more regular time intervals, urine samples were also collected from monkeys A2, A3, A5, and A6. In the latter cases, urine production was stimulated by 3 administering g of mannitol per h (Table 2). The urine volume was measured, and an aliquot was kept for determination of the spiramycin concentration.

At the end of the experiment, baby monkeys B3, B4, and B5 were delivered by cesarean section and immediately sacrificed. The hearts, livers, spleens, brains, and placentas were collected to determine the spiramycin concentrations.

Determination of antibiotic concentrations. Pure spiramycin was kindly provided by Rhône Poulenc, Centre de recherches de Vitry, Alfortville, France. According to the manufacturer, the spiramycin used (lot no. RP5337) contained 90.0% spiramycin I, 0.5% spiramycin II, and 6.3% spiramycin III as determined by high-performance liquid chromatography (HPLC).

Antibiotic concentrations were determined by bioassay

TABLE 2. Collection of samples

Monkey	Sample[a]	Sampling time(s) (min)
A1	Serum	0, 2, 5, 10, 15, 30, 60, 90, 120, 180, 240, 480, 1,440
	Urine	0, 240, 480, 1,440
A2	Serum	0, 2, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 1,320, 1,500, 1,800
	Urine	0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 1,320, 1,500, 1,800
A3	Serum	0, 2, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 375, 1,320, 1,500, 1,680, 1,860
	Urine[b]	0, 15, 30, 60, 90, 120, 210, 240, 270, 300, 330, 360, 375, 1,320, 1,500, 1,680, 1,860
A4	Serum	0, 2, 5, 10, 15, 30, 60, 90, 120, 150, 180, 240, 300, 360, 420, 1,440
A5	Serum	0, 2, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 1,290, 1,470, 1,830, 2,730
	Urine[c]	0, 20, 60, 360, 1,290, 1,470, 1,830, 2,730
A6	Serum	0, 2, 5, 10, 15, 30, 60, 90, 120, 150, 180
	Urine[d]	0, 30
A7	Serum	0, 2, 5, 10, 15, 30, 60, 90, 120, 180, 240, 300, 360
B1	Maternal serum	0, 30, 60, 120, 225, 240, 300
	Fetal serum	0, 30, 60, 120, 225, 240, 300
B2	Maternal serum	0, 30, 60, 120, 180
	Fetal serum	0, 30, 60, 140, 180
B3	Maternal serum	0, 30, 60, 120, 180, 240, 300
	Fetal serum	0, 30, 60, 120, 180, 240, 300
	Amniotic fluid	0, 300
	Fetal tissues	300
B4	Maternal serum	0, 30, 60, 120, 180
	Fetal serum	0, 30, 60, 120, 180
	Amniotic fluid	0, 200
	Fetal tissues	200
B5	Maternal serum	0, 30, 60
	Fetal serum	0, 30
	Amniotic fluid	0, 60
	Fetal tissues	60

[a] Heart, liver, brain, spleen, and placenta tissues were obtained at autopsy.
[b] There was no urine available at 150 and 180 min.
[c] There was no urine available between 60 and 360 min.
[d] There was no urine available after 30 min.

and/or HPLC. In the bioassay, the spiramycin concentration was determined by diffusion in 3% Difco agar medium no. 11 (pH 8.0; Brunschwig Chemie, Amsterdam, The Netherlands), with *Sarcina lutea* ATCC 9341 as the test organism (36). The concentrations of spiramycin in the clinical samples were determined by comparing the inhibition zones to those of the standards. Standards were prepared as follows. A stock solution of 2 mg of pure spiramycin per ml was dissolved in 20% methanol and 80% physiological salt solution. Standards of 10, 5, 2.5, 1.0, 0.5, 0.25, and 0.1 μg/ml were prepared from the stock solution by dilution with physiological salt. A negative control of physiological salt with 0.1% methanol did not inhibit *S. lutea* growth. The sensitivity of the assay was 0.1 μg/ml. The bioassay was linear in the concentration range between 0.25 and 10 μg/ml. Intraday and interday coefficients of variation were found to be 2.0 and 2.6% for 10 μg/ml, 1.5 and 2.7% for 5 μg/ml, 1.7 and 0.7% for 2.5 μg/ml, 3.8 and 5.7% for 1.0 μg/ml, 3.4 and 5.2% for 0.5 μg/ml, 3.7 and 3.8% for 0.25 μg/ml, and 5.1 and 4.3% for 0.1 μg/ml, respectively.

Determination of spiramycin by HPLC was performed on LiChrosorb CP Spher C18 (25 cm by 4.6 mm [inside diameter]) with a solvent of 4 g of H_3PO_4–0.5 g of tetramethylammonium chloride in 500 ml of water mixed with 500 ml of acetonitrile with UV detection at 233 nm and operation at a temperature of 23°C (27). The quantitation level of the HPLC was 0.1 μg/ml. The intraday and interday coefficients of variation were found to be 2.1 and 3.0%, respectively.

Preparation of samples. Tissue samples were homogenized in physiological salt solution (4 ml/g [wet weight] of tissue) with an Ultraturax apparatus (Ystral GmbH, Dottingen, Germany). The crude homogenate was pelleted by 20 min of centrifugation at 1,000 × *g*. The supernatant was used for the bioassay and HPLC determination. The supernatants and serum samples were kept at −20°C until determination. After thawing, 100 μl of the supernatant, serum sample, or urine sample was tested in the bioassay. The samples were diluted with distilled water if necessary.

For HPLC determination, tissue extracts were deproteinized with 0.33 M perchloric acid and centrifuged at 4,000 × *g*. A 200-μl volume of plasma or serum was deproteinized with 200 μl of acetonitrile, vortexed, and centrifuged at 4,000 × *g*. Urine samples were diluted 50 times with distilled water. Aliquots (100 μl) of the prepared samples were injected onto the analytical column.

Pharmacokinetic analysis. The serum concentration-time curves of the experiments with 50- and 250-mg i.v. boluses of spiramycin were fitted best to a linear, open, two-compartment model as determined with the F ratio test. Basic pharmacokinetic parameters were estimated both from single-dose experiments and from steady-state experiments in accordance with standard procedures (17). Renal clearance was estimated by the total-excretion method, in which the cumulative amount of spiramycin excreted in 24 h into the urine was used. Curve fitting was done by least-squares nonlinear regression analysis, in which data were reciprocally weighted ($1/C^2$), with the aid of the PCNONLIN computer program (26).

RESULTS

Determination of spiramycin. In two experiments (monkeys A1 and A4), levels of total spiramycin (I, II, and III) in serum were determined by both bioassay and HPLC. The results found by both detection methods were roughly similar for those serum samples that were collected within 60 min after administration of the dose. For serum samples that were collected after 60 min, the bioassay gave higher concentrations than did HPLC (Table 3).

Examination of spiramycin concentrations in serum by HPLC revealed that mainly component I was measured (93 to 95%). The concentration of spiramycin II was below the quantitation level. Thus, the remaining 5 to 7% was mainly component III.

Dose-finding experiments. Single-dose kinetics were determined after administration of 50 or 250 mg of spiramycin to

TABLE 3. Concentrations of spiramycin in serum measured by bioassay and HPLC

Sampling time (min)	Concn in monkey A1 serum after 50-mg i.v. dose		Concn in monkey A4 serum after 250-mg i.v. dose	
	Bioassay	HPLC	Bioassay	HPLC
0	0	0	0	0
2	18.5	23.7	68	72
5	6.2	9.7	29	24
10	4.4	5.2	28	24
15	3.4	3.9	20.5	14.4
30	1.45	1.65	12	7.2
60	0.8	0.63	7.2	2.6
90	0.58	0.44	4.5	1.3
120	0.4	0.19	4.3	0.96
150			3.75	0.48
180	0.22	0.10	3.75	0.38
240	0.16	<0.10	3.6	0.26
300			3.5	0.16
360			3.45	<0.1
420			3.05	<0.1
1,440	<0.1	<0.1	0.4	<0.1

the rhesus monkeys. The concentrations in serum after administration of a dose of 50 mg are presented in Fig. 1. The serum concentration-time curve followed a two-compartment model. The kinetic parameters are shown in Table 4. After a single dose of 50 mg, 8% of the dose of spiramycin I administered was recovered from the urine of monkey A1 over a period of 24 h. After stimulation of urine production with mannitol, 8 and 14% were recovered from monkeys A2 and A3, respectively. A constant urine flow was induced in monkeys A2 and A3, and the rates were found to be 0.43 and 0.41 ml/min, respectively.

Single-dose kinetics were also determined in monkeys A4, A5, and A6 after administration of an i.v. dose of 250 mg. A prolonged serum elimination half-life of approximately 16.5 h was found, and the total-body clearance decreased to 6.8 ml/kg/min. Despite administration of mannitol to monkey A5, its urine production rate dropped drastically to 0.046 ml/min and a urinary excretion of 0.75% over 24 h was found. A low urine production rate of 0.011 ml/min was also found for monkey A6 under the same experimental conditions (data not shown).

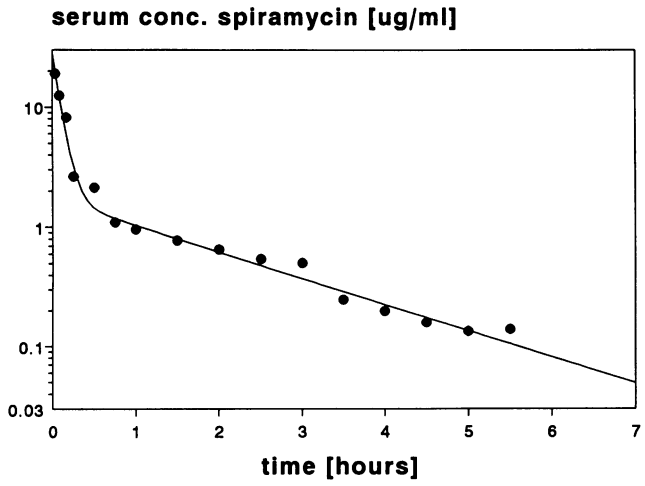

FIG. 1. Concentration (conc.) of spiramycin in monkey A3 serum after a single i.v. dose of 50 mg. The concentrations of spiramycin were measured by bioassay.

A pilot experiment with nonpregnant monkey A7, with a loading dose of 1.75 mg/kg followed by continuous infusion of 0.1 mg/kg/min, showed that a mean steady-state concentration in serum of 3.3 ± 0.47 μg/ml was reached within 10 min (data not shown). The total-body clearance at steady state was 26.2 ml/kg/min (Table 4).

Placental transfer. Transfer of spiramycin from mother to fetus was studied at a target level in serum of 3.0 to 3.5 μg of spiramycin per ml in the mother. The concentrations in serum at steady state are shown in Table 5. As calculated from the steady-state concentrations in serum, total-body clearances of 29.4, 31.4, and 25.0 ml/kg/min were found for monkeys B1, B2, and B3, respectively. These values are in good accordance with those calculated from the single-dose experiments in which 50 mg was given i.v. (Table 4).

Figure 2 shows the concentrations of spiramycin in maternal and fetal sera with constant infusion for monkey B1. In the fetus of monkey B1, 5.5 to 7.9% of the corresponding concentration in the mother's serum was found during a 3-h experiment (also, Table 5). Transplacental transfer was also studied in monkey B2. This monkey had already received a dose of 100 mg daily for 7 days before the continuous infusion was started. The concentration in fetal serum of monkey B2 was also found to be about 6% of the corresponding maternal concentration during the first 2 h. After 2 h, the concentration in fetal serum started to increase and the concentration reached about 22% of the corresponding maternal concentration at 4 and 5 h (Table 5).

Similar kinetics were also found for monkey B3. The predose concentration in the serum of the monkey B3 fetus was below the detection limit, but the fetal levels at 30 and 60 min were higher (14%) than those found in monkey B2 (Fig. 3 and Table 5). At the end of the experiment, the kinetics of monkey B3 differed slightly from the kinetics of monkey B2. The concentration of spiramycin in serum increased in the mother, whereas the fetal concentration decreased (Fig. 3) because the fetus most probably died after collection of the blood sample at 4 h.

After studying placental transfer of spiramycin at a constant level in maternal serum, transfer was studied in monkeys B4 and B5 after a single dose of 50 mg. Prior to the experiment, the monkeys received 50 mg of spiramycin i.v. twice daily for 24 days. The results for monkey B4 are presented in Fig. 4. A trough level of 0.14 μg/ml was found for the fetus. This was 58% of the corresponding maternal concentration (0.24 μg/ml). The concentration in fetal serum increased slightly, to about 0.30 μg/ml, and remained constant for 3 h. The transfer experiment was terminated after 3 h, and an autopsy of fetus B4 was performed. Spiramycin concentrations were determined in fetal tissues. The results are presented in Fig. 5. High spiramycin concentrations were found in the spleen (19 times the concentration in serum), liver (12 times the concentration in serum), and placenta (10 times the concentration in serum). The concentration in the amniotic fluid was relatively steady; 3 h after administration of the final dose, the concentration in amniotic fluid was four times the concentration in fetal serum, but compared with the predose concentration in the amniotic fluid, the concentration was hardly raised. No detectable concentration of spiramycin was found in the brain.

Comparable concentrations in tissue were also found for monkey B5 (Fig. 6). Unfortunately, this monkey turned out to possess only one pair of interplacental blood vessels. As a consequence, the fetus died approximately 30 min after can-

TABLE 4. Pharmacokinetic parameters calculated from single-dose and steady-state experiments

Monkey	Drug regimen	$t_{1/2,z}$[a] (min)	MRT[b] (min)	CL (ml/kg/min)	CL_r[c] (ml/kg/min)	V_1[d] (liters/kg)	V_{ss}[e] (liters/kg)	Urinary excretion (%)
A1	Single 50-mg dose	69	53	45	3.7	0.47	2.4	8
A2	Single 50-mg dose	203	148	35	2.7	0.75	5.1	8
A3	Single 50-mg dose	82	72	34	4.7	0.42	2.3	14
Mean ± SD	Single 50-mg dose	120 ± 70	90 ± 50	38 ± 6	3.7 ± 1	0.55 ± 0.18	3.3 ± 1.6	10 ± 4
A4	Single 250-mg dose	1,140	1,440	8.6		0.73	12.6	
A5	Single 250-mg dose	1,220	1,590	6.4		0.93	10.2	
A6	Single 250-mg dose	640	858	5.5		0.47	4.7	
Mean ± SD	Single 250-mg dose	1,000 ± 310	1,150 ± 350	6.8 ± 1.6		0.71 ± 0.23	9.2 ± 4.1	
A7	Steady-state infusion			26.2				
B1	Steady-state infusion			29.4				
B2	Steady-state infusion			31.4				
B3	Steady-state infusion			25.0				
Mean ± SD	Steady-state infusion			28.0 ± 2.9				

[a] $t_{1/2,z}$, serum elimination half-life.
[b] MRT, mean residence time.
[c] CL_r, renal clearance.
[d] V_1, volume of distribution in the central compartment.
[e] V_{ss}, volume of distribution at steady state.

nulation and the concentrations in serum measured in the experiment were not representative.

DISCUSSION

As determined by HPLC, 93 to 95% of the concentration of spiramycin in serum consisted of component I. At concentrations in serum below 5 μg/ml, only spiramycin I was detectable; spiramycins II and III were below the quantitation level. This is in agreement with the results of Dow et al. (12), who reported the detection of only spiramycin I in plasma from patients given therapeutic doses of spiramycin. The results of the bioassay and HPLC were in agreement for the serum samples obtained within 1 h after administration of the dose. For serum samples collected after 1 h, the bioassay gave higher concentrations than did HPLC. The presence of very low concentrations of components II and III might explain the discrepancy found between the bioassay and HPLC results. It might be possible that components II and III, which cannot be detected by HPLC at very low concentrations, are biologically active and are therefore measured by the microbiological assay. This implies, however, that components II and III would be more potent than component I, which is not logical. The difference between the bioassay and the HPLC results may also mean that a bioactive metabolite that is formed slowly over time is measured. This view is supported by the dose-dependent kinetics observed, indicating the likelihood of formation of metabolites. Since these possible metabolites were active in a bacterial assay, a therapeutic effect on the parasite *T. gondii* may be expected as well. We therefore decided to use the bioassay instead of HPLC because it was our purpose to investigate the effectiveness of spiramycin for treatment of congenital *T. gondii* infections (36).

The distribution of spiramycin, as measured with the bioassay, in rhesus monkeys follows a two-compartment model, as has been found in humans (21, 22). The kinetics appear to be dose dependent, probably as a result of saturable-drug metabolism. Although less obvious than in rhesus monkeys, dose-dependent disposition has also been reported for humans (14).

The urinary excretion of 10% found at a dose of 50 mg in rhesus monkeys is identical to the 5 to 15% reported for humans (22, 23).

TABLE 5. Ratios of spiramycin concentrations in fetal/maternal sera at steady state in mother monkeys B1, B2, and B3

Sampling time (min)	Concn (μg/ml) in maternal/fetal sera (ratio [%])		
	B1	B2	B3
0	0/0	0/0	0.2/0
30	3.35/0.185 (5.5)	2.5/0.12 (4.8)	4.0/0.4 (10)
60	3.65/0.26 (7.1)	2.8/0.15 (5.4)	3.8/0.5 (13)
120	3.3/0.29 (7.9)	3.3/0.26 (7.8)	3.7/0.7 (18.9)
180	2.9/0.195 (6.7)		3.6/1.0 (27.8)
225		3.3/0.54 (16.4)	
240		3.5/0.78 (22.3)	4.0/1.1 (27.5)
300		4.0/0.87 (21.8)	4.8/0.7 (14.6)
Mean[a] ± SD	3.3 ± 0.31/0.23 ± 0.05 (6.8 ± 1.0)	3.2 ± 0.53/0.45 ± 0.33 (13.1 ± 8.1)	4.0 ± 0.43/0.7 ± 0.27 (18.6 ± 7.6)

[a] Calculated from t = 30 min to the end of the experiment.

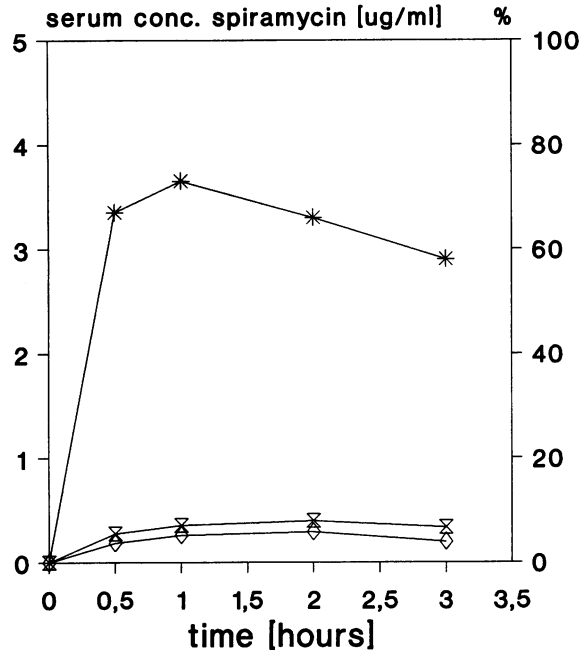

FIG. 2. Concentrations (conc.) of spiramycin in monkey B1 fetal and maternal sera at a constant concentration in maternal serum. Monkey B1 received no spiramycin before the transfer experiment. Symbols: *, maternal; ◇, fetal; ▽△, ratio (percentage).

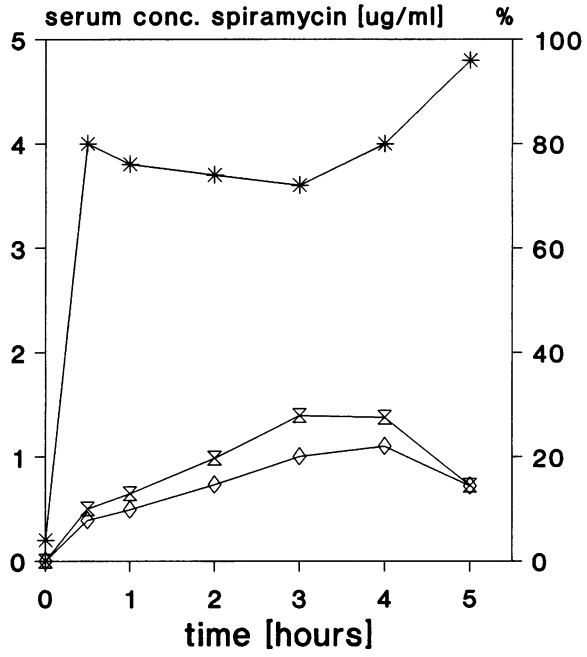

FIG. 3. Concentrations (conc.) of spiramycin in monkey B3 fetal and maternal sera at a constant concentration in maternal serum. Monkey B3 received 100 mg of spiramycin daily in two intermittent doses for 7 days before the experiment, followed by a loading dose of 3.25 mg/kg and continuous infusion of 0.1 mg/kg/min. Symbols: *, maternal; ◇, fetal; ▽△, ratio (percentage).

The renal clearance (15 ± 4 ml/min) is on the order of the glomerular filtration rate of rhesus monkeys (4). Since stimulation of urine production by mannitol did not influence renal clearance, passive reabsorption can be excluded. It seems likely that renal excretion of spiramycin occurs only through glomerular filtration.

Remarkable was the acute oliguric effect observed at the high dose of 250 mg. This phenomenon may explain the low excretion at 250 mg. Several drugs have been reported to cause renal failure (2, 5, 9). Renal failure due to the use of spiramycin or other macrolides has not been reported. Since monkeys A4, A5, and A6 were not sacrificed after the experiment, histological examination of the kidneys was not possible.

The serum elimination half-life of spiramycin in rhesus monkeys was found to be 2 h at a dose of 50 mg. As expected for small animals, this is lower than the value of 5 h found in human volunteers after i.v. administration (14) and the value of 3.8 h reported by Kavi et al. (21) after oral administration.

Studies of the transplacental transfer of spiramycin in humans are poorly documented (13, 15); the duration of treatment and the time interval between the last dose and sampling are not exactly known. Moreover, no data are available on concentrations of spiramycin in fetal tissue. Therefore, an animal model was sought to investigate concentrations in fetal tissue and serum. Since rhesus monkeys possess a hemochorial placenta like humans (34), rhesus monkeys are suitable animals for model studies of transplacental transfer of spiramycin. Placental transfer was measured in monkeys B1 and B2 at a constant maternal spiramycin concentration. During the first 3 h after administration of spiramycin, only 5 to 7% of the corresponding maternal concentration was found in fetal serum. Although the in vivo situation cannot be compared to the in vitro situation, it is remarkable that this percentage is similar to the transfer rate of 9% found by Quentin et al. (33) during a 1-h experiment using a perfusion model of isolated cotyledons. Thus, initially a low concentration was found in fetal serum. Thereafter, the fetal-maternal ratios started to in-

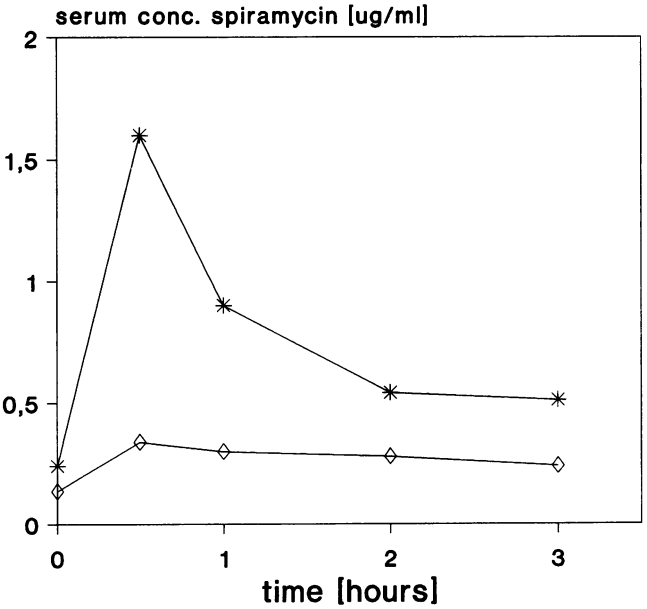

FIG. 4. Concentrations (conc.) of spiramycin in monkey B4 fetal serum after a single dose of 50 mg of spiramycin given i.v. to the mother on day 25. Prior to the transfer experiment, the mother was given 100 mg of spiramycin i.v. daily in two intermittent doses for 24 days. Symbols: *, maternal; ◇, fetal.

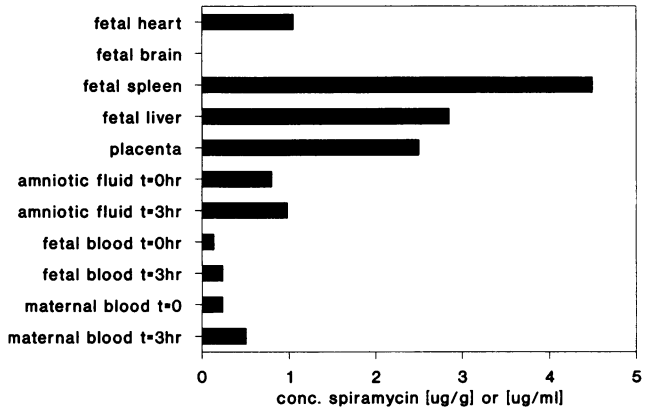

FIG. 5. Concentrations (conc.) of spiramycin in monkey B4 serum, amniotic fluid, and tissue determined 3 h after administration of a final dose of 50 mg.

crease, as found for monkeys B2, B3, and B4. In fact, the fetal-maternal ratio increased to 0.58 in monkey B4, which was given spiramycin for at least 3 weeks. Similar ratios have been found in humans who had been treated for several weeks (13, 15). These data support system hysteresis, indicating that distribution of spiramycin to the fetus takes longer during the first hours after administration of the antibiotic. Tissue and cell distribution probably gradually increases until the tissues and cells become saturated. The results of MacFarlane et al. (24), who found that maximum concentrations of spiramycin in tissue are achieved after 7 days of treatment, support this. Therefore, concentrations in tissue were determined after at least 7 days of treatment (monkeys B3, B4, and B5). Our findings support the results of MacFarlane et al. (24). Drug concentrations in the tissues of monkeys B4 and B5, which were treated for 24 days, were not essentially different from drug concentrations in the tissues of monkey B3, which was treated for 1 week. However, it cannot be concluded from these results whether the maximum levels in tissue are achieved earlier, i.e., before 7 days of treatment.

In rhesus monkeys, as in humans (24) and rats (40), high concentrations of spiramycin are found mainly in tissues such as the liver and spleen. The mechanism by which such high and long-lasting concentrations in tissue are achieved has not been resolved. For the macrolide antibiotic azithromycin, several

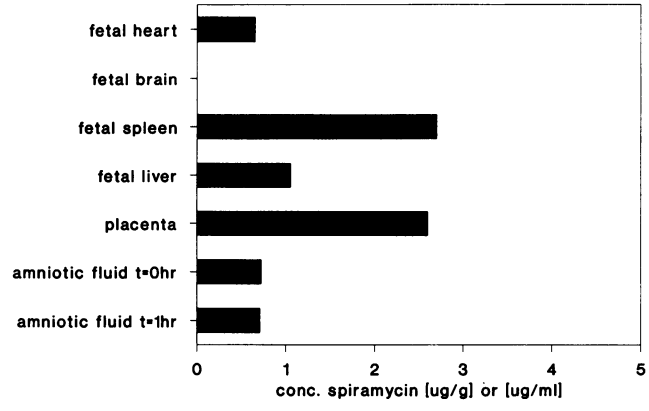

FIG. 6. Concentrations (conc.) of spiramycin in monkey B5 tissue determined 1 h after administration of a final dose of 50 mg.

observations lead to the assumption that phagocytes deliver the antibiotic to cells through uptake, transport, and subsequent release into tissues (18, 25). A similar mechanism may be assumed for spiramycin, since high concentrations of the antibiotic have been found in macrophages (19, 32, 44). Osono and Umezawa (28) have suggested that the levels of spiramycin in tissue are long lasting compared with those of other macrolides because spiramycin is less easily metabolized. Rapid uptake of spiramycin by tissues from serum and slow redistribution from tissues to serum may be another explanation for the long-lasting concentrations in tissue. High concentrations have also been found in the placenta. The finding that concentrations in maternal serum are higher than the concentrations in fetal serum may indicate that the placenta functions as a clearance organ or as a barrier. More research is needed for better insight into the process of transplacental transfer of spiramycin. In vitro studies with isolated placental cotyledons are in progress.

Penetration of spiramycin into cerebrospinal fluid is reported to be poor, but there is little documented evidence (16, 42). In our study, the concentration of spiramycin in the brain was below the detection limit of the bioassay. Since damage of the fetal brain is the most severe consequence, treatment of congenital *T. gondii* infection with spiramycin will be ineffective. It is not clear whether the levels reached in tissues other than the brain are therapeutic. Several investigators have reported concentrations of spiramycin that were effective against *T. gondii* in vitro. Truong et al. (41) found a minimal inhibitory effect at an intracellular concentration of 10 μg/g of tissue. In their in vitro cell culture, the corresponding extracellular concentration was 110 μg/ml. Chamberland et al. (7) found that a concentration of 20 μg/ml had only limited activity (50% inhibition) against *T. gondii* in vitro. Although Derouin et al. (11) reported that inhibition of *Toxoplasma* growth in cell culture was already observed at a concentration of 1 μg/ml, a maximum effect was only achieved at a drug concentration of 250 μg/ml. Such high extracellular concentrations of 200 (20) and 246 (8) μg/ml have been found to inhibit the incorporation of [^{3}H]uracil by *T. gondii* tachyzoites in infected cells but had no significant killing effect. The corresponding intracellular spiramycin concentrations, however, are not exactly known.

Nevertheless, it is difficult to compare the in vitro situation with the in vivo one. Whether the concentrations found in tissue are effective in a congenitally infected fetus needs further investigation. Therefore, we have developed a rhesus monkey model for congenital *T. gondii* infections. The effectiveness of spiramycin has been investigated in this model (36).

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