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ARTICLE

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Treatment of Toxoplasmosis in Pregnancy: Concentrations of Spiramycin and Neospiramycin in Maternal Serum and Amniotic Fluid

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Abstract Toxoplasma infection during pregnancy is widely treated with oral spiramycin to reduce the risk of congenital toxoplasmosis in the infant. Failures of therapy have been observed, however. In this study, a sensitive high-performance liquid chromatography technique was used to measure concentrations of spiramycin and neospiramycin, one of the major metabolites of spiramycin, in maternal serum and amniotic fluid. Samples were obtained from 18 women who underwent amniocentesis for polymerase chain reaction (PCR) diagnosis of fetal infection 5–109 days following the prescription of spiramycin therapy (3 g/day). Concentrations of spiramycin and neospiramycin in both serum and amniotic fluid were highly variable, ranging from nondetectable values to 1 μg/ml. None of the concentrations measured were within the range reported to inhibit growth of the parasite in vitro. Consistent with previous reports, part of the observed variability in maternal and fetal drug concentrations could be explained by individual differences in several pharmacokinetic parameters: intestinal absorption, tissue distribution, cellular uptake, metabolism, transfer across the placenta, drug accumulation in fetal tissue, and maternal and fetal drug elimination. The heterogeneity of the data could also be related to differences in patient compliance with the medication prescribed. By addressing factors that could impair adequate treatment of toxoplasmosis during pregnancy, the data presented call for a larger-scale controlled study to determine individual and diurnal variations in maternal drug levels, patient compliance, and outcomes of the offspring. The activity of neospiramycin against *Toxoplasma gondii* should be assessed.

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Introduction

Primary maternal infection with *Toxoplasma gondii* during pregnancy presents a high risk for fetal infection [1, 2, 3]. Oral treatment with spiramycin is recommended to prevent placental transmission and/or continued fetal infection [4, 5, 6]. This macrolide antibiotic is well tolerated (at a dosage of 1 g t.i.d.) and only rarely causes negative side effects [1, 4, 7, 8]). Spiramycin has been shown to accumulate in placental tissue and pass into the fetal circulation [9]. It is thought to prevent transmission of the infection to the fetus, although this hypothesis is disputed [1, 5, 6]. The reported concentrations in fetal blood and placenta were below those required to inhibit growth of the parasite in vitro [10, 11, 12], an observation also made in a comparative study of rhesus monkeys [13]. Spiramycin may be partially deglycosylated to neospiramycin [14], a compound that exhibits antibacterial activity [15], but the activity of neospiramycin against *Toxoplasma gondii* has not yet been studied.

Congenital toxoplasmosis has been observed during pregnancy, even in cases in which the mother was treated with oral spiramycin, which has raised doubts about the efficacy of this drug [5, 6, 16]. Therefore, the aim of this study was to determine concentrations of spiramycin and neospiramycin in maternal serum and amniotic fluid samples obtained from women who received a standard course of spiramycin therapy for treatment of serologically diagnosed primary maternal infection and who subsequently underwent amniocentesis for diagnosis of fetal infection.

Patients and Methods

Collection of Serum and Amniotic Fluid Samples

From July 1999 to January 2000, serum and amniotic fluid samples were obtained from 18 pregnant women who were receiving

spiramycin treatment due to primary toxoplasma infection. With their consent, the samples of amniotic fluid taken for polymerase chain reaction (PCR) diagnosis of fetal infection were also used for measurement of spiramycin and neospiramycin concentrations using high-performance liquid chromatography (HPLC). After centrifugation at 3,000×*g*, the sediment was used for PCR and the supernatant was frozen at –80°C until use for HPLC.

Experimental Procedures

Spiramycin was a generous gift from the National Institute for Quality Control of Drugs, Vienna, Austria. Neospiramycin was prepared by acid hydrolysis of spiramycin according to Renard et al. [17]. Conversion to neospiramycin exceeded 95%. Calibration solutions for HPLC were prepared by the addition of spiramycin and neospiramycin to serum and amniotic fluid from untreated patients to obtain dilutions of 0.05 μg/ml, 0.1 μg/ml, 0.3 μg/ml, 0.5 μg/ml, and 1 μg/ml. The HPLC system consisted of a LaChrom High Pressure Liquid Chromatograph (Merck, Germany), equipped with an L-7100 pump, an L-7250 autosampler, an L-7300 column oven used at 40°C, an L-7400 UV/Vis Detector (wavelength, 230 nm), a D-7000 interface, and a BDS-C18 analytical column (5 μm, 250×4.6 mm, Hypersil, UK). Samples were prepared on Oasis Extraction Cartridges (Waters Corporation, USA) that had been equilibrated with 1 ml of methanol and 1 ml of distilled water. One milliliter of serum and 4 ml of amniotic fluid were applied, washed with 1 ml of distilled water, and eluted with 1 ml of methanol. Concentrations of spiramycin and neospiramycin were measured using HPLC as described by Renard et al. [17], with the following modifications: in brief, an isocratic mode was used with 70% 10 mM ammonium acetate/acetic acid buffer, pH 4.0, and 30% acetonitril at a flow rate of 1 ml/min. The sample volume injected was 100 μl. The total analyzing time for each sample was 20 min (average retention time: 7.5 min for spiramycin and 5.5 min for neospiramycin). The assay was validated by regression analysis of the relationship between signal and drug concentration at a range from 0.05 μg/ml to 1 μg/ml. R^2 values for spiramycin in serum and amniotic fluid were 0.992 and 0.986, respectively. Calibration of neospiramycin was accomplished by using spiramycin as the external standard and assuming equal extinction coefficients. Detection limits for spiramycin and neospiramycin, defined as a signal-to-noise ratio of 3, ranged from 0.009 to 0.013 μg/ml for serum and from 0.011 to 0.014 μg/ml for amniotic fluid.

Results

Oral spiramycin therapy (1 g t.i.d.) was prescribed to 18 pregnant women with proven or suspected primary toxoplasma infection. In order to confirm or disprove fetal infection, amniocentesis was carried out 5–109 days after initiation of therapy. At this time, concentrations of spiramycin and its metabolite, neospiramycin, were determined in both maternal serum and amniotic fluid. Table 1 summarizes the data obtained. Concentrations of both compounds in serum and amniotic fluid varied greatly, ranging from nondetectable levels up to 1 μg/ml. No correlation was observed between the duration of therapy or gestational age and the concentrations of compounds in either sample. In general, concentrations in serum were higher than those in amniotic fluid (Figs. 1 and 2). None of the concentrations of spiramycin measured were >10 μg/ml, which is considered sufficient to inhibit growth of the parasite in vitro [10, 11, 12, 13]. Furthermore, the relationship between concentrations of spiramycin and neospiramycin did not follow a consistent pattern.

Despite the positive serological diagnosis of primary maternal infection, all newborns were negative for antitoxoplasma IgM antibodies at the time of birth. All exhibited IgG levels that decreased towards undetectable

Table 1 Concentrations of spiramycin and neospiramycin in maternal serum and in amniotic fluid, as determined by HPLC. Samples were obtained after oral therapy with spiramycin had been initiated to treat serologically diagnosed primary maternal toxoplasma infection, i.e. at the time of amniocentesis to prove or disprove fetal toxoplasma infection by PCR

Sample no.	Maternal age (years)	Gestational age (weeks)	Duration of therapy (days)	Drug concentration (ng/ml)			
				Serum		Amniotic fluid	
				SPR	Neo-SPR	SPR	Neo-SPR
1	23	15	20	39	588	81	70
2	31	15	49	368	485	130	245
3	21	16	21	466	n.d.	51	369
4	32	16	29	456	633	96	114
5	18	16	40	54	343	65	224
6	27	16	49	819	348	153	77
7	28	17	13	260	152	70	67
8	28	17	45	93	39	54	94
9	32	17	60	n.d.	n.d.	24	31
10	28	17	109	36	n.d.	87	197
11	38	18	31	83	24	53	166
12	32	18	48	353	n.d.	n.d.	n.d.
13	26	19	6	172	790	67	137
14	28	20	24	642	93	52	17
15	33	20	31	196	1000	389	279
16	29	21	5	34	n.d.	474	332
17	27	24	14	108	n.d.	35	14
18	22	27	77	324	505	414	396

SPR, spiramycin; Neo-SPR, neospiramycin, n.d., not detectable

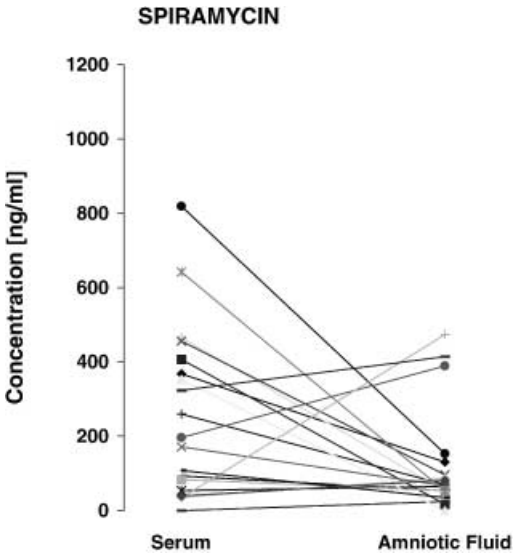

Fig. 1 Correlation between concentrations of spiramycin in serum and amniotic fluid (same patients as in Table 1)

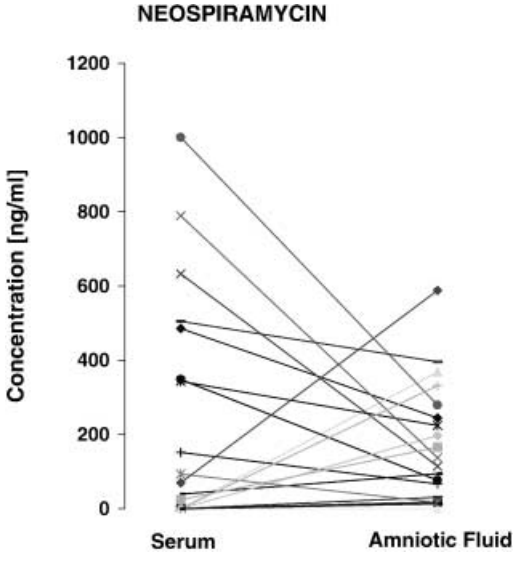

Fig. 2 Correlation between concentrations of neospiramycin in serum and amniotic fluid (same patients as in Table 1)

values during the first year of life (except the offspring of mothers no. 10, 12, and 16, for whom follow-up data were not available). It should be noted, though, that PCR tests for fetal infection were also negative at the time the samples were taken. Thus, it is undetermined whether fetal infection did not occur or whether transmission of the parasite to the fetus was prevented despite the low and variable drug concentrations found in this study.

Discussion

Many studies have addressed the efficacy of prenatal antibiotic treatment of primary toxoplasmosis during pregnancy with respect to both fetal transmission and neonatal sequelae [5, 6]. Though less frequent than at a later gestational age, fetal infection in the first trimester bears a high risk of causing severe organ manifestations. Because of side effects and the risk of teratogenicity, treatment with pyrimethamine and sulfadiazine is restricted to a later gestational age (from week 16 onward), and oral therapy with spiramycin is recommended initially [7, 8]. Despite this treatment, however, organ manifestations have been observed at birth [2, 3, 18]. Therefore, the aim of this study was to determine the concentrations of spiramycin in both maternal serum and amniotic fluid. Samples were obtained from pregnant women serologically diagnosed as having acquired primary toxoplasmosis and subsequently treated with oral spiramycin (1 g t.i.d.) and in whom amniocentesis was performed for diagnosis of fetal infection. A sensitive HPLC technique was used to measure concentrations of spiramycin and its major metabolite, the deglycosylated derivative neospiramycin. As shown in Table 1 and Figs. 1 and 2, concentrations in both maternal serum and amniotic fluid were highly variable. As discussed below, these highly variable concentrations could result from differences between individual patients in the pharmacokinetics of absorption, tissue distribution, metabolism, and excretion of spiramycin. Therefore, effective tissue concentrations may not be reached in some patients, resulting in failure of spiramycin therapy to prevent fetal infection or its progress.

In previous studies, concentrations of spiramycin were generally determined by biological assays. These methods result in the measurement of equivalent concentrations of the drug, which include metabolites that could also exhibit biological activity. Neospiramycin, a metabolite of spiramycin, may have such activity, but concentrations of neospiramycin in humans have not yet been studied. Using a dose of 3 g of spiramycin per day, Garin et al. [19] found mean effective concentrations at birth in maternal serum, fetal serum, and placenta of 1.7 μg/ml, 0.8 μg/ml, and 6.2 μg/ml, respectively. In month 5 of pregnancy, mean maternal and fetal serum concentrations of 0.6 μg/ml and 0.3 μg/ml were reported, but again, individual values showed a great variability, particularly in maternal blood. There was no correlation between fetal and maternal serum concentrations. In another study, mean concentrations in umbilical blood and placental tissue at birth were 0.34 μg/ml and 2.3 μg/ml, respectively [9]. Using an HPLC technique, Bourget et al. [20] measured mean concentrations in amniotic fluid of 0.65 μg/ml. In a rhesus monkey model, comparable fetal-maternal serum concentration ratios of around 0.5 were found, and, compared to fetal serum, concentrations in amniotic fluid were about five times higher, while concentrations in placental tissue were 10–20 times higher [13].

Differences between individual patients in absorption, tissue distribution, metabolism, and elimination of spiramycin may cause some of the variability of the concentrations observed in our study and in the studies reported above. Macrolide antibiotics such as spiramycin are

mainly absorbed in the alkaline intestinal environment [21]. They are acid unstable, and, after oral application, the stomach may be one site for conversion of spiramycin to neospiramycin. Following a single 1 g oral dose, maximal serum concentrations of spiramycin of 0.4–1.4 μg/ml are obtained after approximately 3 h, and bioavailability is estimated to be 30–40%, with a volume of distribution of >300 1 [22, 23, 24]. In accordance with the liposolubility of the compound, this high volume of distribution indicates extensive concentrative uptake into cells such as liver or macrophages [25] but not into brain or cerebrospinal fluid [13, 26]. Cellular uptake appears to explain the "paradox" seen in animal models of infection, i.e. a particular dosage of spiramycin may sufficiently protect the whole animal, while the corresponding serum concentrations remain below the minimum inhibitory concentration determined in vitro [27]. Metabolic modification of spiramycin, including formation of neospiramycin after intravenous administration, has been studied exclusively in animals such as cows [17] and pigs [14]. Excretion in humans is predominantly via the biliary route (approx. 90%). The resulting serum half-life values reported varied widely (3–9 h) and were prolonged with increasing age [22, 21, 23, 24]. Large diurnal variations in serum concentrations (by a factor of 6) were seen in young patients given a single daily dose [22]. No comparable pharmacokinetic data are available for neospiramycin in humans. In cattle, plasma and tissue clearance of neospiramycin appears to be considerably prolonged as compared to spiramycin [17, 28].

It is apparent from these studies that the pharmacokinetic properties of spiramycin exhibit a large interindividual variability that may be attributable to multiple parameters such as the quantity of neospiramycin formed, the rate of absorption and bioavailability, tissue distribution, placental transfer, and the rate of elimination in bile and urine. These variables may in part explain why, in our studies, concentrations found in maternal serum and amniotic fluid are scattered over a wide range (Table 1). Nonetheless, some data appear to indicate that a steady state was not reached in all patients. In particular, in some cases, concentrations in amniotic fluid were higher than in maternal serum (Figs. 1 and 2), indicating that samples were taken a relatively long time after the last administration, when elimination from maternal serum may precede the elimination from the fetal compartment. In addition, very low or nondetectable concentrations are suggestive of insufficient patient compliance with the therapeutic recommendations.

In this study, neospiramycin comprises a considerable but variable fraction of the total drug concentration found in maternal serum or amniotic fluid. Whether this metabolite is effective not only against bacteria [15] but also against the protozoon *Toxoplasma gondii* must still be investigated.

In conclusion, this study took a random "snapshot" of concentrations of spiramycin in maternal serum and amniotic fluid in patients undergoing routine treatment at the time of amniocentesis. The high variability of drug concentrations found could explain the failure of standard spiramycin treatment to prevent fetal infection or progression of infection in some cases. We speculate that this large variability in drug concentrations results partially from individually variable pharmacokinetic parameters. The data also indicate that some patients (e.g. patient 9 in Table 1) did not comply with the therapeutic recommendations. It was surprising to note that none of the concentrations measured were within the range effective in vitro. Even if cellular uptake into the placenta and fetal tissues is assumed, tissue concentrations may not always reach a level sufficient to prevent transmission of the parasite, as initially suggested [1], or to prevent progression of the fetal infection. Within the setting of this study, it was not possible to determine whether the clinical outcome is related to the spiramycin therapy prescribed, but the data presented should raise alertness about the adequacy of treatment. It should lead to a larger scale, controlled study to determine individual and diurnal variations in maternal drug levels, compliance, and outcomes of the offspring. Our data also show that a large fraction of the dose applied may be converted to neospiramycin, whose therapeutic potency against *Toxoplasma gondii* needs to be determined.

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