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Effectiveness of Spiramycin for Treatment of Congenital *Toxoplasma gondii* Infection in Rhesus Monkeys

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The effectiveness of spiramycin for the treatment of rhesus monkey fetuses congenitally infected with *Toxoplasma gondii* was studied. Eight monkeys were infected at day 90 of pregnancy. This is comparable to the second trimester of organogenetic development in humans. Transmission of infection was found prenatally in five of the eight monkeys by detection of the parasite in the amniotic fluid. Treatment with spiramycin (20 mg/kg/day in two intermittent doses given intravenously) was started as soon as fetal infection was proven and was continued until birth. Nine to 14 days after initiation of treatment, the parasite was still detectable in amniotic fluid samples from four of these five cases. However, the parasite was detected only by PCR and not by mouse inoculation. *T. gondii* was also detected only by PCR in the placenta of one monkey that delivered prematurely. This monkey received spiramycin treatment for only 2 weeks. In the four monkeys that received treatment for about 7 weeks, the parasite was not present at birth in the placenta nor in amniotic fluid or neonatal organs. Spiramycin accumulates mainly in maternal tissues. Although concentrations in neonatal tissue were found to be 5 to 28 times higher than the corresponding concentrations in neonatal serum, the concentrations in neonatal tissue were still 11 to 16 times lower than those found in the mothers. However, no spiramycin was found in the fetal brains. Early treatment with spiramycin may prevent transmission of infection to the fetus but most probably cannot interrupt an existing brain infection, which is the most severe outcome of congenital toxoplasmosis in humans.

Congenitally acquired *Toxoplasma gondii* infection can cause severe disorders in a fetus, such as hydrocephalus, microcephalus, deafness, or blindness, or it may even lead to fetal death. The risk of severe abnormalities is high when the fetus becomes infected during the first trimester of pregnancy, but the rate of transmission is relatively low, about 14%. The transmission rate increases to about 60% in the third trimester of pregnancy, whereas the risk of severe damage decreases (12). Therefore, especially early in pregnancy, treatment of the fetus is mandatory to reduce the risk of severe abnormalities. There are several drug regimens available for the treatment of *T. gondii* infections. The recommendations of Couvreur cited by Remington et al. (27, 28) are generally followed for the treatment of congenital *T. gondii* infections. According to these guidelines, pregnant women in whom fetal infection has been proven are treated with pyrimethamine in combination with a sulfonamide, usually sulfadiazine. This drug regimen, however, can cause side effects. Pyrimethamine can cause bone marrow depression and hematologic toxicity (21, 27). At high doses, teratogenic effects have been found in rats (18, 20). Sulfadiazine should not be administered during the last month of pregnancy because it increases the risk of kernicterus (36).

The macrolide antibiotic spiramycin is also used as a drug against *T. gondii*. This drug is well tolerated and safe in pregnancy. Couvreur et al. found that treatment of humans with spiramycin reduced the number of infected placentas, resulting in a reduced risk of congenital toxoplasmosis (7). The effect can probably be explained by the high concentrations of spiramycin found in placental tissue (13, 16). Because spiramycin is thought to create a barrier to transmission of infection, it is given to prevent transplacental passage of the parasite in women with a primary infection.

Recent investigations of transplacental transfer of spiramycin in rhesus monkeys revealed that spiramycin not only accumulates in the placenta but also crosses the placenta and accumulates in the soft tissues of the fetus (31). Concentrations of spiramycin in fetal serum were half of those in maternal serum. Whether the levels that are reached in the serum and tissue of the fetus are effective is not known.

The low frequency of congenital toxoplasmosis makes a placebo-controlled evaluation of treatment of humans with spiramycin impractical. Furthermore, it is considered to be unethical to refrain from treatment of pregnant women with a primary infection. Therefore, we have developed a model for congenital *T. gondii* infection in rhesus monkeys (32). It is based on the assumption that humans and rhesus monkeys—both having a placenta of the hemochorial type (26)—are similar with regard to the process of maternal transmission of the infection and the transfer of antitoxoplasma drugs. By using this animal model, we investigated what concentrations of spiramycin are reached in fetuses and whether these concentrations are effective in congenitally infected fetuses.

MATERIALS AND METHODS

The experiments in the present study were performed in accordance with protocols that have already been described in detail (32, 35). Materials and Methods, therefore, contains only brief descriptions of the original protocols, supplemented with minor modifications.

Monkeys. All monkeys were derived from the Laboratory Animals Breeding Experimental Farm of Shunde Guangdong, Beijing, People's Republic of China. The female monkeys were

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4 to 5 years old and weighed 4.5 to 5.5 kg. Housing, feeding, and breeding of the monkeys occurred exactly as described before (32).

The study group consisted of eight pregnant monkeys (C1 to C8) that were seronegative for immunoglobulin G and immunoglobulin M antibodies to *T. gondii* before the experiment was started. Immunoglobulin G and immunoglobulin M antibodies were measured by enzyme-linked immunosorbent assays (ELISAs) that have been described before (32).

The control group consisted of nine pregnant monkeys (A1 to A9) that received no treatment. The outcome of fetal infection in the control group has been described before (32); the results will be summarized in the present report. The fetuses and babies of the monkeys from the study group and the control group will be referred to as C_f1 to C_f8 and A_f1 to A_f9, respectively.

Experimental design and collection of samples. Fetal infection was tested 10 days after inoculation of the mother and again at birth in the nine monkeys of the control group (32). For the eight monkeys of the study group, the protocol for antenatal screening of fetal infection was extended with the introduction of two additional amniotic punctures 25 and 40 days after inoculation of the mothers. In addition, fetal blood was sampled from an interplacental blood vessel 40 days after inoculation to test for parasitemia (32).

The study design is shown in Fig. 1A. The eight monkeys were inoculated intravenously with 5×10^6 *T. gondii* RH parasites at day 90 of pregnancy (this is comparable to the second trimester of organogenetic development in humans).

At 8, 10, 25, and 40 days after inoculation and during cesarean section (70 days after inoculation), maternal blood was sampled to test for parasitemia. Amniotic fluid samples were obtained by puncture 10, 25, and 40 days after inoculation and tested for the presence of *T. gondii* to monitor fetal infection. At day 160 of pregnancy, a cesarean section was performed. Amniotic fluid, placental tissue, and neonatal blood were collected to test for the presence of the parasite. Baby monkeys C_f1 and C_f2 were prematurely born before day 160. In these cases, the amniotic fluids were lost to examination but the placentas were still available. The neonates were sacrificed, and autopsies were performed. The heart, brain, spleen, liver, and lungs were examined for the presence of *T. gondii*.

Processing of clinical samples. All clinical samples were tested for the presence of *T. gondii* both by mouse inoculation (32) and by nested PCR based on the ribosomal DNA gene (30, 32).

Amniotic fluid samples were collected in time to monitor fetal infection, as indicated above. Six milliliters of amniotic fluid was collected at each amniotic puncture. Two mice were inoculated intraperitoneally with 1 ml of amniotic fluid each to test for the presence of *T. gondii*. The remaining 4 ml was divided into 1-ml portions and centrifuged for 10 min at 800 × *g*. The supernatants were stored at −80°C until determination of the spiramycin concentration. The sediments were each resuspended in 100 μl of physiological salt solution. DNA was isolated from two sediments by guanidinium thiocyanate and silica particles, exactly as described by Boom et al. (4), and tested by PCR. The other two sediments were stored directly at −80°C.

The placenta and maternal and neonatal organs were homogenized in physiological salt solution as described before (35) and tested for the presence of parasites by inoculation of two mice. The protocol for DNA isolation from tissues was modified as follows. DNA was isolated by incubation of the tissue in 6% *p*-aminosalicylic acid–1% NaCl–10 mM EDTA–0.5 μg of proteinase K per ml for 3 h at 37°C. After phenol extraction, the DNA was precipitated overnight at −20°C. One microgram of the tissue DNA was tested for the presence of *T. gondii* by PCR.

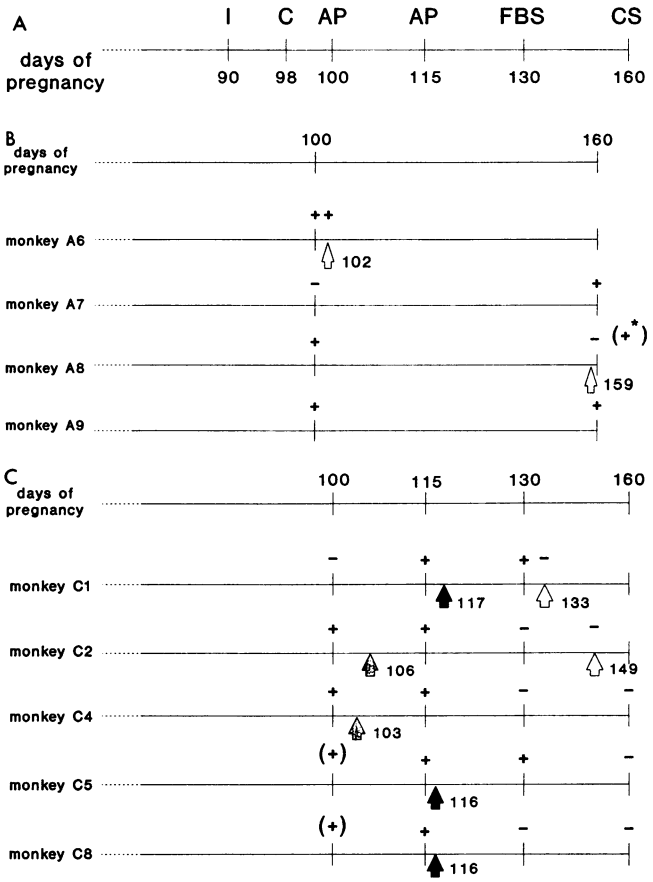

FIG. 1. (A) Time schedule of sample collection during pregnancy. I, infection 90 days after conception; C, control of parasitemia; AP, amniotic puncture; FBS, fetal blood sampling; CS, cesarean section. During fetal blood sampling, both amniotic fluid and fetal blood were collected. During cesarean section, amniotic fluid, neonatal blood, and the placenta were collected. Cesarian section was followed by autopsy of the neonate, and the heart, spleen, brain, and liver were obtained. All samples were tested for the presence of *T. gondii*. (B) Time schedules for the four monkeys in the control group that received no treatment. Detection of *T. gondii* in the amniotic fluid and/or neonatal tissues is indicated by a plus sign. Premature birth is indicated by an arrow, and the pregnancy duration is given in days. (+*), fetal infection proven by demonstration of antibodies in the neonate at serological follow-up. (C) Time schedules for the five monkeys in the study group that received treatment with spiramycin. Detection of *T. gondii* in amniotic fluid is indicated by a plus sign. The day on which spiramycin treatment was started is marked with a dark arrow. Premature birth is indicated by a light arrow, and the pregnancy duration is given in days. (+), result delayed by technical problems and amniotic fluid found positive at retesting.

PCR. All DNA samples were tested for the presence of *T. gondii* by a nested PCR based on the ribosomal DNA gene. The reaction conditions were the same as described before (32), except for the primer set of the first reaction of the nested PCR. This primer set consisted of two *T. gondii*-specific primers which were selected in the second variable region (5′-TGCCTCTTCCCTGGAAGGCA-3′) and the fourth variable region (5′-GTGTGGAGAAATCCAGAAGG-3′) of the

ribosomal DNA gene and generated a 535-bp fragment. The internal primer set for the nested reaction was the same as described before (5′-GTTGACTTCGGTCTGCGACG-3′ and 5′-TTCCAATCACTAGAAATGAA-3′) and generated a *T. gondii*-specific product of 238 bp (30, 32). The fragment was blotted onto a nylon filter and hybridized (30) with a ^{32}P-end-labeled oligonucleotide probe (5′-ATTCCGGAGAAGGAGC CTGA-3′) as described previously (35). To prevent carryover contamination, strict spatial partitioning of the different steps of the PCR was included and the recommendations of Kwok and Higuchi were followed (22).

Treatment with spiramycin. The effectiveness of treatment with spiramycin was studied in a group of five pregnant monkeys (C1, C2, C4, C5, and C8) with a proven fetal infection.

Treatment was started as soon as a congenital infection was proven by detection of *T. gondii* in the amniotic fluid. Treatment was continued until cesarean section or occurrence of a spontaneous birth. The mother was given 20 mg of spiramycin per kg per day intravenously in two intermittent doses. Spiramycin adipate (Rovamycin) for intravenous application was from Rhône Poulenc, Paris, France. Spiramycin was administered intravenously, since it was found for humans that coadministration with food can reduce bioavailability by 50% (14).

The dosing schedule was derived both from recommendations for intravenous administration of spiramycin in humans (29) and from studies of spiramycin pharmacokinetics in rhesus monkeys (31). These studies revealed that the pharmacological behavior of spiramycin in rhesus monkeys was similar to that in humans with regard to the maximum concentrations achieved in serum, tissue distribution, and urinary excretion. As expected for small animals, the serum elimination half-life was lower than in humans.

Determination of antibiotic concentrations. The concentration of spiramycin was determined by a bioassay. In brief, the spiramycin concentration was determined by diffusion in 3% Difco agar medium no. 11 (Brunschwig Chemie, Amsterdam, The Netherlands), pH 8.0, with *Sarcina lutea* ATCC 9341 as the test organism. A stock suspension of *S. lutea* was diluted with distilled water to a test suspension with an optical density at 630 nm of 0.21. The agar medium, at 50°C, was inoculated with 2% of the bacterial suspension and subsequently poured onto a plate on a flat surface. Inhibition zones were measured after incubation at 30°C for 18 h. Spiramycin concentrations were determined in maternal blood, fetal blood, amniotic fluid, the placenta, and organs (heart, spleen, liver, and brain) of the mother and neonate. The blood was centrifuged at 800 × *g* for 10 min. The concentration of spiramycin was determined in a 100-μl sample of either maternal serum, fetal plasma, or amniotic fluid. One gram of tissue was homogenized in 4 ml of physiological salt solution and clarified by centrifugation at 1000 × *g* for 20 min. A 100-μl volume of the supernatant was tested for the concentration of spiramycin per gram of tissue in the bioassay.

Statistical evaluation. Fisher's exact test for comparison of proportions was used to compare the infection probabilities in the study group that received spiramycin and the control group. Furthermore, 80 and 95% confidence intervals were calculated for an infection-free outcome of therapy. The confidence intervals were based on a binomial distribution (2).

RESULTS

Maternal parasitemia that lasted for about 10 days was observed in all eight monkeys of the study group and the nine monkeys of the control group. The parasitemia was detected by mouse inoculation and PCR.

TABLE 1. Pre- and postnatal detection of *T. gondii*

Monkey[a]	Sample	Day of collection[b]	PCR result	Mouse inoculation result
A6	AF[c]	100	+	−
	Plac[d]	102	+	+
	Tissues	102	+	+
A7	AF	160	+	+
	Plac	160	+	+
A8	AF	100	−	+
A9	AF	100	+	−
	AF	160	+	+
	Plac	160	+	+
C1	AF	115	+	−
	AF	130	+	−
	Plac	133	+	−
C2	AF	100	+	+
	AF	115	+	−
C4	AF	100	+	−
	AF	115	+	−
C5	AF	100	+	+
	AF	115	+	−
	AF	130	+	−
C8	AF	100	+	−
	AF	115	+	−

[a] Monkeys A6 to A9 were the control group. Monkeys C1, C2, C4, C5, and C8 were treated with spiramycin.
[b] The day of collection is presented as the number of days after conception. Monkeys were infected at day 90 of pregnancy. Only samples positive for *T. gondii* are specified.
[c] AF, amniotic fluid.
[d] Plac, placenta.

As described in our former report, *T. gondii* was detected antenatally in three of the nine fetuses of the control group (A_f6, A_f8, and A_f9) (32). At birth, four fetuses were found to be congenitally infected. Three infections were diagnosed by detection of the parasite in the amniotic fluid or neonatal organs (A_f6, A_f7, and A_f9), and the fourth was diagnosed by antibody production by the neonate (monkey A_f8). These results were extrapolated to the outcome of the present study for estimation of the effect of treatment. The control group results are shown in Fig. 1B and Table 1.

Prenatally, *T. gondii* was found in the amniotic fluid of five of the eight monkeys of the study group (C_f1, C_f2, C_f4, C_f5, and C_f8). All five cases of transmission were detected by PCR. Mouse inoculation was positive only in monkeys C_f2 and C_f5 (Table 1). Transmission of infection occurred in four of the five cases within 10 days after inoculation of the mother. In monkey C1, infection of the fetus was detected after 25 days (Fig. 1C). Figure 2 shows the PCR result obtained with samples from this monkey.

The first amniotic fluid sample, collected about 12 (range, 9 to 14) days after onset of treatment, was still positive by PCR for monkeys C_f1, C_f2, C_f4, and C_f5 (Fig. 1C). At this time, mouse inoculation was negative for all five cases (Table 1).

At birth, the parasite was detected by PCR in the placenta of

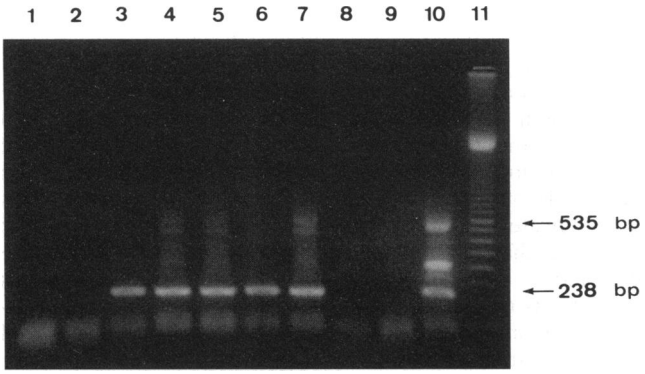

FIG. 2. Ethidium bromide-stained gel showing amniotic fluid samples and the placenta of monkey C1 tested by nested PCR based on the ribosomal DNA gene. Lanes: 1 and 2, amniotic fluid collected on day 100 of pregnancy; 3 and 4, amniotic fluid collected on day 115 of pregnancy; 5 and 6, amniotic fluid samples collected on day 130 of pregnancy; 7, placenta obtained after premature delivery on day 133 of pregnancy; 8, amniotic fluid (negative control); 9, distilled water (negative control); 10, 10 ng of *T. gondii* DNA (positive control); 11, DNA size markers (100-bp ladder).

monkey C1 (Table 1). This monkey delivered prematurely after a pregnancy of 133 days and was treated for only 15 days (Fig. 1C). *T. gondii* was not found in the placentas of the other four monkeys nor in any of the organs of the baby monkeys, including the premature one (monkey C_f1). *T. gondii* was also never found in the fetal blood sampled 40 days after maternal inoculation. Statistical evaluation of these results revealed that the probability of fetal infection in the period from 100 to 160 days after conception in the study group was not significantly different from that of the control group (Table 2; $P = 0.64$). The null hypothesis of equal infection probabilities was not rejected. Given fetal infection, the probability that the infection would still be present at birth was lower in the study group than in the control group (Table 3; $P = 0.048$). The confidence intervals for the outcome after treatment were calculated for this small number of animals. The 80% confidence limits were 0 to 32%, and the 95% confidence limits were 0 to 50%.

Four amniotic fluid samples per monkey were collected over time. For the eight monkeys of the study group, this made a total of 32 amniotic fluid samples. These 32 amniotic fluid samples were tested in duplicate for the presence of *T. gondii* by mouse inoculation and by PCR. When tested by PCR, a discrepancy was found in only 3 (9.4%) of the 32 samples tested in duplicate. In contrast, only one of the two amniotic fluid samples was found positive by mouse inoculation in all cases.

Spiramycin was found to cross the placenta and to accumulate in fetal tissues, but no spiramycin was found in the brain (Table 4). The highest neonatal concentration was measured in the spleen and was found to be 28 times the concentration in neonatal serum. The mean concentration of spiramycin in the placenta was 3.04 ± 0.87 μg/g (Table 4), which was similar to the concentrations reached in the neonatal spleen and liver. These concentrations in tissue, however, did not reach the levels in maternal tissue, which were found to be 11 to 16 times higher than in the neonates.

TABLE 2. Probability of fetal infection in the study and control groups in the period from 100 to 160 days after conception

Infection	No. (%) in:	
	Study group[a]	Control group[b]
Yes	5 (63)	4 (44)[c]
No	3	5[c]
Total	8	9

[a] Treated with spiramycin.
[b] Not treated.
[c] $P = 0.64$.

TABLE 3. Probability that the infection will still be present at birth in the study and control groups

Infection	No. (%) in	
	Study group[a]	Control group[b]
Yes	0 (0%)	3 (75%)[c]
No	5	1[c]
Total	5	4

[a] Treated with spiramycin.
[b] Not treated.
[c] $P = 0.048$.

DISCUSSION

Studies with mice have shown that treatment with spiramycin can prolong survival after lethal infection with *T. gondii* when spiramycin is given in doses of 400 to 950 mg/kg/day. Spiramycin, however, fails to eradicate the parasite from the host (1, 15, 24, 25). The concentrations in serum and tissue at which this prolonged survival has been found are not known. In addition, Beverley et al. (3) have shown that treatment with spiramycin reduces the number of *T. gondii*-induced focal inflammatory lesions in mice. Although they reported spiramycin concentrations in tissue, the concentration in the brain was not determined (3). The concentrations found to inhibit growth of the parasite in vitro vary from 20 to 250 μg/ml, but even high concentrations do not kill the parasite (5, 6, 10, 17, 34).

In a study of humans, Desmonts and Couvreur (11) found a reduced frequency of congenital infection in women treated with spiramycin. This study was, however, hampered by a lack of sufficient matched control patients. In a later study by the same investigators (7), a reduced frequency of placental infection after spiramycin treatment was found compared with an untreated control group. In these studies, the decision to start treatment was based upon serological conversion of the mother and not on transmission of infection to the fetus. Whether spiramycin can cure an already infected fetus is not known, but data show no effect of spiramycin treatment of a pregnant mother on the severity of manifestations in the fetus (27).

In the present study, treatment with spiramycin was not started until congenital infection was proven. The PCR has been used to prove fetal infection. With the PCR, a result can be obtained within 2 or 3 days after receipt of a clinical sample. This provides the possibility of a rapid test result and an informed decision regarding treatment.

From the results it can be concluded that spiramycin is still effective on *T. gondii* after transmission of infection has occurred. Only in case of the premature delivery of monkey C1 was *T. gondii* still present at birth. The parasite was found only in the placenta. This monkey, however, should be considered not to have received adequate therapy since the mother received spiramycin for only 16 days. This argument is derived

TABLE 4. Concentrations of spiramycin in body fluids and tissues after intravenous treatment of the mother with 20 mg/kg/day in two intermittent doses

Start of treatment (day of pregnancy)	Monkey	Spiramycin concn[a] in:													
		Maternal blood (day 115)[b]	Amniotic fluid (day 115)	Maternal blood (day 130)	Amniotic fluid (day 130)	Fetal blood (day 130)	Amniotic fluid at birth[c,d]	Placenta[d]	Neonatal blood[d]	Maternal blood[d]	Neonatal heart	Neonatal brain	Neonatal spleen	Neonatal liver	Maternal heart	Maternal brain	Maternal spleen	Maternal liver
117	C1	NT[e]	NT	1.15	0.76	0.94	NA[f]	3.3	NA	0.28	0.9	0	2.3	2.0	ND[g]	ND	ND	ND
103	C4	1.05	0.8	3.65	1.35	1.4	NA	4.3	0.2	0.34	1.1	0	3.5	0.85	8.25	0	46	10.5
106	C2	1.0	1.15	2.85	0.8	NA	0.65	2.0	0.13	0.25	0.5	0	3.0	2.7	8	0	52	15.5
116	C5	NT	NT	4.2	1.15	1.65	1.15	3.1	0.14	0.24	0.65	0	6.25	3.5	13	0.7	135	67.5
116	C8	NT	NT	3.17	1.1	0.88	1.0	2.5	0.2	0.3	1.0	0	9.5	1.9	7	0	90	18
Mean ± SEM		1.02 ± 0.04	0.98 ± 0.25	3.00 ± 1.15	1.03 ± 0.25	1.22 ± 0.37	0.93 ± 0.26	3.04 ± 0.87	0.17 ± 0.04	0.28 ± 0.04	0.83 ± 0.25	0	4.9 ± 3.0	2.2 ± 1.0	9.1 ± 2.7	0.18 ± 0.35	80.7 ± 41.1	27.8 ± 26.6

[a] Spiramycin concentrations are reported in micrograms per milliliter of body fluid or per gram of tissue.
[b] Blood and amniotic fluid samples were taken approximately 1 h after the last dose was administered.
[c] Spontaneous birth before day 160 or cesarean section at day 160 of pregnancy.
[d] No spiramycin was administered on the day of autopsy; concentrations were measured 25 to 26 h after the last dose was administered.
[e] NT, no treatment at that moment.
[f] NA, not available.
[g] ND, not done.

from Couvreur et al. (7), who considered women not to have been treated if they received spiramycin for less than 15 days.

Monitoring of fetal infection by sequential testing of amniotic fluid samples revealed that spiramycin does slowly reduce the amount of *T. gondii* in the host; the parasite was still present in the amniotic fluid about 12 days after initiation of treatment in four of five cases. However, the parasite was detectable only by PCR and not by mouse inoculation. This may indicate that spiramycin treatment reduces the parasitic load and that the reduced number of parasites cannot be detected by mouse inoculation because this method is not as sensitive as nested PCR. A lower sensitivity of mouse inoculation was also found with amniotic fluid samples collected before treatment was started: only two of five PCR-positive cases were positive by mouse inoculation (monkeys C2 and C5). In 29 (90.6%) of 32 cases, both amniotic fluid samples were found to be positive by the PCR at duplicate testing, whereas only a single sample was found to be positive by mouse inoculation. This finding supports the sensitivity and reproducibility of the PCR.

In our previous report, however, we stated that the nested PCR was as sensitive as mouse inoculation (32). The detection of parasites killed by treatment with spiramycin may be a second explanation for the detection by PCR and not by mouse inoculation. However, Chang and Pechère demonstrated that macrolides have an inhibitory effect but no killing effect on *T. gondii* (6). Moreover, parasites were still viable after they had been incubated with macrolides and subsequently subcultured in medium free of drugs. Since transmission of nonviable parasites is not likely to occur, the finding of *T. gondii* in the amniotic fluid indicates that the fetus had been infected. Detection of *T. gondii* by PCR is therefore of clinical importance, although the PCR cannot discriminate between viable and dead parasites.

The outcome of fetal infection in the control group that received no treatment has been described before (32). This control group therefore seems to have a "historical" character. Statistical analysis of the data revealed that the transmission rate in the control group was not significantly different from that in the study group. This provides support for the use of this historical control group.

When monkeys with a congenital *T. gondii* infection received no treatment, parasites could be isolated at birth in three of the four monkeys. In contrast, no parasites were found in the five monkeys treated with spiramycin. Statistical evaluation revealed that this finding was significant.

The absence of parasites from fetal blood cannot be attributed to spiramycin treatment, because fetal parasitemia was never found in the untreated monkeys. Monitoring of fetal parasitemia is impeded by the limitations of fetal blood sampling in rhesus monkeys. Fetal blood sampling can be performed only once, or occasionally twice, because rhesus monkeys possess a limited number of interplacental blood vessels and disturbance of the fetal blood circulation increases the risk of abortion.

In the human situation, parasites could still be isolated from the placenta despite treatment with spiramycin (7, 11). Isolations of parasites from the placentas of treated monkeys were always negative. The difference cannot be attributed to the concentrations of spiramycin that can be reached in the human placenta, since these equal those found in rhesus monkeys (13, 16). The isolation of parasites from the placenta can probably be explained by the presence of cysts in tissue which are not susceptible to spiramycin treatment. The virulent *Toxoplasma* strain RH that was used probably does not form tissue cysts in rhesus monkeys.

The therapeutic benefit of spiramycin is probably not limited to the action of the antibiotic within the placenta, as suggested by Desmonts and Couvreur (11). Presumably, it also has an effect in fetal tissues, where it reaches concentrations that are similar to those found in the placenta. The present study and a previous study on the pharmacokinetics of spiramycin in rhesus monkeys (31) show that spiramycin does not enter the brain. Since brain damage is the most severe aspect of congenital toxoplasmosis, spiramycin is not the therapy of choice. Infection of the brain was not found in rhesus monkeys, probably because fetal infection was induced not earlier than during the second trimester of pregnancy. However, the possibility that spiramycin does enter the brain if severe inflammation is present cannot be excluded. Inflammation of the brain might explain the low concentration of spiramycin found in the brain of monkey C4. The fact that spiramycin hardly diffuses or does not diffuse into cerebral tissue also explains the failure of spiramycin to prevent neurotoxoplasmosis in immunosuppressed patients (23). Antitoxoplasma drugs that cross the blood-brain barrier, such as pyrimethamine, are more appropriate. Studies with humans have proven that treatment with pyrimethamine and sulfadiazine decreases the number of cases of severe congenital toxoplasmosis (8, 9, 19). The women in these studies, however, were also treated with spiramycin throughout pregnancy. It is not known to what extend spiramycin influenced the outcome of the studies.

Therefore, the effectiveness of combination therapy with pyrimethamine and sulfadiazine is currently being investigated with our model for congenital *T. gondii* infection of rhesus monkeys. In addition, the concentrations of pyrimethamine and sulfadiazine in the mothers and fetuses will be determined.

Under the present experimental conditions, it is shown that spiramycin does have an effect on congenital *T. gondii* infection. The parasitemia which has been experimentally induced by intravenous infection with virulent strain RH cannot be compared to the natural situation, in which an infection is acquired by ingestion of cysts or oocysts of other strains. However, the results indicate that early treatment with spiramycin has a beneficial effect on infected fetuses. Our results and those of Couvreur et al. (7) show a reduction in the number of infected fetuses after spiramycin treatment. These results argue in favor of treatment with spiramycin, as long as the fetus has not been infected. These findings agree with the notion that spiramycin should be used to prevent fetal infection as soon as toxoplasmic seroconversion is discovered in a pregnant woman (27, 33). The findings also favor serological screening in areas with a high incidence of *Toxoplasma* infections in pregnancy. Except in France, serological screening of pregnant women is not routinely performed. In practice, therefore, most cases of congenital infection are diagnosed when transmission of infection to the fetus has already occurred. A prolonged interval between onset of infection and initiation of treatment seems to result in a more severe fetal infection (19). This implies that in most cases, treatment with spiramycin will be started too late to be effective.

In conclusion, the concentrations of spiramycin reached in fetuses have been shown to be effective, although they do not equal the concentrations found to be effective in vitro. Spiramycin most likely reduces the parasitic load. However, spiramycin most probably does not enter the brain. Therefore, treatment with spiramycin will be preventive rather than therapeutic.

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