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Comparative effects of cotrimoxazole (trimethoprim-sulphamethoxazole) and spiramycin in pregnant mice infected with *Toxoplasma gondii* (Beverley strain)

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1 The effects of cotrimoxazole (CTX) and spiramycin (Spir) in mice infected in midpregnancy with the Beverley (Bev) strain of *Toxoplasma gondii* were compared.

2 Therapeutic effectiveness was determined according to the following parameters: rate of successful delivery, litter size, offspring weight and survival.

3 When compared with the uninfected untreated control group, CTX showed a more beneficial therapeutic effect than Spir, with a statistically significant increase in the rate of both successful delivery and offspring survival.

4 Results based on antitoxoplasma antibody determinations in the offspring indicated a better *in utero* control of congenital infection by CTX than by Spir.

Introduction

Chemotherapeutic agents currently in use for treatment of toxoplasmosis in pregnant women still give unsatisfactory results. Although very effective against *Toxoplasma gondii*, the pyrimethamine-sulphonamide combinations are normally contra-indicated during pregnancy because of toxicity and potential teratogenic effects. On the other hand, the safer macrolide antibiotic spiramycin (Spir) does not readily cross the placental barrier (Garin *et al.*, 1968) and therefore may not be effective in treatment of foetuses infected *in utero* (Desmonts & Couvreur, 1979).

In previous studies, we have demonstrated that cotrimoxazole/trimethoprim-sulphamethoxazole, (CTX) was very effective against *T. gondii* trophozoïtes both *in vitro* in cell cultures (Nguyen & Stadtsbaeder, 1975; 1978; Nguyen *et al.*, 1978) and *in vivo* in nonpregnant adult mice (Stadtsbaeder & Calvin-Préval, 1973; Nguyen & Stadtsbaeder, 1983b). Despite its antifolate property (Bushby & Hitchings, 1968), the low toxicity of CTX for humans as compared with that of the pyrimethamine-sulphonamide combinations (Frisch, 1973) suggests its possible use in pregnancy (Nguyen & Stadtsbaeder, 1983a).

There have been no experimental reports dealing with the effectiveness of CTX upon toxoplasmosis in pregnant animals. The present study was designed to compare the antitoxoplasm effects of CTX with that of Spir in female mice infected in midpregnancy with the Beverley (Bev) strain of *T. gondii*.

Methods

Virgin NMRI female mice (25 ± 2 g, 8 to 10 weeks of age from the Animalerie centrale de l'Université Catholique de Louvain, 1200 Brussels, Belgium) were caged in groups of two with a male for 3 days. After this period of time, the males were removed; all the females were pooled, then randomly separated into groups of two.

Subcutaneous inoculation of brain cysts of *T. gondii* was performed 5 days after male removal. Maintenance of the Bev strain of *Toxoplasma* and inoculation technique have been described elsewhere (Stadtsbaeder *et al.*, 1975; Nguyen & Stadtsbaeder, 1983b). Briefly, brains from chronically infected mice were homogenized in saline (1 brain/5 ml). Smears were made to determine the presence of *Toxoplasma* cysts. A volume of 0.5 ml of the brain emulsion was injected subcutaneously in the dorsal area of each test mouse. By previous experience, this inoculum is known to produce a chronic toxoplasma infection in the strain of mice used in the present study (Nguyen & Stadtsbaeder, 1983b).

Treatment with CTX or with Spir was started 5 days after *Toxoplasma* inoculation. The drugs were incorporated as previously described (Nguyen & Stadtsbaeder, 1983b) in drinking water at concentrations of 9.6 mg ml^{-1} for CTX and 10 mg ml^{-1} for Spir. Treatment was maintained until the pregnant mice delivered. The volume of water consumed by each treated group was recorded every 2 days. Groups with a level of consumption below the normal range (2 to 3 ml per day/mouse) were not taken into consideration for calculation of the results.

One day after their birth, offspring born to infected mothers were caged with uninfected mothers to avoid possible transmission of toxoplasmosis via lactation. Survival of the offspring was evaluated every 2 days for 5 months.

Statistically significant differences in delivery and survival rates between test and untreated, uninoculated control groups were calculated by chi-squared analysis with Yates's correction for continuity. All the other parameters (litter size, weight and antibody titers) were compared by Student's t test. In all cases, a value of $P < 0.01$ (two-tailed) was chosen as the level of significance.

Results

The time intervals for Bev inoculation and initiation of treatment were selected as 8 days after male removal and 5 days after inoculation respectively on the basis of results obtained from preliminary experiments, in which the most significant effects were observed at these two times. The results are presented in Table 1.

In the uninoculated, untreated control group delivery occurred in 25/28 of the mated female mice after a gestational period of 21 to 24 days. The average litter size was 10.68 ± 0.31 offspring. All of the offspring survived at 5 months; they reached at that time a mean individual weight of 28.81 ± 0.26 g.

Similar results were obtained in the uninoculated but treated groups regardless of whether they had received CTX or Spir throughout the gestational period, which suggests that the dosages of neither drug had any side effect on pregancy in the present experimental mouse model.

Abnormalities due to Bev inoculation were obvious in the absence of any treatment. Indeed, deliveries occurred in only 8/28 ($\chi^2 = 18.889$, $P < 0.001$) of the mated untreated female mice. The mean litter size was reduced to 6.25 ± 1.25 offspring ($t = 3.43$, $P < 0.01$) with a final survival rate of 20/50 ($\chi^2 = 170.012$, $P < 0.001$). The mean individual weight of the offspring at 5 months was 21.43 ± 1.37 grams ($t = 5.27$, $P < 0.01$). Furthermore, maternal survival during the gestational period was significantly reduced ($\chi^2 = 7.146$, $P < 0.01$) in this group of animals; the rate of mortality reached approximately 30%.

In the group of inoculated mice treated with CTX, successful delivery was observed in 24/28 mated female mice ($\chi^2 = 0.00$, not significant, NS). The mean litter size was 11.13 ± 0.41 offspring ($t = 0.87$, NS). The final survival rate of the offspring at 5 months was 261/267 ($\chi^2 = 4.214$, $P < 0.05$). There were no significant differences in offspring weight or mother survival as compared with control uninfected pregnant mice.

Under the same experimental conditions, treatment with Spir was significantly less effective than with

Table 1 Comparative effects of cotrimoxazole (CTX) and spiramycin (Spir) in female mice inoculated in midpregnancy with brain cysts of *Toxoplasma gondii* (Bev strain)

Toxoplasma inoculation[a]	*Treatment*	*Time interval*[b]	*Delivery*[c]	*Litter size*[d]	*Offspring survival*[e]	*Offspring weight*[f]	*Mother survival*[g]
Uninoculated	Untreated	nil	25/28	10.68 ± 0.31	267/267	28.81 ± 0.26	28/28
	CTX	nil	26/28	11.04 ± 0.26	287/287	28.20 ± 0.34	28/28
	SPIR	nil	25/28	10.04 ± 0.31	251/251	29.23 ± 0.22	28/28
5–8 days after mating	Untreated	nil	8/28*	6.25 ± 1.25+	20/ 50*	21.43 ± 1.37+	20/28+
	CTX	5	24/28	11.13 ± 0.41	261/267‡	29.35 ± 0.20	28/28
	SPIR	5	15/28+	8.73 ± 0.77	120/131*	28.62 ± 0.43	26/28

[a] Mice were inoculated subcutaneously with brain cysts of Bev *Toxoplasma gondii* at 5 to 8 days after mating.
[b] Treatment with CTX or with Spir was started at the indicated time interval (days) after toxoplasma inoculation.
[c] Delivered pregnant mice/total mated mice.
[d] Mean number of offspring per litter ± s.e., at delivery.
[e] Survivors at 5 months/total born offspring.
[f] Mean ± s.e. of offspring weight (g) determined at 5 months after delivery.
[g] Survivors at the time of delivery/total mated female mice.
‡ $P < 0.05$ (two-tailed) from the uninoculated, untreated control.
+ $P < 0.01$, as above.
* $P < 0.001$, as above.

Table 2 Antitoxoplasma antibody response in the offspring (5 months of age) of treated and untreated mother groups which had been inoculated with brain cysts of *Toxoplasma gondii* (Bev strain) in midpregnancy (5 to 8 days after mating)

Mother group	*Reciprocal antitoxoplasma antibody titers in the offspring*[a]					*Total positive/Total tested*
	Negative	64–128	256–512	1024–2048	>4096	
Untreated	5/20[b] (25)	0/20 (0)	1/20 (5)	3/20 (15)	11/20 (55)	15/20 (75)
Cotrimoxazole	197/261 (75.5)	27/261 (10.3)	5/261 (1.9)	4/261 (1.5)	28/261 (10.7)	64/261* (24.5)
Spiramycin	76/120 (63.3)	1/120 (0.8)	1/120 (0.8)	15/120 (12.5)	27/120 (22.5)	44/120+ (36.7)

[a]Antitoxoplasma antibodies were determined by the standard indirect haemagglutination technique with Bio-Mérieux (France) reagents.
[b]Number seronegative or positive/Total tested. Figures in parentheses indicate percentage seronegative or positive.
$^{+}P < 0.01$ (two-tailed) from the untreated group.
$^{*}P < 0.001$, as above.

CTX, especially with regard to the rates of delivery and offspring survival. Successful delivery occurred in only 15/28 mated female mice ($\chi^2 = 7.088$, $P < 0.01$) and 120/131 offspring ($\chi^2 = 20.038$, $P < 0.001$) survived at 5 months. Statistical analysis indicated that these two parameters were significantly lower than those of the uninfected control group. In contrast, the mean litter size at delivery (8.73 ± 0.77 offspring/litter; $t = 2.35$, NS) and the mean offspring weight (28.62 ± 0.43 g/offspring; $t = 0.38$, NS) seemed not to be significantly affected.

In an attempt to determine the effectiveness of the drugs against congenital transmission of toxoplasmosis, antitoxoplasma antibodies were measured in the 5 month-old offspring with the indirect haemagglutination technique. The results are shown in Table 2. Most of the offspring (75%) from untreated mothers were serologically positive; the majority of them (55%) had reciprocal antibody titers greater or equal to 4096. In the treated groups, the percentage of seropositive offspring was 24.5% from CTX-treated mothers ($\chi^2 = 20.99$, $P < 0.001$) and 36.7% from Spir-treated mothers ($\chi^2 = 8.82$, $P < 0.01$). However, high antitoxoplasma antibody titers (HA > 1024) were significantly ($\chi^2 = 25.73$, $P < 0.001$) more frequent in the Spir than in the CTX group.

Discussion

As described in the present study, primary infection in mice with Bev toxoplasma during midpregnancy can result in unsuccessful gestation, or delivery of stillborn offspring; those which were born alive died later during the perinatal period or showed growth retardation. Our previous (unpublished) experience has shown that abnormalities were the most marked when the infection occurred in midpregnancy (5 to 8 days after mating). The above features are typical of acquired toxoplasmosis in pregnancy, as previously reported by other authors in humans (Desmonts, 1982) and in animals (Beverley, 1969; Laugier, 1969). Besides its adverse effects upon pregnancy itself, the results indicated that primary infection with Bev toxoplasma in mice during midpregnancy may also cause maternal death. Earlier studies have indicated that pregnant mice were more susceptible than non-pregnant mice to coxsackievirus (Modlin & Crumpacker, 1982), *Listeria* and *Toxoplasma* (Luft & Remington, 1982). The increase in the susceptibility of pregnant mice to infection may be related to stress and immunosuppression due to pregnancy (Clark *et al.*, 1978a; 1978b; Van Zon Adriaan *et al.*, 1980).

The antitoxoplasm effects of CTX were significantly greater than that of Spir, with regard to mice infected in midpregnancy. Indeed, protection afforded by CTX was complete in that pregnancy developed at a normal rate in the CTX-treated mothers. In contrast, the rate of successful gestation was significantly reduced in the Spir-treated mothers. The difference in efficacy of CTX versus Spir may be accounted for by the lower activity of the latter drug against *T. gondii* trophozoïtes (Nguyen *et al.*, 1978; Nguyen & Stadtsbaeder, 1983b) and by its weak capacity to cross the placental barrier (Garin *et al.*, 1968).

Offspring survivors of either CTX or Spir-treated mice were apparently healthy and showed no external signs of malformations. Experiments are in progress to determine the frequency of congenital toxoplasmosis in both groups of offspring. Preliminary results indicated that seropositive offspring were significantly greater from the group of untreated mothers than from the groups of mothers treated either with CTX or with Spir. On the other hand, CTX appeared to

control foetal infection more efficiently than Spir since high titers of specific antibodies were more frequently found in offspring of Spir than of CTX-treated mothers. More elaborate techniques, i.e., brain cysts quantitation, occurrence of cataract (Hutchison *et al.*, 1982), would be useful to confirm these preliminary data.

In conclusion, the present results strongly suggest that CTX may be of greater therapeutic benefit than Spir in the treatment of acute toxoplasmosis in pregnancy. CTX is officially contra-indicated for use in pregnant women and neonates in the United Kingdom. However, toxoplasmosis is a special risk for this group of people. Therefore, our animal experiments strongly support a review of this policy, at least in this special case.

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