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EFFICACY OF ATOVAQUONE AND SULFADIAZINE IN THE TREATMENT OF MICE INFECTED WITH *TOXOPLASMA GONDII* STRAINS ISOLATED IN BRAZIL

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Summary:

The efficacy of atovaquone and sulfadiazine was examined alone or in combination for the treatment of mice infected with six Brazilian *Toxoplasma gondii* strains previously genotyped using the PCR-RFLP assays of the SAG2 gene, in addition to RH strain. Swiss mice were infected intraperitoneally with 10^2 tachyzoites from each strain of *T. gondii* and treated with 6.25, 12.5, 25 and 50 mg/Kg/day of atovaquone or 40, 80, 160 and 320 mg/Kg/day of sulfadiazine. In a second experiment, mice were treated with the association of previously determined doses of each drug. Treatment started 48 hours post-infection, and lasted 10 days. The susceptibility of *T. gondii* to atovaquone and to sulfadiazine was different according to the parasite strain. It was observed strains that are susceptible to atovaquone, and strains that are resistant to it. Type I strains were more susceptible to the activity of sulfadiazine and more resistant to atovaquone. Yet type III strains were susceptible to atovaquone and to sulfadiazine. Association of atovaquone and sulfadiazine presented a synergic effect in the treatment of mice infected with RH type I strain and an additive effect in the treatment of mice infected with one type I strain and with two type III strains.

KEY WORDS : *Toxoplasma gondii*, atovaquone, sulfadiazine, strain, mice, Brazil.

Résumé : EFFICACITÉ DE L'ATOVAQUONE ET DE LA SULFADIAZINE DANS LE TRAITEMENT DE SOURIS INFECTÉES PAR DES SOUCHES *TOXOPLASMA GONDII* ISOLÉES AU BRÉSIL

L'atovaquone et la sulfadiazine on été évaluées, seules ou en association, quant à leur efficacité dans le traitement de souris infectées par six souches brésiliennes de Toxoplasma gondii, *dont le type génétique a été préalablement déterminé par l'intermédiaire d'une PCR-RFLP du gène SAG2 ; la souche RH a été évaluée conjointement. Des souris Swiss ont été infectées par voie intra-péritonéale avec 102 tachyzoïtes de chaque souche de* T. gondii, *puis traitées avec 6,25, 12,5, 25 et 50 mg/kg/jour d'atovaquone ou 40, 80, 160 et 320 mg/kg/jour de sulfadiazine, pendant dix jours. Dans un second essai, les souris ont été traitées par l'association de ces médicaments à des doses préalablement évaluées. Le traitement des souris a commencé 48 heures après l'infection. La susceptibilité de* T. gondii *à l'atovaquone et à la sulfadiazine a été différente selon la souche du parasite. On a observé des souches sensibles et résistantes à l'atovaquone. Les souches du type I se sont montrées plus susceptibles à la sulfadiazine et plus résistantes à l'atovaquone. Les souches du type III se sont montrées sensibles à l'atovaquone et à la sulfadiazine. L'association de l'atovaquone et de la sulfadiazine a montré un effet synergique dans le traitement des souris infectées par la souche RH du type I et un effet additif dans le traitement des souris infectées avec une souche du type I et deux souches du type III.*

MOTS CLÉS : Toxoplasma gondii, *atovaquone, sulfadiazine, souche, souris, Brésil.*

INTRODUCTION

Toxoplasma gondii (Nicole & Manceaux, 1909) is a protozoan parasite with a cosmopolitan distribution. *T. gondii* infections in immunocompetent individuals are usually assymptomatic, whereas infections in immunocompromised patients or transplacentally infected infants can be life threatening (Sibley *et al.*, 1999). At present, the treatment of choice for toxoplasmosis is the combination of pyrimethamine and sulfonamide, which presents a synergic effect promoting a sequential blockade in the synthesis of the parasite folinic acid (Araújo *et al.,* 1993). This drug association may result in born marrow depression, requiring discontinuation of treatment (Djurkovic *et al.*, 1999). Cysts of *T. gondii* are not affected by treatment with this drug combination (Araújo *et al.*, 1992). Thus, there is a critical need for the development and evaluation of new drugs or drug combinations, which do not present the same problems of the standard therapy. Hydroxynaphthoquinone 566C80 (atovaquone) presented remarkable *in vitro* and *in vivo* activities against tachyzoites and cysts of *T. gondii* (Araújo *et al.,* 1991). The mechanism of action of this drug against *T. gondii* is not known. Hydroxynaphthoquinones, however, act on other protozoa by blocking mitochondrial electron transport, resulting in the inhibition of pyrimidine synthesis (Romand *et al.*, 1996). Atovaquone was one of the most active drugs against cyst of *T. gondii* both *in*

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vivo and *in vitro* assay (Araújo *et al.*, 1992). Most works about experimental toxoplasmosis chemotherapy are carried out with RH strain of *T. gondii*. Little is known about the action of different drugs on parasite strains which are genetically different. In the present report, the efficacy of atovaquone and sulfadiazine was tested, individually and in association, for the treatment of mice infected with six strains of the *Toxoplasma gondii* isolated in Minas Gerais, Brazil and previously genotyped using the PCR-RFLP assays of the SAG2 gene.

MATERIALS AND METHODS

MICE

Outbred female Swiss Webster mice weighing approximately 20 g at the beginning of each experiment were obtained from the Instituto de Ciências Biológicas animal house, and kept in a conventional restricted animal facility. Food and water were available to the animals throughout the experiment.

T. GONDII ISOLATES

Six *T. gondii* strains isolated in Minas Gerais, Brazil and previously genotyped by PCR-RFLP assay of the SAG2 gene were used in this study: SAF, EGS and D4 (type I) and D7, CH1 and CH3 (type III), in addition to the reference RH strain (type I), were used (Ferreira *et al.,* 2004). Type II strains were not used in this study as, so far, no strain of this genotype has been isolated in Brazil. With the exception of RH strain (Sabin, 1941), each strain was isolated in our laboratory from patients with congenital toxoplasmosis (SAF and EGS), from dogs (D4 and D7) and from chickens (CH1 and CH3). Tachyzoites were obtained by inoculating 250 to 500 cysts or 10^5 to 10^6 tachyzoites by intra peritoneal (i.p.) injection in Swiss mice. The peritoneum of these mice was washed three to eight days post inoculation (DPI) and the resulting material was filtered through polycarbonate membrane of 3 µm (Milipore), and diluted for 10^2 tachyzoites/200 µl of phosphate buffered saline (PBS) pH 7.2 and inoculated by i.p. route in 10 mice.

DRUGS

The suspension of atovaquone (Glaxo Wellcome, UK), was prepared according to Araújo *et al.* (1993), with some alterations. 500 mg of micronized atovaquone was dissolved in 25 ml of distilled water containing 0.625 mg of carboxymethylcellulose. The mixture was then sonicated to produce a smooth dispersion and the desired concentrations were prepared in PBS pH 7.2. The suspension of sulfadiazine (Catarinense, Brazil) was prepared by dissolving the drug pulverized in distilled water in appropriate amounts to achieve the desired concentrations. The drugs were administered orally by gavage. Control mice received PBS pH 7.2 only.

EXPERIMENTS OF ATOVAQUONE AND SULFADIAZINE ACTIVITIES

Groups of 10 mice were infected (i.p.) with 10^2 tachyzoites from each strain of *T. gondii*. Treatment was initiated 48 hours post-infection, with 6.25, 12.5, 25 or 50 mg/kg/day of atovaquone or 40, 80, 160 or 320 mg/kg/day of sulfadiazine and continuated for 10 days. A group of 10 infected mice was maintained as non treated control (NTC). From these experiments, a dose of each drug was selected to verify the synergic or addictive effect of the drug association. Doses which increase mice survival time, without preventing mortality, though, were selected (Araújo *et al.,* 1993). In the treatments with survival in all doses of drugs, those lower doses of each drug were selected.

For association experiments groups of 10 mice were infected (i.p.) with 10^2 tachyzoites from each strain of *T. gondii* and treated with the association of previously determined doses of each drug and with the drugs individually. Mice were followed up for 30 days post infection (DPI).

ASSESSMENT OF THERAPEUTIC SCHEMES

To assess the efficacy of therapeutic schemes, mice were followed up for 30 DPI being checked twice daily for death. Survival rates, the presence of brain cysts, specific antibody by ELISA (Fux *et al.,* 2000) and bioassay were analyzed.

The mean number of brain cysts in surviving mice was used as an additional criterion to assess the efficacy of therapeutic schemes. All of the surviving mice were sacrificed by cervical dislocation. Brains were removed and homogenized in 1 ml of PBS pH 7.2 to determine the number of cysts. If no cysts were observed, the brain homogenate was inoculated (i.p.) into one normal mouse (bioassay). The subinoculated mice were also observed for 30 DPI for mortality. Mice surviving the subinoculation of brains were assessed through parasitological tests (tachyzoites in the peritoneal cavity and brain cysts) and ELISA.

STATISTICAL ANALYSIS

The survival rates of mice per experimental group were assessed by Fisher's exact test ($p < 0.05$). The mean number of brain cysts in surviving mice were analyzed by Kruskal-Wallis nonparametric test ($p < 0.05$).

RESULTS

All of the surviving mice infected with each strain of *T. gondii* and treated with sulfadiazine, atovaquone or sulfadiazine plus atovaquone had antibodies for *T. gondii* by ELISA 30 days post infection.

RH STRAIN

All NTC mice died by the 7th day of infection. No mouse treated with different doses of atovaquone survived. Zero, 30, 60 and 100 % of mice survived when treated with 40, 80, 160 or 320 mg/kg/day of sulfadiazine, respectively. The increase of the survival rate of groups treated with 160 or 320 mg/kg/day of sulfadiazine was significant regarding NTC group ($p < 0.05$). No brain cysts were found in any animal. All mice subinoculated with brains of the surviving mice treated with 80 or 160 mg/kg/day of sulfadiazine and six in ten mice treated with 320 mg/kg/day of sulfadiazine died from seven to 11 days after inoculation. Tachyzoites were observed in the peritoneal cavity of all of them. The four surviving subinoculated mice were seronegative for ELISA and had no brain cysts. The selected doses for the drug association were in accordance with predetermined criteria, 40 mg/kg/day of sulfadiazine and 50 mg/kg/day of atovaquone.

All NTC mice and those treated only with 40 mg/kg/day of sulfadiazine or 50 mg/kg/day of atovaquone of the association experiment died during the follow-up period. The drug association treatment of these two doses led to 40 % of mice survival, higher than the simple amount of individual effects of each drug.

This survival increase was significant regarding the other groups ($p < 0.05$), indicating a synergic effect. No brain cysts were found in surviving animals. Bioassay of four surviving mice resulted in three deaths on the 9th day after the inoculation. The surviving mouse presented negative ELISA and absence of brain cysts.

SAF STRAIN

All NTC mice died on the 10th day after infection (Table I). Only the treatment with 50 mg/kg/day of atovaquone promoted a significant survival increase (40 %) regarding NTC. Treatments with 80, 160 or 320 mg/kg/day of sulfadiazine led to 60, 80 and 60 % of survival, respectively. All of the surviving mice presented brain cysts. The mean number of brain cysts in the brain of mice treated with 320 mg/kg/day of sulfadiazine was significantly lower than that of mice treated with 40 mg/kg/day of sulfadiazine ($p < 0.05$), not presenting any differences regarding the other groups of treatment with this drug. The lowest doses of atovaquone (6.25 mg/kg/day) and of sulfadiazine (40 mg/kg/day) tested were selected for the drug association. All NTC mice infected with SAF strain, in the association experiment, died until the 9th day after infection (Table I). Ten, 30 and 20 % of mice treated with 6.25 mg/kg/day of atovaquone, 40 mg/kg/day of sulfadiazine and with the drug association respectively, survived. All of the surviving mice presented brain cysts. Survival rates and the average number of brain cysts in different treatment groups did not present any significant difference, indicating an absence of synergic or addictive effect with the association of sulfadiazine and atovaquone in the treatment of mice infected with SAF strain.

	Strain SAF (type I)	
	Survivors/Total	**Number of cysts**
Non treated control	0/10	–
Atovaquone mg/kg/day		
6.25	1/10	5,800
12.5	1/10	1,800
25	1/10	200
50	4/10	950 ± 619.1
Non treated control	0/10	–
Sulfadiazine mg/kg/day		
40	2/10	3,350
80	6/10	1,283.3 ± 487.5
160	8/10	1,450 ± 1,137.7
320	6/10	933.3 ± 564.5
Non treated control	0/10	–
ATO 6.25 mg/kg/day	1/10	1,000
SUL 40 mg/kg/day	3/10	1,900 ± 1,442.2
ATO + SUL	2/10	550

Table I. – Survival and number of brain cysts in mice infected with SAF strain of *T. gondii* and treated with different doses of atovaquone (ATO), sulfadiazine (SUL) or drug association (ATO + SUL).

EGS STRAIN

All NTC mice died from the 10th to the 12th day after infection (Table II). All mice treated with different doses of atovaquone died. Treatments with 40, 80, 160 or 320 mg/kg/day of sulfadiazine resulted in 0 %, 0 %, 10 % and 20 % of survival, respectively. All surviving animals presented brain cysts. Doses of 80 mg/kg/day of sulfadiazine and 50 mg/kg/day of atovaquone were selected for the drug association experiment. All treatment groups presented 100 % of mortality. No synergic or addictive effect of the association of these two doses of drugs was observed.

D4 STRAIN

90 % of NTC mice inoculated with tachyzoites of D4 strain of *T. gondii* died from the 15th to the 22nd day of the inoculation (Table III). 50, 90, 80 and 80 % mice treated with 6.25, 12.5, 25 or 50 mg/kg/day of atova-

	Strain EGS (type I)	
	Survivors/Total	**Number of cysts**
Non treated control	0/10	–
Atovaquone mg/kg/day		
6.25	0/10	–
12.5	0/10	–
25	0/10	–
50	0/10	–
Non treated control	0/10	–
Sulfadiazine mg/kg/day		
40	0/10	–
80	0/10	–
160	1/10	3,500
320	2/10	350
Non treated control	0/10	–
ATO 6,25 mg/kg/day	0/10	–
SUL 40 mg/kg/day	0/10	–
ATO + SUL	0/10	–

Table II. – Survival and number of brain cysts in mice infected with EGS strain of *T. gondii* and treated with different doses of atovaquone (ATO), sulfadiazine (SUL) or drug association (ATO + SUL).

	Strain D4 (type I)	
	Survivors/Total	**Number of cysts**
Non treated control	1/10	2,200
Atovaquone mg/kg/day		
6.25	5/10	1,180 ± 238.8
12.5	9/10	1,316.7 ± 281.7
25	8/10	1,725 ± 613.5
50	8/10	1,037.5 ± 232.6
Non treated control	1/10	2,200
Sulfadiazine mg/kg/day		
40	7/10	875 ± 150.8
80	10/10	855 ± 348.4
160	9/10	1,005.6 ± 192.8
320	10/10	675 ± 165.4
Non treated control	0/10	–
ATO 6.25 mg/kg/day	4/10	1,212.5 ± 325
SUL 40 mg/kg/day	6/10	908.3 ± 115.8
ATO + SUL	9/10	111.1 ± 85.8

Table III. – Survival and number of brain cysts in mice infected with D4 strain of *T. gondii* and treated with different doses of atovaquone (ATO), sulfadiazine (SUL) or drug association (ATO + SUL).

quone, respectively, survived. Treatments with 40, 80, 160 or 320 mg/kg/day of sulfadiazine resulted in 70, 100, 90 and 100 % of survival, respectively. All surviving mice presented brain cysts (Table III). The group of mice treated with 6.25 mg/kg/day of atovaquone has not presented any significant difference in the survival percentage related to the NTC group. The increase in the survival percentage of the other groups treated with atovaquone and sulfadiazine was significant regarding NTC ($p < 0.05$). Doses of 40 mg/kg/day of sulfadiazine and 6.25 mg/kg/day of atovaquone were selected for the drug association experiment (Table III). All NTC mice died until the 18th day of infection. Treatments with 6.25 mg/kg/day of atovaquone, 40 mg/kg/day of sulfadiazine and with the drug association led to 40, 60 and 90 % of survival, respectively. The survival rate after the treatment with atovaquone associated with sulfadiazine was greater ($p < 0.05$) than that observed in NTC mice or in those treated only with atovaquone (6.25 mg/kg/day) but not regarding mice treated with 40 mg/kg/day of sulfadiazine. All surviving mice presented brain cysts. Mice treated with the drug association presented a significant reduction in the number of cysts ($p < 0.05$) indicating an addictive effect.

D7 STRAIN

Ten percent of NTC mice inoculated with D7 strain tachyzoites survived (Table IV). All animals treated with any doses of atovaquone survived. Treatment with 40, 80, 160 or 320 mg/kg/day of sulfadiazine led to 50, 80, 90 and 90 % of mice survival, respectively. Treatment with 40 mg/kg/day of sulfadiazine resulted in a prolongation of time until death, but it was not significant regarding NTC mice. On the other groups, the survival rate increased significantly ($p < 0.05$).

Seven animals treated with 50 mg/Kg/day of atovaquone did not present any brain cysts and had their brains subinoculated. All subinoculated animals died from the 8th to the 11th day of inoculation and presented tachyzoites in the peritoneal cavity. In all other surviving animals, the presence of brain cysts was verified (Table IV). Doses of 6.25 mg/kg/day of atovaquone and 40 mg/kg/day of sulfadiazine were selected to assess the synergic or addictive effect of the drug association.

	Strain D7 (type III)	
	Survivors/Total	**Number of cysts**
Non treated control	1/10	12,000
Atovaquone mg/kg/day		
6.25	10/10	570 ± 862.2
12.5	10/10	100 ± 124.7
25	10/10	140 ± 128.7
50	10/10	20 ± 35
Non treated control	1/10	12,000
Sulfadiazine mg/kg/day		
40	5/10	3,640 ± 2,219.9
80	8/10	362.5 ± 362.3
160	9/10	1,116.7 ± 812
320	9/10	688.9 ± 778.9
Non treated control	1/10	6,250
ATO 6.25 mg/kg/day	10/10	195 ± 103.9
SUL 40 mg/kg/day	5/10	1,140 ± 341.7
ATO + SUL	10/10	130 ± 88.8

Table IV. – Survival and number of brain cysts in mice infected with D7 strain of *T. gondii* and treated with different doses of atovaquone (ATO), sulfadiazine (SUL) or drug association (ATO + SUL).

In the association experiment, 10 % of NTC mice survived. All mice treated with 6.25 mg/kg/day of atovaquone and 50 % of animals treated with 40 mg/kg/day of sulfadiazine survived. All mice treated with the drug association survived. All surviving mice presented brain cysts (Table IV). Mice treated with the drug association presented a significant reduction in the number of cysts ($p < 0.05$) indicating an addictive effect.

CH1 STRAIN

In this experiment, 20 % of NTC mice survived (Table V). Treatment with 6.25, 12.5, 25 or 50 mg/kg/day of atovaquone led to 60, 80, 80 and 100 % of survival, respectively. The treatment with 40, 80, 160 or 320 mg/kg/day of sulfadiazine led to 60, 80, 100 and 100 % of survival, respectively. The survival rate of mice treated with 12.5, 25 or 50 mg/kg/day of atovaquone and 80, 160 or 320 mg/kg/day of sulfadiazine was higher than that of NTC group ($p < 0.05$). All surviving mice presented brain cysts, except three of 10 mice treated with 320 mg/kg/day of sulfadiazine. After bioassay, all the recipient mice died and presented tachyzoites in the peritoneal cavity. The smaller doses of the two drugs (6.25 mg/kg/day of atovaquone and 40 mg/kg/day of sulfadiazine) were selected to asses the drug association.

In the drug association experiment, 20 % of NTC mice survived. Treatment with 6.25 mg/kg/day of atovaquone, 40 mg/kg/day of sulfadiazine and with the association of these two doses led to 60, 60 and 100 % of survival, respectively (Table V). The increase of the survival rate of animals treated with the drug association, regarding the other groups of the experiment, was significant, indicating an addictive effect. Mice treated with drug association presented a lower number of brain cysts ($p < 0.05$).

	Strain CH1 (type III)	
	Survivors/Total	**Number of cysts**
Non treated control	2/10	2,875
Atovaquone mg/kg/day		
6.25	6/10	483.3 ± 194.1
12.5	8/10	143.8 ± 72.9
25	8/10	162.5 ± 87.6
50	8/10	165 ± 105.5
Non treated control	2/10	2,875
Sulfadiazine mg/kg/day		
40	6/10	466.7 ± 267.7
80	8/10	343.8 ± 207.8
160	10/10	100 ± 62.4
320	10/10	80 ± 85.6
Non treated control	2/10	3,825
ATO 6.25 mg/kg/day	6/10	533.3 ± 260.1
SUL 40 mg/kg/day	6/10	1,012.5 ± 312.1
ATO + SUL	10/10	150 ± 100

Table V. – Survival and number of brain cysts in mice infected with CH1 strain of *T. gondii* and treated with different doses of atovaquone (ATO), sulfadiazine (SUL) or drug association (ATO + SUL).

CH3 STRAIN

All NTC mice died up to the 9th day after infection (Table VI). Treatment with 6.25, 12.5, 25 or 50 mg/kg/day of atovaquone led to 0, 60, 80 and 90 % of survival, respectively. Treatment with 40, 80, 160 or 320 mg/kg/day of sulfadiazine led to 10, 40, 60 and 80 % of survival, respectively. Increase in the survival rate of mice treated with 12.5, 25 or 50 mg/kg/day of atovaquone and with 80, 160 or 320 mg/kg/day of sulfadiazine was significant regarding NTC mice ($p < 0.05$). All surviving mice presented brain cysts (Table VI). Doses selected for use in combination were 6.25 mg/kg/day of atovaquone and 40 mg/kg/day of sulfadiazine.

	Strain CH3 (type III)	
	Survivors/Total	**Number of cysts**
Non treated control	0/10	–
Atovaquone mg/kg/day		
6.25	0/10	–
12.5	6/10	1,116.7 ± 932.6
25	8/10	1,200 ± 882.4
50	9/10	250 ± 82.9
Non treated control	0/10	–
Sulfadiazine mg/kg/day		
40	1/10	1,000
80	4/10	625 ± 206.2
160	6/10	283.3 ± 292.7
320	8/10	225 ± 155.8
Non treated control	0/10	–
ATO 6.25 mg/kg/day	0/10	–
SUL 40 mg/kg/day	1/10	2,300
ATO + SUL	1/10	1,150

Table VI. – Survival and number of brain cysts in mice infected with CH3 strain of *T. gondii* and treated with different doses of atovaquone (ATO), sulfadiazine (SUL) or drug association (ATO + SUL).

In the drug association experiment, all NTC mice died. Treatment with 6.25 mg/kg/day of atovaquone, 40 mg/kg/day of sulfadiazine and with association of these two drugs led to 0, 10 and 10 % of mice survival, respectively (Table VI).

DISCUSSION

Up to the moment, no study has been carried out to assess the efficacy of atovaquone and sulfadiazine in mice experimentally infected with strains of *T. gondii* isolated in Brazil. In addition, little is known about the activity of these drugs against

strains that belong to different genetic types. In this work, the behavior of six strains isolated in the state of Minas Gerais, Brazil, besides RH strain, was evaluated regarding the action of atovaquone and sulfadiazine action, with differences being found in the response pattern. Our results have shown that *T. gondii* sensitivity to the treatment with atovaquone or sulfadiazine is different according to the parasite's strain, varying from totally atovaquone-sensitive strains to totally atovaquone-resistant strains. These results are in accordance with those found by Araújo *et al.* (1991) and Sordet *et al.* (1998), who also identified these response differences among *T. gondii* strains.

The response pattern of type I strains regarding drugs was different when compared to type III strains. RH, SAF and EGS strains (type I) were more susceptible to sulfadiazine action and more resistant to atovaquone. D4 (type I), CH1 and CH3 (type III) strains presented a similar susceptibility to atovaquone and to sulfadiazine. D7 strain was more susceptible to atovaquone. In this study, determining strains resistant to drugs action indicates the likelihood of occurring alterations in genes encoding these drugs targets, leading to resistance. Aspinall *et al.* (2002) identified five polymorphic nucleotides that are associated with three different alleles of the gene encoding dihydrophalate synthase enzyme, which is sulfadiazine's target, among samples of 37 natural populations of *T. gondii*. This alteration offered resistance of these populations to sulfadiazine. Atovaquone, however, is likely to act inhibiting the mitochondrial electron transport in the parasite. This drug is a structural analog of Coenzyme Q, inhibiting bc1 cytochrome activity as it binds to its Q domain. In a study carried out by McFadden *et al.* (2000), alterations in this domain have been identified to likely lead to resistance to atovaquone. Thus, further studies aiming at determining alterations in studied strains, which may offer resistance to the tested drugs, are needed. The finding of naturally resistant populations may explain the fact that 10 % of patients with toxoplasmic encephalitis do not respond to the treatment with sulfadiazine (Aspinall *et al.*, 2002).

Our results for RH strain are in accordance with data in the literature (Araújo *et al.*, 1993; Romand *et al.*, 1993). Both these authors and this present work have shown the existence of a synergic effect of atovaquone-sulfadiazine association in the treatment of murine toxoplasmosis in mice infected with RH strain.

Additive effect of atovaquone and sulfadiazine association was observed in treatment of mice infected with D4, D7 and CH1, but not in mice infected with SAF, EGS and CH3 strains. The survival rate and the mean number of tissue cysts in mice infected with CH1 (type I) strain and treated with the drug association have shown a significant difference regarding the other groups (NTC and drugs not associated). Survival rate of mice infected with D4 (type I) strain and treated with the drug association was not different from animals treated with 40 mg/kg/day of sulfadiazine. However, the mean number of cysts found in mice treated with the association was significantly lower. The analysis of the mean number of brain cysts also allow defining that the treatment of mice infected with D7 strain, with the association, presented additive effect. The present work has allowed the assessment of efficacy of atovaquone and sulfadiazine association in the treatment of mice infected with different *T. gondii* strains, showing that this activity is variable according to the strain.

There is a need of extending this study with a greater number of *T. gondii* strains from different genetic types besides assessing the efficacy of other drugs or drug combinations in the treatment of mice infected with those strains. These results, associated to an extensive knowledge of the populational structure of *T. gondii*, will allow the establishment of appropriate therapeutic schemes.

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