LACK OF ACTIVITY OF ARTEMETHER FOR PROPHYLAXIS AND TREATMENT OF *TOXOPLASMA GONDII* AND *PNEUMOCYSTIS CARINII* INFECTIONS IN RAT

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Summary :

Artemisinin and its derivatives have been found effective *in vivo* against *Plasmodium* and *in vitro* against *Toxoplasma gondii* and *Pneumocystis carinii*. We tested the activity of artemether for prophylaxis and treatment in the rat model of concurrent *T. gondii* and *P. carinii* infection. Artemether at doses of 18 and 100 mg/kg administered (s.c.) in prophylaxis did not prevent toxoplasmosis or pneumocystosis, while trimethoprim-sulfamethoxazole (reference treatment) was effective for prevention of both infections. Similar results were obtained in curative studies. These results do not support the use of artemether for prevention or treatment of toxoplasmosis or pneumocystosis.

KEY WORDS : *Toxoplasma gondii*, *Pneumocystis carinii*, artemether, prophylaxis, treatment.

Résumé : INEFFICACITÉ DE L'ARTÉMETHER DANS LA PRÉVENTION ET LE TRAITEMENT DE LA TOXOPLASMOSE ET DE LA PNEUMOCYTOSE CHEZ LE RAT CO-INFECTÉ

L'artémisine et ses dérivés ont une activité anti-*Plasmodium* *in vivo* et une activité anti-*Toxoplasma gondii* et anti-*Pneumocystis carinii* *in vitro*. L'artémether a été évalué en prophylaxie et en traitement de la toxoplasmose et de la pneumocystose chez le rat co-infecté. Contrairement au triméthoprim-sulfaméthoxazole (traitement de référence), l'artémether, aux doses de 18 ou 100 mg/kg (administré par voie sous-cutanée) ne permet ni la prévention ni le traitement de la pneumocystose et de la toxoplasmose.

MOTS CLÉS : *Toxoplasma gondii*, *Pneumocystis carinii*, artemether, prophylaxie, traitement.

INTRODUCTION

New approaches for therapy and prophylaxis of toxoplasmosis and pneumocystosis are urgently needed because of the high frequency of side effects associated with the administration of sulfonamides and folates inhibitors. In this context, artemisinin and its derivatives may be of potential interest as these compounds, which have activity against *Plasmodium* in experimental models and in humans (Peters *et al.*, 1986; White, 1994) have been also found effective *in vitro* against *T. gondii* and *P. carinii* (Merali & Meshnick, 1991; Ou-Yang *et al.*,

1990). However, Chang *et al.* (1988) reported that arteether was ineffective in the treatment of acute toxoplasmosis, but no data is available for prophylaxis of toxoplasmosis or pneumocystosis. In this study, we took advantage of the use of a rat model of concurrent *T. gondii*/*P. carinii* (Brun-Pascaud *et al.*, 1994) to evaluate the efficacy of artemether for treatment and prevention of both infections.

MATERIALS AND METHODS

The experimental design has been described in detail elsewhere (Brun-Pascaud *et al.*, 1994). Briefly, *P. carinii* infection was induced in Wistar rats by 25 mg of cortisone acetate (Hydrocortisone Roussel®; Hoechst-Roussel, Paris, France) injected subcutaneously twice weekly and a low-protein (8 %) diet (Usine Alimentation Rationnelle, Villemoisson, France). After 5 weeks of immunosuppression, rats were inoculated intraperitoneally with 10⁷ tachyzoites of the virulent RH strain of *T. gondii*. For prophylactic experiments, rats were treated from the initiation of the corticosteroid treatment. In the reference prophylactic group, rats received *per os* trime-

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This study was done in accordance with prevailing regulations regarding care and use of laboratory animals in European Communities (*Journal officiel des Communautés Européennes*, 18 Décembre 1986, L358).

thoprim-sulfamethoxazole (TMP-SMX) (Bactrim®, Pediatric suspension; Roche, Neuilly-sur-Seine, France) 5 days per week then every day after *T. gondii* inoculation at daily dose of 20 and 100 mg/kg respectively, and rats in the control group received no therapy. All surviving rats were sacrificed after 7 weeks of corticosteroid administration.

As preliminary experiments showed that a high dose (100 mg/kg, s.c., three times a week) of artemether (Paluther®, Rhône-Poulenc Rorer Doma, Antony, France) induced acute toxicity, artemether was subcutaneously (s.c.) administered using three different schedules as follows: *i*) 18 mg/kg/day 2 days per week then every day after *T. gondii* inoculation, *ii*) 18 mg/kg/day 5 days per week and *iii*) 100 mg/kg/day 2 days per week then every day after *T. gondii* inoculation in prophylactic experiments. In curative experiments, the same daily dosages were administered every day from the second day following the intraperitoneal inoculation of *T. gondii* until death or sacrifice.

P. carinii cyst counts were determined in lung tissue after enzymatic digestion and Toluidine blue O staining. *T. gondii* infection was quantified in target organs by an indirect immunofluorescence assay following tissue culture (Brun-Pascaud *et al.*, 1994).

P. carinii cysts counts and *T. gondii* parasitic burdens were processed using one-way analysis of variance, and each interesting pair of groups was compared using Bonferroni's adjusted *t* test (Godfrey, 1985). Dif-

ferences in values were considered significant if the *P* value was ≤ 0.05 or 0.05 divided by the number of tests made. Data were expressed as means ± 1 standard deviation.

RESULTS

Results of prophylactic experiments are summarized in Table I. Active infection with *P. carinii* and *T. gondii* was found in all control rats. 15 of 17 rats treated with artemether died within 8 days (range 4-8, mean: 5.8 ± 1.1) post *T. gondii* inoculation and all rats exhibited active infection with *T. gondii* and *P. carinii* even at the higher dosage. In contrast, all rats treated with TMP-SMX were alive 14 days after *T. gondii* inoculation and were then sacrificed: neither pleural fluid effusion nor *T. gondii* was found in organs and the level of *P. carinii* cyst counts reflected a latent infection as usually demonstrated (Brun-Pascaud *et al.*, 1994).

No curative effectiveness of artemether could also be demonstrated: 8 of 10 treated rats died at 6.1 ± 2.6 post *T. gondii* inoculation. The means *T. gondii* burdens were $\log 4.2 \pm 0.8$ and $\log 4.7 \pm 0.4$ in lung, $\log 2.1 \pm 1.2$ and 1.6 ± 1.6 in brain, 6.2 ± 0.6 and 4.5 ± 2.6 in spleen and $\log 5.7 \pm 1.6$ and 5.4 ± 2.1 in liver, the *P. carinii* counts were $\log 5.4 \pm 0.8$ and 5.0 ± 0.7 in rats treated respectively with 18 mg/kg/day and 100 mg/kg/day. *T. gondii* and *P. carinii* infections were

Groups	Untreated controls	Prophylactic treatment ^a			
		Artemether 18 mg/kg	Artemether 100 mg/kg	TMP-SMX 20-100 mg/kg	
Treatment day/week		2/week	5/week	2/week	5/week
(Number of rats)	(10)	(6)	(5)	(6)	(5)
Day of death or sacrifice post <i>T. gondii</i> infection (number of rats)	death 5.7 ± 2.8 (8/10)	death 6.2 ± 0.5 (4/6)	death 5.7 ± 1.0 (5)	death 7.5 ± 3.7 (6)	sacrifice 14 (5)
Pleural fluid volume (ml)	5.5 ± 1.7	3.2 ± 1.7	5.4 ± 2.2	3.3 ± 2.0	0
<i>T. gondii</i> (log 10)					
pleural fluid	2.1 ± 1.8	2.2 ± 1.5	3.4 ± 0.8	2.6 ± 2.0	—
lung	4.7 ± 0.8	4.7 ± 0.4	4.6 ± 0.5	4.5 ± 0.9	0*
brain	2.2 ± 1.1	0.8 ± 1.7	3.8 ± 0.7	2.7 ± 1.6	0*
spleen	4.7 ± 0.9	4.7 ± 0.8	5.5 ± 0.4	5.6 ± 1.0	0*
liver	5.9 ± 0.4	5.8 ± 0.4	6.1 ± 0.3	6.4 ± 0.2	0*
<i>P. carinii</i> (log 10)					
lung	6.3 ± 0.8	5.3 ± 0.9	6.6 ± 0.3	5.7 ± 1.0	$2.6 \pm 0.6^*$

^a Drugs were given daily after *T. gondii* inoculation. * $p < 0.001$ vs untreated controls.

Table I. — Prophylactic activity of artemether against *P. carinii* and *T. gondii* infections.

not any different compared to untreated controls. TMP-SMX reference combination was fully effective on both infections, all rats were alive after 14 days of treatment; at autopsy, tissue cultures for *T. gondii* were negative in all organs, and the mean number of *P. carinii* cysts was $\log 3.6 \pm 0.3$.

CONCLUSION

Our results show that artemether was ineffective for the treatment or prevention of *T. gondii* and *P. carinii* infections, even using the higher dosages that can be tolerated in rats. These results, which are in agreement with those reported by Chang and Pechere (1988) with the related compound arteether in the treatment of acute murine toxoplasmosis, are rather disappointing in regard to the excellent *in vitro* activity of these compounds (Holfels *et al.*, 1994; Ou-Yang *et al.*, 1990), and do not support the use of these drugs in humans for treatment or prevention of both toxoplasmosis and pneumocystosis.

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