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Short Report: Activity of Artemether and Mefloquine against Juvenile and Adult *Schistosoma mansoni* in Athymic and Immunocompetent NMRI Mice

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Abstract. Immune effector mechanisms can enhance the activity of antischistosomal drugs. We examined the *in vivo* effect of single oral doses of the antimalarials artemether (400 mg/kg) and mefloquine (200 mg/kg), recently described to have promising antischistosomal properties, against juvenile and adult *Schistosoma mansoni* in T cell-deficient and in comparably infected age- and sex-matched immunologically intact control mice. Artemether and mefloquine are equally effective in athymic and immunocompetent mice. Artemether treatment resulted in total and female worm burden reductions ranging between 71.1% and 85.3%, whereas mefloquine achieved total and female worm burden reductions of 80.4–97.8%. In conclusion, artemether and mefloquine act T-cell independently and no synergistic interaction with the immune response was involved.

The antimalarials artemether and mefloquine have promising antischistosomal properties.[1–3] However, the mechanisms of action of these schistosomicides are not yet known. Mefloquine might inhibit hemozoin formation,[3] and it has been shown that artemether interacts with haemin to exert a toxic effect on schistosomes.[4] Interestingly, artemether also exhibits immunosuppressive activity.[5]

Several antischistosomal drugs were found to have a reduced efficacy in immunosuppressed mice and immune effector mechanisms, particularly antibody, can enhance the activity of antischistosomal drugs.[6–8] The relevance of these experimental observations in mice to human disease is not known.

The aim of this study was to assess whether the schistosomicidal activities of artemether and mefloquine also depend on immune responsiveness. We studied the efficacy of single oral doses of artemether and mefloquine against juvenile and adult *Schistosoma mansoni* in both T-cell deprived mice and in age- and sex-matched immunologically intact control mice.

Our animal studies were approved and conducted in accordance with national and cantonal regulations on animal welfare (permission no. 2070). Mefloquine was obtained from Mepha AG (Aesch, Switzerland) and artemether was obtained from Kunming Pharmaceutical Cooperation (Kunming, China). Each drug was suspended homogenously in a vehicle containing 7% Tween-80, 3% ethanol, and water shortly before oral administration.

Cercariae of *S. mansoni* were obtained from infected intermediate host snails following routine procedures in our laboratories.

Female NMRI mice (N = 30, age: 3 weeks, weight: ~20 g) and female NMRI-nude (Foxn1) mice (N = 30, age: 3 weeks, weight: ~20 g) were purchased from Charles River (Sulzfeld, Germany). Hence, in contrast to previous studies on the immune dependence of antischistosomal drugs, which used mice deprived of their T-cells by thymectomy and injections with thymocyte antiserum[6] or B cell-deficient mice,[8] we used a mutant mouse strain. One possible disadvantage of our approach is that the background of the NMRI and NMRI nude strains used in our study is not fully identical.

Mice were kept in groups of 10 in Macrolon cages in environmentally controlled conditions (temperature: ~25°C; humidity: ~70%; 12 h light and 12 h dark cycle) and acclimatized for 1 week. They had free access to water and food.

Each mouse was infected subcutaneously with ~80 *S. mansoni* cercariae. Twenty-one days (pre-patent infection) or 49 days (patent infection) after the experimental infection, groups of 5 mice were treated orally with single oral artemether (400 mg/kg) or single oral mefloquine (200 mg/kg). Two groups of 10 NMRI nude and 10 NMRI mice served as controls. At 21 days post-treatment, mice were killed using CO_2. The liver of each mouse was removed, compressed between 2 glass plates, and all *S. mansoni* worms were removed, sexed, and counted using a stereoscopic microscope. The mesenteric tissue was placed in a Petri dish and examined using a stereoscopic microscope. All *S. mansoni* were removed, sexed, and counted. For statistical analysis Statsdirect statistical software was used (version 2.4.5; Cheshire, United Kingdom). The Kruskal-Wallis (KW) test, which compares the medians of the responses between the treatment and control groups, was used. A difference in median was considered to be significant at a significance level of 5%.

Six out of 20 nude mice died during the course of a patent *S. mansoni* infection, while the infection was tolerated by NMRI mice. Athymic mice are well known to exhibit a high susceptibility to bacterial, virus, or parasitic infections. In particular, nude and other immunosuppressed mice infected with *S. mansoni* suffer from an egg-induced hepatoxicity reaction.[9–11] Potentially fatal exacerbations of disease have also been observed in *S. mansoni*-infected mice treated with the immunosuppressant cyclosporine,[12] and recent research indicates that during infection with *S. mansoni* T cell-dependent immunoregulatory mechanisms may help to control morbidity.[13]

The respective antischistosomal activities of artemether (400 mg/kg) and mefloquine (200 mg/kg) against juvenile *S. mansoni* in nude NMRI and NMRI mice are summarized in Table 1. We assessed total and female worm burden reductions, including changes in worm distributions. Statistically significant worm burden reductions were achieved with both drugs in the two mouse strains. The differences in total and female worm burden reductions between nude and immunologically intact mice were not statistically significant for both drugs. Artemether treatment resulted in total and female worm burden reductions of 75.6–85.3%. Total and female worm burden reductions of 87.3–97.8% were achieved with mefloquine in

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TABLE 1

Effect of artemether and mefloquine against juvenile *Schistosoma mansoni* harbored in NMRI and NMRI nude mice*

Mouse strain	Drug (dosage [mg/kg])	No. of mice investigated	No. of mice cured	Mean number of worms (SD)					Total worm burden reduction (%)	KW	*P* value	Female worm burden reduction (%)	KW	*P* value
				Liver	Mesenteric veins	Total	Males	Females						
NMRI	–	9†	0	1.0 (1.3)	17.0 (10.6)	18.0 (10.3)	9.4 (5.1)	8.6 (5.5)	–	–	–	–	–	–
	Artemether (400)	5	1	0	4.4 (4.3)	4.4 (4.3)	2.4 (2.3)	2.0 (2.0)	75.6	6.78	0.009	76.8	6.85	0.009
	Mefloquine (200)	5	4	0	0.4 (0.9)	0.4 (0.9)	0.2 (0.4)	0.2 (0.4)	97.8	9.20	0.002	97.7	9.24	0.002
NMRI nude	–	6‡	0	2.8 (1.9)	21.7 (6.9)	24.5 (7.9)	13.5 (4.6)	11.0 (3.5)	–	–	–	–	–	–
	Artemether (400)	5	1	1.8 (2.7)	1.8 (1.5)	3.6 (3.2)	1.6 (1.7)	2.0 (1.6)	85.3	7.50	0.006	81.8	7.56	0.006
	Mefloquine (200)	5	1	1.6 (1.5)	1.0 (1.7)	2.6 (3.1)	1.2 (1.6)	1.4 (1.5)	89.4	7.53	0.006	87.3	7.63	0.005

* SD = standard deviation; KW = Kruskal Wallis.
† One mouse died during the course of infection.
‡ Two mice died during the course of infection.

NMRI and NMRI nude mice. These findings are in line with previous studies.[3,14]

Table 2 summarizes the activity of artemether and mefloquine when given to mice harboring adult *S. mansoni* infections. Again, statistically highly significant total and female worm burden reductions were obtained: artemether achieved total and female worm burden reductions of 71.1–81.4%. Interestingly, the activities of artemether against adult *S. mansoni* reported here are higher than in previous studies,[15,16] which might be caused by mouse strain differences or slightly lower infection intensities. Total and female worm burden reductions of 77.3–96.5% were observed after treatment of mice with mefloquine. The differences in total and female worm burdens between athymic NMRI and NMRI mice after artemether and mefloquine treatment were not statistically significant.

The antischistosomal drugs praziquantel, oxamniquine, hycanthone, and antimony are less effective in T-cell deprived mice[7] and the efficacy of praziquantel was found to be reduced in B-cell depleted mice.[8] An activation of drugs by the immune system has not only been described for antischistosomal drugs but also for other anti-parasitic treatments: for example, although the lack of B-cells did not impair the effect of the experimental drug toltrazuril in mice infected with *Neospora caninum*, T-cell immunity was found to be essential for full efficacy of treatment.[17]

In contrast, we found that artemether and mefloquine are equally effective against *S. mansoni* in athymic and immunocompetent mice. Hence, mefloquine and artemether act T-cell independently and do not involve synergistic interaction with the immune response for efficacy on *S. mansoni*. Another experimental antischistosomal drug, amoscanate was also not influenced by the absence of T cell-mediated immune responsiveness in mice.[7]

It has been suggested that after praziquantel treatment, synergistically active antibodies gain access to key components of the morphologically damaged worm surface (mainly the membrane over the tubercles on the male worms), which is denied them by the normal undamaged worm surface.[7,18] The effects of mefloquine and artemether on the tegument of *S. mansoni*, on the other hand, show distinct differences when compared with praziquantel,[19,20] and antibodies may therefore not be able to interact with the tegument of mefloquine- and artemether-treated worms. Studies using indirect immunofluoresence might be useful to confirm our hypothesis. In addition, the question whether innate or T-cell independent humoral immune responses are required to support the activity of mefloquine and artemether on schistosomes has not yet been addressed and might be part of future studies on the antischistosomal properties of these antimalarials.

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TABLE 2

Effect of artemether and mefloquine against adult *Schistosoma mansoni* harbored in NMRI and NMRI nude mice*

Mouse strain	Drug (dosage [mg/kg])	No. of mice investigated	No. of mice cured	Mean number of worms (SD)				Total worm burden reduction (%)	KW	*P* value	Female worm burden reduction (%)	KW	*P* value
				Liver	Mesenteric veins	Total	Males	Females					
NMRI	–	9†	0	1.0 (1.3)	17.0 (10.6)	18.0 (10.3)	9.4 (5.1)	8.6 (5.5)	–	–	–	–	–
	Artemether (400)	5	0	3.6 (2.3)	1.6 (1.7)	5.2 (3.3)	3.6 (1.9)	1.6 (1.5)	71.1	6.76	0.009	81.4	7.52	0.006
	Mefloquine (200)	4†	1	2.3 (1.7)	0	2.3 (1.7)	2.0 (1.8)	0.3 (0.5)	87.2	7.74	0.006	96.5	7.84	0.005
NMRI nude	–	6‡	0	2.8 (1.9)	21.7 (6.9)	24.5 (7.9)	13.5 (4.6)	11.0 (3.5)	–	–	–	–	–	–
	Artemether (400)	4†	0	5.5 (3.1)	0.5 (1.0)	6.0 (3.4)	3.0 (1.4)	3.0 (2.0)	75.5	6.59	0.01	72.7	6.70	0.009
	Mefloquine (200)	4§	1	4.8 (3.2)	0	4.8 (3.2)	2.3 (1.5)	2.5 (1.7)	80.4	6.59	0.01	77.3	6.70	0.009

* SD = standard deviation; KW = Kruskal Wallis.
† One mouse died during the course of infection.
‡ Two mice died during the course of infection.
§ Three mice died during the course of infection.

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