In Vitro and In Vivo Treatments of *Echinococcus* Protoscoleces and Metacestodes with Artemisinin and Artemisinin Derivatives

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In vitro treatment of *Echinococcus multilocularis* and *Echinococcus granulosus* larval stages with the antimalarials dihydroartemisinin and artesunate (10 to 40 μM) exhibited promising results, while 6 weeks of in vivo treatment of mice infected with *E. multilocularis* metacestodes (200 mg/kg of body weight/day) had no effect. However, combination treatments of both drugs with albendazole led to a substantial but statistically not significant reduction in parasite weight compared to results with albendazole alone.

Cystic echinococcosis, caused by *Echinococcus granulosus*, is distributed worldwide. Alveolar echinococcosis, caused by *Echinococcus multilocularis*, is generally confined to the northeastern hemisphere (2). Growth and/or proliferation of *Echinococcus* metacestodes, mainly in the liver but also in the lungs and other organs, leads to the development of space-occupying lesions and organ malfunction and will eventually cause death (10, 23). The preferred treatment option is radical resection of the parasitic mass. Surgery is accompanied by chemotherapy, and in inoperable cases, chemotherapy is the only option. Albendazole and mebendazole are currently used (8, 10). For alveolar echinococcosis, these compounds were shown to act parasitostatically rather than parasitocidally, with high recurrence rates after interruption of therapy. Improved drug treatments are needed (8, 24).

Most countries to which malaria is endemic have now adopted artemisinin-based combination therapy as a first-line treatment for *Plasmodium falciparum* infection (34), and activities of artemisinins against other protozoans have been reported (1, 12). Trematodes, including schistosomes (31) and others, have proven susceptible to artemisinins and semisynthetic derivatives (13–16, 26), and antitumor activities of artemisinins have been reported (11, 18, 35). *E. multilocularis* metacestodes also exhibit tumor-like properties, including potentially unlimited growth and proliferation (17). These findings have prompted us to investigate the potential of artemisinins for antiechinococcal treatment.

We first assessed the in vitro activities of artemisinin, artesunate, artemether, and dihydroartemisinin (DHA) against *E. granulosus* and *E. multilocularis* larval stages. These were evaluated in comparison to albendazole and nitazoxanide as reference drugs (8). All compounds were dissolved as stock solutions of 10 mM in dimethylsulfoxide.

*E. granulosus* protoscoleces were isolated, maintained, and tested in vitro as described earlier (21, 32). Compounds were added at 4, 10, and 40 μM. Viability of protoscoleces was assessed microscopically by using a trypan blue exclusion test (Fig. 1). At 40 μM, artesunate and DHA exhibited activities similar to that of nitazoxanide (32), but the action of DHA was delayed by 2 days (with a 90% reduction in viability occurring on day 6.) Artemisinin and artemether were ineffective (data not shown). At 10 μM, artesunate and DHA showed strongly decreased efficacies compared to that of nitazoxanide (Fig. 1).

*E. multilocularis* metacestode drug assays were carried out as previously described (7, 9, 21, 27, 28, 30). Artemisinins and albendazole were added to the cultures at a concentration of 40 μM. During the 12-day treatment, 200 μL of culture supernatant was collected daily and stored at −20°C to measure *E. multilocularis* alkaline phosphatase (EmAP) activity (30). Artesunate treatment led to a rapid increase of EmAP activity in medium supernatants within 4 days (Fig. 2). DHA exhibited a delayed effect, with an increased EmAP activity coming up at day 8. Artemisinin and artemether treatments did not result in a high-level EmAP release (Fig. 2), as earlier reported by Reuter et al. (25). No elevated EmAP levels were observed at 10 μM drug concentrations (data not shown). EmAP activity has been identified earlier as a marker indicating the loss of viability of in vitro drug-treated vesicles (28, 30). Our findings correlated well with scanning and transmission electron microscopy analyses (6), confirming that in vitro exposure of *E. multilocularis* metacestodes to artesunate and DHA resulted in profound tissue alterations and loss of the characteristic multicellular structure of the germinal layer (data not shown). Similar observations were made when *E. granulosus* metacestodes were exposed to these compounds (M. Spicher and A. Hemphill, unpublished).

The effects of artesunate and DHA were further evaluated...
with the experimental BALB/c mouse model (27, 29). Mice were separated into 6 treatment groups of 10 animals each. Drug suspensions were prepared in 0.5% carboxymethylcellulose (CMC) and were applied as follows: (i) artesunate at 200 mg/kg of body weight (bw), (ii) a combination of artesunate (200 mg/kg bw) and albendazole (50 mg/kg bw), (iii) DHA (200 mg/kg bw), (iv) a combination of DHA (200 mg/kg bw) and albendazole (50 mg/kg bw), (v) albendazole at 200 mg/kg bw, and (vi) 0.5% CMC alone (control group). Treatment began at 8 weeks postinfection, and the drug and control suspensions were applied by intragastric inoculation (100 μl/mouse/day) for 6 weeks. Finally, mice were sacrificed by CO2 euthanasia, and parasite tissue was removed from the peritoneal cavity, and the parasite weight was determined (Fig. 3). Parasite weights within the CMC control (5.71 ± 1.79 g), artesunate (4.60 ± 2.28 g), and DHA (4.11 ± 2.03 g) groups were consistently high, with minor differences. As expected, continuous treatment of mice with albendazole (2.96 ± 1.10 g) resulted in a significant reduction in parasite weight. In addition, the combination of artesunate and albendazole (1.39 ± 0.81 g) and the combination of DHA and albendazole (1.38 ± 1.25 g) resulted in an even more pronounced reduction of the parasite weights compared to the control (Fig. 3). The improvements obtained with albendazole, artesunate-albendazole, and DHA-albendazole were highly significant (in one-way analysis of variance, $F = 44.66; P = 0.0000$). The artesunate-albendazole and DHA-albendazole treatments resulted in lower mean parasite weights than those obtained with the albendazole treatment alone, but the differences narrowly missed statistical significance (Kruskal-Wallis multiple-comparison $z$-value test; differences were considered significant if the $z$ value was $>1.96$; the 

![FIG. 1. Protoscolicidal activity of artemisinin derivatives. *E. granulosus* protoscoleces were cultured in vitro in the presence of artesunate, DHA, and nitazoxanide (NTZ) as a positive control (10 and 40 μM). Note the dose-dependent killing of protoscoleces by both artesunate and DHA. This experiment was repeated three times with virtually identical outcomes. One representative result is shown. DMSO, dimethylsulfoxide.](https://example.com/fig1)

![FIG. 2. EmAP activity in culture supernatant of drug-treated *E. multilocularis* metacestodes. Artesunate, DHA, artemisinin, and artemether were applied to in vitro-cultured vesicles at 40 μM, and EmAP activity was measured in culture supernatants at different time points as indicated. Albendazole (ABZ) and corresponding amounts of the solvent dimethylsulfoxide (DMSO) were added as positive and negative controls, respectively.](https://example.com/fig2)

![FIG. 3. Experimental chemotherapy with *E. multilocularis*-infected mice. In vivo treatment of *E. multilocularis*-infected mice was carried out with albendazole (ABZ), artesunate (AS), dihydroartemisinin (DHA), and combinations of ABZ-AS and ABZ-DHA. CMC is the solvent control (0.5% CMC in phosphate-buffered saline). The box plots indicate the distribution of parasite weights in the different treatment groups. Significant reductions in parasite weights in relation to those in the CMC control group were achieved by treatment with ABZ, ABZ-AS, and ABZ-DHA. Although the combination treatments were the most efficient, the reduction in both groups in relation to ABZ alone was not significant (Kruskal-Wallis multiple-comparison z-value test; difference was significant with a $z$ value of $>1.96$; $z$ value for artesunate-ABZ was 1.89; $z$ value for DHA-ABZ was 1.92).](https://example.com/fig3)
z value for artemunate-albendazole was 1.89; the z value for DHA-albendazole was 1.92).

No adverse effects were observed in the drug-treated groups, with the exception of one mouse that was found dead in the DHA-albendazole group at day 30 and one mouse that was found dead in the artemunate-albendazole group at day 32. The deaths of these two mice could potentially be attributed to the described toxicity and neurotoxicity of artemisinin derivatives in laboratory animals (3, 4, 20, 22). However, none of the mice exhibited any aberrant behavior during the treatments, and histopathological examination of liver, kidney, and brain tissue did not show any signs of toxicity, indicating that the deaths of these two mice could possibly be attributed to other causes.

The promising in vitro results that were achieved with artemunate and DHA (Fig. 1 and 2) could not be completely translated to the in vivo mouse model (Fig. 3). There are several potential explanations for this. First, artemisinins are primarily evaluated in the in vivo mouse model (Fig. 3). There are several "sultane and DHA (Fig. 1 and 2) could not be completely trans-

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