We examined the effects of praziquantel and the artemisinins on adult *Echinostoma caproni*. In vitro, both praziquantel and the artemisinins exhibited exposure-response relationships. In vivo, worm burden reductions of 100% were achieved with single oral doses of praziquantel, artesunate, and artemether at 50, 700, and 1,100 mg/kg of body weight, respectively.

Food-borne trematodiasis is an emerging public health problem (6). The current arsenal for treatment and morbidity control of food-borne trematodiasis consists of only two drugs, namely, praziquantel and triclabendazole (4, 5), and hence new drugs are urgently needed. We studied the trematocidal properties of the artemisinins against adult *Echinostoma caproni* in vitro and in vivo. For comparison, the effect of praziquantel was also examined.

Approval of our animal studies was obtained according to local government regulations. Artesunate was obtained from Mepha (Aesch, Switzerland); artemether, arteether, and praziquantel were obtained from Kunming Pharmaceutical Cooperation (Kunming, China); and artemisinin and dihydroartemisinin were obtained from Hoffman-La Roche (Basel, Switzerland). The chemical structures of the drugs investigated are depicted in Fig. 1. Drugs were prepared in homogenous suspensions in 7% Tween 80 and 3% ethanol before oral administration. Metacercariae of *E. caproni* were obtained from infected *Biomphalaria glabrata* following routine procedures in our laboratories.

Female NMRI mice (*n* = 94; age, 6 weeks) were purchased from RCC (Itingen, Switzerland). Mice were kept in groups of 10 in Macrolon cages under controlled environmental conditions (temperature, ~25°C; humidity, ~70%; light-dark cycle, 12 h-12 h) and acclimatized for 1 week. They had free access to water and food. For in vitro studies, five mice were infected orally with 35 metacercariae. At 2 weeks postinfection, mice were killed. Trematodes harvested from the excised small intestines were washed and incubated in 24-well microtiter plates (Costar) containing NCTC-135 culture medium (Gibco) supplemented with 50 μg/ml streptomycin and 50 U/ml penicillin (Gibco). Five trematodes were used for each control and experimental group. Stock solutions of praziquantel, artemisinin, artemether, arteether, artesunate, and dihydroartemisinin at 10 mg/ml were prepared with 60% dimethyl sulfoxide. The flukes were incubated with 100, 10, and 1 μg/ml drug for 72 h. The control well contained the highest concentration of solvent, 0.6% dimethyl sulfoxide. Cultures were kept at 37°C in an atmosphere of 5% CO₂ and observed immediately and at 1, 3, 6, 24, 48, and 72 h under a dissecting microscope.

For in vivo studies, 94 mice were each infected orally with 30 to 35 metacercariae of *E. caproni*. At 2 weeks postinfection, groups with four mice each were orally administered praziquantel at a dose of 12.5, 25, 50, or 100 mg/kg or one of four artemisinins at a...
single oral dose ranging from 400 to 1,500 mg/kg. Two groups with 8 and 10 untreated mice served as controls. At 3 days post-treatment, mice were euthanized with CO2. At necropsy, all E. caproni metacercariae were removed from the small intestine and counted. Drug efficacy was assessed by comparing the mean number of trematodes in any treatment group with that for the corresponding control group. Differences were tested for significance using an unpaired two-tailed Student t test, allowing for unequal variance. The data were considered significant if the P value was below 0.05 (STATA software, version 8.0; StataCorp., College Station, TX).

Table 1 summarizes the observed mortality of adult E. caproni after exposure to either praziquantel or any of the artemisinins at different concentrations in vitro. Trematodes exposed to praziquantel at concentrations of 1 to 100 μg/ml contracted immediately. They had a coiled appearance for at least 48 h and did not regain movement during the 72-h observation period. With the exception of the 1- and 10-μg/ml concentrations of artemisinin, exposure to any of the other artemisinins resulted in the death of E. caproni in vitro, with distinctive concentration- and exposure time-response relationships. Dihydroartemisin was the fastest acting artemisinin derivative. The motor activity of E. caproni decreased 3 h after incubation at 100 μg/ml; after exposure for 6 h at this concentration, all five specimens were dead. After 24 h of exposure, all flukes incubated with 100 μg/ml of artemesinate were dead. Another 24 h later, E. caproni organisms exposed to the highest concentrations of artemether and arteether were dead, displaying vesicles on their teguments. An effect of artemisinin on E. caproni was only observed after 72 h of incubation at the highest concentration, when three of five flukes were dead.

Praziquantel, the current drug of choice against intestinal flukes (5), was included as a benchmark. The drug’s dose-response relationship against adult E. caproni in mice is presented in Table 2. Single doses of praziquantel at 50 or 100 mg/kg resulted in killing of all trematodes. When praziquantel was administered at 25 mg/kg, a worm burden reduction of 83% was achieved (P = 0.005). At half this dose, the observed worm burden reduction was only 32% (P = 0.543). Our results not only confirm the excellent therapeutic potential of praziquantel against this intestinal trematode but also demonstrate the suitability of the E. caproni-mouse model for screening compounds for trematocidal activity.

The effects of the artemisinins against adult E. caproni harbored in mice are summarized in Table 3. Single oral doses of artemesinate (400 mg/kg), arteether (500 mg/kg), artemether (500 mg/kg), and artemisinin (600 mg/kg) were not effective, yielding no or only small worm burden reductions (up to 22%). However, 100% worm burden reductions were observed following administration of artemesinate or artemether at single oral doses of 700 and 1,100 mg/kg, respectively. Interestingly, these are much higher doses (sevenfold higher in the case of artemesinate) than those needed to obtain a cure of Plasmodium berghei infection in the mouse (5). A worm burden reduction of 99% was achieved with a single oral dose of 1,500 mg/kg artemisinin. Three of four mice were cured with 1,300 mg/kg arteether.

The administration of artemesinate at 500 or 700 mg/kg yielded significantly higher worm burden reductions than those obtained with the other artemisinins. The differences in the dose-response relationships between the various artemisinin derivatives investigated might be explained by the rate of met-
abolic conversion into dihydroartemisinin. A previous study with rats showed that the percentage of an oral dose of 10 mg/kg that converted into dihydroartemisinin was 72.7% for artesunate, while biotransformation was less complete in the case of arteether (15.9%) or artemether (12.4%) (7). Artemisinin is not metabolized into dihydroartemisinin (9). Our in vitro studies support this speculation, since dihydroartemisinin displayed the promptest trematocidal effect. However, it is likely that the mother compounds also contributed to the in vivo activity, as they all exhibited activity in vitro. Chemical properties might also play a role in the different trematocidal activities of artemisinin derivatives; in contrast to the water-soluble artesunate, artemether and arteether are lipophilic and hence have different absorption and distribution identities. The comparative pharmacokinetic knowledge of the absorption phases of the artemisinins is incomplete.

The artemisinins are emerging as a major drug class with a broad spectrum of activity against *Plasmodium* (1, 2), *Schistosoma* (8, 10), and, as shown here, the intestinal fluke *E. caproni*. Our results call for broader investigations of other food-borne trematodes in laboratory studies and sequentially in clinical trials.

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