

Our research team is interested in identifying and developing novel, broad-spectrum, orally active drugs for helminthic diseases. Helminths (parasitic worms) include cestodes (tapeworms), nematodes (roundworms) and trematodes (flukes). A main focus of our work so far has been on trematodes, but efforts are ongoing to establish and to work with nematode rodent models in the near future. We collaborate closely with the Parasite Chemotherapy unit (see section 4), exchanging ideas, compounds and techniques.

Soil-transmitted helminthiasis, schistosomiasis and food-borne trematodiasis affect hundreds of millions of people, particularly those in poor rural communities of the developing world. The global burden of these diseases is considerable and accounts for the loss of several million disability-adjusted life years annually. In addition, a number of helminths are of great veterinary importance, and some are significant zoonoses (e.g. *Schistosoma japonicum* and *Fasciola hepatica*). Vaccines for preventing these diseases are not available now and are unlikely to be soon. Accordingly, therapeutic drugs are the only practical means of controlling morbidity. However, effective control of helminthiasis relies on regular administration of such drugs, and very few are currently available. For example, only two drugs are presently used to treat trematode infections (praziquantel for fluke infections, with the exception of *Fasciola* spp., for which triclabendazole is used). Given the clear danger in relying on only a few drugs, efforts are under way to develop new, safe and efficacious anthelmintics.

The main research foci of our work are the following:

- To develop in vitro assays and in vivo helminth rodent models and to screen promising molecules for drug discovery.
- To investigate the pharmacokinetic (PK) and metabolic parameters of drug development candidates in rodents experimentally infected with different helminths. This work is pivotal, as several helminthic infections, e.g. schistosomiasis and fascioliasis, are a common cause of altered drug disposition kinetics. In addition, the assessment of physicochemical and pharmacological properties at early stages of drug discovery can accelerate the conversion of hits and leads into candidates for further development. We collaborate closely with the Division of Pharmacology and Toxicology at the University Hospital Basel and the Fachhochschule Nordwest-schweiz, both of which offer excellent analytical facilities (LC/MS-MS).
- To assess the activity of promising drug development candidates, including PK parameters in large animals (e.g. sheep infected with *F. hepatica*).
- To contribute to elucidating the mechanism of action of novel drugs through use of scanning electron microscopy and transmission electron microscopy, and uptake studies (in collaboration with the Microscopy Centre at the University of Basel).
- To design and assist with human clinical trials in endemic countries, working together with the Pharmaceutical Medicine unit (see section 12, page 85), medi-

cal department and the Ecosystem Health Sciences unit (see section 13).



Helminth Drug Development research team. (Photo S. Wittlin)

Over the past 3 years we have worked with three main compounds and compound classes: the artemisinins and synthetic peroxides, mefloquine and the Chinese anti-helminthic drug tribendimidine. To illustrate our research activities, we summarise below the results obtained with the artemisinins and synthetic peroxides.

Trematocidal properties of the artemisinins and 1,2,4-trioxolanes

In vivo studies

We have investigated the in vitro and in vivo activities of selected artemisinins and the synthetic trioxolane OZ78 against major food-borne trematodes. We selected OZ78 based on its weak antimalarial properties, lack of mutagenicity, excellent PK properties and a toxicological profile similar to that of artesunate. The activities of artesunate, artemether and OZ78 were determined against juvenile and adult *F. hepatica* and *Clonorchis sinensis* flukes in the rat model. Artemether and artesunate were also screened in the chronic *Ophistorchis viverrini* infection model in the hamster. Single 200–400-mg/kg oral doses of artesunate and artemether completely cured chronic infections of *F. hepatica*. Administration of artesunate and artemether at a dose of 200-mg/kg to rats harbouring juvenile *F. hepatica* flukes resulted in worm burden reductions of 46 and 82%, respectively. Finally, a single 100-mg/kg oral dose of OZ78 administered to rats with an acute or a chronic *F. hepatica* infection resulted in worm burden reductions of 100%. Artesunate and artemether were also highly effective against adult *C. sinensis*; worm burden reductions of 99–100% were found in *C. sinensis*-infected rats following a single 150-mg/kg oral dose of artesunate and artemether. A twofold higher dose of OZ78 (i.e. 300-mg/kg) was necessary to achieve significant worm burden reductions against juvenile and adult *C. sinensis* flukes. Experiments carried out with hamsters infected with adult *O. viverrini* showed that artesunate and artemether, administered at a single 400-mg/kg oral dose, resulted in worm burden reductions of 78 and 66%, respectively.

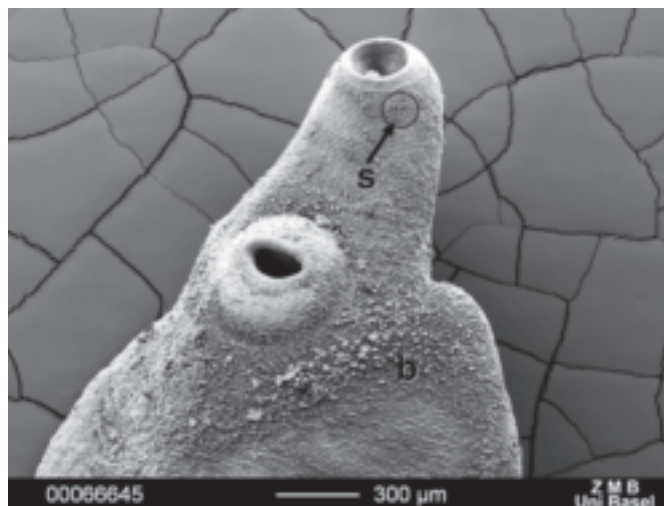
In addition, the effect of selected OZs was investigated in detail against the main schistosome species. Single 200-mg/kg oral doses of OZ78, OZ209 and OZ288 resulted in worm burden reductions of 82.0–95.4% against 21-day-old juvenile *Schistosoma mansoni* harboured in mice. Somewhat lower but still significant worm burden reductions (up to 52.2%), were observed against 49-day-old adult *S. mansoni* in the mouse model. In hamsters infected with either juvenile or adult *S. mansoni*, single 200-mg/kg doses of OZ78 and OZ288 achieved worm burden reductions of 71.7–86.5%. These in vivo results were confirmed for *S. japonicum*, and preliminary work suggests that the OZ compounds are also active against the third major schistosome species, *S. haematobium*.

Pharmacokinetic studies

PK parameters should be studied during the early phases of drug development to identify potential concerns about drug concentration-related toxicity. PK studies also aid in selecting ideal trematocidal peroxidic drug development candidates. We determined the PK parameters of artesunate and its main metabolite dihydroartemisinin (DHA) using LC-MS/MS (liquid chromatography-tandem mass spectrometry) in rats infected with hepatic and biliary stages of *F. hepatica* and compared the results to those in uninfected rats after single intragastric (100-mg/kg) and intravenous doses (10-mg/kg). Our data suggest that *F. hepatica* infections may strongly influence the disposition kinetics of the artemisinins: rats harbouring juvenile and adult *F. hepatica* infections showed considerable changes in PK parameters of artesunate and DHA. Following oral administration the area under the curve (AUC) (a measure of drug concentration in a set period of time) and maximum plasma concentrations (C_{max}) of artesunate and dihydroartemisinin were 1.7–4.4-fold higher in infected rats. An opposite trend was observed after intravenous injection. The elimination half-life ($t_{1/2}$) of artesunate and DHA was altered in infected rats following oral and intravenous administration of artesunate. Further PK studies with a variety of peroxidic compounds in different hosts are ongoing in our laboratories.

Scanning electron microscopy

We have undertaken in vivo and in vitro studies to assess the tegumental changes in adult *F. hepatica* induced by artemether and artesunate and OZ78 by means of scanning electron microscopy (SEM). SEM analysis of flukes incubated in the presence of the drugs without a supplementation of the medium with haemin showed only minor and localised damage of the tegument. In the presence of haemin, extensive tegumental damage – including sloughing, blebbing and eruptions, particularly in the ventral and dorsal mid-body and tail region – was evident. After 24 hours in vivo, disruption of the tegument was seen in the treated flukes, and the damage increased in severity 48–72 hours post-treatment. Sloughing, swelling and extensive furrowing of the tegument was observed in several flukes, in particular in the tail region and the ventral apical cone region. Similar strong disruptions of the tegument were also observed in analysing SEM pictures taken from *C. sinensis* recovered from rats after treatment with OZ78.



Scanning electron microscopy image of an adult *F. hepatica* 48 h post-treatment with a single 200-mg/kg oral dose of artemether. Sloughing (s) and blebbing (b) are visible in the apical cone region.

Studies in sheep infected with *F. hepatica*

In collaboration with our partners at the University of Naples, we assessed the efficacy and safety of artemether in sheep with a natural *F. hepatica* infection. Artemether was administered orally or intramuscularly, sheep were monitored for 8 hours post-treatment and then once daily for adverse events, and drug efficacy was estimated by faecal egg count reductions and worm burden reductions. Single 40- and 80-mg/kg oral doses of artemether showed no effect on *F. hepatica* egg and worm burden. Treatment with a single 160-mg/kg intramuscular dose of artemether significantly reduced the egg burden (64.9%) and worm burden (91.3%). At half this dose, a worm burden reduction of 65.3% was obtained, which was still statistically significant. The lowest intramuscular dose of artemether investigated (40 mg/kg) had no effect on worm burden and egg counts. There were no adverse events due to artemether; however, two abortions were observed 7 days post-treatment. In conclusion, artemether shows interesting fasciocidal properties in sheep, but embryotoxicity is a concern. Further studies are ongoing to assess the potential of OZ78 and artesunate for treating *Fasciola* infections in different ruminants.



Proof-of-concept trial with artemether in *F. hepatica*-infected sheep in the Campania region of Italy. (Photo J. Keiser)

Proof-of-concept study with artemether in *F. hepatica*-infected patients in Egypt

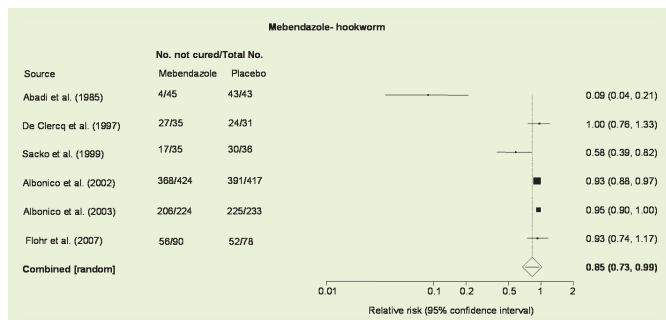
The aim of this study was to assess the safety and efficacy of oral artemether in an exploratory phase II trial in adult Egyptian patients infected with *F. hepatica*, *F. gigantica* or a combination of both. The study was carried out as a field study at Beheira governorate, lower Egypt, and included patients with parasitologically confirmed infection with *Fasciola* spp. In an initial study, oral artemether was administered to 20 patients as a 3-day treatment regimen that closely mirrors the common antimalarial treatment regimen, consisting of a 6 doses of 2 capsules (a total of 80 mg of artemether) given as a single dose daily for 3 days mornings and evenings after food intake. Adverse events were closely monitored. We found that artemether was well tolerated, and 8 out of 20 patients were cured. A follow-up study has been launched with a slightly higher dose of artemether, administering 3 doses of 200 mg of artemether within 12 hours, a regimen that has also already been used to treat malaria.



Examination of stool samples using the Kato-Katz method for the presence of *F. hepatica* eggs in the Beheira region of Egypt in the framework of our proof-of-concept trial with artemether in *F. hepatica*-infected patients.

Systematic literature reviews

We have produced several comprehensive literature reviews summarising advances in trematocidal drug development, especially the artemisinins. We have furthermore conducted a meta-analysis on the efficacy of common drugs against soil-transmitted helminthiases. We found that single oral doses of albendazole, mebendazole and pyrantel pamoate are highly efficacious against *Ascaris lumbricoides*. For hookworm infection,



Risk ratio estimate and pooled random risk estimate of randomised, placebo-controlled trials of mebendazole against hookworm infections. Rectangles indicate risk ratios, and sizes of the rectangles represent the weight given to each study in the meta-analysis. Diamond and vertical dashed line indicate the combined risk ratio; horizontal lines indicate 95% confidence intervals.

albendazole is superior to mebendazole and pyrantel pamoate. Treatment of *Trichuris trichiura* with single oral doses of current anthelmintics is unsatisfactory. Our findings demonstrate the urgent need for new anthelmintics.

Scientists: J. Keiser, J. Utzinger, M. Tanner, Ch. Hatz, P. Odermatt, J. Chollet
 Students: U. Duthaler, E. Furger, Y. Haggemüller, T. Manneck
 Collaboration: National Institute of Parasitic Diseases, Shanghai, China (S. H. Xiao); University of Nebraska Medical Center, Omaha (J. Vennerstrom); University of Naples (G. Cringoli, L. Rinaldi); Microscopy Centre, University of Basel; University of Basel Hospital (S. Krähenbühl); Fachhochschule Nordwestschweiz (J. Huvwyler)
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