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Activity of artemether and OZ78 against triclabendazole-resistant *Fasciola hepatica*

Jennifer Keiser[a,*], Jürg Utzinger[b], Jonathan L. Vennerstrom[c], Yuxiang Dong[c], Gerry Brennan[d], Ian Fairweather[d]

[a] *Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, P.O. Box, CH—4002 Basel, Switzerland*
[b] *Department of Public Health and Epidemiology, Swiss Tropical Institute, P.O. Box, CH—4002 Basel, Switzerland*
[c] *College of Pharmacy, University of Nebraska Medical Center, Nebraska, NE 68198-6025, USA*
[d] *Medical Biology Centre, The Queens University of Belfast, Belfast, Northern Ireland*

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Resistance

Summary Triclabendazole is the drug of choice against *Fasciola hepatica* infections in humans and animals. However, parasite resistance against triclabendazole is spreading in the veterinary field, and there are no drugs of comparable activity currently available for the treatment and control of fascioliasis. We investigated the efficacy of single oral doses of artemether and OZ78 against adult triclabendazole-resistant *F. hepatica* harboured in rats, and compared the results with triclabendazole administered at two different doses. Single oral doses of 100 mg/kg OZ78 and 200 mg/kg artemether resulted in worm burden reductions of 100%. Whereas a single 10 mg/kg dose of triclabendazole achieved a worm burden reduction of only 4.0%, a five-fold higher dose yielded a significant worm burden reduction of 60.9%. However, the lower dose of triclabendazole administered to rats harbouring a triclabendazole-sensitive *F. hepatica* isolate resulted in a worm burden reduction of 95.3%. Our findings confirm that artemether and OZ78 possess good fasciocidal properties, even against a triclabendazole-resistant *F. hepatica* isolate, and hence these drugs might become useful in areas where triclabendazole resistance is common.
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1. Introduction

Fascioliasis is a zoonotic disease caused by an infection with the liver flukes *Fasciola hepatica* and/or *F. gigantica*. Although fascioliasis belongs to the so-called neglected tropical diseases (Hotez et al., 2006), it is a considerable public health problem and of even greater veterinary importance. An estimated 91 million people are at risk of fascioliasis, and up to 17 million people might be infected (Keiser and Utzinger, 2005; WHO, 1995). In livestock, there are significant economic losses due to growth retardation, reduced milk yield and impaired animal fertility (Schweizer et al., 2005).

* Corresponding author. Tel.: +41 61 284 8218; fax: +41 61 284 8105.
E-mail address: jennifer.keiser@unibas.ch (J. Keiser).

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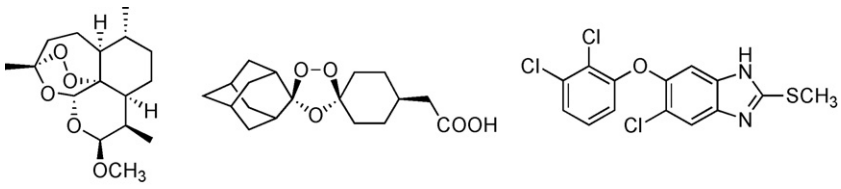

Figure 1 Chemical structures of artemether, OZ78 and triclabendazole.

In veterinary medicine, triclabendazole is the most commonly used drug against fascioliasis, due to the high activity of the drug against both juvenile and adult stages of the parasite. Other veterinary fasciolicides, such as clorsulon, closantel, nitroxynil and albendazole, lack activity against the juvenile stages of *F. hepatica* and *F. gigantica* (Fairweather and Boray, 1999). The young developing *F. hepatica* migrate through the liver parenchyma and thus cause extensive haemorrhaging and fibrosis of the liver (Behm and Sangster, 1999). Treatment of human fascioliasis relies primarily on triclabendazole, but the drug is registered in only four countries (Keiser et al., 2005).

Field isolates of *F. hepatica* resistant to triclabendazole were first described in Australian livestock in the mid-1990s, and resistance is now spreading across Europe. Most recently, triclabendazole resistance has been reported in Spain (Alvarez-Sanchez et al., 2006; Fairweather, 2005; Keiser et al., 2005). Although triclabendazole resistance has not been reported in human infections thus far, it might just be a matter of time, as humans are likely to become infected with *Fasciola* metacercariae of animal origin (Coles, 2006).

There is a pressing need to develop new fasciocidal drugs that are active against all parasite stages. A few promising compounds have been discovered in the recent past. For example, a derivative of triclabendazole, namely 5-chloro-2methylthio-6-(1-naphthyloxy)-1*H*-benzimidazole (compound alpha) showed high activity in vitro and in vivo (Ibarra et al., 2004; Vera Montenegro et al., 2004). Compound alpha, however, failed to show any activity against a triclabendazole-resistant *F. hepatica* infection in lambs (McCoy et al., 2006). We have recently reported the fasciocidal properties of the antimalarials artemether and artesunate and of a synthetic 1,2,4-trioxolane (OZ78); in the *F. hepatica*—rat model. We found that single oral doses of 200 mg/kg artemether and 100 mg/kg OZ78 resulted in worm burden reductions of 100% against both juvenile and adult *F. hepatica* (Keiser et al., 2006a, 2006b).

The aim of the present study was to determine the efficacy of single oral artemether and OZ78 against adult triclabendazole-resistant flukes harboured in rats. Our results are compared with those for triclabendazole, administered at two different doses.

2. Materials and methods

2.1. Drugs

OZ78 was synthesized at the College of Pharmacy, University of Nebraska Medical Center (Nebraska, NE, USA). Artemether was the gift of Kunming Pharmaceutical Co-operation (Kunming, China). Triclabendazole was obtained from Novartis Animal Health Ltd (Basel, Switzerland) and the commercial drench (5% Fasinex, Novartis Animal Health Ltd) was used. The chemical structures of the three drugs are presented in Figure 1. Compounds were prepared as suspensions in 7% (v/v) Tween-80 and 3% (v/v) ethanol before oral administration.

2.2. Ethical clearance, parasites and host—parasite models

All animal studies presented here were approved by national regulatory authorities in Switzerland and the UK. Female Wistar rats ($n = 24$; age, 5 weeks; weight, ~100 g) were purchased from RCC (Itingen, Switzerland). Adult male Sprague-Dawley rats ($n = 16$; age, 9 weeks; weight, ~320 g) were bred in the Animal House at Queen's University of Belfast (Belfast, Northern Ireland). Rats were housed in groups of no more than four in Macrolon cages under environmentally controlled conditions (temperature, ~25 °C; humidity, ~70%; 12 h light/dark cycle); they were acclimatized for 1 week and had free access to water and rodent food.

Metacercariae (Oberon isolate) of *F. hepatica* were produced from infections of the snail *Galba truncatula* using routine procedures in our laboratories at Queen's University of Belfast, Belfast, Northern Ireland (Walker et al., 2006). The metacercariae used for the experiments were from the same batch and were stored no longer than 16 weeks prior to use. The Oberon isolate was identified in 1999 in Oberon, Australia, where low cure rates had been reported in sheep following triclabendazole treatment. It has since been kept in the laboratory. Metacercariae of the Cullompton isolate (triclabendazole-sensitive) were purchased from Mr G. Graham (Addlestone, UK).

Thirty and ten rats were each infected intragastrically with 20—25 metacercarial cysts of the Oberon and Cullompton isolates of *F. hepatica*, respectively.

Twelve to fifteen weeks post-infection, rats infected with the Oberon isolate were treated orally with artemether (200 mg/kg) and OZ78 (100 mg/kg). Two groups of rats received triclabendazole at 10 mg/kg and 50 mg/kg, respectively. Four (for triclabendazole) and six (for artemether and OZ78) untreated rats, respectively, served as control groups.

Four rats infected with the Cullompton isolate of *F. hepatica* received triclabendazole at a single 10 mg/kg oral dose 12 weeks post-infection. Six rats infected with the same isolate remained untreated, and hence served as controls.

Table 1 Worm burden reductions of adult *Fasciola hepatica* harboured in rats (Cullompton isolate) following the administration of triclabendazole

Treatment	Dose (mg/kg)	No. rats investigated	No. rats cured[a]	Mean worm burden (SD)	Total worm burden reduction (%)	Kruskal-Wallis test	*P*-value
Control	–	6	–	5.3 (1.9)	–	–	–
Triclabendazole	10	4	3	0.25 (0.5)	95.3	6.75	0.009

[a] The number of rats without flukes.

Ten to 17 days post-treatment, rats were euthanized by CO_2. At necropsy *F. hepatica* were harvested from the excised bile ducts and counted.

2.3. Statistical analysis

Statistical analysis was performed with version 2.4.5 of Statsdirect statistical software (Statsdirect Ltd, Cheshire, UK). Average worm burdens were expressed as arithmetic means, including values of zero for animals with no worms. We used the Kruskal-Wallis (KW) test to compare the medians of the responses between the treatment and the matching control group. A difference in median was considered to be significant at a level of 5%.

3. Results and discussion

Table 1 shows that administration of a single 10 mg/kg oral dose of triclabendazole to rats harbouring an adult triclabendazole-sensitive *F. hepatica* infection, resulted in a worm burden reduction of 95.3% (KW = 6.75; $P = 0.009$). This finding is in accordance with previous studies with triclabendazole-susceptible *F. hepatica* isolates, which reported complete worm burden reductions with 10 mg/kg single oral doses of triclabendazole (Boray et al., 1983; Turner et al., 1984).

However, triclabendazole at a single 10 mg/kg oral dose failed to show a significant effect against the triclabendazole-resistant Oberon isolate of *F. hepatica*; a low worm burden reduction of only 4.0% was observed (Table 2). At a five-fold higher dose of triclabendazole, we observed a statistically significant worm burden reduction of 60.9% in rats infected with the triclabendazole-resistant *F. hepatica* isolate (KW = 5.40; $P = 0.020$).

The lack of activity of triclabendazole against the Oberon isolate in rats with a standard dose of 10 mg/kg confirms recent findings obtained in sheep; a single 10 mg/kg oral dose of triclabendazole administered to sheep infected with this triclabendazole-resistant strain of *F. hepatica* showed an efficacy below 5% against 2- and 4-week-old flukes (Walker et al., 2004).

Importantly, we found that both artemether and OZ78 were highly efficacious against this triclabendazole-resistant isolate of *F. hepatica*; administration of single oral doses of 100 mg/kg OZ78 or 200 mg/kg artemether resulted in worm burden reductions of 100% (KW = 7.2; $P = 0.007$). In view of our recent findings that single oral doses of 100 mg/kg OZ78 and 200 mg/kg artemether administered to rats infected with a triclabendazole-sensitive *F. hepatica* isolate achieved 100% worm burden reductions (Keiser et al., 2006a, 2006b), there is no apparent difference between the fasciocidal efficacy of artemether and OZ78 against the triclabendazole-resistant and the triclabendazole-sensitive isolates. The most likely explanation of this finding arises by different mechanisms of actions of these peroxidic drugs when compared with triclabendazole. It has been demonstrated that triclabendazole disrupts microtubule-based secretory processes in the liver flukes (Fairweather, 2005; Fairweather and Boray, 1999). The mechanism of action of the artemisinins on trematodes remains to be elucidated, but in vitro studies with *F. hepatica* and schistosomes suggest that haemin plays a role (Keiser and Utzinger, in press; Xiao et al., 2001). A long-standing hypothesis to account for the antimalarial specificity of the artemisinins is that the peroxide bond undergoes reductive activation by haem

Table 2 Worm burden reductions of adult *Fasciola hepatica* harboured in rats (Oberon isolate) following the administration of triclabendazole, OZ78 and artemether

Treatment	Dose (mg/kg)	No. rats investigated	No. rats cured[a]	Mean worm burden (SD)	Total worm burden reduction (%)	Kruskal-Wallis test	*P*-value
Control 1	–	4[b]	–	6.25 (1.9)	–	–	–
Triclabendazole	10	4[b]	0	6.0 (2.2)	4.0	0.09	0.764
Control 2	–	4[c]	–	5.75 (1.7)	–	–	–
Triclabendazole	50	4[c]	0	2.25 (1.0)	60.9	5.40	0.020
Control 3	–	6[d]	–	4.2 (1.7)	–	–	–
OZ78	100	4[d]	4	0	100	7.20	0.007
Artemether	200	4[d]	4	0	100	7.20	0.007

[a] The number of rats without flukes.
[b] First experiment.
[c] Second experiment.
[d] Third experiment.

released by parasite Hb digestion producing carbon-centred free radicals (Golenser et al., 2006). Another likely target of the artemisinins is the PfATP6, a SERCA-type Ca^{2+}-ATPase (Eckstein-Ludwig et al., 2003). As *Plasmodium*, *Schistosoma* spp. and *Fasciola* spp. degrade Hb to generate free haem, a similar mechanism of action of the artemisinins might be involved in the death of these parasites.

In conclusion, we have shown that artemether and OZ78 have good activities against a triclabendazole-resistant *F. hepatica* isolate. If studies in larger animals and exploratory clinical trials confirm the fasciocidal properties of artemether and OZ78, these drugs might emerge as alternative broad-spectrum fasciocidal drugs, which would be particularly useful in areas where triclabendazole resistance is common.

Authors' contributions: JK, JU, GB and IF designed the study protocol, carried out the experiments and analysed and interpreted the data; JLV and YD synthesized OZ78 and contributed to the study protocol; JK, JU, JLV and IF drafted the manuscript. All authors read and approved the final manuscript. JK and IF are guarantors of the paper.

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Conflicts of interest: None declared.

Ethical approval: Not required.

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