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Efficacy, metabolism and pharmacokinetics of Ro 15-5458, a forgotten schistosomicidal 9-acridanone hydrazone

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Background: Treatment of schistosomiasis, a neglected disease, relies on just one partially effective drug, praziquantel. We revisited the 9-acridanone hydrazone, Ro 15-5458, a largely forgotten antischistosomal lead compound.

Methods: Ro 15-5458 was evaluated in juvenile and adult *Schistosoma mansoni*-infected mice. We studied dose–response, hepatic shift and stage specificity. The metabolic stability of Ro 15-5458 was measured in the presence of human and mouse liver microsomes, and human hepatocytes; the latter also served to identify metabolites. Pharmacokinetic parameters were measured in naive mice. The efficacy of Ro 15-5458 was also assessed in *S. haematobium*-infected hamsters and *S. japonicum*-infected mice.

Results: Ro 15-5458 had single-dose ED_{50} values of 15 and 5.3 mg/kg in mice harbouring juvenile and adult *S. mansoni* infections, respectively. An ED_{50} value of 17 mg/kg was measured in *S. haematobium*-infected hamsters; however, the compound was inactive at up to 100 mg/kg in *S. japonicum*-infected mice. The drug-induced hepatic shift occurred between 48 and 66 h post treatment. A single oral dose of 50 mg/kg of Ro 15-5458 had high activity against all tested *S. mansoni* stages (1-, 7-, 14-, 21- and 49-day-old). *In vitro*, human hepatocytes produced *N*-desethyl and glucuronide metabolites; otherwise Ro 15-5458 was metabolically stable in the presence of microsomes or whole hepatocytes. The maximum plasma concentration was approximately 8.13 μg/mL 3 h after a 50 mg/kg oral dose and the half-life was approximately 4.9 h.

Conclusions: Ro 15-5458 has high activity against *S. mansoni* and *S. haematobium*, yet lacks activity against *S. japonicum*, which is striking. This will require further investigation, as a broad-spectrum antischistosomal drug is desirable.

Introduction

Schistosomiasis is a neglected tropical disease caused by blood-dwelling flatworms of the *Schistosoma* genus. Out of the six medically important species, *Schistosoma haematobium*, *Schistosoma japonicum* and *Schistosoma mansoni* account for the highest burden of disease.[1] Disabling morbidities such as anaemia, malnutrition and impaired child development, as well as increased susceptibility to co-infection with other parasitic diseases, pose significant health problems.[2–4] Control and treatment of schistosomiasis have been entirely dependent upon praziquantel for the past four decades. Although active against all medically important schistosomes, praziquantel is rarely curative, and has little to no effect on developing parasites. Further, its increasing use might trigger drug resistance.[5,6] Thus, there is a need for new drugs.

In the 1980s, 9-acridanone hydrazones were investigated by Hoffmann La-Roche for the treatment of schistosomiasis. Ro 15-5458 was the lead candidate selected for further development.

Ro 15-5458 had ED_{90} values below 100 mg/kg in mice and hamsters infected with *S. mansoni*, *S. haematobium* and *S. japonicum*.[7] The excellent *in vivo* efficacy against *S. mansoni* was confirmed in baboons[8,9] and Cebus monkeys.[10] Although studies on the biochemical characterization and possible mode of action of Ro 15-5458 were carried out,[11,12] considerable gaps in our understanding of this intriguing antischistosomal lead compound remain. In spite of its remarkable activity, Ro 15-5458 was not further investigated, probably because of the successful introduction of praziquantel as antischistosomal treatment in the market at that time. The aim of the present study was to further characterize and define the strengths and weaknesses of Ro 15-5458.

Materials and methods

Ethics

In vivo efficacy studies were carried out in accordance with Swiss national and cantonal regulations on animal welfare at the Swiss Tropical and Public Health Institute (Basel, Switzerland) under permission number 2070. At the University of California San Diego, use of hamsters was approved by the university's Institutional Animal Care and Use Committee. Pharmacokinetic (PK) studies were conducted at the Center for Drug Candidate Optimization, Monash University in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Study protocols were reviewed and approved by the Monash Institute of Pharmaceutical Sciences Animal Ethics Committee.

Vertebrate animals and parasites

For *in vivo* efficacy studies, female mice (NMRI strain; 3 weeks old; weight ~20–22 g) were purchased from Charles River, Germany. Golden Syrian LVG hamsters (Charles River, USA), exposed to 350 *S. haematobium* (Egyptian strain) cercariae and Swiss Webster mice (Taconic Inc, NY) exposed to 40 *S. japonicum* cercariae (Philippine strain) were ordered from the US NIH's Biomedical Research Institute (BRI). Rodents were kept under environmentally controlled conditions (temperature ~25°C; humidity ~70%; 12 h light, 12 h dark cycle) with free access to water and rodent diet, and acclimatized for 1 week before infection. Cercariae of *S. mansoni* (Liberian strain) were obtained from infected intermediate host snails (*Biomphalaria glabrata*) and mechanically transformed to newly transformed schistosomula (NTS) as described previously.[13] Mice were infected by subcutaneously injecting approximately 100 *S. mansoni* cercariae.

For PK studies, the systemic exposure of Ro 15-5458 was studied in male Swiss outbred mice weighing 25.6–30.4 g. Mice had access to food and water *ad libitum* throughout the pre- and post-dose sampling period.

Ro 15-5458

Ro 15-5458 was synthesized as described (Material S1, available as Supplementary data at *JAC* Online).[14] For *in vitro* assays, the compound was dissolved in DMSO (Sigma–Aldrich, Switzerland) at a concentration of 10 mM. For *in vivo* efficacy and PK studies, on the day of dosing, solid compound was dispersed in Tween-80 [7% (v/v) final], after which ethanol [3% (v/v) final] and Milli-Q water were added. The formulation was then vortexed and sonicated.

In vitro studies

Phenotypic screening of NTS and adult worms

For *S. mansoni* NTS and adults, and adult *S. japonicum* worms, transparent flat-bottom 96- and 24-well plates were used, respectively (Sarstedt, Switzerland). Approximately 100 NTS per well were incubated in the presence of 1.56–100 μM Ro 15-5458 in 250 μL of M199 medium (Gibco, USA) supplemented with 5% (v/v) FCS (Bioconcept AG, Switzerland), 1% (v/v) penicillin/streptomycin solution (Sigma–Aldrich, Switzerland) and 1% (v/v) antibacterial/antifungal[13] solution for up to 72 h at 37°C and 5% CO_2. At least three adult worms (both sexes) were incubated with 10 and 100 μM Ro 15-5458 in 2 mL of RPMI 1640 (Gibco, USA) supplemented with 5% (v/v) FCS and 1% (v/v) penicillin/streptomycin for 72 h at 37°C and 5% CO_2.[13]

The activity of Ro 15-5458 on NTS and adults was judged by scoring the overall viability of the parasites.[13,15] The possible influence of protein binding on activity was tested by supplementing the medium with 45 g/L BSA (AlbuMax II Lipid-Rich BSA, Gibco).[16] Also, any long-term effect of Ro 15-5458 on adult worms was tested by incubating the worms at 10 and 100 μM for 48 h and, after extensive washing, continuing the incubation in medium only for a further 10 days.

The *ex vivo* assessment of viability of adult *S. mansoni* post exposure to Ro 15-5548 *in vivo* was also studied. After their perfusion from Ro 15-5458-treated mice, worms were washed and incubated in Basch medium[17] supplemented with 5% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin for up to 14 days.[15] For all experiments, the highest concentration of DMSO served as a negative control. Experiments were conducted in duplicate and repeated at least once.

Metabolic stability of Ro 15-5458 incubated with mouse and human liver microsomes or human hepatocytes

The metabolic stability study of Ro 15-5458 in the presence of mouse and human liver microsomes (both obtained from Xenotech) was performed at Monash University. Ro 15-5458 was incubated at a final concentration of 1 μM at 37°C and a 0.4 mg/mL protein concentration. The metabolic reaction was initiated by the addition of an NADPH-regenerating system. Over a 60 min incubation period, the reaction was quenched at different timepoints by adding acetonitrile containing diazepam as an internal standard followed by centrifugation to pellet the precipitated material (4500 **g**, 4 min, 21°C). Control samples (containing no NADPH) were included and quenched at 2, 30 and 60 min to monitor for potential degradation in the absence of the cofactor. The samples were analysed by LC-MS (Waters Xevo G2 QTOF instrument equipped with an Acquity UPLC system) under positive electrospray ionization, and MS data were acquired in the mass range of 80 to 1200 Da. The *in vitro* intrinsic clearance values (expressed as μL/min/mg protein) were calculated using the first-order degradation rate constant (h^{-1}) and the microsomal protein concentration (mg/μL). The metabolic stability of Ro 15-5458 in the presence of human hepatocytes was assessed by the contract research organization, WuXi AppTec (Shanghai, China), according to its standard protocols and under the auspices of the CDIPD-UCSD (Method S1).

Metabolite profiling following incubation of Ro 15-5458 with human hepatocytes in vitro

This study was performed by WuXi AppTec under the auspices of the CDIPD-UCSD. Cryopreserved human hepatocytes (In Vitro Technologies) were thawed and incubated for 120 min with 10 μM Ro 15-5458 in 200 μL of William's E medium containing 1×10^6 cells/mL at 37°C, 5% CO_2. Samples were precipitated with a 2-fold volume of acetonitrile (containing 0.1% formic acid) and centrifuged at 3220 **g** for 20 min. The supernatant was removed and dried with N_2 gas at room temperature. The residue was reconstituted in 200 μL of 10% acetonitrile/0.1% formic acid and 15 μL was injected onto an Acquity UPLC HSS T3 column (1.8 μm, 2.1×100 mm) under the control of a Waters Xevo G2 QToF MS system. MS/MS analyses were performed in positive electrospray ionization mode, and UPLC/MS^E was used as scan mode. The metabolites of Ro 15-5458 were detected and further characterized by LC-MS^n (n = 1–2). The metabolite structures were elucidated by comparative analysis of fragments between the parent and individual

metabolites. Samples were also monitored by UV absorbance for the purpose of estimating relative metabolite concentrations.

In vivo efficacy studies

S. mansoni

To investigate the dose–response relationship of Ro 15-5458, selected single doses (6.25, 12.5, 25, 50 and 100 mg/kg) were administered to groups of four *S. mansoni*-infected mice by oral gavage at 21 days (juvenile infection) or 49 days (adult infection) post infection. To determine the onset of action of Ro 15-5458 (hepatic shift), four mice infected with *S. mansoni* were treated with 17.5 mg/kg Ro 15-5458 on day 49 post infection. After 8, 24, 48 and 72 h, one mouse was euthanized and the worms in the mesenteric vein system were removed by picking.[18] The livers were excised, placed between two transparent plastic layers and pressed (liver squash);[13] worms were sexed and counted. A confirmation experiment with four mice was conducted in the same way, employing the post treatment timepoints of 60, 66, 78 and 85 h. To study whether the bioactivity of Ro 15-5458 was dependent on the development of the parasite, groups of four *S. mansoni*-infected mice were given single oral doses of 12.5 or 50 mg/kg Ro 15-5458 at days −2, 0, 1, 7, 14, 21, 28, 35, 42 and 49 post infection. Infected but untreated mice served as controls in all experiments.

S. haematobium and S. japonicum

To study the dose–response relationship of Ro 15-5458 in adult *S. haematobium* infections, groups of three to four hamsters were treated orally with single doses of 20 and 25 mg/kg, 6 months after infection. For *S. japonicum*, on day 35 post infection, 50 and 100 mg/kg mg/kg Ro 15-5458 were administered to groups of four mice by oral gavage. Sixteen and 21 days after treatment, respectively, hamsters and mice were euthanized with CO_2. Worms were removed by picking, sexed and counted, and the worm burden reduction was calculated.[19] Infected but untreated animals served as controls in all experiments.

In vivo PK studies in mice

Ro 15-5458 (50 mg/kg) was orally administered to non-fasted Swiss outbred mice ($n = 6$) at a dose volume of 10 mL/kg. Blood samples were collected via submandibular bleeding (approximately 120 μL; conscious sampling) at 1, 3, 7, 24, 30 and 48 h with three mice per timepoint and a maximum of three samples from each mouse. Blood was collected in polypropylene Eppendorf tubes containing heparin as an anticoagulant and a stabilization cocktail (Complete, Sigma–Aldrich) to minimize the potential for *ex vivo* compound degradation in blood/plasma samples. Once collected, blood samples were centrifuged (14 100 **g**) and the supernatant plasma was removed and stored at −80°C until analysis by LC-MS.

Samples were assayed against a 12-point calibration curve prepared in blank mouse plasma and processed together with the study samples. Samples and calibration standards were precipitated with a 2-fold volume of acetonitrile followed by vortex mixing and centrifugation (14 100 **g**, 3 min). The supernatant was separated and 2 μL was injected onto the column [Phenomenex Kinetex PFP column (50×2.1 mm, 2.6 μm)] for LC-MS analysis. A Waters Xevo TQD coupled to a Waters Acquity UPLC with positive electrospray ionization in multiple-reaction monitoring mode was used for detection. A gradient cycle time of 4 min and a flow rate of 0.4 mL/min were employed. The mobile phase consisted of an acetonitrile/water gradient with 0.005 M ammonium formate. The plasma concentration versus time profile was defined by the average plasma concentration at each sample time.

Data analysis

The *in vitro* activity against NTS and adult *S. mansoni* was calculated in Microsoft Excel using the mean viability values (±SD) of Ro 15-5458 in relation to the control values. CompuSyn software (version 1.0; ComboSyn Inc., 2007) was used to calculate EC_{50} values. The worm burden reduction (WBR) (*in vivo*) was determined based on the percentage of worm burden in infected, treated rodents compared with infected, untreated rodents, which served as controls,[20] and a Kruskal–Wallis test was employed for statistical significance in R (version 3.5.1). GraphPad Prism (Version 8.2.1) was used to calculate ED_{50} values and to generate graphs. PK parameters were calculated using non-compartmental methods (PKSolver Version 2.0).

Results

In vitro activity of Ro 15-5458 against S. mansoni and S. japonicum

NTS exposed to 100 μM Ro 15-5458 for 72 h died; however, lower concentrations were not lethal. An EC_{50} of 33 μM was calculated. Adult *S. mansoni* worms were exposed to 10 and 100 μM Ro 15-5458 with and without the addition of albumin (45 g/L) for 72 h. EC_{50} values of 85 and >100 μM were calculated for worms incubated without and with albumin, respectively, suggesting a decrease in activity due to protein binding. An EC_{50} of 65 μM was obtained when performing a phenotypic readout on adult *S. japonicum* worms (without albumin). Results are summarized in Table 1.

When *S. mansoni* and *S. japonicum* were incubated for 48 h with 100 μM Ro 15-5458, then washed, and left in medium alone, the worms died after an additional 7 days. At 10 μM, the same experiment induced slowed motility and an inability of the worms to adhere to the plate over the remaining 10 days of the incubation.

Ro 15-5458 is stable when incubated with mouse and human microsomes, and human hepatocytes

In both test systems, the *in vitro* intrinsic clearance data suggested that the hepatic clearance of Ro 15-5458 is very low (Tables 2 and 3), according to commonly used classification bands to categorize compounds as low, medium or high clearance.

Table 1. *In vitro* results

Species, development stage	Concentration(s) tested (μM)	Outcome
S. mansoni, NTS	1–100 μM (6 dilutions)	EC_{50}=33 μM
S. mansoni, adult worms	100, 10, 0 μM	EC_{50}=85 μM
S. mansoni, adult worms (+ albumin)	100, 10, 0 μM	EC_{50}>100 μM
S. mansoni, adult worms (long term)	100 μM for 48 h, then medium only	Worms died after an additional 7 days of incubation in medium only.
S. japonicum, adult worms	100, 10, 0 μM	EC_{50}=65 μM

Identification of glucuronidated and de-ethylated metabolites produced by human hepatocytes in vitro

Metabolic profiling of Ro 15-5458 incubated with human hepatocytes identified two metabolites, a glucuronidated product (mol. wt = 569.67 Da, P + $C_6H_8O_6$) and a de-ethylated product, (mol. wt = 365.50 Da, P − C_2H_4) (Figure 1). The relative concentration of the metabolites was estimated based on the UV peak area relative to that of the parent peak (Figure S1). The de-ethylated metabolite accounted for approximately 6.6% of the total peak area, with the parent Ro 15-5458 accounting for approximately 90% of the total peak area (Table 4).

In vivo dose–response relationship against S. mansoni, S. haematobium and S. japonicum

Single oral doses of Ro 15-5458 were significantly effective in mice harbouring *S. mansoni* (juvenile, $P = 0.0024$ for all doses tested; adult, $P = 0.0016$ for all doses tested) and hamsters infected with *S. haematobium* ($P = 0.0076$ for all doses tested) (Table 5). Against juvenile *S. mansoni*, of the five doses tested, the highest, 50 and 100 mg/kg, produced WBRs of 93.5% and 99%, respectively. Lower doses were ineffective. The ED_{50} value was 15 mg/kg. Better activity was observed in animals harbouring adult parasites. Doses of 12.5 and 25 mg/kg resulted in total WBRs of 100% and 98.7%, respectively, with an ED_{50} value of 5.3 mg/kg. For *S. haematobium*, doses of 20 and 25 mg/kg resulted in total WBRs of 68% and 87%, respectively. In contrast, in mice infected with adult *S. japonicum* Ro 15-5458 lacked activity after a dose of 50 mg/kg. In the 100 mg/kg treatment group ($n = 4$), three mice died 4 h after treatment. In two subsequent experiments at the same dose, low efficacy was observed, but without the previously noted toxicity.

Table 2. Metabolic stability in mouse and human microsomes

Microsome species	$t_{1/2}$ (min)[a]	$CL_{int,\ in\ vitro}$ (μL/min/mg protein)[b]	Clearance classification
Human	>255	<7	low
Mouse	>255	<7	low

[a]$t_{1/2}$ (degradation half-life)=$\ln_2/k$.
[b]CL_{int} = CL_{int}, *in vitro* × liver mass (g)/body weight (kg) × microsomal protein mass (mg)/liver mass (g).

Hepatic shift (S. mansoni)

The onset of the efficacy of Ro 15-5458, indicated by a shift in the distribution of worms from the mesenteric veins to the liver, is illustrated in Figure 2. In the first experiment, Ro 15-5458 acted slowly; at 8, 24, 48 h after a 17.5 mg/kg dose, all worms were still present in the mesenteric veins. By 72 h, the worm count in the mesenteric veins had decreased by 80% relative to non-treated controls. In the second experiment using the same dose, no worms had shifted to the liver 60 h after treatment. By 66 h, only a few worms had shifted to the liver (dead), while most of the worms were still

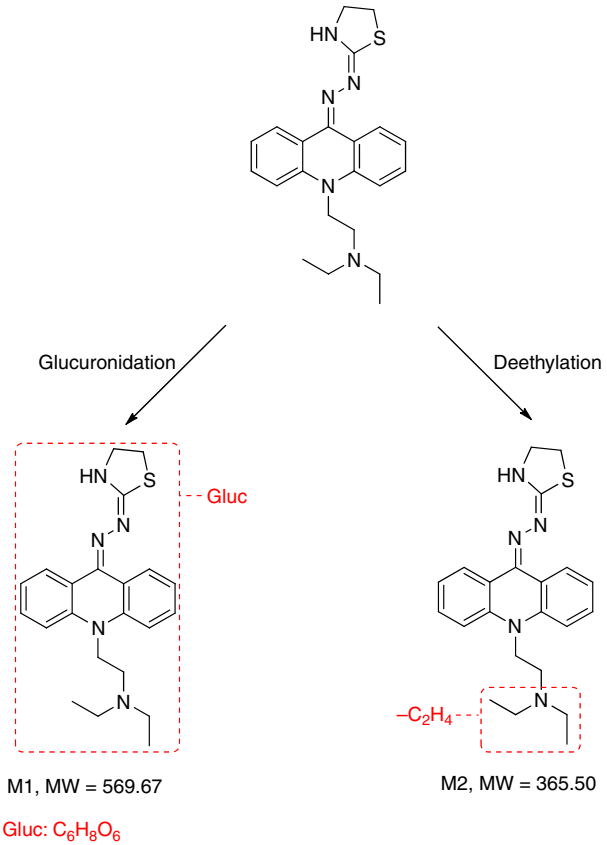

Figure 1. Metabolic products of Ro 15-5458 after incubation with human hepatocytes *in vitro*. This figure appears in colour in the online version of *JAC* and in black and white in the printed version of *JAC*.

Table 3. Metabolic stability in human hepatocytes

		Human hepatocytes		
Compound ID	Cell density (×10^6 cells/mL)	k_e[a]	$t_{1/2}$ (min)	*in vitro* CL_{int} (μL/min/10^6 cells)
Ro 15-5458	0.5	0.0076	91.2	15.2
7-Ethoxycoumarin[b]		0.0229	30.3	45.7
7-Hydroxycoumarin[c]		0.0372	18.6	74.4

[a]k_e is the elimination rate constant.
[b]7-Ethoxycoumarin is a cytochrome P450 substrate.
[c]7-Hydroxycoumarin is a metabolite standard (the enzymatic product of 7-ethoxycoumarin).

Table 4. Metabolites of Ro 15-5458 in human hepatocytes

Metabolite code	$[M + H]^+$ *m/z*	LC-MS retention time (min)	Relative abundance (UV peak area)[a]	Metabolic pathway
M1	570.24	7.38	2.87%	glucuronidation, (P + $C_6H_8O_6$)
M2	366.17	10.38	6.63%	de-ethylation, (P – C_2H_4)
Parent (Ro-15-5458)	394.21	10.99	90.50%	NA

NA, not applicable.
[a]Semi-quantitative data calculated by UV peak areas of the metabolites and parent in samples under UV wavelength at 254–460 nm.

Table 5. Dose–response relationships of Ro 15-5458 against various species and developmental stages of the schistosome parasite

				Mean number of worms (SD)						
Stage (age of) infection	Dose (mg/kg)	No. of animals used	No. of animals cured	liver	MV	total	males	females	total WBR % (SD)	*P* value (Kruskal–Wallis)
S. mansoni	control 1	8	–	0.6 (1.4)	33.8 (9.1)	34.4 (9.9)	17.4 (5.0)	17.0 (5.1)	–	–
juvenile	control 2[c]	4	–	NA	NA	43.6 (5.3)	21.8 (5.3)	21.8 (5.3)	–	–
(21 days old)	6.25	4	0	1.3 (1.5)	18.0 (2.9)	19.3 (3.3)	10.8 (2.1)	8.5 (1.9)	44.0 (9.6)	0.002
	12.5	4	0	0.0 (0.0)	19.3 (5.6)	19.3 (5.6)	9.8 (2.6)	9.5 (3.0)	44.0 (16.4)	
	25	4[a]	0	2.7 (2.1)	26.3 (10.0)	29.0 (10.8)	17.0 (5.0)	12.0 (6.1)	19.2 (27.4)	
	50	4	2	1.0 (1.4)	1.3 (1.9)	2.3 (2.6)	0.5 (1.0)	1.8 (2.1)	93.5 (7.7)	
	100[c]	4	2	NA	NA	0.5 (0.6)	0.5 (0.6)	0.0 (0.0)	99.0 (0.5)	0.02
S. mansoni adult	control	8	–	0.0 (0.0)	19.9 (8.1)	19.9 (8.1)	10.0 (4.1)	9.9 (4.1)	–	–
(49 days old)	6.25	4	0	3.0 (2.2)	10.0 (10.7)	13.0 (9.7)	5.8 (3.8)	7.3 (6.0)	43.6 (32.0)	0.002
	12.5	4	4	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	100.0 (0.0)	
	25	4	4	0.0 (0.0)	0.3 (0.5)	0.3 (0.5)	0.0 (0.0)	0.3 (0.5)	98.7 (2.5)	
S. haematobium	control	4	–	4.0 (3.3)	36.0 (13.0)	18.5 (8.3)	21.5 (5.8)	18.5 (8.3)	–	–
adult	20	4	1	0.5 (1.0)	12.3 (10.2)	12.8 (10.2)	6.5 (5.3)	6.3 (4.9)	68.1 (25.6)	0.025
(6 months old)	25	3	2	0.0 (0.0)	5.3 (9.3)	5.3 (9.3)	2.7 (4.6)	2.7 (4.6)	86.7 (23.1)	
S. japonicum adult	control	6	–	0.3 (0.8)	17.5 (7.1)	17.8 (6.6)	8.8 (3.1)	9.0 (3.9)	–	–
(35 days old)	control[d]	4	–	4.0 (4.3)	25.5 (10.0)	29.5 (13.4)	14.8 (6.7)	14.8 (6.7)	–	–
	control[e]	4[a]	–	0.0 (0.0)	18.0 (6.9)	18.0 (6.9)	9.0 (3.5)	9.0 (3.5)	–	–
	50	4	0	4.8 (1.0)	21.0 (7.0)	25.8 (7.8)	12.5 (3.4)	13.3 (4.6)	0.0 (0.0)	>0.05
	100	4[b]	0	NA	NA	NA	NA	NA	NA	NA
	100[d]	5	0	2.2 (3.4)	14.6 (11.0)	16.8 (13.8)	8.6 (7.0)	8.2 (6.8)	43.0 (46.9)	>0.05
	100[e]	5	0	3.5 (1.9)	11.8 (4.2)	15.3 (5.9)	7.8 (3.0)	7.5 (2.9)	20.8 (25.0)	>0.05

MV, mesenteric veins; NA, not applicable. Total WBR (%)=100% − 100% × ($WB_{treated\ animals}/WB_{control\ animals}$), whereby negative WBR values were set to zero before averaging.
A *P* value of ≤0.05 was considered as significant.
[a]One mouse was not infected and was excluded from the analysis.
[b]Three mice died 4 h after treatment; the last mouse was sacrificed at the same timepoint because of poor general condition.
[c]Experiment was performed at the CDIPD-UCSD; worms were recovered by reverse perfusion of the hepatic portal and mesenteric veins.
[d]Second experiment, new animal batch used.
[e]Third experiment, new animal batch used.

in the mesenteric veins (alive). The worm numbers in the mesenteric veins had decreased by 44% and 76% relative to non-treated controls 78 and 85 h after treatment.

Effect of Ro 15-5458 on S. mansoni adults ex vivo

Mice infected with 42-day-old adult *S. mansoni* were administered a single oral dose of 15 mg/kg Ro 15-5458. After 22 h, and before the hepatic shift had commenced, worms were recovered and incubated *in vitro*, as described.[15] From the ninth day onwards, exposed adult male worms displayed a progressively more intense corkscrew-like coiling along the long axis (Video S1) relative to non-treated controls (Video S2). Also, both the oral and ventral sucker, and the intervening neck region became withered and rigid (Figure S2) in contrast to non-treated controls (Figure S3).

Stage specificity of single oral 12.5 and 50 mg/kg Ro 15-5458 in mice infected with S. mansoni

Doses of 50 and 12.5 mg/kg Ro 15-5458 were tested to understand the compound's efficacy against the parasite residing in the skin (day 1), lungs (day 7) and in the mesenteric system as juveniles (days 14 and 21) and adults (day 49) (Figure 3). At 50 mg/kg, all developmental stages of *S. mansoni* were affected. High total WBRs of 82%, 100%, 92%, 94% and 99% were observed for treatments occurring on days 1, 7, 14, 21 and 49 post infection. Lower efficacy was measured when 12.5 mg/kg was administered. Nevertheless, statistically significant worm burden reductions for all development stages were recorded for treatment doses of 12.5 mg/kg ($P = 0.019$) and 50 mg/kg ($P < 0.001$) when compared with untreated control animals.

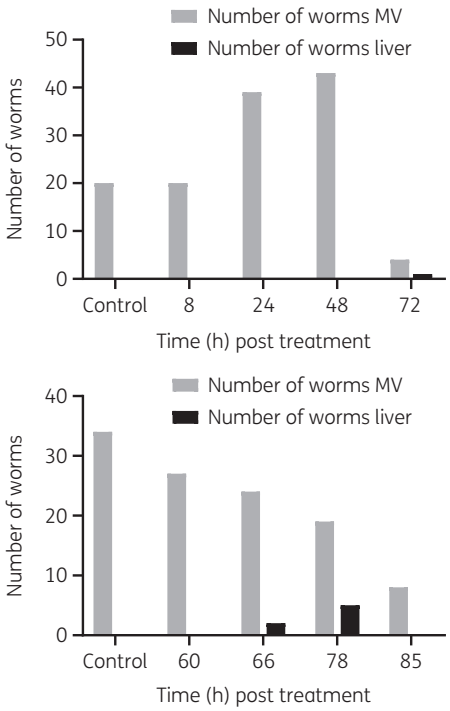

Figure 2. Hepatic shift of *S. mansoni* in mice 49 days after treatment with 17.5 mg/kg Ro 15-5458. Numbers of worms alive in the mesenteric veins (MV) and number of dead worms in the liver are shown from two individual experiments. Each bar shows the number of worms found in one mouse.

PK in mice

Following oral administration of Ro 15-5458 (50 mg/kg) to male Swiss outbred mice, high plasma concentrations were detectable at the first sampling timepoint (1 h) post-dose, indicating rapid absorption (Figure 4). The maximum plasma concentration (C_{max}) of 20.66 μM (8.13 μg/mL) was measured at 3 h post-dose (t_{max}) and the apparent elimination half-life was approximately 5 h. The area under the plasma concentration–time curve (AUC_{0-48h}) was approximately 254 μM·h (100 μg·h/mL) and the clearance (CL/F) was 8.3 mL/min/kg (Table 6). Additionally, to determine the extent of protein binding, an experiment using mouse plasma was performed (Method S2). The unbound fraction (f_u) was low and had a value of 0.021±0.001, which correlates well with the observed clearance *in vivo*.[21]

Discussion

Praziquantel is globally employed in preventive chemotherapy campaigns to millions of people each year.[22] A recent review[5]

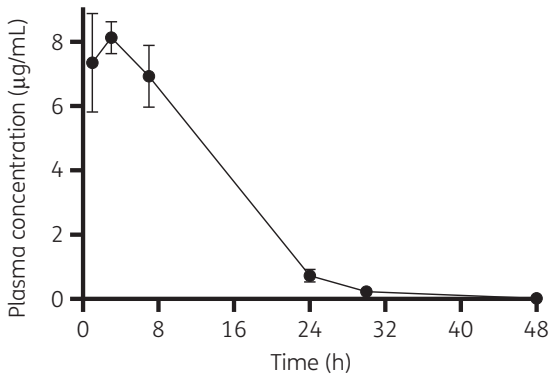

Figure 4. Plasma concentration–time curve of Ro 15-5458 in male Swiss outbred mice following oral administration of 50 mg/kg. Means and SD from three mice are shown.

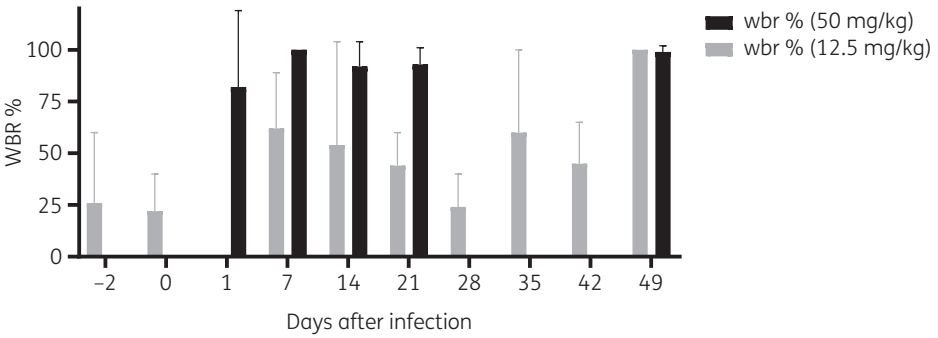

Figure 3. Stage-specific efficacy of Ro 15-5458 in *S. mansoni*-infected mice. Mice ($n = 4$) were treated once orally with 12.5 mg/kg Ro 15-5458 on days −2, 0, 7, 14, 21, 28, 35, 42 and 49, and with 50 mg/kg on days 1, 7, 14, 21 and 49 post infection. Treatment on days 21 and 49 post infection was performed in the course of the dose–response study. For the *S. mansoni*-infected mice group treated on day 49 post infection, 25 mg/kg instead of 50 mg/kg served as the highest dose. Acquired data were integrated in the stage specificity study for the statistical analysis and generation of the displayed figure. Average worm burden reductions (WBRs %) were calculated 21–49 days post treatment (means and SD are presented).

Table 6. PK parameters in non-infected mice

PO dose	C_{max} (μM/μg/mL)	t_{max} (h)	AUC (μM·h/μg·h/mL)	$t_{1/2}$ (h)	CL/F (mL/min/kg)
50 mg/kg (*n*=6)	20.66/8.13	3	254/100	4.9	8.3

Data presented are based on the mean concentration versus time data.
Experiments were performed at Monash University; mice were not fasted prior to drug administration.

stated the pressing need for new treatment options, but lamented the fact that none seemed to be on the horizon. It is against this background that we re-examined the *in vivo* efficacy of the largely forgotten Ro 15-5458, which was discovered in the early 1980s.[7] 9-Acridanone hydrazones were reported to be active in rodents infected with *S. mansoni*,[23–25] *S. haematobium*[26] and *S. japonicum*.[7] Three doses of Ro 15-5458 (10, 15 and 20 mg/kg) were tested in a mouse model of chronic *S. mansoni* infection and each was effective (WBR >83.6%).[27] Subsequently, Ro 15-5458 was evaluated in primates, revealing high efficacy with no apparent toxicity.[9,10,24,28,29] Baboons were cured of *S. mansoni* infections when treated with 50 and 25 mg/kg, whereas 12.5 mg/kg led to a WBR of 35%.[8,9] In Cebus monkeys, doses of 50, 25 and 12.5 mg/kg cured infection.[10] In contrast, higher and multiple doses of praziquantel were required to achieve cure in infected primates. For example, in *S. japonicum*-infected Vervet monkeys, cure was only obtained with five daily doses of 50 mg/kg praziquantel.[30]

Here, we confirmed the high efficacy of Ro 15-5458 against *S. mansoni* in the mouse model. Adult *S. mansoni* infections were more susceptible to Ro 15-5458 than juvenile worm infections, with respective ED_{50} values of 5.3 mg/kg and 15 mg/kg. Nevertheless, Ro 15-5458 had significant activity against immature parasites: high WBRs of 80%–100% were obtained when infected mice were treated with 50 mg/kg on days 1, 7, 14 and 21 after infection. Thus, in contrast to the well-established variable efficacy of praziquantel,[31,32] Ro 15-5458 is active throughout the parasite's development.

We also confirmed activity in *S. haematobium*-infected hamsters and determined an ED_{50} value of 17 mg/kg. We expected Ro 15-5458 to act in a similar manner in *S. japonicum*-infected mice. However, at doses up to 100 mg/kg, no significant WBRs were noted. Higher doses could not be tested as toxicity was observed. These findings are contrary to published results which showed significant efficacy.[7] It is possible that different strains of *S. japonicum* are variously susceptible to treatment. Moreover, of the three medically important schistosomes studied here, *S. japonicum* infections are the most pathogenic[33] and this might have exacerbated any underlying toxicity of Ro 15-5458.

The *in vitro* data for *S. mansoni* NTS and adults, and *S. japonicum* adult worms indicate that Ro 15-5458 is a slow-acting compound and that high concentrations are necessary for worm death. Worms collected from mice after a single oral dose of 15 mg/kg Ro 15-5458 demonstrated a progressive corkscrew phenotype with withered suckers and neck region, which *in vivo* would conceivably hinder the parasite's ability to maintain its position in the mesenteric system as well as feed on host blood. The low levels of metabolism observed with both microsomes and hepatocytes, and the low clearance after oral administration, do not support a role for active metabolites in the mode of action of Ro 15-5458.

We investigated the hepatic shift caused by Ro 15-5458 by dissecting *S. mansoni*-infected mice at different timepoints after treatment. The shift only became apparent at the 66 h timepoint and was notable for the loss of worms from the mesenteric system. The slowness of onset of the liver shift induced by Ro 15-5458 contrasts markedly with that for praziquantel which occurs 30 min after drug treatment.[34]

We conclude that Ro 15-5458 has excellent antischistosomal properties against all development stages of *S. mansoni* and a favourable PK profile that supports single oral dosing. The lack of activity against *S. japonicum* identified here requires further investigation, given that a broad-spectrum antischistosomal drug is desirable.

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Transparency declarations

None to declare.

Supplementary data

Material S1, Methods S1 and S2, Figures S1, S2 and S3 and Videos S1 and S2 are available as Supplementary data at *JAC* Online.

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