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ORIGINAL PAPER

Effect of single-dose oral mefloquine on the morphology of adult *Schistosoma japonicum* in mice

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Abstract It has been recently documented that the antimalarial drug mefloquine shows in vivo activity against schistosomes. In the present study, we assessed the effect of mefloquine on the morphology of adult *Schistosoma japonicum* worms. Mice were infected with *S. japonicum* cercariae for 35 days and then treated with a single 400-mg/kg oral dose of mefloquine. Groups of mice were killed between 24 h and 14 days post-treatment and worms were recovered from the liver and mesenteric veins, fixed in 70% alcohol, stained with acid carmine, and examined under a light microscope. Worms obtained from nontreated mice served as controls. *S. japonicum* recovered from mice 24 h post-treatment had severely dilated guts and the entire worm body was swollen. Meanwhile, reproductive glands, including the testis, ovary, and vitelline gland, showed signs of degeneration. Damage further progressed, particularly among vitelline glands, which resulted in disturbance of ova formation and cessation of oviposition 3 days post-treatment. Three to 7 days after mefloquine administration, adherence of host leukocytes on the damaged tegument was observed. Our results confirm that mefloquine possesses antischistosomal properties, exhibiting a rapid onset of action and causing extensive morphologic damage to adult *S. japonicum*.

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Introduction

Praziquantel is virtually the only drug available for the treatment and control of schistosomiasis, one of the so-called neglected tropical diseases, that nevertheless affects over 200 million individuals in the developing world (Steinmann et al. 2006; Caffrey 2007; Doenhoff et al. 2008). The success of praziquantel is explained by its good safety and efficacy profile against all schistosome species parasitizing humans. Administered at a single 40-mg/kg oral dose, praziquantel usually results in egg reduction rates of 90% and above and cure rates of at least 60–70% (Raso et al. 2004; Chen 2005; Danso-Appiah et al. 2008; Black et al. 2009). Moreover, the price of praziquantel has plummeted; the treatment of a school-aged child now costs less than US$0.20 (Fenwick et al. 2003; Doenhoff et al. 2008). While praziquantel effectively kills adult schistosomes and the very young stages shortly after skin penetration, an important shortcoming of praziquantel is the lack of efficacy against schistosomula, the

young developing stages of the parasite (Yue et al. 1985; Sabah et al. 1986; Xiao et al. 1987). This issue might explain the low observed "cure" rates and rapid "reinfection" rates in areas of heavy schistosomiasis transmission where patients are likely to be infected with juvenile and adult parasites concurrently (Wu et al. 1993; Dabo et al. 2000; Danso-Appiah and de Vlas 2002; N'Goran et al. 2003).

There is a need to develop new antischistosomal drugs with a broad spectrum of activity against all stages of the parasite (Keiser and Utzinger 2007). Progress has indeed been made lately. Worth mentioning are promising results reported for the artemisinins (for a recent review, see Utzinger et al. 2007), the synthetic trioxolanes (Xiao et al. 2007), an inhibitor of thioredoxin glutathione reductase, i.e., 4-phenyl-1,2,5-oxadiazole-3-carbonitrile-2-oxide (Kuntz et al. 2007; Sayed et al. 2008), and the cysteine protease inhibitor K11777 (Abdulla et al. 2007). Additionally, recent in vivo studies found that the antimalarial drug mefloquine—an arylaminoalcohol compound (Ohnmacht et al. 1971)—possesses antischistosomal properties. Research thus far focused on experimental therapy, egg fecundity, and histopathologic investigations in adult schistosomes (Van Nassauw et al. 2008; Keiser et al. 2009; Zhang et al. 2009). In this study, we report the dynamics of morphologic changes of adult *Schistosoma japonicum* recovered from mice after treatment with a single oral dose of mefloquine.

Materials and methods

Host–parasite model and infection

Fifteen mice (Kunming strain), weighing ~20 g, were purchased from Shanghai Experimental Animal Center, Chinese Academy of Sciences (Shanghai, China). Mice were maintained on rodent food and water ad libitum. After an acclimatization period of 1 week, each mouse was infected percutaneously with ~40 *S. japonicum* cercariae (Anhui isolate) freshly shed from *Oncomelania hupensis* snails.

Mefloquine

Mefloquine was kindly provided by F. Hoffmann-La Roche (Basel, Switzerland). The drug was suspended in 7% Tween-80 and 3% alcohol at a concentration of 40 g/L and administered to mice at a dose of 10 mL/kg.

S. japonicum recovery and morphologic investigations

Thirty-five days after experimental infection of mice with *S. japonicum* cercariae, mice were randomly allocated to five groups of three animals each. Four groups of mice were treated with a single 400-mg/kg oral dose of mefloquine and killed by bloodletting 24 h, 3 days, 7 days, and 14 days post-treatment. The three remaining mice were left untreated and schistosomes recovered from this group served as controls. Schistosomes residing in the liver and mesenteric veins of mice were recovered by the perfusion technique (Yolles et al. 1947) using ice-cold normal saline. Additionally, worms adhering to the liver vessels—a phenomenon observed particularly with female worms 7 and 14 days post-treatment—were collected by forceps after tearing apart the liver into small pieces.

Worms were fixed in 70% alcohol, stained with acid carmine, and then mounted on microscope slides with Arabic gum and a cover slip. Schistosomes were analyzed under a light microscope equipped with an ocular micrometer (Leica DM2500; Wetzlar, Germany). Emphasis was placed on morphologic alterations and changes in size, particularly worm body, ovary in female worms, and testis in male specimens.

Statistical analysis

Mean values were calculated for worm body size and lengths of ovaries and testes according to treatment groups. Differences between worms recovered from mice treated with mefloquine and untreated control specimens were tested using a two-sided *t*-test in Microsoft Office Excel version 2003.

Results

Control worms

Adult female and male *S. japonicum*, recovered from untreated mice, showed normal morphologic features. The internal organ structures of the worms, including the esophagus, gut starting point, the two gut branches, gut blind end, testis, ova, uterus, ovary, and vitelline glands are depicted in Figs. 1, 2, 3, 4, 5, and 6.

In brief, female controls were characterized by pigment-filled gut branches and gut blind ends (Figs. 1 and 3). Moreover, the posterior part of the body was filled with vitelline glands (Fig. 2) and the uterus was filled with many ova (Fig. 3). Figures 4 and 5 show the esophagus, which is the connection between the oral sucker and the oral cavity, of adult male controls. The esophagus is surrounded by esophagus glands and its distal end is connected with the two gut starting points (Figs. 4 and 5). Finally, control male *S. japonicum* are characterized by seven testes, which are arranged either in tandem form (Fig. 6) or nontandem form (no picture shown; Fan and Lin 1976).

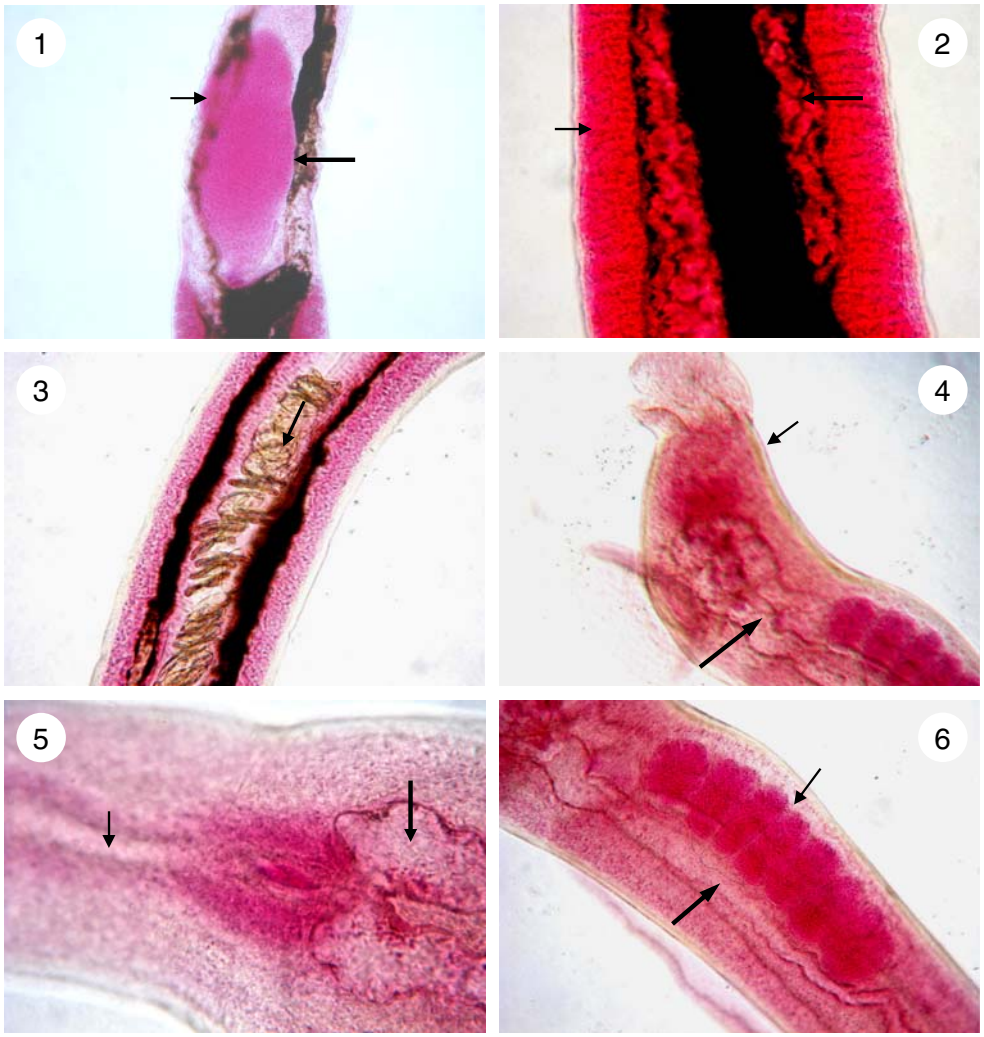

Fig. 1 Female adult *S. japonicum* worm harbored in an untreated control mouse, showing ovary (*small arrow*) and gut (*large arrow*; ×100)
Fig. 2 Female adult *S. japonicum* worm harbored in an untreated control mouse, showing vitelline glands (*small arrow*) and gut (*large arrow*; ×200)
Fig. 3 Female adult *S. japonicum* worm harbored in an untreated control mouse, showing ova in the uterus (*arrow*; ×200)
Fig. 4 Male adult *S. japonicum* worm harbored in an untreated control mouse, showing anterior end of the worm body (*small arrow*) and gut starting points (*large arrow*; ×100)
Fig. 5 Male adult *S. japonicum* worm harbored in an untreated control mouse, showing esophagus (*small arrow*) and two gut starting points (*large arrow*; ×200)
Fig. 6 Male adult *S. japonicum* worm harbored in an untreated control mouse, showing testes (*small arrow*) and gut (*large arrow*; ×100)

Female *S. japonicum* following mefloquine treatment

Worm body size

Mefloquine exhibited a rapid onset of action on female *S. japonicum* harbored in mice. Table 1 shows that, 24 h post-treatment, the body size of female worms was significantly shorter when compared to untreated control females ($P<0.05$). Three days post-treatment, the worm body sizes were further shortened to almost half of the untreated control worms. Moreover, schistosomes showed a somewhat reduced width compared to untreated control

Table 1 Changes in the size of worm body of 35-day-old adult female and male *S. japonicum* worms recovered from mice treated orally with a single dose of mefloquine (400 mg/kg)

Time post-treatment	Female worms (mm)		Male worms (mm)	
	Length (mean±SD) [no. of worms examined]	Width (mean±SD) [no. of worms examined]	Length (mean±SD) [no. of worms examined]	Width (mean±SD) [no. of worms examined]
Control	13.50±2.24 [10]	0.21±0.03 [10]	11.15±2.08 [10]	0.40±0.04 [10]
24 h	10.64±1.78 [10]**	0.21±0.02 [10]*	8.67±1.36 [10]**	0.37±0.06 [10]*
3 days	7.67±1.67 [6]***	0.16±0.02 [6]*	6.16±1.06 [10]***	0.26±0.05 [10]***
7 days	–	–	5.54±0.69 [10]***	0.24±0.09 [10]***
14 days	–	–	6.32±1.13 [10]***	0.28±0.05 [10]***

SD standard deviation

*$P>0.05$ versus control; **$P<0.05$ versus control; ***$P<0.01$ versus control

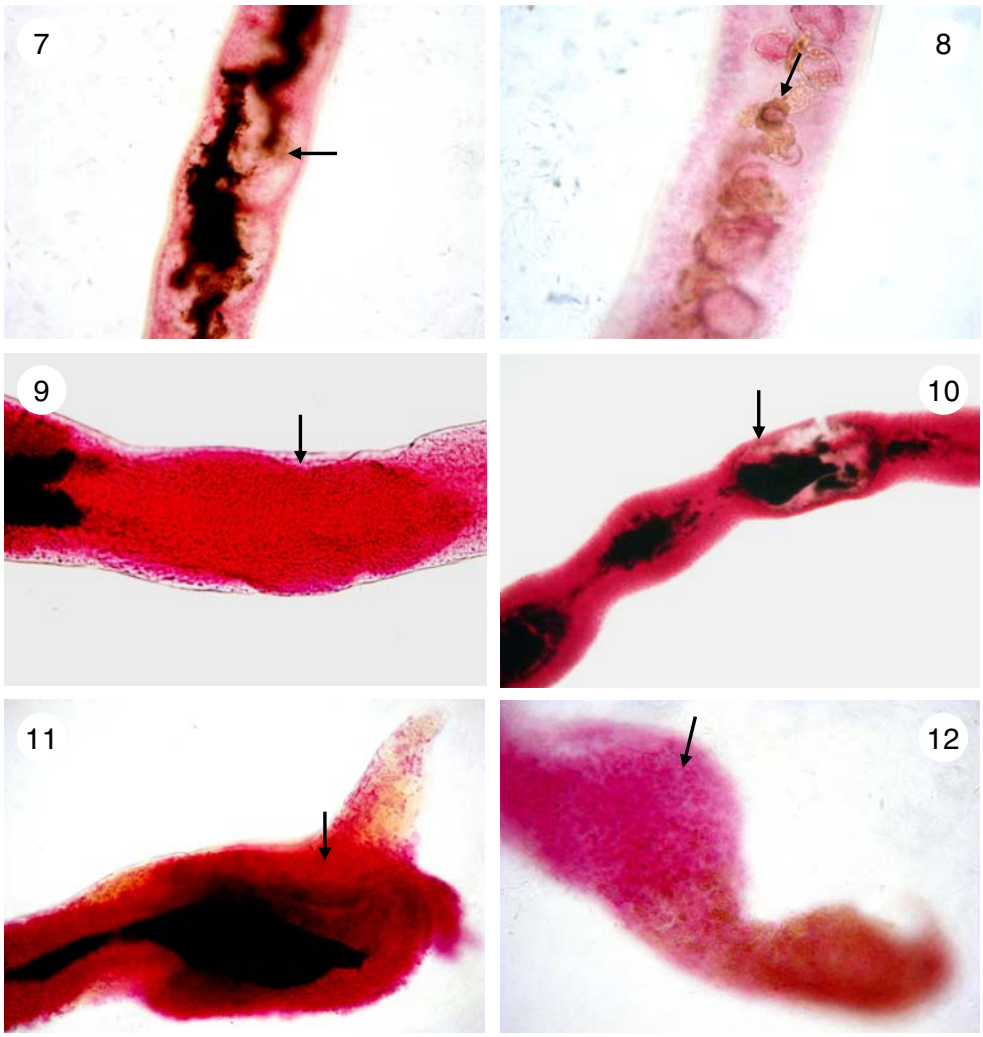

Fig. 7 Swelling of gut in a female adult *S. japonicum* worm harbored in a mouse 3 days after administration of a single 400-mg/kg oral dose of mefloquine (*arrow*; ×200)
Fig. 8 Female adult *S. japonicum* worm harbored in a mouse 3 days after mefloquine treatment with a single oral dose of 400 mg/kg, showing abnormal ova components (*arrow*; ×400)
Fig. 9 Female adult *S. japonicum* worm harbored in a mouse 24 h after administration of a single 400-mg/kg oral dose of mefloquine, showing swelling of ovary (*arrow*; ×100)
Fig. 10 Focal swelling of posterior portion of a female adult *S. japonicum* worm harbored in a mouse 24 h after administration of mefloquine at a single oral dose of 400 mg/kg (*arrow*; ×100)
Fig. 11 Female adult *S. japonicum* worm harbored in a mouse 3 days after mefloquine dosing, showing swelling of vitelline glands and adherence of host leukocytes on the collapsed surface above the vitelline glands (*arrow*; ×100)
Fig. 12 Female adult *S. japonicum* worm harbored in a mouse 7 days after the administration of mefloquine at a single oral dose of 400 mg/kg, showing swelling of posterior portion of the worm body and adherence of numerous host leukocytes on the damaged tegument (*arrow*; ×200)

specimens. The size of female worms collected 7 or 14 days post-treatment could not be measured any longer, as only fragments of schistosomes could be recovered.

Gut

Twenty-four hours after mefloquine administration, adult female worms recovered from the mesenteric veins showed a dilated gut blind end. Many female worms obtained from the liver displayed a dilatation of gut branches and the gut blind ends. Three to 7 days after mefloquine dosing, most worms revealed a dilatation of gut either in its branches or the blind ends. In some instances, the dilated gut occupied the anterior body lumen (Fig. 7). Additionally, in several female worms, a strong dilatation of the focal anterior portion of the gut was observed.

While pigment-filled gut branches and gut blind ends were characteristic for control females (Figs. 1 and 3), worms recovered from the mesenteric veins of mice treated with mefloquine showed signs of depigmentation and a decrease

of pigment in the two gut branches and gut blind ends. Most of the female worms recovered from the liver completely lacked or possessed only very small amounts of pigment in the gut branches; however, pigment and depigmentation was still observed in the gut blind ends. Three and 7 days after mefloquine dosing, all adult female worms recovered from the liver either lacked or exhibited only trace amounts of pigment in the gut branches and gut blind ends.

Formation of ova

Twenty-four hours after mefloquine dosing, intact ova were visible in the uterus of female worms recovered from the mesenteric veins. However, in several female worms, which had been lodged in the liver, abnormal ova components, consisting of ootid, vitelline cells, and vitelline particles, were observed. Three days post-treatment, most female worms examined lacked normal ova and only abnormal ova components were seen (Fig. 8).

Alteration of ovaries

Twenty-four hours after mefloquine administration, the lengths and widths of ovaries from adult female *S. japonicum* were somewhat shorter and wider than ovaries examined from control females. However, the differences in these parameters between treated and nontreated specimens showed no statistical significance (Table 2). At this time point, some ovaries lost their definition and were swollen (see Fig. 9 and compare with Fig. 1). Three days post-treatment, the mean size of the ovaries was significantly reduced both in length ($P<0.01$) and in width ($P<0.05$) compared to the control specimens. Finally, 7 days post-treatment, a further reduction of lengths and widths of the ovaries was noted; in some worms, the length of the ovaries was only about a fourth of the lengths measured in the respective controls.

Posterior parts of worm body and vitelline glands

Twenty-four hours after mefloquine administration, approximately half of the adult female *S. japonicum* recovered from the mesenteric veins and all the female worms recovered from the liver showed focal swelling of the worm body, especially visible in the posterior part of the body (Fig. 10). Degeneration of vitelline glands, and an indistinct and irregular arrangement of vitelline lobules was also noted. In contrast, in control females, the posterior part of the body (i.e., the part of the worm body behind the ovary) was filled with vitelline glands on both sides (Fig. 2). Three to 7 days post-treatment, all female worms examined showed severe focal swelling in the posterior part of the worm body. Several vitelline lobules lost their definition resulting in sparseness or atrophy. There was focal swelling of the tegument above the damaged vitelline glands, and host leukocytes adhered to the surface (Fig. 11). The entire schistosome tails were covered with numerous host leukocytes (Fig. 12).

Male *S. japonicum* following mefloquine treatment

Worm body size

The mean body length of male *S. japonicum* recovered from mice treated with mefloquine was significantly shorter than that of the controls, already 24 h after treatment ($P<0.05$) (Table 1). While several paired worms could be recovered from the mesenteric veins 24 h post-treatment, all worms had shifted to the liver and most of them were unpaired at 3 and 7 days post-treatment. At these observation time points, body lengths and widths of the male worms were significantly shorter when compared to the untreated control specimens ($P<0.01$). Fourteen days post-treatment, most of the male worms were still found in the liver. The few surviving worms that had shifted back to the mesenteric veins were slightly longer than 7 days post-

Table 2 Changes in size of ovary in female and testis in male *S. japonicum* worms recovered from mice treated orally with a single dose of mefloquine (400 mg/kg)

Time post-treatment	Ovary of female worms (μm)		Testis of male worms (μm)	
	Length (mean±SD) [no. of ovaries examined]	Width (mean±SD) [no. of ovaries examined]	Length (mean±SD) [no. of testes examined]	Width (mean±SD) [no. of testes examined]
Control	584±56 [10]	157±25 [10]	141±21 [20]	97±18 [20]
24 h	536±74 [15]*	178±30 [15]*	150±18 [26]*	106±16 [26]*
3 days	392±76 [16]***	123±18 [16]**	119±28 [22]***	87±16 [22]*
7 days	313±54 [6]***	108±23 [6]***	99±17 [25]***	62±14 [25]***
14 days	–	–	104±19 [27]***	46±12 [27]***

SD standard deviation

*$P>0.05$ versus control; **$P<0.05$ versus control; ***$P<0.01$ versus control

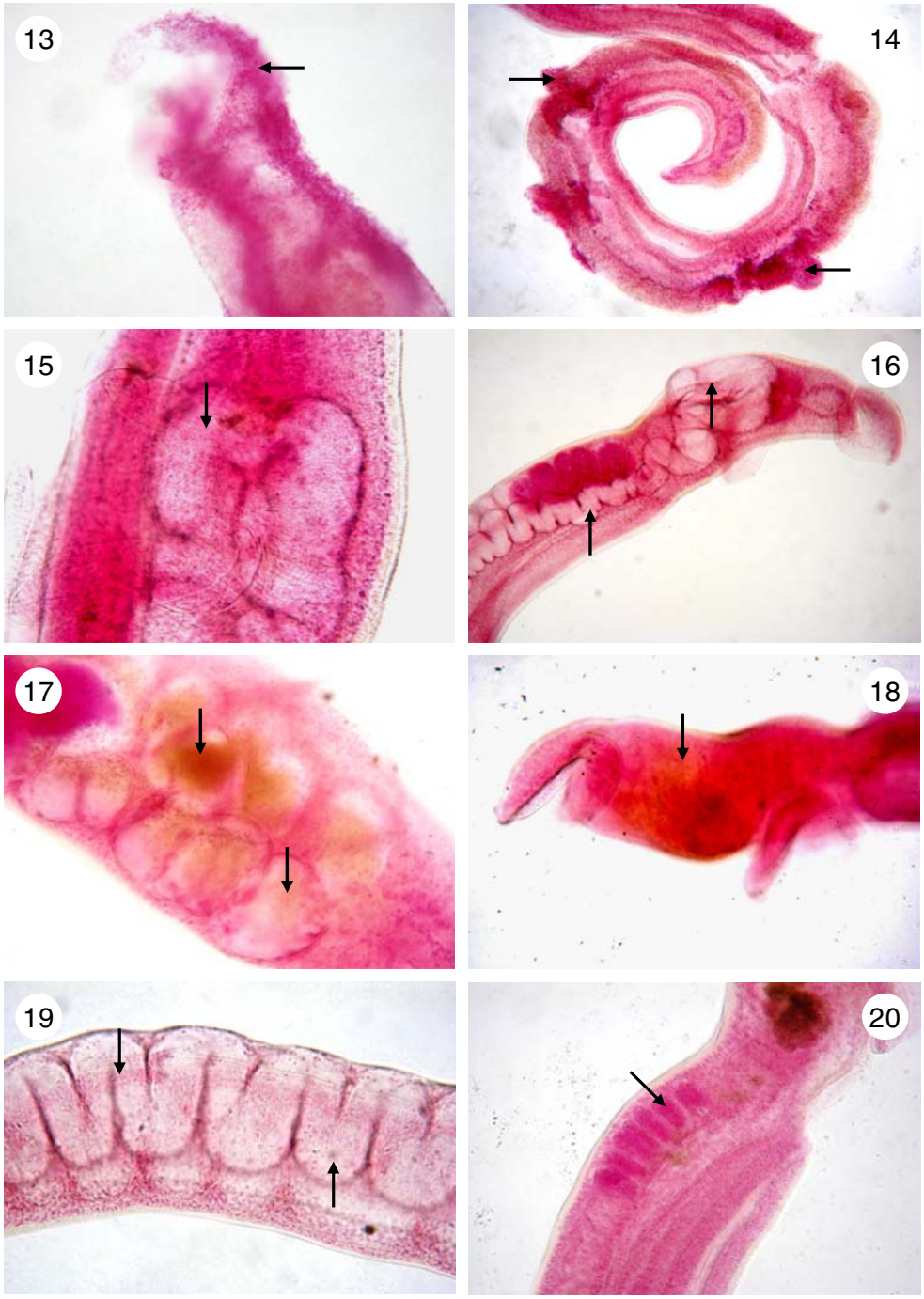

Fig. 13 Male adult *S. japonicum* worm harbored in a mouse 3 days after mefloquine treatment, showing collapse and adherence of host leukocytes at the anterior end of the worm body (*arrow*; ×100)
Fig. 14 Male adult *S. japonicum* worm harbored in a mouse 7 days after mefloquine treatment, showing extensive damage of worm body with adherence of host leukocytes on the damaged tegument (*arrow*; ×50)
Fig. 15 Swelling of the two gut starting points in a male adult *S. japonicum* worm harbored in a mouse 24 h after administration of a single 400-mg/kg oral dose of mefloquine (*arrow*; ×200)
Fig. 16 Extensive swelling of the gut in a male adult *S. japonicum* worm harbored in a mouse 24 h after administration of a single 400-mg/kg oral dose of mefloquine (*arrow*; ×50)
Fig. 17 Male adult *S. japonicum* worm harbored in a mouse 7 days after mefloquine dosing, showing severely dilated gut branches in the anterior part of the worm (*arrow*; ×200)
Fig. 18 Male adult *S. japonicum* worm harbored in a mouse 7 days after administration of a single 400-mg/kg oral dose of mefloquine, showing swelling of the portion between the oral and the ventral suckers (*arrow*; ×100)
Fig. 19 Severely dilated gut occupying the whole body lumen in the posterior region of a male adult *S. japonicum* harbored in a mouse 14 days after administration of mefloquine at a single oral dose of 400 mg/kg (*arrow*; ×200)
Fig. 20 Male adult *S. japonicum* worm harbored in a mouse 14 days after mefloquine dosing, showing atrophy of the testes (*arrow*; ×100)

treatment, but their body size was still significantly shorter than the control worms.

Tegumental alteration

Focal swelling was an important feature seen on the tegument of most of the adult male *S. japonicum* recovered from the mesenteric veins and the liver of mice treated with mefloquine. The focal swelling was apparent at each observation time point, hence up to 14 days post-treatment. Three to 7 days after mefloquine dosing, numerous host leukocytes adhered to the sucker (Fig. 13) or to other parts of the worm body surface, giving the worm a cloudy appearance (Fig. 14).

Esophagus and gut

Twenty-four hours after mefloquine administration, approximately half of the adult male *S. japonicum* lodged in the mesenteric veins of the mice showed dilated gut starting

points, but only minor dilatations were visible in other parts of the gut. The guts of male worms recovered from mice liver were more affected, showing not only dilatation of the two gut starting points (Fig. 15), but many worms also revealed extensive dilatation in the two gut branches and the gut blind end. Some male *S. japonicum* showed dilatation of the entire gut, which gave the worm a transparent appearance (Fig. 16). At 3, 7, and 14 days post-treatment, in addition to dilated starting points of the gut, there was also extensive dilatation of the gut branches (Fig. 17) and blind ends. Further expansion of the gut starting points combined with severe swelling of the tegument resulted in the head portion to appear unusually large (Fig. 18). Fourteen days after mefloquine administration, adult male *S. japonicum* surviving the treatment had migrated back to the mesenteric veins. These worms were still characterized by pronounced dilatation of the gut. In one highly degenerated worm recovered from the mouse liver, the dilated gut almost filled the entire body lumen (Fig. 19).

Testis

Twenty-four hours after mefloquine administration, the testes showed light swelling and the mean size of the testes measured was slightly but not significantly larger than that of the control worms (Table 2). Three days post-treatment, the testes were significantly reduced in length compared with the controls ($P<0.01$). The mean width of the testes was slightly reduced. In several worms, the testes started to overlap and fused together. Seven to 14 days post-treatment, the mean width and length of the testes of *S. japonicum* showed a further and significant reduction (Fig. 20).

Discussion

We documented the dynamics of morphologic changes in adult female and male *S. japonicum* worms recovered from mice after treatment with a single 400-mg/kg oral dose of mefloquine. Our study complements recent in vivo investigations pertaining to the antischistosomal properties of mefloquine (Van Nassauw et al. 2008; Keiser et al. 2009; Zhang et al. 2009) and might aid to further elucidate the mechanism of action of this antimalarial drug on schistosomes.

Exposure of adult *S. japonicum* to mefloquine in vivo resulted in extensive damage of the worm's digestive system, tegument, musculature, parenchymal tissues, and reproductive system, the latter including the testes, ovaries, vitelline glands, and egg formation. Our observations are consistent with recent findings derived from a histopathologic investigation of *S. japonicum* harbored in mice treated with a single oral dose of mefloquine (Zhang et al. 2009). The most pronounced alteration observed in adult female and male worms was the severe damage of the gut. Twenty-four hours post-treatment, the gut starting points were already dilated, which further progressed to dilatation of gut branches and the blind ends. Similar to *Plasmodium* spp., schistosomes feed on ingested erythrocytes, which is essential for parasite development, growth, and reproduction. Cathepsin D plays an important role in the digestion of hemoglobin, released from the erythrocytes into proteins, amino acids, and pigment (Kloetzel and Lewert 1966; Lawrence 1973; Brindley et al. 2001, Morales et al. 2008). It has been speculated that an interference with hemoglobin digestion is involved in the mechanisms of action of mefloquine against *Plasmodium* (Foley and Tilley 1997, 1998). Although we have documented severe gut damage in adult *S. japonicum* in the present study and in our preceding investigations, which suggest an interference with hemoglobin degradation, new research is necessary to deepen our understanding of how exactly the gut of schistosomes is damaged following mefloquine administration and whether hemoglobin digestion is a drug target. Gut dilatation was also universally seen in schistosomes exposed to an artemisinin derivative (i.e., artemether) in vivo (Xiao et al. 2004). However, the intensity of dilatation induced by mefloquine was more severe than that caused by artemether. Interestingly, administration of praziquantel did not result in gut damage, although extensive vesiculation on the tegument was a common feature observed on schistosomes treated with praziquantel (Andrews et al. 1983; Xiao et al. 1984; Andrews 1985). Vesicles protruding from the tegumental surface following praziquantel treatment could be seen in both histologic sections of schistosomes and when examining intact worm samples (Xiao et al. 1980, 1984). Vesiculation was not observed in mefloquine-treated worms as shown in the present study. On the other hand, small vesicles beneath the tegument were seen in histologic sections of male *S. japonicum* in a previous study (Zhang et al. 2009). Hence, the mechanism of vesicle formation induced by mefloquine seems to be different when compared to praziquantel.

Focal swelling of the worm body was a common feature revealed both in adult male and female worms, indicating that the tegument, muscle, parenchymal tissues, and vitelline glands were affected by mefloquine. Interestingly, adherence of host leukocytes was observed following focal swelling and collapse of the body surface or the vitelline glands. A release of worm antigens and antibody response might be involved in this process. Hence, the mechanism of action of mefloquine against schistosomes might be immune-dependent. The mechanism of actions of several antischistosomals, such as praziquantel, involves a synergy

between humoral immune response and the drug (Sabah et al. 1985; Brindley and Sher 1987). Studies are underway in our laboratories to elucidate whether the host antibody response is involved in the antischistosomal action of mefloquine.

In conclusion, our findings further demonstrate that mefloquine exhibits in vivo efficacy against adult *S. japonicum* worms with a rapid onset of action. Further laboratory investigations are warranted to elucidate the possible mechanism of action of mefloquine against schistosomiasis. Moreover, in areas where malaria and schistosomiasis coexist and mefloquine is employed as an antimalarial drug, the potential ancillary benefits of mefloquine against schistosomiasis should be investigated with no further delay.

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