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ORIGINAL PAPER

Transmission electron microscopic observation on ultrastructural alterations in *Schistosoma japonicum* caused by mefloquine

Shu-hua Xiao · Jian Xue · Binggui Shen

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Abstract The purpose of the study was to explore the ultrastructural alterations of adult *Schistosoma japonicum* induced by mefloquine. Eight out of ten mice infected with 60–80 *S. japonuicum* cercariae for 35 days were treated orally with mefloquine at a single dose of 400 mg/kg. Four groups of two mice were killed at 8 h, 24 h, 3 days, and 7 days post-treatment, and schistosomes were collected by perfusion technique, fixed, and examined under a transmission electron microscope. Schistosomes obtained from the remaining two mice served as control. Eight hours after mefloquine 400 mg/kg was administered to the infected mice, various alterations in the tegument and subtegument tissues of both male and female worms were seen, which included focal lysis of tegmental matrix resulted in vacuole formation, decrease in rod-like and discoid-like secretary bodies, light swelling or focal lysis of musculature, extensive lysis of internal structure of sensory organelles, and appearance of vacuole or myelin-like structure in perinuclear cytoplasm of syncytium and epithelial cells. In vitelline cells of female worms, the most significant alteration was extensive lysis or fusion of vitelline balls in vitelline droplets and decrease in granular endoplasmic reticulum Twenty-four hours post-treatment, damage to the tegument and subtegument tissues had increased in severity. In male worms, the most prominent alterations were emergence of large vacuoles in the tegument, detachment of cytoplasmic process from the tegumental surface, focal collapse of internal structure of sensory organelle, and loss of definition of syncytium and gut epithelial cell. In female worms, focal lysis in tegumental matrix, musculatures, and parenchymal tissues resulted in emergence of vacuole or myelin-like structure, reduction of nucleoli, fusion of partial nuclear membrane together with cytoplasm in epithelial cell, and lysis of interstitial tissues among the vitelline cells which were universal. Three and 7 days post-treatment, besides the aforementioned alterations, the significant damage to the male worms were disrupted outer plasma membrane detached from the cytoplasmic process, swelling of individual cytoplasmic process, extensive swelling and focal lysis in the musculature, parenchymal tissues and perinuclear cytoplasm of syncytium, accompanied by emergence of swollen mitochondria, vacuoles, and myelin-like structure, and severe damage to gut epithelial cell. In female worms, apart from disruption of outer plasmic membrane in cytoplasmic process, severe swelling of tegumental matrix accompanied by emergence of vacuoles, swollen mitochondria and myelin-like structure, focal lysis of heterochromatin and nucleoli, disappearance of microvilli in gut epithelial cells, and emergence of myelin-like structures in vitelline cells were observed. The results indicate that administration of mefloquine to mice infected with adult *S. japonicum* exhibits an extensive damage to the ultrastructure in tegument and subtegument tissues including syncytium, gut epithelial cells, parenchymal tissues, and vitelline cells of schistosomes.

S.-h. Xiao (✉) · J. Xue · B. Shen
National Institute of Parasitic Diseases,
Chinese Center for Disease Control and Prevention,
Key Laboratory of Parasite and Vector Biology, MOH,
WHO Collaborating Centre for Malaria,
Schistosomiasis and Filariasis,
Shanghai 200025, People's Republic of China
e-mail: shxiao4@hotmail.com

J. Xue
e-mail: xuejian74@yahoo.com.cn

Introduction

Mefloquine, an amino alcohol antimalaria drug, was synthesized in 1971 (Ohnmacht et al. 1971). This antima-

larial drug has been released to the market and used extensively in clinical treatment for more than 30 years. In 2008, mefloquine was reported to be effective in the significant reduction of egg production in *Schistosoma mansoni*-infected mice after administration of a single oral dose of 150 mg/kg, but the drug had no effect on worm burden (Van Nassauw et al. 2008). In 2007, through screening test, mefloquine had been found to be effective against both juvenile and adult *S. mansoni*. Afterwards, the effect of mefloquine against *Schistosoma japonicum* was also confirmed (Keiser et al. 2009). The finding of mefloquine against schistosomes possesses important significance because this is the first drug which exhibits similar efficacy against developing stages of juvenile and adult schistosomes under the same dose levels which is different from praziquantel and artemisinins. It is already recognized that praziquantel exhibits potential effect against adult schistosomes and very early stage of schistosomula penetrating into the host's skin for a few hours, but much poorer efficacy against young developing stages of schistosomula (Yue et al. 1985; Sabah et al. 1986; Xiao et al. 1987, 2009a). On the contrary, a series of experimental studies indicated that juvenile stages of the parasite were more susceptible to artemisinins (artemether and artesunate) than the adults (Le et al. 1982; Xiao et al. 1995; Li et al. 1996). For these reasons, praziquatnel and artemisinins are only used for therapy and prophylaxis of schistosomiasis, respectively. Therefore, in evaluation by the experimental study angle, mefloquine or its analogous compounds may possess the possibility to develop an ideal antischistosomal drug used for treatment and prevention.

In order to understand the antischistosomal properties of mefloquine, morphological and histopathological alterations of adult schistosomes in mice following oral administration of mefloquine have been studied. Morphological observation indicated that mefloquine exhibited a rapid onset of action and caused extensive morphologic damage to adult *S. japonicum* which included severe dilatation of gut, focal swelling of worm body, damage to reproductive glands (ovary, vitelline gland, and testis), and interference with ova formation (Xiao et al. 2009b). Histopathological study provided further evidence of in vivo activity of mefloquine against adult schistosomes, which is revealed in focal roughing and vesiculation of the tegument, swelling of muscles and parenchymal tissues, dilatation of the gut accompanied by decrease in pigment particles, focal desquamation of gut epithelial cells, and degeneration of oocytes as well as vitelline gland cells. Consequently, the damaged worms were surrounded by inflammatory cells or the cells penetrated into the worm body through the damaged tegument which resulted in worm death and further developed to dead worm abscesses and dead worm granuloma (Zhang et al. 2009). According to the aforementioned data, we macroscopically understand the impact on worm digestive and reproductive functions and the process of action on the worms induced by mefloquine at cell level. Therefore, it is necessary to further investigate the action of mefloquine on schistosomes using electron microscopy in order to reveal the essence and feature of mefloquine-induced alterations.

Recently, tegumental alterations of schistosomes induced by mefloquine has been studied using scanning electron microscopy, indicating extensive and severe damage to the tegument which included focal swelling of worm body, focal swelling, fusion and peeling of tegumental ridge, and injury of oral sucker as well as sensory structures. But no vesiculation similar to that induced by praziquantel and artemether along the tegument was seen (Xiao et al. 2010). In this paper, we report the mefloquine-induced ultrastructural alteration of schistosomes using transmission electron microscopy (TEM).

Materials and methods

Drug

Mefloquine hydrochloride purchased from Libang Pharmaceutical Co. Ltd (Xian, China) was suspended in 7% Tween 80 and 3% ethanol at a final concentration of 40 g/L (free base). The volume administered to the mice was 10 mL/kg.

Host and parasites

Ten female mice (Kunming strain), weighing 20–22 g, were provided by Shanghai Experimental Animal Center, Chinese Academy of Sciences (Shanghai, China). Animals were acclimatized for 1 week before infection, and they had free access to water and food. *S. japonicum* cercariae (Anhui isolate) were freshly shed from intermediate host, *Oncomelania hupensis* snails.

Infection, treatment, and collection of schistosomes

Ten mice were infected with 60–80 *S. japonicum* cercariae via shaved abdomen skin. At 35 days post-infection, eight mice were treated orally with mefloquine at a single dose of 400 mg/kg, and groups of two mice were killed by bloodletting 8 h, 24 h, 3 days, and 7 days. The remaining two mice left untreated and schistosomes recovered from this group served as control. Schistosomes lodging in the liver of mice were recovered by the perfusion technique (Yolles et al. 1947) using ice-cold Hanks' balanced salt solution (HBSS).

Transmission electron microscopic observation

Worms were rinsed three times with ice-cold HBSS and fixed in buffered 2.5% glutaraldehyde phosphate (0.2 mol/L,

pH 7.4). Post-fixation was proceeded in buffered osmium tetraoxide, followed by dehydration before embedding in spur resin. Ultrathin sections of the schistosomes were stained with uranyl acetate and lead citrate. Finally, the adult schistosome specimens were examined by a Philips CM210.

Results

The ultrastructural features of the tegument and subtegumental structures, including musculature, syncytium, gut epithelial cells, sensory organelle, and vitelline cells, in adult *S. japonicum* recovered from untreated control mice were shown in Figs. 1, 2, 3, and 4, which were similar to those described in the previous literature (Xiao et al. 1981, 1996).

Eight hours after mice infected with 35-day-old *S. japonicum* were treated orally with mefloquine at a single dose of 400 mg/kg; various alterations in the tegument and subtegument tissues of both male and female worms were seen. In male worms, the most significant changes observed in the tegument included focal lysis of tegumetnal matrix which resulted in the formation of vacuoles with various sizes (Fig. 5), decrease in rod-like and discoid-like secretary bodies, and indistinction of basal membrane. At this time point, swelling and focal lysis of underlying muscle bundles accompanied by degenerated mitochondria were usually seen (Fig. 6). The sensory organelles distributed on the tegumental surface showed extensive lysis of internal structure which resulted in an emptying of the internal cavity of organelle or disappearance of bilayer membrane in the internal wall with focal swelling and collapse and lysis of matrix which resulted in the formation of vacuoles (Fig. 7). In the subtegument, the major alterations of syncytium were swelling of nucleus, disappearance of gap between nucleus and perinuclear cytoplasm, and extensive lysis of cytoplasm which resulted in vacuole formation (Figs. 7 and 8). In addition, extensive swelling and lysis were also seen in parenchymal tissues (Figs. 5 and 7). In female worms, the usually ultrastructural alterations observed in tegument were indistinction of tegumental matrix accompanied by disappearance of rod-like secretary bodies, decrease in discoid secretary bodies, and focal detachment of external cytoplasmic membrane from the top of distal cytoplasmic processes. No apparent alteration on sensory organelle distributed on the tegument was seen. In the subtegument, the circular muscle bundles usually showed normal appearance, while light swelling of longitudinal muscle bundles with enlarged mitochondria was universal. During this time point, no apparent damage to the syncytium and parenchymal tissues was observed. In gut epithelial cells, some of them lost their definition with indistinct nucleus, disappearance of gap between the nucleus and cytoplasm, expansion of granular endoplasmic reticulum, and focal lysis of cytoplasm resulted in vacuole formation (Fig. 9). Meanwhile, the microvilli stretched out from the gut epithelial cell were various in their length or expanded in their distal end. In vitelline cells of female worms, the most significant alteration was decrease in granular endoplasmic reticulum and extensive lysis or fusion of vitelline balls in vitelline droplets which resulted in the formation of many vacuoles containing residual bodies or large black balls (Fig. 10). In some sections, the vitelline balls were released to the cytoplasm of vitelline cell because of the collapse of vitelline droplets.

Twenty-four hours post-treatment, damage to the tegument and subtegument had increased in severity. In male worms, extensive lysis of tegumental matrix resulted in the formation of some large vacuoles and emergence of swollen mitochondria. In some male specimens, the tegumental matrix revealed indistinction with disappearance of rod-like and discoid-like secretary bodies, and focal lysis of matrix resulted in the formation of vacuoles, emergence of some membrane-like inclusion bodies, and disruption of basal membrane (Fig. 11). At this observation time point, some of the male worms showed the most severe tegumental damage, i.e., detachment of damaged and collapsed cytoplasmic processes from the tegumental surface (Fig. 12). In sensory organelles, some of them showed intact or disruption of bilayer membrane structure, accompanied by lysis of internal structures and emergence of swollen mitochondria. In one sensory organelle which suffered serious damage, focal collapse of its internal wall was observed (Fig. 13). The musculatures underneath the tegument usually showed swelling with various degrees, and focal lysis of the muscle bundles resulted in the formation of many vacuoles which was also universal (Figs. 11 and 12). The damage to the syncytium underneath the musculature was characterized by loss of cell definition, partial disruption of bilayer nuclear membrane, swollen nucleus, focal lysis of chromatin, hetrochromatin and perinuclear cytoplasm accompanied by vacuole formation, and concentrated granular endoplasmic reticulum. In gut epithelial cells, the prominent alterations included swollen nucleus, fusion of partial nuclear membrane together with the cytoplasm, decrease in granular endoplasmic reticulum, emergence of many vacuoles in the cytoplasm, and some microvilli with expansion in the distal end. In the serious one, the nucleus lost its definition and fused together with the cytoplasm accompanied by release of some heterochromatins to the cytoplasm (Fig. 14). In female worms, the main alterations in tegument were disappearance of rod-like secretary bodies, decrease in discoid-like secretary bodies, and focal lysis of matrix which resulted in the formation of vacuoles or emergence of swollen mitochondria. The underneath musculatures revealed swelling and focal lysis, while in parenchymal tissues, focal lysis resulted in the emergence of some myelin-like structures (Fig. 15). In

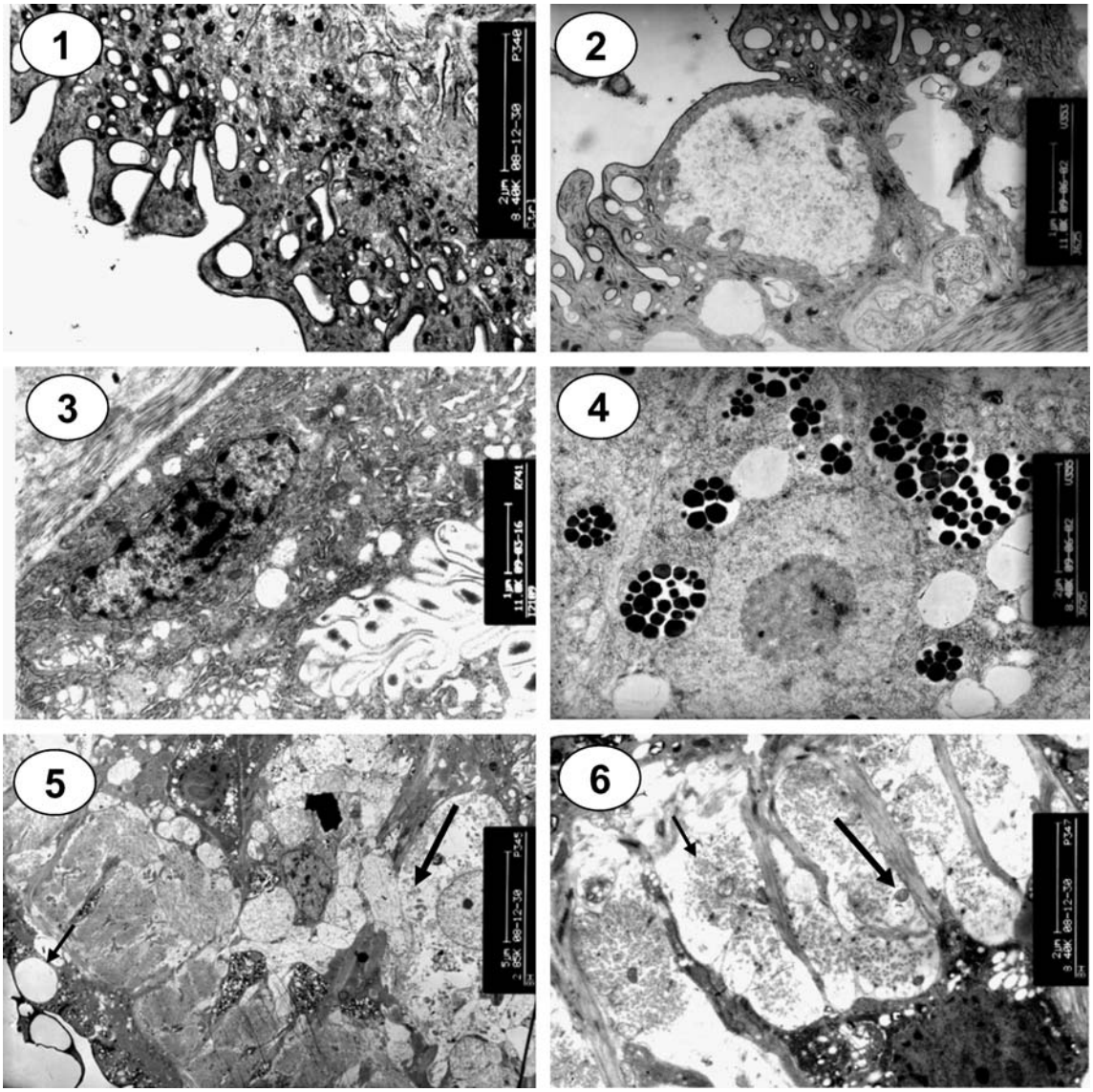

Fig. 1 Tegument of male adult *S. japonicum* worm harbored in an untreated control mouse (×7,400)
Fig. 2 Sensory organelle of male adult *S. japonicum* worm harbored in an untreated control mouse (×9,700)
Fig. 3 Gut epithelial cell of female adult *S. japonicum* worm harbored in an untreated control mouse (×9,700)
Fig. 4 Vitelline cell of female adult *S. japonicum* worm harbored in an untreated control mouse (×9,700)
Fig. 5 Male adult *S. japonicum* worm harbored in a mouse 8 h after administration of a single 400 mg/kg oral dose of mefloquine, showing vacuole formation in tegument (*small arrow*) and extensive lysis in parenchymal tissues (*large arrow*, ×2,500)
Fig. 6 Swelling and focal lysis of muscle bundles (*small arrow*) accompanied by degenerated mitochondria (*large arrow*) underlined the tegument in a male adult *S. japonicum* harbored in a mouse 8 h after mefloquine treatment with a single oral dose of 400 mg/kg (×7,400)

gut epithelial cells, expansion of granular endoplasmic reticulum, degenerated mitochondria, loss of definition of cytoplasm accompanied by formation of vacuoles containing residual bodies and emergence of myelin-like structures, and disorder arrangement of microvilli were seen. Other prominent alterations included reduction of nucleoli and fusion of partial nuclear membrane together with cytoplasm. In vitelline cells of female worms, the main alterations were similar to those at the previous time point. But in some vitelline cells, many vitelline droplets with or without vitelline balls were fused together, with decrease of granular endoplasmic reticulum, and disappearance of nucleoli in individual cell (Fig. 16). In addition, lysis of interstitial tissues among the vitelline cells was a common feature.

Three and 7 days after administration of mefloquine, male and female worms still showed severe damage to the tegument and subtegument. In male worms, the typical features included focal lysis of the tegumental matrix which resulted in the formation of vacuoles accompanied by disruption or disappearance of basal membrane (Fig. 17), a row of size similar vacuoles usually revealed along the tegument, the disrupted outer plasmic membrane detached from the

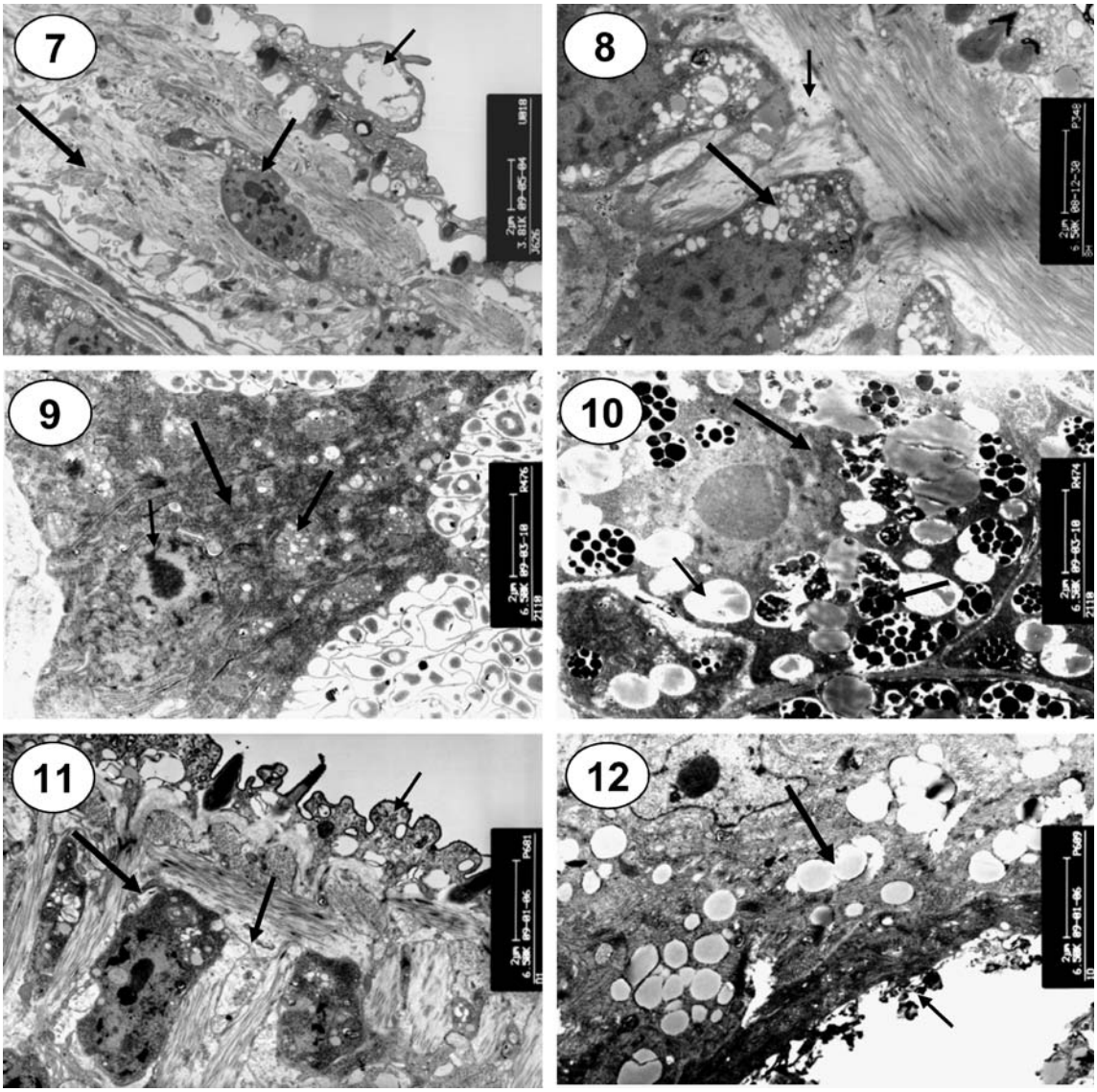

Fig. 7 Male adult *S. japonicum* harbored in a mouse 8 h after mefloquine treatment, showing destruction of damaged sensory organelle (*small arrow*), swollen nucleus of syncytium (*moderate arrow*), and focal lysis of parenchymal tissues (×3,400)

Fig. 8 Male adult *S. japonicum* harbored in a mouse 8 h after mefloquine dosing, showing swelling and focal lysis of muscle bundles (*small arrow*), disappearance of gap between nucleus and perinuclear cytoplasm, and formation of vacuole in cytoplasm (*large arrow*) of a syncytium (×5,800)

Fig. 9 Female adult *S. japonicum* harbored in a mouse 8 h after administration of mefloquine at a single oral dose of 400 mg/kg, showing a damaged gut epithelial cell including indistinct nucleus (*small arrow*), emergence of vacuole in cytoplasm (*moderate arrow*), and expansion of granular endoplasmic reticulum (*large arrow*, ×5,800)

Fig. 10 Vitelline cell of female adult *S. japonicum* harbored in a mouse 8 h after mefloquine dosing, showing lysis of vitelline balls in vitelline droplet resulted in vacuole formation (*small arrow*), fusion of vitelline balls in vitelline droplets (*moderate arrow*), and decrease of granular endoplasmic reticulum (*large arrow*, ×5,800)

Fig. 11 Male adult *S. japonicum* harbored in a mouse 24 h after mefloquine treatment, showing emergence of some membrane-like inclusion bodies (*small arrow*) in tegument, swelling, and focal lysis of muscle bundles (*moderate arrow*) and damaged syncytium (*large arrow*, ×5,800)

Fig. 12 Male adult *S. japonicum* harbored in a mouse 24 h after mefloquine dosing, showing detachment of severely damaged tegument from the worm surface (*small arrow*) and vacuoles formed by lysed muscle bundles (*large arrow*, ×9,700)

cytoplasmic process, and the swollen cytoplasmic process, filled with vacuoles containing residual bodies, and membrane-like inclusion bodies, hanged on the tegument (Fig. 18). Meanwhile, prominent changes observed under the tegument were extensive swelling and focal lysis in the musculature, parenchymal tissues and perinuclear cytoplasm of syncytium, accompanied by the emergence of lysosomes, swollen mitochondria, vacuoles, and myelin-like structures (Figs. 17 and 19). The sensory organelles also showed disruption of bilayer membrane and focal lysis of their

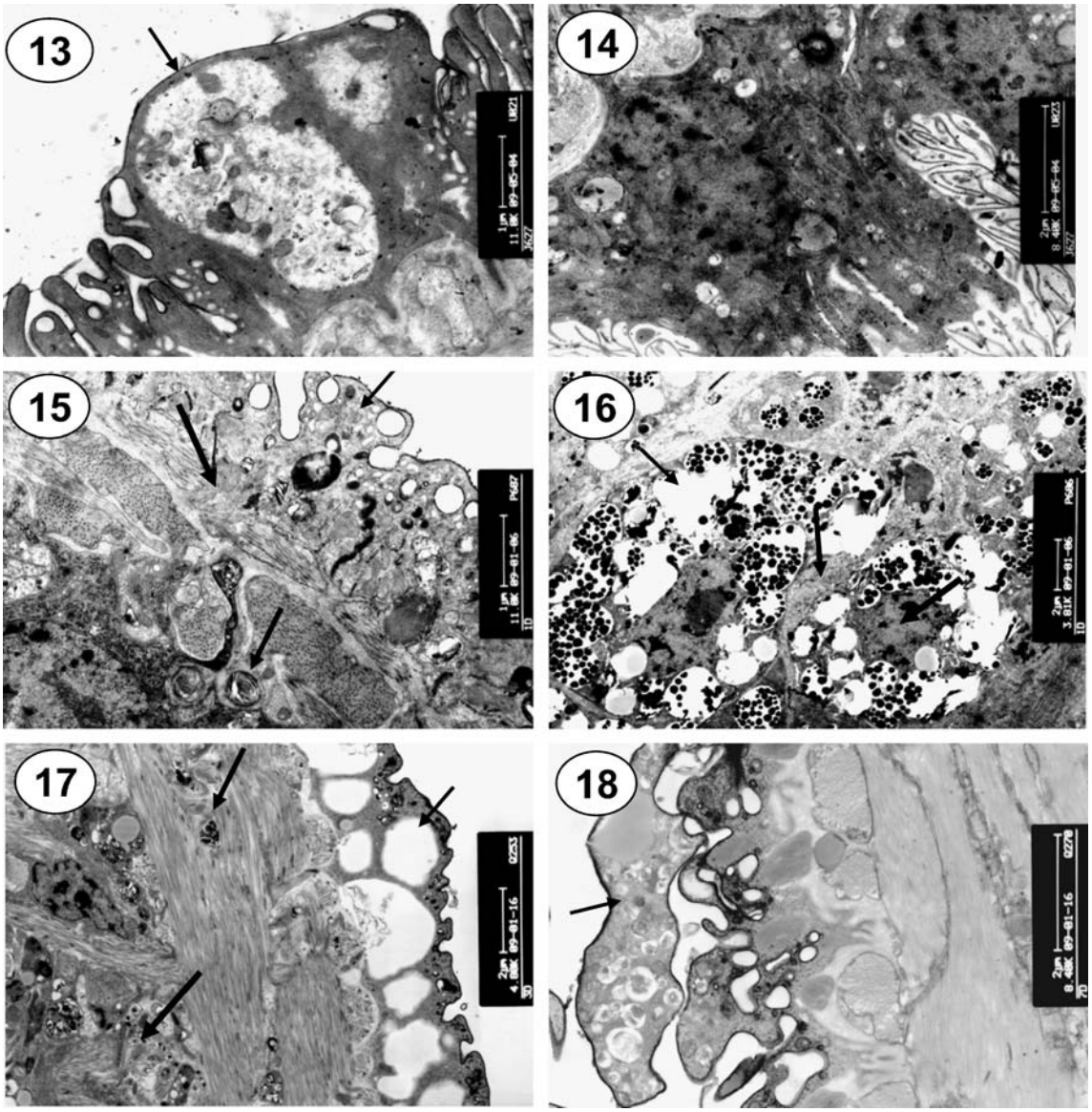

Fig. 13 Damaged sensory organelle (*small arrow*) distributed on the tegument of a male adult *S. japonicum* harbored in a mouse 24 h after administration of mefloquine (×9,700)

Fig. 14 Male adult *S. japonicum* harbored in a mouse 24 h after mefloquine administration showing severely damaged gut epithelial cell (×7,400)

Fig. 15 Female adult *S. japonicum* harbored in a mouse 24 h after mefloquine treatment showing emergence of membrane-like inclusion bodies (*small arrow*) in tegumental matrix and myelin-like structure (*moderate arrow*) in parenchymal tissues, as well as swelling and focal lysis of muscle bundles (*large arrow*, ×9,700)

Fig. 16 Vitelline cell of female adult *S. japonicum* harbored in a mouse after mefloquine administration, showing fusion of vitelline droplets (*small arrow*), decrease of granular endoplasmic reticulum (*moderate arrow*), and disappearance of nucleoli (*large arrow*, ×3,400)

Fig. 17 Male adult *S. japonicum* harbored in a mouse 3 days after administration of 400 mg/kg mefloquine, showing large vacuoles in tegument (*small arrow*), emergence of lysosome in muscle (*moderate arrow*), and destruction of parenchymal tissues (*large arrow*, ×4,200)

Fig. 18 Male adult *S. japonicum* harbored in a mouse 7 days after mefloquine dosing, showing severe swelling of cytoplasmic process (*small arrow*), filled with vacuoles containing residual bodies, and some membrane-like inclusion bodies, hanged on the tegument (×7,400)

internal structures which resulted in the formation of vacuoles or membrane-like inclusion bodies (Fig. 19). At this observation time point, severe damage to the gut epithelial cells was seen which revealed in loss of cell definition with blurred nucleus and cytoplasm filled with vacuoles, emergence of myelin-like structures, and almost disappearance of microvilli (Fig. 20). Similar ultrastructural alterations were also seen in female worms. In the section of female worms, the major tegumental changes were the vacuole formation due to lysis of tegumental matrix, focal disruption of outer plasmic membrane, or damaged outer plasmic membrane detached from the cytoplasmic processes to form vacuoles (Fig. 21). In another worm specimen, the cytoplasmic processes of tegument fused together and the

swollen matrix filled with small membrane-bound cyst-like structures, degenerated mitochondria, and myelin-like structures (Fig. 22). The musculatures underneath the tegument also showed swelling and focal lysis. In the syncytium, focally disrupted cell membrane, myelin-like structures, swollen mitochondria, and vacuoles of varying size were observed. In gut epithelial cells, decrease of granular endoplasmic reticulum, focal lysis of heterochromatin and nucleoli, emergence of vacuoles and degenerated mitochondria, and disappearance of microvilli were observed (Fig. 23). The prominent alterations in vitelline cells 7 days post-treatment were similar to those seen at the previous time

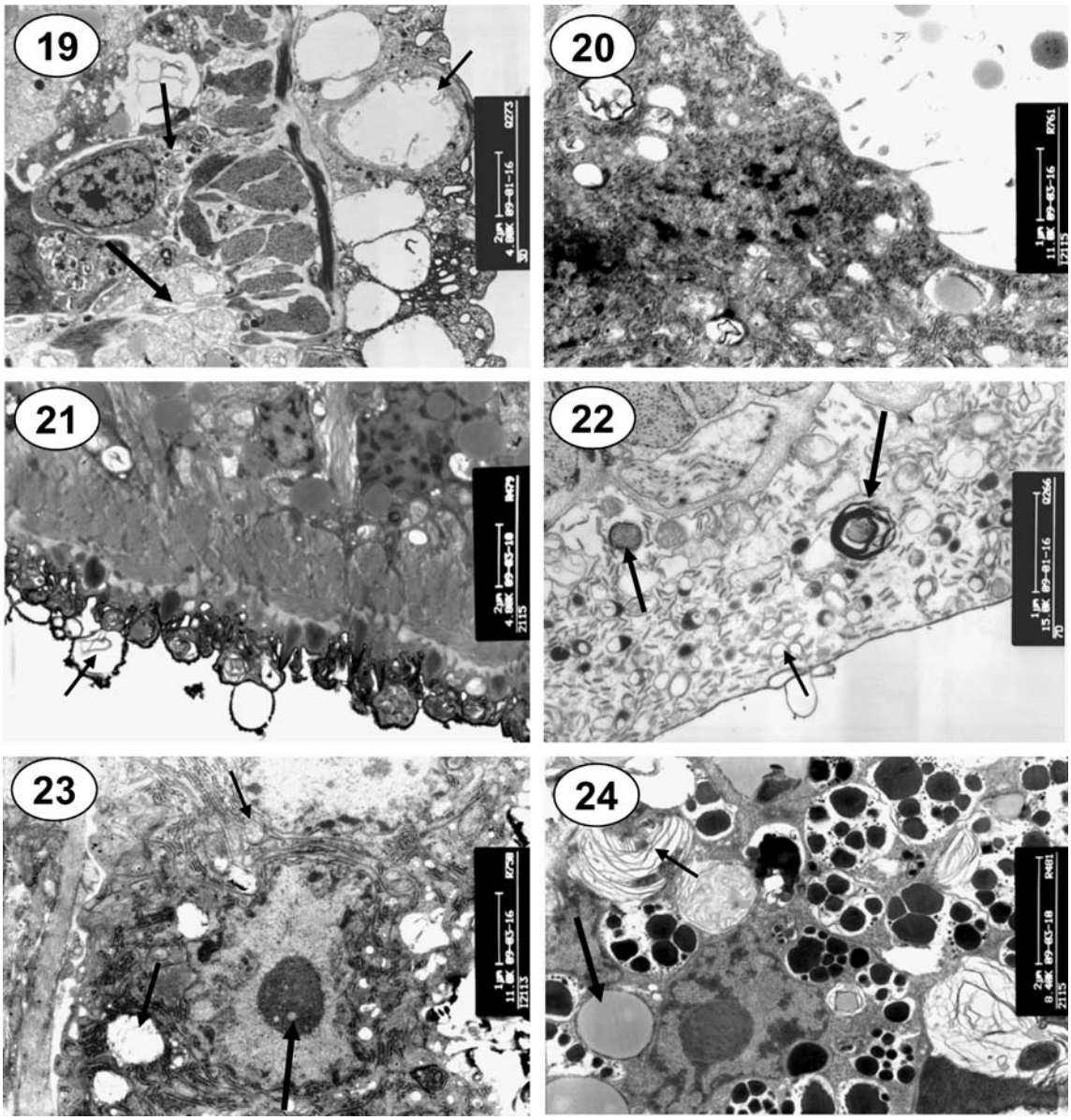

Fig. 19 Male adult *S. japonicum* harbored in a mouse 3 days after administration of mefloquine, showing disrupted sensory organelle (*small arrow*), syncytium (*moderate arrow*), and parenchymal tissues (*large arrow*, ×4,200)
Fig. 20 Male adult *S. japonicum* harbored in a mouse 7 days after mefloquine treatment showing a destroyed gut epithelial cell (×9,700)
Fig. 21 Tegument of female adult *S. japonicum* harbored in a mouse 7 days after mefloquine treatment, showing vacuoles formed by damaged outer plasmic membrane detached from cytoplasmic processes (*arrow*, ×4,200)
Fig. 22 Tegument of female adult *S. japonicum* harbored in a mouse 7 days after administration of mefloquine showing swelling of tegumental matrix filled with small membrane-bound cyst-like structures (*small arrow*), degenerated mitochondria (*moderate arrow*), and myelin-like structure (*large arrow*, ×13,500)
Fig. 23 Gut epithelial cell of female adult *S. japonicum* harbored in a mouse 3 days after mefloquine dosing, showing swollen mitochondria (*small arrow*), vacuoles in cytoplasm (*moderate arrow*), and focal lysis of nucleus (*large arrow*, ×9,700)
Fig. 24 Female adult *S. japonicum* harbored in a mouse 3 days after mefloquine administration, showing damaged vitelline cell with many myeline-like structures (*small arrow*) and lipid droplets (*large arrow*, ×5,800)

point with the exception that many myelin-like structures were observed (Fig. 24).

Discussion

In a previous paper, we reported that in mice infected with adult *S. japonicum* and treated orally with mefloquine at a single dose of 400 mg/kg, only 47.3% of the worms shifted to the liver 24 h post-treatment (Keiser et al. 2009). TEM observation demonstrated that 8 h after mefloquine was administered to the infected mice, prominently ultrastructural alterations were seen in both male and female worms which are consistent with the SEM observation on the tegument of schistosomes harbored in mice following treatment with mefloquine (Xiao et al. 2009b).

Since the tegument of schistosome is the only interface that contacted the host and displays important role in the absorption of nutrients and defense function to escape the host immune response (Pappas and Read 1975; Capron et al. 1987; Pearce and Sher 1987; Halton 1997), the damage to the tegument should impact on the provision of nutrition and disrupt the immunological "disguise" of the worm which results in the exposure of worm surface antigen and leads to being vulnerable to attack of the worm by host immune response. It is already recognized that many antischistosomal drugs, such as praziquantel, artemether, etc., also show extensive damage to the tegument, indicating that tegument of schistosome is one of the key important targets attacked by the drugs. Although different types of antischistosomal drugs may exert various modes of action on the worms, induction of similar damage patterns of tegument is usually seen. The present study reveals that the patterns of damage to the tegument and subtegument of schistosomes induced by mefloquine, such as blurred tegumental matrix, decrease or disappearance of rod-like and discoid-like secretary bodies, and focal lysis of tegumental matrix, resulted in vacuole formation, collapse of focal damaged tegument and disruption or disappearance of basal membrane, extensive swelling and focal lysis of musculature, and destruction of syncytium which are basically similar to those induced by praziquantel and artemether (Becker et al. 1980; Mehlhorn et al. 1981; Xiao et al. 1981, 2002, 2006). Particularly, mefloquine and artemether exhibit very high similarity in damage to the tegument of schistosomes. It was found that after the action of both drugs, lysis of tegument matrix was usually revealed in the portion close to the basal membrane which resulted in the formation of larger vacuoles just above the basal membrane. On the other hand, a difference was also seen in that severe swelling of distal cytoplasm process was usually observed in the tegument induced by artemether, which resulted in the formation of many vacuoles protruded from the tegumental surface seen by SEM, TEM, and light microscopic observation (Wu et al. 1983; Xiao et al. 2002, 2004, 2006). No such alterations were seen in mefloquine-damaged tegument of schistosomes. The results could explain why no vacuole protruded on the tegumental surface examined by SEM. But in histopathological observation on the worms shifted to the liver of mice treated with mefloquine, vacuoles, which formed by focal lysis of musculature, were only observed beneath the tegument, and no other vacuoles protruded from the tegumental surface, which was quite different from artemether (Wu et al. 1983; Xiao et al. 2002, 2004, 2006).

Mefloquine and artemether are different types of antimalarials. Although the action of mechanism against malaria is still not expounded, interference with hemoglobin metabolism may display the important role of the two drugs. Since mefloquine exhibits a potential and fast killing effect on schistosome in vitro, it is necessary to further study whether mefloquine exerts its action on other targets apart from hemoglobin metabolism. As to praziquantel, it is only effective against helminthes, including trematodes and cestodes. When these three drugs are comparatively assessed for their efficacy against adult *S. japonicum* in experimental chemotherapy, significantly higher worm burden reductions are obtained with mefloquine and praziquantel than artemether. In addition, the efficacy of praziquantel against adult schistosomes is antibody-dependent (Doenhoff et al. 1987). All these data indicate that apart from damage to the tegument and subtegument tissues, other factors, such as energy metabolism, antioxidant system, host immune situation, etc., are also important in killing the worms following drug administration.

In addition to the ultrastructural alterations in tegument, musculature, syncytium, and parenchymal tissues, significant changes in gut epithelial cells and vitelline cells of female worms are also seen, which may disturb the digestive function and block the oviposition of female worms. But these lesions might not be the cause of worm death induced by mefloquine because in worms that survived the treatment with mefloquine, severe dilatation of gut is universal and could recover to normal gradually post-treatment. Recovery of ova formation and oviposition was also observed in female worms that survived the mefloquine treatment.

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Referrences

Becker B, Mehlhorn H, Andrews P, Thomas H, Eckert J (1980) Light and electron microscopic studies on the effect of praziquantel on *Schistosoma mansoni, Dicrocoelium dendriticum,* and *Fasciola hepatica* (Trematoda) in vitro. Z Parasitenkd 63:113–128

Capron A, Dessaint JP, Caporn M, Ouma JH, Butterworth AE (1987) Immunity to schistosomes: progress toward vaccine. Science 238:1065–1072

Doenhoff MJ, Sabah AA, Fletcher C, Webbe G, Bain J (1987) Evidence for an immune-dependent action of praziquantel on *Schistosoma mansoni* in mice. Trans R Soc Trop Med Hyg 81:947–51

Halton DW (1997) Nutritional adaptations to parasitism within the platyhelminthes. Int J Parasitol 27:693–704

Keiser J, Chollet J, Xiao SH, Mei JY, Jiao PY, Utzinger J, Tanner M (2009) Mefloquine—an aminoalcohol with promising antischistosomal properties in mice. PLoS Negl Trop Dis 3:e350

Le WJ, You JQ, Yang YQ, Mei JY, Guo HF, Yang HZ, Zhang CW (1982) Studies on the efficacy of artemether in experimental schistosomiasis. Acta Pharmaceutica Sin 17:187–193

Li SW, Wu LJ, Liu ZD, Hu LS, Xu YX, Liu YM, Liu X, Fang JT (1996) Studies on prophylactic effect of artesunate on *Schistosomiasis japonica*. Chin Med J 109:848–853

Mehlhorn H, Becker B, Andrews P, Thomas H, Frenkel JK (1981) In vivo and in vitro experiments on the effects of praziquantel on *Schistosoma mansoni*. A light and electron microscopic study. Arzneimittelforschung 31(3a):544–554

Ohnmacht CJ, Patel AR, Lutz RE (1971) Antimalarials. 7. Bis (trifluoromethyl)-(2-piperidyl)-4-quinolinemethanols. J Med Chem 14:926–928

Pappas PW, Read CP (1975) Membrane transport in helminth parasites: a review. Exp Parasitol 37:469–530

Pearce EJ, Sher A (1987) Mechanisms of immune evasion in schistosomiasis. Contrib Microbiol Immunol 8:219–232

Sabah AA, Fletcher C, Webbe G, Doenhoff MJ (1986) *Schistosoma mansoni:* chemotherapy of infections of different ages. Exp Parasitol 61:294–303

Van Nassauw L, Toovey S, Op V, den Bosch J, Timmermans JP, Vercruysse J (2008) Schistosomicidal activity of the antimalarial drug, mefloquine, in *Schistosoma mansoni*-infected mice. Travel Med Infect Dis 6:253–258

Wu LJ, Yang HZ, Yang YQ (1983) Histological and histochemical changes of *Schistosoma japonicum* and host liver caused by artemether. Yao Xue Xue Bao 18:7–14

Xiao Sh, Yang YQ, Shu YS, Wang ZW (1981) Ultrastructural changes of tegument, syncytium, vitelline cells and muscles of *Schistosoman japonicum* caused by pyquiton. Acta Zool Sin 27:305–309

Xiao SH, Yue WJ, Yang YQ, You JQ (1987) Susceptibility of *Schistosoma japonicum* of different developmental stages to praziquantel. Chin Med J 100:759–768

Xiao SH, You JQ, Yang YQ, Wang CZ (1995) Experimental studies on early treatment of schistosomal infection with artemether. Southeast Asian J Trop Med Public Health 26(2):306–18

Xiao SH, Shen BG, Catto BA (1996) Effect of artemether on ultrastructure of *Schistosoma japonicum*. Chin J Pparasitol Parasitic Dis 14:181–187

Xiao S, Shen B, Utzinger J, Chollet J, Tanner M (2002) Ultrastructural alterations in adult *Schistosoma mansoni* caused by artemether. Mem Inst Oswaldo Cruz 97:717–24

Xiao SH, Guo J, Chollet J, Wu JT, Tanner M, Utzinger J (2004) Effect of artemether on *Schistosoma mansoni*: dose–efficacy relationship, and changes in worm morphology and histopathology. Chin J Parasitol Parasitic Dis 22:148–153

Xiao SH, Tanner M, Shen BG, Utzinger J, Chollet J (2006) Effect of artemether on the tegument of adult *Schistosoma haematobium* recovered from mice. Chin J Parasitol Parasitic Dis 24:425–432

Xiao Sh, Mei JY, Jian PY (2009a) Further study on mefloquine concerning several aspects in experimental treatment of mice and hamsters infected with *Schistosoma japonicum*. Parasitol Res 106:131–138

Xiao SH, Chollet J, Utzinger J, Mei JY, Jiao PY, Keiser J, Tanner M (2009b) Effect of single-dose oral mefloquine on the morphology of adult *Schistosoma japonicum* in mice. Parasitol Res 105:853–861

Xiao SH, Xue J, Shen BG (2010) Tegumental alterations of adult *Schuistosoma japonicum* harbored in mice treated with a single oral dose of mefloquine. Chin J Parasitol Parasitic Dis 28:1–7

Yolles TK, Morre DV, DeGusti DL, Ripsom CA, Meleney MS (1947) A technique for the perfusion of laboratory animals for the recovery of schistosomes. J Parasitol 33:419–426

Yue WJ, You JQ, Mei JY (1985) Prophylactic activity of praziquantel in animals infected with *Schistosoma japonicum*. Acta Pharmacol Sin 6:186–188

Zhang CW, Xiao SH, Utzinger J, Cholle J, Keiser J, Tanner M (2009) Histopathological changes in adult *Schistosoma japonicum* harbored in mice following treatment with mefloquine. Parasitol Res 104:1407–1416