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Tegument changes of *Schistosoma japonicum* and *Schistosoma mansoni* in mice treated with artemether[1]

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KEY WORDS *Schistosoma japonicum*; *Schistosoma mansoni*; artemether; scanning electron microscopy

AIM: To study the effect of artemether (Art) on the tegument of schistosomes. **METHODS**: Mice infected with *S japonicum* cercariae for 7 and 35 d, or with *S mansoni* cercariae for 49 d were treated intragastrically with Art 200 - 300 mg·kg^{-1}·d^{-1} for 2 d. *Schistosomes* were collected in groups of 2 mice at various intervals after medication for scanning electron microscopic observation. **RESULTS**: The tegumental changes induced by Art appeared to be similar in *S japonicum* and *S mansoni*: swelling and fusion of tegumental surfaces, vesicle formation and collapse of discoid-like sensory structures. In *S japonicum* the emergence of tegumental alterations was earlier in 7-d-old schistosomulae than that in 35-d-old adult worms. **CONCLUSION**: Art injured the teguments of *S japonicum* and *S mansoni*.

Artemether (Art), β-methyl ether of artemisinin[1], was therapeutically active against *Schistosoma japonicum*[2,3] and *S mansoni*, particularly in d 7 - 21 developmental stages[4]. In this study a scanning electron microscopic examination was done on *S japonicum* and *S mansoni* from artemether-treated mice.

MATERIALS AND METHODS

Drug For *S japonicum* studies, Art was made by Kunming Pharmaceutical Factory (lot No 880701) and suspended in 5 % starch solution to make up a final concentration of 20 g·L^{-1}. For *S mansoni* studies, Art was provided by Shanghai Institute of Material Medica, Chinese Academy of Sciences and dissolved in polyethylene glycol (PEG 400) at a concentration of 30 g·L^{-1}.

Parasites *S japonicum* cercariae (Anhui isolate), obtained from infected *Oncomelania hupensis* snails, were provided by the Department of Vector Biology and Control in the Institute of Parasitic Diseases (Shanghai, China). *S mansoni* cercariae (KEB strain), obtained from *Biomphalaria glabrata* snails, were provided by Dr Raymond DAMIAN, Department of Zoology, University of GEORGIA, Athens, GA, USA.

Scanning electron microscopic studies *S japonicum*: NIH strain mice, ♀, weighing 20 - 24 g were maintained on rodent diet and given water *ad lib* in the animal care facilities of the Institute of Parasitic Diseases (Shanghai, China). Each mouse was infected with 50 or 100 cercariae via shaved abdominal skin. Seven or 35 d after infection the mice were treated via intragastric gavage ig with Art 200 mg·kg^{-1} daily for 2 d. At 8 - 24 h after the first dose and 1 - 28 d after the second dose, 2 mice from each group were killed. The worms were collected by perfusion[5] with ice-cold Hanks' balanced salt solution (HBSS) and fixed in 2.5 % glutaraldehyde-phosphate buffer (0.1 mol·L^{-1}, pH 7.4).

After fixation, worms were processed according to the standard methods[6] and examined with a Joel JSM-820 scanning electron microscope. Five to 6 ♂ and ♀ worms in each group of 35-d-old (d 35) adult worms, or 8 - 11 worms in each group of 7-d-old schistosomulae were examined. The teguments of control untreated 7-d-old schistosomulae and 35-d-old adult worms were comparable to those previously described[6].

S mansoni: C3H mice, ♂, weighing 18 - 22 g, obtained under National Institute of Health (USA) contract were each inoculated subcutaneously with 150 *S mansoni* cercariae (KEB strain). Infected mice were maintained on rodent diet and water *ad lib* in the animal care facilities of the Veterans Administration Hospital (Augusta GA, USA). Seven weeks after infection the mice were treated ig with Art 300 mg·kg^{-1} daily for 2 d and killed at different intervals after the treatment. The worms were recovered by perfusion

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with iced HBSS and fixed in 3.4 % glutaraldehyde in 0.1 mol $\cdot L^{-1}$ cacodylate buffer (pH 7.0). After fixation, samples were rinsed in cacodylate buffer for 20 min. Dehydration was accomplished in successive rinses with 50 %, 75 %, 95 %, and 100 % ethanol; 2 changes each, 20 min per step. Final drying was accomplished in a critical-point dryer (Blazers Union, Vaduz, Liech-temtein) with CO_2 as the transitional solvent. The dried samples were mounted on 9-mm aluminum stubs and coated with gold in a technic Hummer Model V sputtering apparatus (Technic, Alexandria VA, USA). All samples were examined in a Joel Model JSM-35 CF II Scanning electron microscope (JEOL, Peabody MA, USA). The photo-micrographs were taken on polaroid type 55 positive/negative film (Polaroid, Cambridge MA, USA). Control worms were obtained by perfusion from infected mice treated ig with the polyethylene glycol 10 mL $\cdot kg^{-1} \cdot d^{-1}$ for 2 d. The tegumental surface of untreated control ♂ and ♀ adult S *mansoni* were comparable to those described by others[7].

RESULTS

S japonicum The 7-d-old schistosomulae: at 8 h after the 1st dose of Art, all schistosomulae showed mild or moderate swelling of tegumental ridges which usually resulted in loss of definition of spines and segmental annular troughs (Fig 1A *vs* 1B, Plate 1). At 24 h, swelling of ridges was seen along the whole worm body. On d 1 – 3 after the 2nd dose, severe swelling and fusion of ridges were seen in part of worms. In some worms, swelling created a smooth surface and most spines, sensory structures and segmental annular troughs disappeared (Fig 1C, D), or located in anterior part of the worm (Fig 1E). On d 14 – 28 after treatment, the residual worms were free from damages.

The 35-d-old adult worms: at 8 h after the 1st dose of Art, a few ♂ worms showed fusion of ridges accompanied by enlargement of discoid-like sensory structure (Fig 1F *vs* 1G). At 24 h, tegumental damage spread over the surface of the worms. Many small vesicles appeared with focal erosion and peeling of tegumental ridges which resulted in exposure of underlying tissues (Fig 1H).

At 8 – 24 h after the 1st dose, tegumental damage along the surface of ♀ worms was severer than that of ♂ worms. The common effect was pronounced swelling and fusion of ridges. Focal erosion and peeling were noted on the anterior or middle part of the worm surface (Fig 1I).

At 24 h after the 2nd dose, the tegumental damage spread to the area between the oral and ventral suckers. Focal erosion and peeling of tegument were seen in some worms. In these foci, the host leukocytes usually attached on the damaged surface (Fig 1J). Severe swelling and fusion of ridges appeared in different parts of the tegument, resulting in a flattened surface of the worm (Fig 1K).

On d 3 – 7 after treatment, no further changes were detected in most ♂ worms, although a few of them still showed extensive erosion, peeling, vesiculation (Fig 1L) and attachment of host leukocytes to the damaged surface usually in the middle or tail part of the worm. In ♀ worms, the main alterations were extensive erosion and peeling accompanied by exposure of underlying tissue, formation of vesicles, extensive fusion or wrinkling of ridges.

At 2 – 4 wk after treatment, most of the residual ♂ worms appeared to be recovered (Fig 1M).

S mansoni Male worms (49-d-old): about 24 h after the 1st or 2nd dose of Art, no major alteration of tegument was seen except for some small vesicles and slight swelling in focal regions of tegument. Focal areas of tegument showed fusion and disappearance of spined-tubercles and sensory bulbs; the tail surface showed its abnormal appearance due to swelling fusion, and a smooth appearance (Fig 2A *vs* 2B, Plate 2). On d 3 – 7 after treatment all worms showed some damages to their surfaces, including swelling, flattening of spined tubercles and vesicle formation in areas between tubercles (Fig 2C *vs* 2D). In a few worms, swelling and fusion of the tegument surface were very severe. On d 14 – 28 d after treatment, a few worms became normal but some still showed slight or moderate damage to focal regions.

Female worms (49-d-old): About 24 h after the 1st dose of Art, no apparent damage to the worm surface was seen. The first damage was detected 1 d after the 2nd dose. The tegument showed swelling, fusion of ridges, and less prominence of spines (Fig 2E *vs* 2F). On d 7 after

treatment, the most frequent damages were swelling, fusion, and wrinkling of the entire surface. The swelling was accompanied by diminution of sensory bulbs and spines (Fig 2G *vs* 2H). A sponge-like appearance emerged around the neck area and large cloudy and "boss-like" masses protruded from the surface (Fig 2I). Tegumental damage reached maximal by d 7 – 14 after treatment. By d 21 – 28, a few worms still demonstrated severe surface damage.

DISCUSSION

Tegumental changes induced by artemether appear to be similar in *S mansoni* and *S japonicum* as assessed by scanning electron microscopy. When compared with praziquantel, artemether exhibits a slow onset of action on *S mansoni* and *S japonicum*. When infected mice were treated orally with praziquantel, over 90 % of adult worms were found in the liver within 30 min of dose administration[8], and drug-induced tegumental damage was seen within 15 min[9]. With artemether, maximal shift occurred 3 – 7 d after treatment[4]. Furthermore, differences in the nature of tegumental damage induced by the 2 drugs were observed. Praziquantel-induced vesiculation is most prominent[9] and is severer in ♂ worms. Artementer-induced damage is characterized by swelling of the tegument and occurred most rapidly in 7-d-old schistosomulae. While praziquantel, on the other hand, exhibits little effect on 7-d-old schistosomulae of *S mansoni*[9] and *S japonicum*[10].

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蒿甲醚引起小鼠体内日本血吸虫和曼氏血吸虫皮层变化[1]

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关键词 日本血吸虫；曼氏血吸虫；蒿甲醚；扫描电子显微镜检查

目的：研究蒿甲醚(Art)对血吸虫皮层的作用。**方法**：小鼠于感染日本血吸虫尾蚴后 7 d 和 35 d 或感染曼氏血吸虫尾蚴后 49 d，每 d ig Art 200 – 300 mg·kg^{-1}，共 2 d，并于治后不同时间取虫作扫描电镜观察。**结果**：Art 引起日本血吸虫和曼氏血吸虫皮层的变化相仿，主要是皮层褶嵴肿胀和融合，皮层表面的糜烂与剥落，以及空泡的形成和盘状感觉器的破溃，且日本血吸虫 7 d 童虫开始出现皮层损害的时间较 35 d 成虫的迅速。**结论**：Art 对日本血吸虫和曼氏血吸虫皮层有损害作用。

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