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# Scanning electron microscope observation on tegumental damage of 21-d-old *Schistosoma japonicum* induced by praziquantel[1]

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**AIM:** To study the effect of praziquantel (Pra) on the tegument of 21-d-old schistosomula, mice infected with *Schistosoma japonicum* cercariae for 21 d were treated ig with Pra at a single dose. **METHOD:** Groups of mice were killed at different intervals within 48 h, and the worms were collected by perfusion for scanning electron microscopic observation. **RESULTS:** When the dose used was 300 mg·kg$^{-1}$, the worms showed mild or moderate swelling, fusion or even erosion and collapsed of the tegumental ridges, which was characterized by swelling of the discoid sensory structures. At higher dose of 500 mg·kg$^{-1}$, similar alterations in the worm surface were seen, but more extensive and serious. When Pra 500 mg·kg$^{-1}$ was given daily for 3 d, severe swelling, erosion and peeling of the tegument accompanied by the attachment of the host leukocytes on the damaged surface were seen. **CONCLUSION:** Pra exhibited a direct killing effect on 21-d-old schistosomula.

**KEY WORDS** *Schistosoma japonicum*; scanning electron microscopy; praziquantel

When mice harbored with 21-d-old schistosomula were treated with intragastric gavage (ig) of praziquantel (Pra), the damage of the worm tegumetn could be detected, but no apparent therapeutic effect was seen[1,2]. After adminstration of Pra 500 mg·kg$^{-1}$·d$^{-1}$ × 1 − 3 d, the worm reduction rates was 43.8 %−90.4 %[3]. Nevertheless, the oviposition of 21-d-old female schistosomula was inhibited at least 2 wk after a single dose of Pra was given to the infected mice. In view of the above-mentioned difference, appropriate regimens of Pra for prevention purpose were designed[4]. In this paper the Pra-induced tegument damage of 21-d-old schistosomula was studied.

## MATERIALS AND METHODS

**Drug** Pra was purchased form Shanghai the 6th Pharmaceutical Factory. The drug was suspended in 1 % tragacanth to a concentration of 30−50 g·L$^{-1}$.

**Parasite** Anhui strain of *Schistosoma japonicum* cercariae released from artificially infected *Oncomelania hupensis* snails, were provided by our Institute.

**Mice** Female NIH strain mice ($n$=60) weighing 20±$s$ 2 g were maintained on a rodent chow and given water *ad lib*. Each mouse was inoculated with 60−80 cercariae *via* the shaved abdominal skin. Twenty-one days after inoculation, the mice were treated ig with Pra 300 mg·kg$^{-1}$ or 500 mg·kg$^{-1}$·d$^{-1}$×1−3 d. After 1, 4, 24, 48, and 72 h, 2 mice of each group were killed. The worms were collected by perfusion with ice-cold Hank's balanced salt solution (HBSS) and fixed in 2.5 % glutaraldehyde-phosphate buffer (0.1 mol·L$^{-1}$, pH 7.4).

**Scanning electron microscopy** The fixed worms were processed routinely[5] and examined with a Joel JSM-820 scanning electron microscope. In each group 5−6 ♂ and ♀ schistosomula were examined.

## RESULTS

The morphological features of tegumental

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surface of untreated 21-d-old schistosomula (Fig 1A, Plate 3,4) were comparable to those previously described[2]. Since the tegumental alterations of ♂ and ♀ worms induced by Pra were similar, the results were described together.

300 mg·kg$^{-1}$ group: One hour after the ig Pra, all worms showed tegumental damages: mild or moderate swelling of tegumental ridges and formation of small and dispersed vesicles. The discoid sensory structures usually appeared in swelling or fusion. In some cases, the discoid sensory structure protruded from the surface like a trumpet. Some discoid sensory structures showed severe swelling and many small holes on its surface (Fig 1B, Plate 3,4). Detachment of sensory cilia was usually seen. After 4 h, the enlarged discoid sensory structures were immersed in the swollen tegumental ridges with hole-like appearance (Fig 1C, Plate 3,4), and some sensory structures were collapsed. After 24 h, no further aggravation of tegumental damage was seen. In some discoid sensory structures erosion and collapse appeared on their surface or the tegumental ridges (Fig 1D, Plate 3,4). After 48 h, most of the tegumental surface became normal appearance; only local tegument and part of the discoid sensory structures still exhibited swelling and fusion of tegumental ridges (Fig 1E, Plate 3,4).

500 mg·kg$^{-1}$ group: After 1 h, apparent damage of tegumetn appeared in all worms. The severe swollen ridges fused together. The alteration in sensory structures was similar to that described above. After 4 h, many vesicles appeared on the tegumental surface. Some vesicles were resulted from severe swelling of discoid sensory structures (Fig 1F, Plate 3, 4). A few of the swollen discoid sensory structures showed erosion and collapse. No apparent alteration of papilla-like sensory structure was seen. After 24-48 h, larger fused mass of swollen tegumental ridges was seen in local portions of some worms. In some portions of the worm surface the damage of tegument was not evident, but the discoid sensory structures distributed in the tegumental ridges showed severe swelling, erosion, and collapse, or even peeling with many host leukocytes attached on the damaged surface (Fig 1G. Plate 3,4), especially at the tail of worms.

500 mg·kg$^{-1}$·d$^{-1}$×3 d group: One to 4 h after the last medication, extensive damage of tegument was seen in all worms, but normal tegumental ridges were still found. The damages of tegument detected were focal or extensive swelling and fusion or erosion and collapse of the tegumental ridges (Fig 1H, Plate 3,4). Meantime, numerous host leukocytes attached on the damaged tegument. No further aggravated damage of the discoid sensory structures was seen and the papilla-like sensory structures still appeared to be normal. After 24—48 h, all worms showed extensive and severe damage on the tegument. Some worms showed swelling, fusion, erosion, and peeling on its surface (Fig 1I, Plate 3,4), with numerous host leukocytes attached (Fig 1J, Plate 3,4). After 3—7 d, severe erosion and peeling appeared in most of the worms and a few of the worms showed apparent atrophy and looked like cotton flocculus.

## DISCUSSION

Previous paper indicated that less efficacy of Pra on 21-d-old schistosomula might be ascribed to the low level of antischistosomal antibody presented in the host during 3 wk after infection, ie the demaged schistosomula did not suffer enough attack from the host immune response[6]. therefore, when the host was immunized by adult worm homogenates or infected simultaneously with 7-wk-old adult

worms, the effect of Pra on d 21 schistosomula increased significantly[16]. the present results demonstrated again that a curative dose of Pra was sufficient to induce apparent effect on the tegumental surface of 21-d-old schistosomula. Of interesting was that the discoid but not the papilla-like sensory structures was more susceptible to the drug, although the outer portion of the both structures seemed to be developed from the tegument. The mechanism was still unknown.

When the single dose of Pra was raised up, the damage of worm tegument was more severe than the lower single dose. Therefore, the higher dose of Pra not only suppressed the oxiosition of the female worms[4], but also led to the attachment of the host leukocytes on the damaged suface of a few worms. After prolongation of the treatment course to 3 d, all of the worms examined showed attachment of numerous host leukocytes on their damaged surface, demonstrating that, besided the antibody-dependent effect of Pra on 21-d-old schistosomula, Pra also possessed a direct killing action on d 21 schistosomes which provided the possibility of using Pra in early treatmetn of schistosomal infection in order to control acute schistosomiasis or reduce intensity of schistosomal infection.

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## 吡喹酮对21-d 日本血吸虫童虫皮层损害的扫描电镜观察

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**目的**：观察吡喹酮对21-d 童虫皮层的作用. **方法**：小鼠于感染日本血吸虫尾蚴达21 d 时，ig 1剂吡喹酮，并在治疗后1—48 h 的不同时间内剖杀取虫，作扫描电镜观察. **结果**：吡喹酮的剂量为300 mg·kg$^{-1}$时，宿主体内的21-d 童虫示有轻度或中度的皮层褶峰肿胀、融合、糜烂或破溃，且以盘状感觉器的肿胀为特征. 用吡喹酮的较高剂量500 mg·kg$^{-1}$治疗，虫的体表亦有相似的变化，但较广泛和严重. 若每 d ig 吡喹酮500 mg·kg$^{-1}$，连给3 d，则虫的皮层严重肿、糜烂和剥落，并伴有宿主的白细胞附着. **结论**：结果表明，吡喹酮对21-d 童虫有直接杀死作用.

**关键词** 日本血吸虫；血吸虫童虫；扫描电子显微镜检查术；皮层；吡喹酮