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Early treatment of schistosomal infection with praziquantel in mice[1]

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ABSTRACT The first single dose of praziquantel (Pra) was given ig to mice on the day of infection with *Schistosoma japonicum* cercariae, or 7 d, 14 d, and 21 d after infection. Afterwards, the same dose of Pra was given once at 1—3 wk intervals for 2—3 times. The prophylactic effect was estimated by the reduction of average number of total and ♀ worms, the number of mice without ♀ worm, and the gross change of the liver. When mice were treated with Pra 300—500 mg $\cdot kg^{-1}$ initially on d 21 after infection and repeated once every 1—2 wk for 2—3 times, almost all the ♀ worms lodged in the host were killed, showing that either the host was protected from the infection of schistosomes or a great decrease in the intensity of the infection resulted.

KEY WORDS *Schistosoma japonicum*; schistosomula; praziquantel; artemether; combination drug therapy

The effect of praziquantel (Pra) on different developmental stages of *Schistosoma japonicum* has been studies. Apart from its effect on adult worms, Pra was exhibited to have effect on cercariae invading the skin of the host, 3 h-old and 21-d-old schistosomulae[1-4]. Based on the antischistosomal activities of Pra, we suggest if Pra is given once at appropriate intervals for several times to the host started at early stage of infection, the majority or even all of the ♀ worms are expected to be killed before their sexual maturity. Thus, the appropriate regimen could be selected and used for control of acute schistosomiasis or reduced the intensity of infection. Besides Pra, studies on the effect of artemether (Art), a derivative of qinghaosu, indicated that Art was more effective against 7-d-old schistosomula[5]. Therefore, the combined treatment with Pra and Art was also tested in early treatment of schistosomal infection to measure the possibility of synergic action of the two drugs.

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MATERIALS AND METHODS

Parasites *Schistosoma japonicum* cercariae (Anhui isolate), obtained from infected *Oncomelania hupensis*, was provided by our Institute.

Mice Kunming strain mice of either sex weighing $18 \pm s$ 2 g were maintained on a rodent feed and water *ad lib* in the animal care facilities of the Institute.

Infection and therapy Each mouse was infected with 48—52 cercariae via the shaved abdominal skin and treated ig with Pra at 1—3 wk intervals for 1—5 times. The mice were killed 4—5 wk after treatment for collection of residual worms by perfusion. The therapeutic efficacy was evaluated by total worm reduction rate, ♀ worm reduction rate, the number of mice without ♀ worm, and the gross change of the liver.

Effect on egg production of ♀ worm Mice infected with cercariae for 21 d were treated ig with Pra 500 mg $\cdot kg^{-1}$. Groups of 2—3 mice were killed 3, 7, 14, and 21 d after treatment and the schistosomes lodged in the host were flushed out with ice cold Hank's balanced salt solution (HBSS) from the liver and mesenteric veins. The worms were fixed in 70% ethanol and stained with acid carmine. The reproductive system of the ♀ worms was examined under a light microscope and the eggs present in the uterus were counted. In untreated mice on d_{21}, d_{28}, and d_{35} ♀ worms were examined as controls.

Statistical method All data obtained from the experiments were analyzed with *t* test.

RESULTS

Treatment started on d$_0$ When mice were treated ig with Pra 500 mg·kg^{-1} on d$_0$, the total and ♀ worm reduction rates were 80.3% and 83.7%, respectively. 4/20 mice were free from ♀ worm, but none of the mice was cured. In other groups, mice were treated ig with Pra 500 mg·kg^{-1} on d$_0$ and then once at 1—3 wk intervals for 2—4 times. The average numbers of total and ♀ worm in each group were similar, but much less than those of the above-mentioned group treated only once with Pra. After the mice were treated ig with Pra for several times, part of the livers showed normal appearance, purplish red in color and soft consistency, while other parts were light red in color with some dispersed egg tubercles on the surface. In these 3 treated groups over half of the mice was free from ♀ worms and a few of them were cured (Tab 1).

Treatment started on 1—3 wk after infection When Pra was given ig to mice 1 wk after infection at a daily dose of 500 mg·kg^{-1} for 3 d, no apparent efficacy was seen. In above-mentioned mice treated ig with Pra 500 mg·kg^{-1} weekly for 3 wk, the average number of total worms was less than that in the control, but which was not the case in the average number of ♀ worms. In other 2 groups of mice, the 2nd and the 3rd doses of Pra were given at 2—3 wk intervals following the first dosing which took place 1 wk after infection, resulting in an apparent lowering of the average number of total worms and ♀ worms as compared with the above-mentioned 2 groups,

Tab 1. Mice infected with *Schistosoma japonicum* cercariae and treated with ig praziquantel 500 mg·kg^{-1} given on different days after infection. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs control.

Day of medication	Mice cured	Mice without ♀ worms	Total worms	WRR/%	Female worms	FWRR/%	Liver alteration
Control	0/20	0/20	31.5±9.0	—	14.0±9.0	—	+−++
d_0	0/20	0/20	6.2±4.4^c	80.3	2.3±1.0^c	83.7	±−+
$d_{0,7,14,21,28,35}$	2/14	8/14	2.1±1.5^c	93.3	0.5±0.7^c	96.5	−−+
$d_{0,14,28,42}$	5/13	8/13	1.4±1.5^c	95.6	0.5±0.7^c	96.5	−−+
$d_{0,21,28,35}$	6/17	10/17	1.9±2.6^c	94.0	0.6±1.1^c	95.7	−−+
Control	0/20	0/20	29.0±7.0	—	9.8±2.8	—	+−++
$d_{7,8,9}$	0/14	0/14	31.4±8.0^a	—	10.0±3.7^a	—	+−++
$d_{7,14,21}$	0/15	0/15	23.5±3.9^b	18.4	8.5±2.1^a	13.3	+−++
$d_{7,21,35}$	2/16	7/16	4.1±3.5^c	85.8	0.7±0.9^c	92.9	±−+
$d_{7,28,49}$	1/12	10/12	3.3±2.3^c	88.5	0.2±0.4^c	98.0	±−+
$d_{14,15,16}$	0/16	0/16	28.0±7.1^a	2.8	9.3±3.5^a	5.1	+−++
$d_{14,21,28}$	0/16	0/16	11.9±3.6^c	58.7	3.2±1.8^c	67.3	+−++
$d_{14,28,42}$	2/16	12/16	3.2±2.9^c	88.9	0.3±0.4^c	96.9	±−+
$d_{14,35,56}$	6/16	12/16	1.6±1.7^c	94.4	0.3±0.4^c	96.9	±−+
$d_{21,22,23}$	0/15	5/15	8.7±4.8^c	69.8	1.7±1.6^c	82.7	±−+
$d_{21,28,35}$	2/17	12/17	4.4±5.4^c	84.7	0.5±1.0^c	94.9	−−+
$d_{21,35,49}$	8/17	14/17	1.6±2.6^c	94.4	0.2±0.4^c	98.0	−−+
$d_{21,42,63}$	12/17	17/17	0.6±1.1^c	97.9	0^c	100.0	±−+

WRR: worm reduction rate; FWRR: female worm reduction rate.
−: normal; ±: normal color, needle point-like egg tubercles seen occasionally in 1—2 lobes of the liver; +: light red color, egg tubercles larger than needle point dispersed on the whole liver surface; ++: marked red color, egg tubercles fused together with millet-like in size distributed extensively on the liver surface.

and half of the mice being free from ♀ worms (Tab 1).

In mice treated ig with Pra 500 mg·kg^{-1} daily for 3 d started 14 d after infection, no apparent effect was found. When Pra was given weekly for 3 wk started on d 14, the average numbers of total worms and ♀ worms were significantly lower than those of the control with a ♀ worm reduction rate of 67.3%. In other 2 groups, the 2nd and 3rd doses of Pra were given at 2—3 wk intervals following the first dosing started on d 14, the average numbers of total and ♀ worms were less than those of the above-mentioned 2 groups with the same ♀ worms reduction rates of 96.9%. Nevertheless, 75% of mice treated in these 2 groups were free from ♀ worms (Tab 1).

When mice were treated ig with Pra at 3 wk after infection at 500 mg·kg^{-1}·d^{-1}×3 d, the total and ♀ worm reduction rates were 69.8% and 82.7%, respectively with one-third of mice without ♀ worms. In mice treated ig with Pra 500 mg·kg^{-1} once a week for 3 wk started on 3 wk after infection, the average numbers of total and ♀ worms were less than those of the above-mentioned group and the ♀ worm reduction rate was 94.9%. When Pra was given once every 2—3 wk for 3 times, the total worm reduction rate was still higher and the ♀ worm reduction rate was 98—100%. No apparent difference in the average number of ♀ worms was seen between the groups treated with Pra once every 1 or 2 wk (Tab 1). In groups treated ig with Pra at 1 or 2 wk intervals, most of the livers were soft and red in color and only 1/6 of them showed sparse fine egg tubercles. The livers in the group treated with Pra at 3 wk intervals were slightly harder with some fibrous egg tubercles (Tab 1).

Effect of various doses At 3 wk after infection the mice were treated ig with Pra 300 or 500 mg·kg^{-1}. Afterwards, the same dose of Pra was given weekly for twice. The total and ♀ worm reduction rates of 300 mg·kg^{-1} group were 70.8% and 80.8%, respectively, but the average numbers of total and ♀ worms in this group were significantly higher and the numbers of mice without ♀ worm or cured were less than those in the 500 mg·kg^{-1} group. In the 300 mg·kg^{-1} group, about half of the mice showed normal livers, while others manifested some larger egg tubercles in the dark red livers. In the 500 mg·kg^{-1} group, most of the mice showed normal livers apart from some fine egg tubercles in a few of mice. When the 2nd and the 3rd doses of Pra 300 mg·kg^{-1} were given to the mice at 3 wk intervals after the first dosing started on 3 wk after infection, the average numbers of total and ♀ worms were still higher than those of the 500 mg·kg^{-1} group. In this group, 9/13 mice were free from ♀ worm with a total worm reduction rate of 96.2% and the gross pathological changes of the liver were similar to those of the 500 mg·kg^{-1} group (Tab 2).

In another experiment, mice were treated ig with Pra 300 mg·kg^{-1} at 1—3 intervals and a single dose (200 mg·kg^{-1}) of artemether (Art) was given on d_7 after infection. No apparent increase of efficacy was noted. On the other hand, when a dose of Pra was added in the regimen of d_{21}, d_{28}, d_{35}, and given on d_{42} after infection, the efficacy of Pra increased significantly, as shown by the finding that ♀ worm reduction rate reached 98.6% and ♀ worms disappeared in 13/17 mice (Tab 2).

Alternative administration of Pra and artemether When mice were treated ig with Pra 500 mg·kg^{-1} or Art 300 mg·kg^{-1} on d_0 and d_7, respectively, the average numbers of total worms in these 2 groups were similar, but less than that in the control. In mice treated ig with Pra on d_0 and Art on d_7 or d_{14},

Tab 2. Mice infected with *Schistosoma japonicum* cercariae and treated with ig praziquantel (Pra) or in combination with artemether (Art) ig on different days after infection. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs the corresponding ▲ group.

Day of medication	Drug dose ($mg\cdot kg^{-1}$) Art	Pra	Mice cured	Mice without ♀ worms	Total worms	WRR/%	Female worms	WRR/%	Liver alteration
Control	0	0	0/20	0/20	24.0±8.0	—	10.4±3.4	—	+−++
$d_{21,28,35}$	0	300	2/16	6/16	7.0±6.4[c]	70.8	2.0±2.2[c]	80.8	±−++
$d_{21,28,35}$▲	0	500	8/17	14/17	0.9±1.1	96.2	0.2±0.4	98.1	−−±
$d_{21,42,63}$	0	300	3/13	9/13	2.9±2.6[c]	87.9	0.4±0.7[b]	96.2	±−+
$d_{21,42,63}$▲	0	500	6/16	16/16	1.0±1.0	95.8	0	100	±−+
Control	0	0	0/13	0/13	28.0±11.0	—	13.9±4.3	—	+−++
d_7	200	0	4/16	6/16	3.4±3.0[c]	87.9	1.0±1.1[b]	92.8	−−+
$d_{21,28,35}$	0	300							
d_7	200	0	4/14	7/14	2.9±2.7[b]	89.7	0.9±1.1[b]	93.5	−−+
$d_{21,35,49}$									
$d_{21,28,35}$	0	300	3/16	6/16	4.2±3.5[c]	85.1	1.6±1.5[c]	88.5	−−++
$d_{21,35,49}$	0	300	3/16	5/16	4.6±4.2[c]	83.6	1.5±1.7[c]	89.2	−−+
$d_{21,28,35,42}$▲	0	300	8/17	13/17	0.9±1.1	96.8	0.2±0.4	98.6	−−±

WRR: worm reduction rate; FWRR: female worm reduction rate.
−: normal; ±: normal color, needle point-like egg tubercles seen occasionally in 1−2 lobes of the liver; +: light red color, egg tubercles larger than needle point dispersed on the whole liver surface; ++: marked red color, egg tubercles fused together with millet-like in size distributed extensively on the liver surface.

no apparent increase in efficacy was seen. When mice were treated ig with Art on d_7 and Pra on d_{21}, less average numbers of total and ♀ worms were obtained, but the difference was not significant *vs* the group treated with either Pra or Art alone (Tab 3).

Tab 3. Effects of praziquantel (Pra) combined with artemether (Art) given alternately to mice on different days after infection with *Schistosoma japonicum* cercariae. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs ▲ group.

Day of medication	Drug dose ($mg\cdot kg^{-1}$) Art	Pra	Mice cured	Mice without ♀ worms	Total worms	WRR/%	Female worms	FWRR/%
Control	0	0	0/20	0/20	31.5±8.9	—	14.2±4.6	—
d_0	0	500	0/20	4/20	6.2±4.4[a]	80.3	2.3±1.9[a]	83.7
d_7	300	0	0/19	0/19	9.5±4.4[b]	69.8	3.2±2.0[a]	77.3
d_0▲	0	500	0/20	4/20	4.6±2.8	85.4	1.6±1.1	88.7
d_7	300	0						
Control	0	0	0/15	0/15	38.1±5.3	—	17.2±2.7	—
d_0	0	500	0/10	0/10	10.9±7.6[a]	71.4	3.6±3.0[a]	79.1
d_7	300	0	0/10	0/10	10.7±4.5[a]	71.9	3.5±1.9[a]	79.7
d_0▲	0	500	0/10	0/10	9.3±4.2	75.6	3.2±1.7	81.4
d_7	300	0						
d_0	0	500	0/10	0/10	9.1±4.8[a]	76.1	4.2±2.6[a]	75.6
d_{14}	300	0						
d_7	300	0	0/8	0/8	7.5±5.3[a]	80.3	2.3±1.2[a]	86.8
d_{21}	0	500						

Effect of Pra on egg production of d_{21} ♀ worm When mice were treated ig with Pra 500 mg·kg^{-1} on d_{21} after infection, 3 to 7 d later, the ♀ worms showed apparent shrinkage in size, depigmentation of the intestine, degeneration of the vitelline gland, atrophy of the ovary, and disappearance of eggs in uterus. In d_{28} control ♀ worms, eggs were seen in all of the worms examined with an average number of 84±47/♀ worm. On d 14 after treatment, 21/34 ♀ worms (61.8%) examined showed no egg in their uteri or no apparent recovery of their damaged reproductive glands. The other 13 ♀ worms showed recovery of their reproductive glands in various degrees and the eggs were found in the uterus with an average number of 40±45/♀ worm, which was significantly less than that of d_{35} control ♀ worm with an egg reduction rate of 80.4%. On 21 d after treatment, most of the residual ♀ worms showed apparent recovery of their reproductive glands with an average egg number of 93±55/♀ worm, which was reduced to 54.5% of that found in the control ♀ worms (Tab 4).

Tab 4. Egg production of ♀ worms harbored in mice infected with *Schistosoma japonicum cercariae* for 21 d and treated with ig praziquantel at a single dose of 500 mg·kg^{-1}. $\bar{x}\pm s$. $^{c}P<0.01$ *vs* **35-d control.** ^{d}*vs* **28-d control.**

Days after treatment	female worms	♀ worms without egg in uterus	Egg number	Egg reduction rate/%
3	11	11	0	—
7[d]	17	17	0[c]	100.0
14	34	21	40±45[c]	80.4
21	20	0	93±55[c]	54.4
21-d control	10	10	0	—
28-d control	19	0	84±47	—
35-d control	17	0	204±69	—

DISCUSSION

Although higher total and ♀ worm reduction rates were seen when a single dose of Pra was given to mice on d_0, less efficacy was found in rabbits treated with Pra on the same time after infection (To be published). In view of the above-mentioned results and the inaccessibility of delivery of the drug to the pilot area on the day of infection, the regimen has not been further studied.

When mice were treated ig with Pra on d_7, or d_{14} after infection at a daily dose for 3 d, none or only a slight effect was seen. The same was true when the same dose of Pra was given weekly. However, if the second and the third doses were given at 2—3 wk intervals, higher ♀ worm reduction rates were seen. Since d_7 and d_{14} schistosomula were unsusceptible to Pra[2], only the drug given on d_{28}—d_{56} might display the lethal effect on adult worms, ie. the ♀ worms would mature and lay eggs before they were affected by the drug. Therefore, in spite of higher ♀ worm reduction rate and about half of the animals treated were free from ♀ worms, the liver of each mouse still showed traces of damage caused by eggs.

Among the regimens tested, the most promising one was that Pra was given initially to the host on d_{21} after infection, followed by the repeated dosing at 1—2 wk intervals for 2—3 times. With this early treatment regimen the average numbers of total and ♀ worms, and the number of mice without ♀ worm were somewhat less than those in 3 wk group. Although all mice were free from ♀ worm in the latter group, traces of gross pathological changes of the liver induced by eggs was seen in each mouse. On the contrary, in 1 and 2 wk group traces of egg tubercles could hardly be found in the liver in most of the mice with

an apperance similar to that of the non-infected mice. the study on the effect of Pra on egg production of d_{21} ♀ worm noted that the oviposition of ♀ worms was inhibited significantly or even completely during the 2 wk after treatment, indicating the rationality of above-mentioned recommended regimens.

A previous paper indicated that combined Pra and Art did not show any synergetic effect even at higher doses and with longer treatment courses[4]. Since d_7, and d_0, d_{21} schistosomula were more susceptible to respective Art and Pra while given separately[4], the 2 drugs were then given alternatively. Using this regimen, the efficacy only increased negligibly and it seemed meaningless to use this regimen in practice.

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用吡喹酮早期治疗小鼠的血吸虫感染

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摘要 小鼠于感染血吸虫尾蚴后不同时间给予首剂吡喹酮(Pra) 300—500 mg·kg^{-1}，然后每隔1—3 wk ig 1次相同剂量的Pra，共给2—3次，并根据残存虫数和肝脏变化评价疗效. 结果认为宿主自感染后d_{21}开始用Pra治疗，每隔1—2 wk给药1次，共给2—3次时，可杀灭宿主体内绝大部分或全部♀虫，从而达到保护宿主或降低宿主感程度的目的.

关键词 日本血吸虫；血吸虫童虫；吡喹酮；蒿甲醚；联合药物疗法

《第四届全国生物医药色谱学术会议》通知

中国化学会色谱委员会和中国色谱学会决定于1994年9月23—27日在西安召开《第四届全国生物医药色谱学术会议》，通知如下：

1 征文内容：液相色谱、气相色谱、毛细管色谱、薄层色谱、超临界流体色谱、毛细管电泳等在生物医药学方面的理论、有关技术及其应用. 已在全国性会议上及刊物上发表过的论文，请勿提交.

2 征文截止日期：1994年3月31日(以邮戳日期为准).

3 征文要求：应征文稿请写成1500字以内的详细摘要(包括必要的数据、图表). 字迹应清晰工整，有绘图纸按正式出版物要求绘制插图，一式三份. 挂号邮寄至：北京大学化学系爱今收，邮编：100871. 未录取稿件不予退回. 会议期间同时举行产品展销会，欢迎有关厂商参展.