

吡喹酮对日本血吸虫感染小鼠和兔的疗效与体液免疫水平的关系

乐文菊、肖树华、梅静艳 (中国预防医学科学院寄生虫病研究所¹, 上海 200025, 中国)Relationship between the effects of praziquantel on mice and rabbits with different intensities of *Schistosoma japonicum* infection and humoral immunity levels of the host

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ABSTRACT Rabbits were infected with 48-52, 198-202 and 498-502 cercariae of *Schistosoma japonicum* and treated ig with single doses of praziquantel (40 mg/kg) 4 and 8 wk post infection. The worm reduction rates of the 8 wk groups were 87.9, 92.2 and 97%, respectively. These values were significantly higher than 48.5, 52.3 and 58.6% for the corresponding 4 wk groups. The serum antibody titres of the 8 wk post infection rabbits were apparently higher than those of the 4 wk rabbits. Meanwhile, for the same duration of infection, the specific antibody levels in the heavy worm burden groups were also higher than those in the light worm burden groups. The results obtained in the mice infected with *S. japonicum* at different intensities and treated ig with single doses of praziquantel at 300 mg/kg at different durations were similar to the results in the rabbits. The correlation between efficacy and specific antibody level was confirmed by calculating the coefficient of correlation with r values of 0.454-0.983 ($P < 0.01$).

The results also indicated that heavy infections with *S. japonicum* were still more responsive to praziquantel treatment. In addition, the effect of praziquantel on worms was closely related to the specific

antibody response in the host.

KEY WORDS praziquantel; *Schistosoma japonicum*; antibody formation

摘要 小鼠和兔感染不同感染度的日本血吸虫后, 在不同时间分别 ig 吡喹酮治疗, 疗效以感染后 4 wk 组为最差。小鼠感染后 5-7 wk 和兔感染后 8 wk 的疗效均明显增加。这一结果与宿主特异性抗体的增长相一致。重感染动物的体液抗体滴度较高。认为吡喹酮的抗虫作用除药物的直接作用外, 与宿主体内特异性抗体水平的高低亦有密切关系。

关键词 吡喹酮, 日本血吸虫, 抗体形成

临床研究指出, 用吡喹酮治疗感染度较重地区的日本血吸虫病患者的疗效逊于感染度较轻地区的⁽¹⁻³⁾, 这一差异究竟是因药物对不同虫负荷患者的疗效不同, 抑或是由于在感染较重地区内受治患者易于重复感染, 是值得探讨的一个问题。本文介绍吡喹酮治疗不同感染度的小鼠和兔血吸虫病的结果, 借作临床参考。

MATERIALS AND METHODS

吡喹酮由本所合成, 用 1% 西黄蓍胶配制成 20, 30 和 40 mg/ml 的混悬液。昆明系小鼠体重 $20.3 \pm SD 1.5$ g 或 NIH 系小鼠 20.1 ± 1.4 g (均由本所繁殖); 新西兰种兔 (购自昆山种兔场) 2.7 ± 0.2 kg。均♀♂兼用。

疗效试验 昆明系小鼠经腹部皮肤分别感

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染 23-27, 48-52 或 98-102 条血吸虫尾蚴, 于感染后 d 33 单次 ig 吡喹酮 400 mg/kg 治疗; NIH 系小鼠感染 23-27 或 73-77 条血吸虫尾蚴, 分别于感染后 4, 5, 6 和 7 wk 单次 ig 吡喹酮 300 mg/kg。兔感染 48-52, 198-202 或 498-502 条血吸虫尾蚴后 4 和 8 wk 单次 ig 吡喹酮 40 mg/kg。上述动物于治毕后 4-5 wk 解剖, 根据残存虫数评价疗效。

宿主免疫水平的测定 上述两种感染度的 NIH 系小鼠和 3 种感染度的兔, 于吡喹酮治疗前, 小鼠于摘除眼球放血 (每组 5 鼠) 和自兔耳缘静脉滴血 (每组 6-7 兔) 2-3 ml, 分离血清, 应用环卵沉淀试验 (COP)⁽⁴⁾, 胶乳凝集试验 (LA)⁽⁵⁾ 和间接血凝试验 (IHA)⁽⁶⁾ 等方法测定宿主的体液免疫水平。COP 按常规法进行。LA 所用共价结合的日本血吸虫卵胶乳抗原由本所与上海市医学化验所合作生产, 批号 86311。血清用生理盐水作倍比稀释, 起始稀释度为 1:10, 若出现凝集, 即为阳性。IHA 的致敏红细胞由中国人民解放军 163 医院生物诊断制品研究室提供, 批号 85014。取血清作 1:5 稀释, 出现 1+ 反应, 即作为阳性。

RESULTS

吡喹酮治疗小鼠血吸虫病的疗效 小鼠感染 25, 50 和 100 条血吸虫尾蚴后 d 33 单次 ig 吡喹酮 400 mg/kg 治疗, 每鼠平均虫数依次为 5 ± 3 , 11 ± 6 和 18 ± 12 条, 与相应对照组比较, 减虫率分别为 62, 68, 74%。即药物对不同感染度小鼠的疗效, 差别不明显 ($P > 0.05$) (Tab 1)。吡喹酮 300 mg/kg 单次 ig 治疗感染 5 wk 的 25 和 75 条小鼠, 每鼠平均虫数分别为 4 ± 3 和 6 ± 5 条, 均明显低于 4 wk 治疗组 ($P < 0.01$), 其中 25 条组 6 和 7 wk 治疗组的每鼠平均虫数又明显低于 5 wk 治疗组 ($P < 0.01$) (Tab 1)。上述两种感染度小鼠于感染后 4 wk 用 COP 和 LA 检测时, 已呈阳性反应; 感染后 5-7 wk, 小鼠体液的抗体滴度增高 (Tab 2)。

Tab 1. Effects of praziquantel on mice with different intensity of *Schistosoma japonicum* infection and at different periods post infection $\bar{x} \pm SD$. *** $P < 0.01$ vs corresponding control group, † $P > 0.05$, †† $P < 0.01$ vs 7-wk group infected with 25 cercariae, or 5-wk group infected with 75 cercariae. %: worm reduction rate.

Cer-cariae	Time after infection	Prazi-quantel (mg/kg)	Mice	Worms	%
25	33 d	0	13	13 ± 4	
25		400	18	$5 \pm 3^{***}$	62
50	33 d	0	13	34 ± 6	
50		400	18	$11 \pm 6^{***}$	67
100	33 d	0	17	69 ± 14	
100		400	13	$18 \pm 12^{***}$	73
25		0	35	13 ± 5	
25	4 wk	300	23	$8 \pm 4^{\dagger\dagger}$	34
25	5 wk	300	28	$4 \pm 3^{\dagger\dagger}$	67
25	6 wk	300	26	$0.8 \pm 1.4^{\dagger}$	94
25	7 wk	300	21	1.0 ± 1.3	92
75		0	29	42 ± 10	
75	4 wk	300	24	$20 \pm 10^{\dagger\dagger\dagger}$	52
75	5 wk	300	15	6 ± 5	86

Tab 2. Antibody reaction level of mice at various periods after being infected with *S. japonicum* cercariae. COP = circumoval precipitin test, LA = latex assay

Number of cercariae	Weeks after infection	Ova showing precipitates (%) (COP)	Reciprocal of serum titre (LA)
25	4	25	160-320
25	5	30	160-320
25	6	42	1280-2560
25	7	41	160-5120
75	4	28	160-640
75	5	40	160-1280

吡喹酮治疗兔血吸虫病的疗效 吡喹酮 40 mg/kg 单次 ig 治疗 50, 200 和 500 条感染兔, 4 wk 治疗组每兔平均虫数依次为 17 ± 4 , 61 ± 27 和 138 ± 52 条, 明显高于 8 wk 治疗组 ($P < 0.01$)。在感染后相同时间内, 药物对 3 种不同感染度兔的疗效, 均无明显差别 ($P > 0.05$) (Tab 3)。兔于感染 4 wk 后, 用 COP,

Tab 3. Effects of praziquantel 40 mg/kg ig on rabbits with different intensity of *S. japonicum* infection and at different periods post infection. $\bar{x} \pm SD$. *** $P < 0.01$ vs corresponding 8-wk group.

Cercariae wk	n	Worms	%	Rabbits without ♀ worm
50	0	9	33 ± 8	0
50	4	9	17 ± 4***	48
50	8	10	4 ± 3	88
200	0	11	128 ± 21	0
200	4	11	61 ± 27***	52
200	8	12	10 ± 9	92
500	0	11	333 ± 50	0
500	4	12	138 ± 52***	59
500	8	11	10 ± 10	97

wk, weeks after infection; n, rabbits;
%, worm reduction rate

Tab 4. Antibody reaction level of rabbits at various periods after being infected with *S. japonicum* cercariae. PRN = positive rabbit number; PESP = % of ova showing precipitates; RST = reciprocal of serum titre.

Number of cercariae	Wk after infection	Circumoval PRN	precipitin test PESP	Latex assay PRN	RST	Indirect haemoagglutination PRN	RST
50	4	6/6	1-3	4/6	20-40	5/6	20-160
50	8	7/7	64-74	7/7	80-320	7/7	160-5120
200	4	6/6	2-22	6/6	20-40	6/6	40-160
200	8	6/6	62-76	6/6	160-1280	6/6	1280-20480
500	4	3/6	1-3	5/6	20-80	5/6	10-40
500	8	7/7	64-84	7/7	320-2560	7/7	1280-40960

Tab 5. Relationship between the effects of praziquantel 40 mg/kg ig on rabbits with *S. japonicum* infection and antibody reaction level of the host. n = 34, *** $P < 0.01$.

Cercariae wk	Ova showing precipitates (COP)	Reciprocal of serum titre (LA)	(IHA)	Worm reduction rate (%)	
50	4	2.2 (1-3)	26 (10-40)	100 (20-160)	48 (38-66)
50	8	68.3 (64-74)	173 (80-320)	2160 (160-5120)	88 (72-100)
200	4	7.7 (2-22)	33 (20-40)	57 (20-160)	52 (27-75)
200	8	70.0 (62-76)	480 (160-1280)	9287 (1280-20480)	92 (88-100)
500	4	1.3 (1-3)	48 (20-80)	24 (10-40)	59 (47-72)
500	8	74.9 (71-84)	1173 (320-2560)	14467 (1280-40960)	97 (93-99)
		r = 0.983***	r = 0.500***	r = 0.454***	

wk = weeks after infection, COP = circumoval precipitin test, LA = latex assay, IHA = indirect haemoagglutination, r = correlation coefficient with the therapeutic effect.

LA 和 IHA 检测时, 50, 200 和 500 条 3 组实验兔体内的抗体大部分呈阳性反应, 但血清滴度不高; 感染 8 wk 后, 3 种感染兔的血清抗体皆呈阳性反应, 且抗体滴度增高, COP 的环沉率, LA 和 IHA 阳性反应所需的血清滴度, 均随感染兔的虫负荷增加而增高, 即抗体的效价与感染度成正比 (Tab 4)。用统计学检测不同感染度兔用 3 种方法测定的抗体含量或滴度与药物对相应的受治兔的疗效的相关系数, r 值依次为 0.983, 0.500 和 0.454 ($n = 34$), 差别明显 ($P < 0.01$), 表示其间的关系显著 (Tab 5)。

DISCUSSION

临床上用药物治疗血吸虫病患者, 其疗效受多种因素的制约, 特别是患者虫负荷的多寡以及是否有重复感染的可能性。本文结果表

明, 感染度不同的小鼠或兔, 于感染后相同时间内, 用相同剂量的吡喹酮治疗, 减虫率均无明显差别, 充分说明, 至少在感染度相差10倍的情况下, 并不影响吡喹酮的疗效。因此, 在血吸虫感染较重的流行地区用吡喹酮治疗时, 疗效较差并非由于宿主的虫负荷量, 而很可能是重复感染的结果。

近年来的研究结果指出, 体外试验中, 在有免疫血清存在时, 吡喹酮杀血吸虫的作用强度明显增加^(7,8), 另一方面, 吡喹酮对缺乏抗体的感染小鼠的疗效明显降低, 对该鼠被动输入免疫血清后再用吡喹酮治疗, 则杀虫作用明显增加⁽⁹⁾, 同时, 用吡喹酮相同的剂量治疗同一感染度的兔血吸虫病, 其疗效以感染时间较长、宿主抗体滴度较高者为较好⁽¹⁰⁾。本文进一步用吡喹酮治疗不同感染度小鼠和兔血吸虫病的结果表明, 感染度不同的宿主在感染后的相同时间内, 其抗体水平随感染度的加重而增加, 而药物对轻度和重度感染动物的疗效则无明显差别, 再次表明, 吡喹酮的杀虫作用与宿主抗体的水平高低密切相关。因此认为, 吡喹酮治疗血吸虫病的疗效在很大程度上受宿主抗体水平的制约。

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