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Susceptibility of various animals to *Pneumocystis carinii* infection

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Abstract: *Pneumocystis carinii* (Pc) is an important opportunistic pathogen of immune compromised hosts, and is known to infect various animals. The present study observed the infection status of 6 mammals and 3 strains of albino rats with Pc after suppression of their immunity. Methyl-prednisolone was injected once a week and tetracycline was supplied with water for 5 to 21 weeks. Hamsters, guinea pigs, rabbits, dogs, cats and pigs were negative by impression smear, and only the rats were found infected by Pc. All of the three strains of rats, Sprague-Dawley(SD), Wistar(W) and Fisher(F), were infected by Pc but W rats showed heavier degree of infection in earlier period than F or SD rats. The present findings suggest that W rat is the best among the animals used in the present study for production of Pc.

Key words: *Pneumocystis carinii*, albino rats, Wistar, Fisher, Sprague-Dawley, mammals, susceptibility

INTRODUCTION

The most serious hindrance on researches of *Pneumocystis carinii*(Pc) is the difficulty to get enough purified organisms. Various trials of *in vitro* cultivation of Pc showed only 10 fold growth on cultured cells(Cushion *et al.*, 1985) and also found low yield of Pc organisms in broth media(Blumenfeld and Griffiss, 1989; Cushion and Ebbets, 1990). Since the cultivation products are not satisfactory, the organisms are now supplied only from the animals experimentally suppressed of their immunity(Walzer and Young, 1984). Most of the studies have used rats for the animal model of Pc production (Walzer *et al.*, 1989; Hong *et al.*, 1992a). However, *in vivo* production of Pc has some demerits. At first, contamination with other microorganisms is inevitable even in negligible amount. Also contamination of the purified Pc preparation with host protein or DNA makes the results difficult to interpret. Furthermore purification of a specific stage of Pc is impossible because the trophic form and the cystic form are always mixed in the host lungs and no method is available at present to exclude one form completely. Additionally, maintenance of the animal colonies drains a major part of research expenses.

Induction of Pc infection in rats is a combined measure of corticosteroid injection, low protein diet and antibiotic medication(Walzer *et al.*, 1984). The measure is also effective to other animals such as rabbits(Yamada *et al.*, 1984; Soulez *et al.*, 1988), guinea pigs(Yamada *et al.*, 1984) and mice(Powles *et al.*, 1992).

Establishing an efficient animal model helps the research on Pc upgrade the quality and quantity. The large animals have the larger lungs than rats, and eventually produce more organisms. The present study intended to screen the susceptibility of several mammals to Pc infection, and also to observe the strain

difference of albino rats.

MATERIALS AND METHODS

1. Induction of *P. carinii* infection in mammals

Table 1 summarizes the scheme of animals used for experimental induction of Pc infection. The animals were injected of methyl-prednisolone (Depomedrol®, Korea Upjohn Ltd.) of 2 to 10 mg/kg doses. Tap water mixed with ampicillin 1 mg/ml was supplied without limitation. Six animals other than rats, and 3 strains of albino rats, Sprague-Dawley(SD), Fisher(F) and Wistar(W), were included for the present experiment.

2. Determination of intensity degree of Pc infection

The animals were anesthesized with ether, and the lungs were isolated. Three impression smears were made from the lungs of an animal, and stained in Diff Quik solution. The smears were microscopically screened and the intensity of infection was determined by the number of cysts in 20 high power fields; no cyst was graded as 0, 1 to 9 cysts as 0.5+, 10 to 19 as 1+, 20 to 99 as 2+, 100 or more as 3+. Grade 0 is equivalent with less than 10^4 nuclei, 0.5+ with 10^5, 1+ with 10^6, 2+ with 10^{7-8}, and 3+ with 10^9 nuclei after purification from the whole lungs of a rat by the experience in our laboratory.

Table 1. Scheme of the immune suppression of various animals

Animals	No. of exam.	Dose(mg) of m-pd*		Duration (week)
		1-2wk	3-21wk	
Rat	318	4	2	—
Sprague-Dawley	136	—	—	13
Fisher	76	—	—	13
Wistar	106	—	—	10
Guinea pig	4	8	4	21
Hamster	5	8	4	7
Cat	3	30	15	8
Dog	3	30	15	6
Rabbit	4	12	6	10
Pig	2	300	150	13

* Methyl-prednisolone(Depomedrol®, Korea Upjohn Ltd.)

RESULTS

All of the animals other than rats; hamsters, guinea pigs, dogs, rabbits and pigs, were negative for Pc by impression smears. The rats were found infected by Pc from 2 weeks in F strain, and most of the 3 strains became infected from 5 weeks. The longer the duration of immune suppression, the more the organisms in the lungs(Table 2). However, the intensity of infection doesn't exactly correlate with the duration of immune-suppression, and a wide range of infection intensity was observed even in the rats of the same group. The intensity of infection fluctuated severely especially in SD rats. As a statistic analysis, Mantel-Haenszel (MH) chi-square test adjusting the week of immune-suppression was applied to assess association between intensity of infection and the rat strain. The test showed the difference in the infection intensity by the rat strains was significant in general, and most significant in weeks from 5 to 8. In weeks from 1 to 4 and from 9 to 13, too many cells were 0 in number, and thus the MH test was not valid.

DISCUSSION

In Korea, Pc is commonly prevalent among the experimental rats (Hong *et al.*, 1992a & b), and also seems to be actively transmitted among humans(Hong, 1991). Nonetheless, establishment of the animal model with larger mammals than rats for Pc infection fails through the present study scheme using guinea pigs, hamsters, dogs, cats, rabbits and pigs. The rabbits, guinea pigs and ferrets were already found to be experimentally infected by Pc(Yamada *et al.*, 1984; Soulez *et al.*, 1988). Matsumoto(1987) suggested that the monkeys were naturally infected and therefore they could also be a candidate of the experimental model.

Table 2. Infection of *P. carinii* in the rats by the week of experiment

Strains	Intensity of* infection	No. of rats at the weeks of immune suppression**													Total (%)
		1	2	3	4	5	6	7	8	9	10	11	12	13	
Sprague-Dawley	0	0	0	10	0	9	17	0	1	6	0	4	3	0	50(36.8%)
	0.5+	0	0	0	1	7	5	2	1	3	0	6	0	0	25(18.4%)
	1+	0	0	0	2	12	4	2	1	4	0	4	5	4	38(27.9%)
	2+	0	0	0	0	4	1	0	1	4	0	2	2	2	16(11.8%)
	3+	0	0	0	0	0	0	0	0	1	2	2	0	2	7(5.1%)
	Subtotal	0	0	10	3	32	27	4	4	18	2	18	10	8	136(100%)
Fisher	0	0	3	3	3	0	0	0	1	0	0	0	2	1	13(18.3%)
	0.5+	0	2	0	0	0	0	2	6	1	0	0	5	2	18(25.4%)
	1.0+	0	0	0	0	0	11	0	3	0	0	0	2	2	18(25.4%)
	2.0+	0	0	0	0	4	1	0	1	4	0	2	2	2	16(22.5%)
	3.0+	0	0	0	0	0	0	0	4	0	2	0	0	0	6(8.5%)
	Subtotal	0	5	3	3	4	12	2	15	5	2	2	11	7	71(100%)
Wistar	0	5	3	5	0	9	5	0	0	2	0	0	0	0	29(27.4%)
	0.5+	0	0	4	2	1	0	0	0	1	1	0	0	0	9(8.5%)
	1.0+	0	0	3	1	10	2	2	0	6	0	0	0	0	24(22.6%)
	2.0+	0	0	0	0	5	6	3	2	4	0	0	0	0	20(18.9%)
	3.0+	0	0	0	0	4	4	5	4	2	5	0	0	0	24(22.6%)
	Subtotal	5	3	12	3	29	17	10	6	15	6	0	0	0	106(100%)

* Intensity of infection was graded by the number of cysts in 20 high power fields; 0, no cyst; 0.5+, 1 to 9 cysts; 1+, 10 to 19 cysts; 2+, 20 to 99 cysts; 3+, 100 or more cysts.

** $\chi^2=4.24$, df=4, not significant in 1 to 4 weeks; $\chi^2=51.0$, df=8, $p<0.01$ in 5 to 8 week; $\chi^2=16.0$, df=8, $p<0.05$ in 9 to 13 weeks; $\chi^2_{MH}=49.0$, df=8, $p<0.01$ in general.

Several points should be considered to interpret the low susceptibility of those mammals in this study. The first is the possibility of failure of immune-suppression. The dose of prednisolone ranged from 2 to 10 mg/kg and the duration was 5 to 21 weeks. Its therapeutic dose is 1 mg/kg in humans, but that may be differed by the animals. The successful report on the rabbits and guinea pigs used steroid 35 mg/kg and 25 mg/kg with low protein diet to deprive the immune response efficiently(Yamada *et al.*, 1984). The present study used only steroid in less doses to prevent unexpected death, but the rabbits, guinea pigs or pigs were looking emaciated and gained no body weight during the experiment which represented the steroid effect. However, we still hardly exclude the possibility of failed suppression of their immunity. It is necessary to convince that they were truely compromised of their immunity.

Second, we don't have any evidence whether those animals had latent infection of Pc in their lungs before the experiment. There was no neighboring severely infected animals which might be an important infection source(Hong *et al.*, 1992b). Also the exact mode of natural transmission among different animals is not yet known, and thus any conclusion related with this may be hasty. Induction of Pc infection to the animals with rich sources of Pc will give the answer.

The third plausible explanation is that the animals really have very low susceptibility to Pc infection. Walzer(1986) observed different infectivity of Pc between rats and hamsters, and discussed different host susceptibility. The hamster was suggested to have natural resistance to Pc infection even in severely immunocompromised condition. There have been no records on Pc infection of dogs, cats and pigs. They may be possibly resistant to Pc infection just like the hamster. However, since the numbers

of those animals are not enough to derive any conclusion in the present study, we can only find that injection of methyl-prednisolone with regular diet is not so good a regimen for Pc infection of guinea pigs, rabbits, dogs, cats and pigs.

The rat is the most common animal model for Pc infection(Walzer, 1986; Hughes, 1989; Hong *et al.*, 1992a). The present result shows that all of the 3 strains of albino rats are susceptible to Pc infection with a little difference by the strain. A significant difference was found in the intensity of infection between the strains. By the present data, the Wistar rat is the best animal model because of the earlier and severer infection than Sprague-Dawley or Fisher rats. The differed susceptibility of Pc by the strain of mice was also observed(Walzer *et al.*, 1977; Powles *et al.*, 1992).

The infection of Pc may depend upon both parameters, the susceptibility of the host and the infectivity of the organisms. Of course various hosts may be differently susceptible to Pc, and none is known for the variation of infectivity or virulence by strain of Pc. The karyotypes are suggestive enough that Pc is a group of genetically complexed organisms(Hong *et al.*, 1990, 1992a & b). Still whether the same Pc strain infects wide spectrum of host species or not is under debate. Therefore no answer is available to the question that Pc from rats can infect other mammals including humans. Further studies on the nature of Pc are strongly required.

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=국문초록=

카리니주폐포자충 감염에 대한 여러 포유동물 및 흰쥐 계통에 따른 감수성

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국내에서 카리니주폐포자충(*Pneumocystis carinii*, 이하 Pc) 연구에 적합한 동물모델을 개발하고자, 총 7종류의 실험동물(흰쥐, 기니픽, 햄스터, 개, 고양이, 토끼 및 돼지)에 methyl prednisolone(Depomedrol®, Korea Upjohn Ltd.)을 주사하여 면역억제를 유발하고 폐를 슬라이드 글라스에 문질러 도말표본을 만들었다. 도말표본을 Diff Quik 염색 후 광학현미경으로 관찰하여 Pc의 감염정도를 음성 (0), 0.5+, 1+, 2+ 및 3+의 5등급으로 구분하였다. 실험동물 중 흰쥐에서만 Pc 폐염이 유발되고 다른 동물에서는 Pc를 관찰하지 못하였다. 대부분의 동물은 전신쇠약의 증세를 보였으며, 대체로 면역억제 6주경부터 사망하기 시작하였다. 흰쥐의 경우 실험한 세 계통 모두에서 감염을 확인하였다. Sprague-Dawley 흰쥐의 경우 총 136마리 중 86마리(63.2%)가 Pc에 감염되었고, Fisher 흰쥐는 76마리 중 63마리(82.9%)에서, Wistar 흰쥐는 106마리 중 77마리(72.6%)에서 Pc 감염이 양성이었다. 감염정도와 시기에서도 차이가 있어서 Wistar 흰쥐에서 더 이른 시기에 높은 감염정도로 관찰되었다. 이상의 결과에 의하면 Pc 병원체를 얻기에는 흰쥐 세 계통 중 Wistar 흰쥐가 Pc 연구에 가장 적합한 실험동물이고 그외의 실험동물은 Pc 감염에 적합하지 않음을 확인하였다.

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