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Differences in susceptibility to infection with *Fasciola hepatica* between strains and sexes of the rat

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랫트의 스트레인과 성 간의 간질에 대한 감수성의 차이

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초 록 : 랫트는 간질에 높은 감수성을 나타내기 때문에 간질의 연구에 실험실 모델로 많이 이용된다. 그러나 간질의 피낭유충을 랫트에 감염시킨 다음 랫트의 분변에서 충란을 검출해보면 충란의 검출률은 100%에 훨씬 못미쳐서 실험의 설계에 어려움을 겪는 경우가 흔하다. 이 연구에서는 우리나라에서 많이 사육되고 있는 4가지 스트레인(Sprague-Dawley, Fisher 344, the spontaneously hypertensive rats 그리고 Wistar-Kyoto)의 암·수 랫트에 간질의 피낭유충을 경구적으로 접종하고 분변검사와 부검을 실시하여 감염여부를 확인하였다. 충체회수율은 랫트의 성과 스트레인 간에 유의성 있는 차이를 보였는데 Sprague-Dawley계의 랫트가 가장 높은 충체회수율을 보였으며 평균 충체회수율은 암컷보다 수컷에서 높았다. 이 연구의 결과는 간질에 관한 연구 특히 간질구충제의 효능을 검정하는 시험에 유용한 지침이 될 것으로 사료된다.

Key words : *Fasciola hepatica*, rats, strain, sex, susceptibility.

Introduction

The use of rats as a laboratory model in the study of *Fasciola hepatica* is well established. They survive the acute infection when given metacercariae(MC) and are economical to maintain in the laboratory, compared to other animal species such as cattle, sheep, and rabbits. During the 1960s in mamy laboratories the *F hepatica*-infected rat replaced the rabbit, especially in controlled tests on the anthelminthic efficacy of fasciolicides[1].

When the anthelminthic activity of fasciolicides, especially those effective compounds against adult *F hepatica*, is tested, a patency rate of 100% is considered desirable because this would result in a reduction in the number of experimental animals per dose group. The patency rate in the

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rat, however, does not reach 100% when they are artificially infected[2,3]. It was also found that there was a significant difference in the fluke burdens in different sexes and strains of mice[4,5] and rats[6,7] infected with *F hepatica*.

In the present communication, male and female rats of four different strains were experimentally infected with *F hepatica* MC and their susceptibility to infection was evaluated.

Materials and methods

Rats : Male and female rats of the spontaneously hypertensive rats(SHR), Sprague-Dawley(SD), Fischer 344(F 344), and Wistar-Kyoto(WKY) strains were kindly supplied by the Hallym Experimental Animal Center, Hallym University, Chunchon, Korea. These rats were inbred strains and they were approximately nine weeks old at the start of the experiment.

Infection : The methods used to culture *Lymnaea viridis* snails and to produce *F hepatica* MC were as described by Lee *et al*[8,9]. Each rat was infected *per os* with 10 MC by using 1ml plastic syringe with an intubation needle bent in the middle[10].

Egg counts : Starting five weeks after infection, the feces from each rat were examined three times a week, using Flukefinder™(Visual Difference, Moscow, ID, USA). Once the patency period was established, egg counts were carried out at weekly intervals.

Fluke recovery : All rats including the non-patent rats were killed by cervical dislocation at 21 weeks post-infection and their livers were examined for fluke recovery. Rats that died before patency were necropsied; the thoracic and abdominal viscera and cavities were examined and thoroughly rinsed with water to recover any worms in these sites.

Statistical analysis : An analysis of variance was performed on the number of flukes recovered from the rats that died before the termination of experiment or killed at 21 weeks post-infection. The factors incorporated in the model were strain, sex and interaction between strain and sex. Differences between the average fluke burden of rats of different strains and sexes were tested using the Student's *t*-test.

Table 1. Effect of sex and strain on susceptibility to *Fasciola hepatica* in the rat infected with 10 metacercariae each

Strain	Sex	Patency rate(%)	Number of flukes recovered from individual rats	Total	Mean
SHR	M(8)	100.0	4,4[a],2,2,1,1,1,1,	16	2.0
	F(7)	57.0	3,2,2,1,,0,0,0	8,	1.1
SD	M(9)	88.9	5.4.4[b],3,3,2,2,1,1,	25	2.8
	F(6)	16.7	1,0,0,0,0,0,	1	0.2
F344	M(10)	30.0	2,1,1,0,0,0,0,0,0,0	4	0.4
	F(8)	25.0	3,3,0,0,0,0,0,0	6	0.8
WKY[c]	M(7)	0	3,3,3,2,2,2,2	17	2.4
	F(8)	0	3,2,2,2,1,0,0,0	10	1.3

[a]Died at 11 weeks after infection.
[b]Died at 7 weeks after infection.
[c]All rats died between 4 and 8 weeks after infection except the 3 rats.

Results

The patency rates of each strain and the number of flukes recovered from individual rats are shown in Table 1. The prepatent period in SHR, SD and F344 strains was very consistent, ranging from eight to nine weeks. Although not significant, the patency rates of the three strains were higher in males than in females. All rats of WKY strain died before they became patent except three rats, which were non-patent and survived until the termination of the experiment.

Statistical analysis showed that there was a significant difference in the fluke burdens between both sexes($p < 0.001$) and strains($p < 0.01$). In addition, there was a significant interaction between sex and strains($p < 0.01$). The overall fluke burden was higher in male rats than in female rats. Investigation of the interaction between sex and strain revealed that the difference between sexes was cofined to rats of SD and WKY strains. The average fluke buren of F 344 rats was significantly lower than that of the other three strains of rats.

Discussion

The rats, which were patent, discharged *F hepatica* eggs in the feces consistently and the eggs per gram of feces

reached its peak at about 18 weeks after infection[1]. The adult flukes were recovered from the bile ducts of all the rats; there was a marked overlap in sizes of the flukes. The patency rates in the three rat strains and overall fluke burdens in all rat strains were higher in males than in females. There was also a difference in fluke burdens between rat strains (Table 1).

Dobson[11] has shown that the sex of the rat can influence *Nematospiroides dubius* infections, with the males harbouring more parasites than the females. It was also found that more *F hepatica* developed to maturity in male rats than in females[6,7]. One of the authors[12] infected male and female SD rats eight weeks old with 10 MC each and attained patency rates of 80.9 and 52.3%, respectively. The results in the present study support these previous findings.

Rats are known to be highly susceptible to infection with *F hepatica*[1,13]. However, unlike cattle or goats, the natural hosts of the parasite[14,15], rats rarely show a patency rate of 100% when infected experimentally with *F hepatica* MC[3,12]. In the present study all rats of WKY strain died before the patency period was established. Overall patency rate of the other three rat strains was 52.9%, with males showing higher rates. Chun and Min[3] obtained a patency rates of 73.3% when they infected male SD rats weighing 150g with 10 MC each. These results together with the higher fluke burden in males indicate that male rats are better to get higher patency rate with the fluke.

The anthelminthic acitivity of new fasciolicides cannot always be tested in the parasite's natural hosts, e.g. sheep and cattle. Thus, model infections have to be used. When rats and mice are used as a laboratory model in the study of *F hepatica* it is not possible to get an infection rate of 100%[2,5,16]. The aninals which survived the acute infections with the parasite are later found to be free from infection. In the present study we deterimined the patency rates of males and females of four different rat strains. Known patency rates of the rats would help design the future experiments.

Conclusion

Male and female rats of four different strains(SHR, SD, F 344, ad WKY) were experimentally infected with 10 *Fasciola hepatica* metacercariae each, and their susceptibility to infection was determined. There was a significant difference in the fluke burdens between both sexes and strains. The overall fluke burden was higher in male rats than in females.

Investigation of the interaction between sex and strain revealed that the difference between sexes was confined to rats of SD and WKY strains. The average fluke burden of F 344 rats was significantly lower than that of the other three strains of rats.

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