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Susceptibility of experimental animals to reinfection with *Clonorchis sinensis*

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Abstract: The present study observed the resistance to reinfection with *Clonorchis sinensis* in various experimental animals including mice, guinea pigs, rabbits, and dogs, as well as rats and hamsters. The resistance rates to reinfection in rats, mice, hamsters, guinea pigs, rabbits, and dogs were 79.7%, 58.0%, -12.6%, 54.8%, 62.6%, and 6.0%, respectively. Worms recovered from reinfected rats and mice were immature, and significantly smaller than those from the primarily infected ($P < 0.01$), whereas those from other animals were fully matured to adults. These findings indicate that the protective response against reinfection with *C. sinensis* is prominent in rats and mice, and that they may be a good animal model to investigate the mechanism of resistance to reinfection with *C. sinensis*.

Key words: *Clonorchis sinensis*, liver fluke, reinfection, resistance, rats, mice

Based on epidemiological studies, humans are known to be repeatedly infected with *C. sinensis* by frequent consumption of raw freshwater fish in endemic areas (Hong et al., 1994), and reinfection or superinfection readily occurs in human with ongoing exposure, resulting in serious morbidity if untreated. On the contrary, rats develop high level of protection against reinfection with *C. sinensis*, which dramatically increased by higher worm burden or longer duration of the primary infection (Chung et al., 2004). However, hamsters are not resistant to reinfection with *C. sinensis* (Chung et al., 2004). These findings suggest that the resistance to reinfection with *C. sinensis* should vary according to the host species. The present study was undertaken to investigate the resistance to reinfection with *C. sinensis* in various experimental animals including mice, guinea pigs, rabbits, and dogs, as well as rats and hamsters.

Metacercariae of *C. sinensis* were isolated from the naturally infected freshwater fish, *Pseudorasbora parva*, collected in a pond of Jinju-si, Gyeongsangnam-do (Province), Korea. Experimental animals were all males and 5-week old, except for rats (4-week old). They were Sprague-Dawley rats, FVB mice, Syrian golden hamsters, guinea pigs, New Zealand white rabbits and dogs, and divided into 2 experimental groups; reinfection control, and reinfection groups. Animals in the reinfection groups were orally infected with metacercariae using a gavage needle according to the experimental schedule (Fig. 1), and treated with praziquantel (Distocide®, Shinpoong Pharmaceutical Co., Seoul, Korea) 4 weeks after the primary infection.

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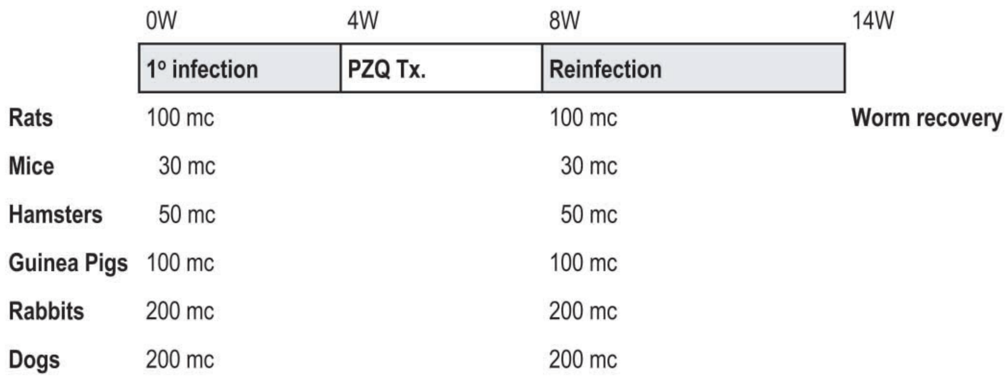

Fig. 1. Experimental scheme for observation of resistance to reinfection with *Clonorchis sinensis* in various experimental animals. 1° infection, primary infection; PZQ Tx, praziquantel treatment; mc, metacercariae.

Animals in both reinfection control and reinfection groups were infected with the same number of metacercariae, and then sacrificed by deep anesthesia and cervical dislocation at 6 week post-reinfection. Adult worms were recovered from the liver of animals under a stereomicroscope, and worm recovery and resistance rates were determined as described previously (Chung et al., 2004). The body size of recovered worms was measured after acetocarmine staining.

To guarantee the same degree of metacercarial infectivity between the 2 experimental groups, animals in reinfection control groups were infected with the same batch of *C. sinensis* metacercariae that were used for reinfection groups. Therefore, they were actually primarily infected animals, and their worm recovery rates were compared with those of reinfection groups. The determined resistance rates to reinfection in rats, mice, hamsters, guinea pigs, rabbits, and dogs were 79.7%, 58.0%, -12.6%, 54.8%, 62.6%, and 6.0%, respectively (Table 1). When the body size of worms recovered from reinfected animals were compared to those from the primarily infected, the flukes from hamsters, guinea pigs, rabbits, and dogs were similar in size (data not shown). However, the flukes from reinfected rats and mice were significantly smaller than those from the primarily infected. The average body size of recovered worms from primarily infected rats was 2.47 x 1.0 mm, while that of worms from reinfected rats was 0.58 x 0.28 mm ($P < 0.01$), and all of them were immature.

The present study confirms again that rats are highly resistant to reinfection with *C. sinensis*, which corresponds well with the previous report (Chung et al., 2004). In addition to rats, mice, guinea pigs and rabbits also showed decreased worm recovery rates in reinfection with *C. sinensis*, while hamsters and dogs developed no protective response against reinfection.

Rats are also known to be resistant to reinfection with *Fasciola hepatica* and *Schistosoma mansoni*, with which acquired immunity is closely related (Doy et al., 1978; Cetre et al., 1999). The mechanism of resistance to reinfection with *C. sinensis* in rats is not clear, however, the immunity has been suggested to be related with resistance to reinfection (Chung et al., 2004). When the immunity of infected rats was suppressed by methylprednisolone after chemotherapy, the worm recovery rate of the reinfected immunosuppressed rats was restored to that of the primarily infected rats, and worms were fully matured to adults. In the present study, poor development of worms was found in reinfected resistant rats, which suggests that protective immunity induced by the primary infection in the reinfected rats severely impairs the development of *C. sinensis* at reinfection (Chung et al., 2004). However, it remains to be explored which components of the host immunity are responsible for the resistance in rats reinfected with *C. sinensis*.

Interestingly, hamsters showed opposite responses in reinfection, depending on the species of 2 closely related liver flukes, *C. sinensis* and *Opisthorchis viverri-*

Table 1. Worm recovery rates and resistance rates to reinfection with *Clonorchis sinensis* in experimental animals

Animals	Experimental groups	No. of animals	Worm recovery rate (%)[a)]	Resistance rate (%)
Rats	Reinfection control	10	63.9 ± 20.4	-
	Reinfection	8	13.0 ± 6.9[b)]	79.7
Mice	Reinfection control	11	17.6 ± 13.0	-
	Reinfection	13	7.4 ± 6.1[b)]	58.0
Hamsters	Reinfection control	9	68.0 ± 15.0	-
	Reinfection	9	77.8 ± 30.2	-12.6
Guinea pigs	Reinfection control	10	34.7 ± 13.7	-
	Reinfection	7	15.7 ± 12.2[b)]	54.8
Rabbits	Reinfection control	7	35.0 ± 7.6	-
	Reinfection	7	13.1 ± 8.2[b)]	62.6
Dogs	Reinfection control	3	41.6 ± 14.5	-
	Reinfection	4	39.1 ± 14.1	6.0

[a)]mean ± SD.
[b)]significantly different from the reinfection control, $P < 0.05$.

ni. Hamsters were reported resistant to reinfection with *O. viverrini* (Flavell, 1982), but not to reinfection with *C. sinensis* (Chung et al., 2004). This phenomenon was confirmed again in the present study; however, it is difficult to explain why hamsters develop different responses to reinfection of the 2 liver flukes. Furthermore, several studies reported contradictory data on the protective immunity against reinfection with *O. viverrini* in hamsters. Prior infection with *O. viverrini* did not protect hamsters against reinfection by the same parasite (Sirisinha et al., 1983), and adoptive transfer of spleen cells and sera from infected hamsters produced a moderate, but insignificant reduction in the worm burden in recipient hamsters (Flavell et al., 1980).

In clonorchiasis of mice, the susceptibility of mice to *C. sinensis* infection is different according to the mouse strain (Choi et al., 2003). FVB mice are susceptible to *C. sinensis* infection, while BALB/c mice are relatively unsusceptible. This susceptibility variation has been suggested to be associated with Th2 cytokine production, especially IL-4 in FVB mice, and FVB mice showed significantly higher serum IgE level than BALB/c mice (Choi et al., 2003). According to the susceptibility, FVB mice were selected to find out the presence of resistance to reinfection in mice in this study. The FVB mice showed reduced worm recovery and poor development of recovered worms, suggesting significant resistance to reinfection with *C. sinensis*, although it was not as high as that of rats.

A reduced worm burden, delayed development of worms and decreased fecundity are indices of resistance in animals infected with helminths (Garcia et al., 1983; Claerebout and Vercruysse, 2000; Chung et al., 2004). In the present study, guinea pigs and rabbits showed significantly lower worm recovery in reinfection than in the primary infection, but the recovered worms developed well. Guinea pigs and rabbits acquired resistance to reinfection, but the effect was limited only to the recovery of worms. That is different from the dual effects of resistance to reinfection in rats and mice. Therefore, it is clear that the resistance to reinfection with *C. sinensis* is extremely variable by host species. This discrepancy between recovery and development of worms has been also found in pigs which were repeatedly infected with *Oesophagostomum quadrispinulatum*, a porcine nodular worm. The pigs showed a lower worm burden, but normally developed worms (Talvik et al., 1998). It needs further investigation to clarify the relationship between the resistance to reinfection and worm development.

In conclusion, rats and mice are resistant to reinfection with *C. sinensis* by low recovery and impaired development of reinfected worms. However, guinea pigs and rabbits show only low recovery. Rats and mice are good animal models for investigation of resistance mechanism to reinfection with *C. sinensis*.

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