

Study on *Metagonimus yokogawai* (Katsurada, 1912) in Korea*

VII. Susceptibility of Various Strains of Mice to *Metagonimus* Infection and Effect of Prednisolone

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INTRODUCTION

Metagonimus yokogawai, one of the heterophyid flukes occurring in intestine of mammals and man, is prevalent along many riverside areas in Korea (Seo *et al.*, 1981), so that human infection with this fluke is one of the major health problems in endemic areas.

For laboratory infection to study the biological and pathological characteristics of this fluke infection, many kinds of animals such as dogs, cats and rodents including mice have been used (Ito, 1964; Yokogawa *et Sano*, 1968; Hong *et Seo*, 1969; Kagei *et Kihata*, 1970; Chai, 1979). However, the susceptibility of those animals in terms of worm recovery rate, growth of worms, or longevity in host is known to be much variable by kinds of host and even among the same species of animals. Especially in mice, the recovery rate of worms at 6~10 days after infection has been in some wide range, 0.7~60.5%, according to different authors (Yokogawa *et Sano*, 1968; Hong *et Seo*, 1969; Kagei *et Kihata*, 1970). Such difference is considered due to different strains of mice used in each experiment which may reveal different levels of natural resistance to this fluke infection.

Difference in resistance of host animals to certain parasitic infections has been studied by many investigators. In *Schistosoma mansoni* infec-

tion, for example, different stocks of the snail intermediate host, *Biomphalaria glabrata*, were reported to be variable in the susceptibility to infection with the miracidia (Richards *et Merritt*, 1972; Richards, 1973). Variations in susceptibility of mouse strains to *S. mansoni* infection with the cercariae were also reported (Stirewalt *et al.*, 1965; Colley, 1972). Furthermore, the difference of mouse strains has made variable results in induction of acquired resistance after vaccination with antigens of *S. mansoni* (Murrell *et al.*, 1979) or *Nematospiroides dubius* (Cypess *et Zidan*, 1975).

This study was undertaken to know the difference in susceptibility of 5 strains of mice to experimental *M. yokogawai* infection by observing the worm recovery rate and the dimension of worms. It was also planned to study the effect of prednisolone, an immunosuppressive and anti-inflammatory agent, on the susceptibility of ICR strain mice to infection with this fluke during the course of 6 hours to 35 days after the infection.

MATERIALS AND METHODS

The metacercariae of *M. yokogawai* were obtained from the flesh of the sweetfish, *Plecoglossus altivelis*, caught at Tamjin river basin where is one of the known endemic areas of metagonimiasis in this country. The mortar-ground fish flesh was mixed with 10-fold volume of artificial gastric juice and the mixture

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incubated at 37°C for longer than 12 hours. The freed metacercariae were collected and counted to groups, so that the metacercarial number to be infected were prepared.

A total of 89 male mice, aged 4~5 weeks and weighing 20~25 grams, were used for two experimental purposes. For the observation of variability in susceptibility of different strains of mice to *M. yokogawai* infection, 60 mice of 5 strains (CBH, A, DBA, C⁵⁷BL and KK), 12 for each strain, were fed with the metacercariae through polyethylene intragastric tubes under slight anesthesia with ether. Each mouse was infected with 300 metacercariae in number. These mice were kept for 7 days by feeding with regular normal diet and water. They were sacrificed on 7th day by cervical dislocation and examined for the presence of adult flukes in their intestinal tract especially in small intestine. The intestinal lumen was opened with a pair of scissors and dipped in cold saline for 6~12 hours to free many worms from mucosa. The freed or still attached worms to mucosa were all collected and counted in each intestinal segment of each experimental mouse. All these experiments were simultaneously performed on 5 strains to reduce bias.

In order to compare the developmental status or size of the worms recovered from different strains of mice, 30 worms from each strain were fixed by 10% formalin solution under slight pressure and their length and width measured.

Twenty-nine mice of ICR strain were divided into the same numbers of two groups and the chronological pattern of worm recovery and the effect of prednisolone injection were observed. One group was *M. yokogawai*-infected but not treated, and another was prednisolone-injected mice every other day from 1 week before infection until sacrificed. Prednisolone was subcutaneously injected to inner thigh of mice with the dose of 10 mg/kg of body weight each time. The number of metacercariae infected was 1,800 per mouse in both treated and untreated groups. The method of metacercarial infection

to mice was as already described. After the infection the experimental mice in both groups were simultaneously sacrificed at intervals from 6 hours to 35 days (Table 4). The small intestine was resected and divided into three parts, *i.e.*, duodenum, jejunum and ileum, and the worms in each part were collected. The results of worm recovery in two groups were compared.

In order to assure the immunosuppressive effect of prednisolone in ICR mice, blood pictures especially the differential counts of leucocytes were examined and compared with those in untreated. The blood was obtained by heart puncture of each mouse under anesthesia directly before sacrifice for worm recovery from the intestine.

RESULTS

1. Susceptibility of Five Strains of Mice to *M. yokogawai* Infection

The experimental infection of mice with *M. yokogawai* resulted in success in 33 out of 60 mice used when they were examined 7 days after the infection (Table 1). But the success rates were considerably different by strains with the range from 25.0% (CBH strain) to 83.3% (KK strain). The strains of mice in the order from higher success rate in infection were KK, DBA, C⁵⁷BL, A and CBH. However, the statistical test revealed that the difference in rate was significant only between KK and

Table 1. The rate of success in experimental infection of *M. yokogawai* to 5 strains of mice

Mouse strain	No. mice used	No. mice infected	Success rate (%) in exp. infection
CBH	12	3	25.0
A	12	5	41.7
C ⁵⁷ BL	12	7	58.3
DBA	12	8	66.7
KK	12	10	83.3

* Statistically significant difference in success rate was met between KK and A ($p < 0.05$), KK and CBH ($p < 0.01$), DBA and CBH ($p < 0.05$) and C⁵⁷BL and CBH strains ($p < 0.05$).

Table 2. The result of worm recovery (*M. yokogawai*) from 5 strains of experimental mice

Mouse strain	No. total metacerc. given	No. worms recovered from mouse intestine		
		proximal	distal	total(%)*
CBH	3,600	1	43	44 (1.2)
A	3,600	56	69	125 (3.5)
C ⁵⁷ BL	3,600	146	192	338 (9.4)
DBA	3,600	0	149	149 (4.1)
KK	3,600	358	321	679(18.9)

* Per cent to number of metacercariae given

+ The difference in worm recovery rates was statistically significant among all strains of mice except between A and DBA strains ($p > 0.05$).

Table 3. The measurements of *M. yokogawai* recovered from 5 strains of experimental mice

Mouse strain	No. worms measured	Worm size(average+S.D.)	
		length(mm)	width(mm)
CBH	30	0.554±0.092	0.216±0.040
A	30	0.573±0.132	0.239±0.039
C ⁵⁷ BL	30	0.683±0.111	0.244±0.032
DBA	30	0.573±0.102	0.214±0.034
KK	30	0.637±0.110	0.229±0.039

+ The length of worms recovered from mouse strains was significantly different except between CBH and A, CBH and DBA, and C⁵⁷BL and KK strains ($p > 0.05$).

CBH, DBA and CBH, and C⁵⁷BL and CBH strains (Table 1).

When the success in experimental infection was numerically expressed by counting the parasitized worms in mouse intestine, the results were also different by strains of mice. In KK strain, all but two mice used being infected with adult flukes, the largest number of worms (18.9 % in worm recovery rate; No. recovered worms/No. metacercariae infected) were recovered from the intestine (Table 2). On the other hand, from CBH and A strains, which revealed lower success rate in number of mice, much smaller number of worms were recovered and the recovery rates 1.2 and 3.5% respectively. The mouse strains in the order from higher worm recovery rate were KK, C⁵⁷BL, DBA, A and CBH. The difference in rate between strains

was statistically significant in all cases except between A and DBA strains (Table 2).

The length and width of *M. yokogawai* were also considerably different by strains of mice from which the flukes were recovered (Table 3). The largest flukes were from C⁵⁷BL strain and they were 0.683 mm in average length (with 0.111 mm of standard deviation, S.D.) and 0.244 mm in average width (with 0.032 mm of S.D.). The smallest flukes were 0.554 mm (S.D. 0.092 mm) in length and 0.216 mm (S.D. 0.040 mm) in width, and were collected from CBH strain. Mouse strains in the order from larger length of worms recovered were C⁵⁷BL, KK, A, DBA and CBH and statistically significant differences were met in all occasions except between CBH and DBA, CBH and A, C⁵⁷BL and KK strains (Table 3).

In combination of the three results for estimation of susceptibility to *M. yokogawai* infection, it seems that KK and C⁵⁷BL strains of mice are more susceptible than CBH or A strains.

2. Effect of Prednisolone Injection on the Susceptibility of ICR Mice to *M. yokogawai* Infection

In ICR mice to which the metacercariae of *M. yokogawai* were given but not treated with prednisolone before or after the infection, the chronological worm recovery rate from 6-12 hours to 35 days following infection was of much peculiar pattern (Table 4 and Fig. 1). Until 1 day after infection, as high proportion as 38.4 or 66.3% of introduced metacercariae were recovered from the small intestine. But after 3 days only 0.1% were found to remain in the intestine and thereafter up to 35 days 0.0-0.7% were recovered as juveniles or adult flukes. This result suggests that ICR mice are not highly susceptible to *M. yokogawai* infection.

However, prednisolone injection to ICR mice brought about much different results and it elevated the susceptibility (Table 4 and Fig. 1). The worm recovery rate up to 1 day after infection was relatively low, 29.1-29.8%, but those after 3 days were consistently higher than

Table 4. The chronological worm recovery of *M. yokogawai* in two groups of mice (ICR) by location of worms in small intestine

Time after infection to sacrifice	No. worms recovered from							
	Untreated mice				Prednisolone-treated mice			
	Duodenum	Jejunum	Ileum	Total (%)**	Duodenum	Jejunum	Ileum	Total (%)**
6~12 hours*	231	1,122	28	1,381 (38.4)	771	263	15	1,049 (29.1)
1 day*	182	1,114	1,092	2,388 (66.3)	152	572	347	1,071 (29.8)
3 days*	1	2	1	4 (0.1)	1,120	1,547	4	2,671 (74.2)
6 days*	0	1	0	1 ^a (0.06)	157	1,440	609	2,206 (61.3)
7 days*	1	1	0	2 ^a (0.2)	87	1,327	21	1,435 ^a (79.7)
14 days*	0	2	25	27 (0.7)	193	534	212	939 (26.2)
21 days*	0	0	0	0 (0.0)	33	500	36	569 (15.8)
35 days*	0	3	0	3 (0.1)	0	0	0	0 (0.0)
Total	415	2,245	1,146	3,806	2,513	6,183	1,244	9,940

* Data from two mice (1 mouse for 'a'), each given 1,800 metacercariae

** Worm recovery rate (% to total number infected)

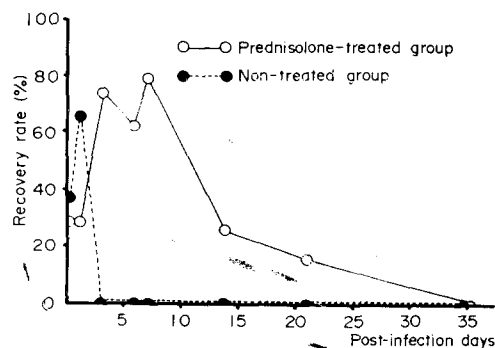


Fig. 1. Comparative worm recovery rate (*M. yokogawai*) in prednisolone-treated and untreated ICR mice.

those in untreated ICR mice up to 21 days. The peak in recovery rate, 61.3-79.7%, was obtained during 3-7 days after infection but the rate decreased thereafter to 26.2% on 14th day, 15.8% on 21st day and 0% on 35th day.

It is also noteworthy that the parasitic location in host intestine of two groups shows some significant feature (Table 4). For example, after 1 day in untreated mice, as much as 46% (1,092 juveniles in number) of flukes were recovered from ileum and others were mostly from jejunum. This result provides a suggestion that in ICR mice many *M. yokogawai* juveniles could not retain their favorable habitat of upper or middle portion of small intestine

later than 1 or 2 days. After 3 days, in practice, only 1, 2 and 1 worms were found to remain in upper, middle and lower portions of small intestine respectively. Finding the majority of worms (25 out of 27) in ileum after 14 days supports the above suggestion. Quite comparably in ICR mice injected with prednisolone, about two-thirds of the juvenile of adult flukes were consistently found from jejunum through whole experimental period. This suggests higher susceptibility of the drug-treated mice to *M. yokogawai* infection than untreated.

Blood picture especially the differential count of leucocytes appeared to be remarkably different between prednisolone-treated and untreated ICR mice. In untreated group, the eosinophils

Table 5. Differential leucocyte counts in ICR mice infected with *M. yokogawai* in comparison with prednisolone-treated group

	D.f. counts(*) in <i>M. yokogawai</i> infected mice	
	Untreated group	Prednisolone-treated group
Eosinophil	5.0 (1-10)	0.7 (0-4)
Neutrophil	39.3(24-69)	56.9(22-73)
Lymphocyte	51.0(19-63)	35.5(19-72)
Monocyte	4.7 (2-10)	6.9 (2-16)

* Range

* Checked at the time of sacrifice

were 1-10% (average 5.0%) through the experimental period with *M. yokogawai* (Table 5). On the other hand, in prednisolone-treated mice, the eosinophils were always less than 1% except for one mouse sacrificed on 21st day (it revealed 4% eosinophils and from which only 29 adult flukes were recovered). There were relative neutrophilia and lymphocytopenia in prednisolone-treated mice (Table 5).

DISCUSSION

In assessment of host susceptibility to certain helminthic infections, the parasitic burden after infection as well as worm development or longevity in each host are important items to be taken into accounts. Extent of damage or hazardous effect on the host should be considered too, however, it is practically difficult to estimate especially in laboratory animals. For this reason the former three items were mainly concerned in the present study.

The parasitic burden after experimental infection, being expressed as worm recovery rate, is the most commonly used parameter among the three items. It greatly depends on the host susceptibility to infection but may as well on some parasite-side factors such as maturity or infectivity of larvae. In case of *M. yokogawai*, Kagei *et al.* (1970) reported that the metacercariae collected from the sweetfish showed some differences in their maturity according to season and those in various seasons produced different worm recovery rates (1.8-24.9%) in the same ICR mice on 10-11th post-infection day. The highest rate was obtained from the experiment with the metacercariae collected in September. In this study all of the metacercariae used were from one group of sweetfish caught at one area in October and experiments were simultaneously performed, so that the possibility of bias due to difference in metacercarial infectivity is neglected.

As already stated, the susceptibility to *M. yokogawai* is known to be different among the laboratory animals. In cats, dogs, or hamsters,

the development of worms is good, the recovery rate of worms high and their maintenance of parasitism fairly long (Takahashi, 1929; Koga, 1938; Yokogawa *et al.*, 1968; Kang *et al.*, 1983). But all these features of susceptibility are not so good in small laboratory animals such as mice, rats and guinea pigs (Koga, 1938; Gushima, 1939; Yokogawa *et al.*, 1968; Kagei *et al.*, 1970; Chai, 1979). According to their reports and the results of this study, the susceptibility is different even among the same mouse host of different strains.

In some helminthic infections, the parasitic burden sometimes spontaneously decreases as infection time goes by. This phenomenon is called "spontaneous cure by the host" (Smithers, 1976). It is especially evident in less susceptible animals, in which the longevity of worms is considerably short. As a result the worm recovery rate after experimental infection may be relatively high at early stage but gradually decreases and eventually becomes zero within a short time.

The phenomenon of spontaneous cure has been observed in *M. yokogawai* infection especially in rodent hosts. In albino rats, for example, the number of worms was reduced later than 1 week after infection up to 4th week of observation period (Chai, 1979). Also in mice the recovery rate of worms was significantly reduced and became 0% after 56 days (Kagei *et al.*, 1970). For this reason, in this study, the recovery rate of worms from 5 strains of mice was compared at the same time (7th day after infection) and the chronological pattern of the recovery rate was observed in ICR mice from 6 hours to 35 post-infection days.

In ICR mice, *M. yokogawai* did not successfully maintain its parasitism in this study. Many worms (up to 66% of infected) were found to remain in intestine until 1 day after infection but only a few number (less than 0.7%) remained there after 3 days, so that the susceptibility of ICR mice to *M. yokogawai* infection is estimated to be very low. This is quite different from the report of Kagei *et al.* (1970) with ICR

mice in Japan. They observed over 15% of worm recovery rate until 11–15th day after infection with gradual decrease thereafter. This discrepancy is difficult to explain. A possible speculation is that there may be differences in genetic characters between ICR mice having been bred in two different localities.

Not many reports are available on the nature and base of different susceptibility of host strains to certain parasitic infection. However, it was described by Murrell *et al.* (1979) that important differences in response to defined parasite antigens exist among mouse strains and these variations may be based on differences in genes composing the major histocompatibility complex. The difference in susceptibility of snail intermediate host of *S. mansoni* was also studied by Richards *et Merritt* (1972) and Richards (1973) and they concluded that the susceptibility of juvenile and adult snails was regulated by a complex of four or more genetic factors.

There are many reports on the effect of adrenocorticosteroid hormones on elevation of the susceptibility of animals to parasitic infections. The examples of parasites are *Trichinella spiralis* (Stoner *et Godwin*, 1953; Coker, 1955 & 1956), *Nematospiroides dubius* (Cross, 1960), *Necator americanus* (Miller, 1966), *Paragonimus westermani* or *P. iloktsuenensis* (Tada, 1967; Lee *et Chyu*, 1967; Lee *et al.*, 1976) and *Clonorchis sinensis* (Lee, 1967; Lee, 1968).

The adrenocorticosteroids are known to have various effects when administered to animals and man. Especially to immune systems, the drug has influences on both humoral (Germuth *et al.*, 1950; Fischel *et al.*, 1952) and cellular reactions (Gilman *et al.*, 1980). As a result, in *T. spiralis* infection, for example, the treatment of mice with cortisone lowered immunity against a primary infection with the larvae and there was a long persistence of more adult worms in the intestine and the establishment of much larger number of larvae in the musculature than in control mice (Coker, 1955). The drug effect was also evident in previously immunized mice not to resist or respond to

further entrance of larvae (Coker, 1956).

In conclusion of this study, it is suggested that immunological characters depending on some genetic factors of various strains of mice may be responsible for the different susceptibility to infection with *M. yokogawai*, so that they would become more susceptible by injecting adrenocorticosteroids such as prednisolone. Further study to demonstrate any lymphoid cells or antibodies such as IgA attached to the worms in untreated mice while not in prednisolone-treated ones may provide a more detailed information on action of the drug.

SUMMARY

An experimental study was undertaken to observe the difference in susceptibility of mouse strains to *Metagonimus yokogawai* infection by estimating it from worm recovery rate and dimension of worms. It was also studied the effects of prednisolone injection on the chronological pattern of worm recovery in ICR mice. The metacercariae were obtained from sweetfish and 300 in each number were given to 5 strains (CBH, A, DBA, C⁵⁷BL and KK) of mice, and after 7 days period, the worms were collected from their intestine. Prednisolone at the dose of 10 mg/kg was injected to ICR mice every other day from 7 days prior to infection until sacrificed at 6 hours to 35th post-infection day. ICR mice infected with *M. yokogawai* but untreated were used for controls.

The success rate in infection of mice ranged 25.0–83.3% by strains, the worm recovery rate 1.2–18.9%, and the average size of worms 0.554–0.683 mm long and 0.214–0.244 mm wide. The higher rates and larger size of worms were observed in KK and C⁵⁷BL strains than others and the difference was statistically significant. In ICR mice for control, the worm recovery rate until 1 day after infection was relatively high (38–66%) but it became much lower (less than 0.7%) during 1–35 days. However, prednisolone injection brought about persistently high recovery rates (16–80%) until

21 days. It was concluded that the susceptibility to *M. yokogawai* infection is different by strains of mice but it can be elevated by prednisolone injection probably due to suppression of immune responses in ICR mice.

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=국문초록=

요꼬가와흡충에 관한 研究

VII. 마우스 Strain別 感受性 및 Prednisolone의 영향

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마우스에 대한 요꼬가와흡충 實驗感染에 있어서 蟲體回收率 등 感受性(susceptibility)이 實驗者에 따라 매우 다른 것으로 報告되어 있다. 著者等은 이것이 마우스 strain에 따른 差異일 것으로 생각하고 이 研究를 통하여 확인하고자 하였으며, 또 prednisolone 주입으로 宿主의 면역반응을 低下시킬 때 蟲體回收率이 어떻게 變動하는가를 관찰하고자 하였다.

요꼬가와흡충 被囊幼蟲은 銀魚의 근육으로부터 분리한 것을 사용하였으며 마우스 5個 strain (A, DBA, CBH, C⁵⁷BL, KK) 총 60마리에 대해서 300個씩의 被囊幼蟲을 感染시키고 1주일 후에 도살하여 蟲體回收率 및 蟲體의 크기를 測定하였다. 또 ICR系 마우스 29마리에는 각각 1,800個씩의 被囊幼蟲을 感染시키되 그중 15마리에 대해서는 感染 1週前부터 도살시까지 격일로 prednisolone 10mg/kg를 주입하였고 각각 感染 6시간부터 35일까지의 蟲體回收率을 관찰하였다.

결과는 다음과 같다.

1. 마우스 5개 strain에 있어서 感染成功率은 25.0~83.3%의 범위에서 차이를 보였고, 蟲體回收率은 1.2~18.9%, 回收蟲體의 크기는 平均 길이 0.554~0.683mm, 폭 0.214~0.244mm이었다. 이들 數値는 KK 및 C⁵⁷BL strain에서 다른 3가지 strain보다 높았으며 有意한 差異를 보였다.

2. ICR系 마우스에서 시간별로 관찰한 蟲體回收率은 감염 1일내에는 38~66%의 높은 率을 보였으나 1일 이후에는 35일까지 0.7%이하의 낮은 回收率을 보였다. 그러나 prednisolone을 투여한 群에서는 感染後 21일까지도 16~80%의 높은 蟲體回收率을 유지하였다.

이상의 결과로 미루어 보아 마우스는 strain에 따라 요꼬가와흡충 感染에 대한 感受성이 다르다는 것을 알 수 있었다. 또한 感受성이 낮은 ICR系 마우스도 prednisolone을 주입하면 감염 3週까지 높은 蟲體回收率을 얻을 수 있음을 알게 되었다.