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Effects of gamma-irradiation on the survival and development of *Gymnophalloides seoi* in C3H mice

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Abstract: An experimental study was carried out to observe the effects of radiation on the infectivity of metacercariae of *Gymnophalloides seoi* to C3H mice. Oysters, the second intermediate host, were collected from an endemic area, and non-irradiated control, metacercaria-irradiation, and oyster-irradiation groups were prepared. One hundred metacercariae were infected orally to each mouse, and worm recovery rate was compared by groups at 7th day post-infection. In the metacercaria-irradiation group, the worm recovery rate was significantly reduced at radiation doses higher than 200 Gy, and the number of intrauterine eggs significantly reduced at doses over 50 Gy. In the oyster-irradiation group, 50 Gy significantly reduced both the worm recovery rate and number of uterine eggs. In the two groups, no worm was recovered at 1,000 Gy irradiation. Conclusively, irradiation of oysters with 200-1,000 Gy was effective to control infectivity of metacercariae to C3H mice, and could be adopted as a control measure for gymnophalloidiasis.

Key words: *Gymnophalloides seoi*, metacercaria, oysters, irradiation, worm recovery rate

INTRODUCTION

Gymnophalloides seoi Lee, Chai and Hong, 1993 is a new human intestinal trematode in Korea. Its first human infection was discovered in 1988 (Lee *et al.*, 1993), and high prevalence was proved among the seashore people on a southwestern coastal island (Lee *et al.*, 1994). The oysters, *Crassostrea gigas*, were proved to be the source of infection (Lee *et al.*, 1995a). Recently, *G. seoi* infection was also discovered from a patient who lived in Inchon City, far from Shinan-gun (Lee *et al.*, 1995b). Therefore, a nationwide survey is needed to properly understand the distribution of gymnophalloidiasis.

The normal habitat of flukes belonging to the family Gymnophallidae is the small intestine, but it is known that they can migrate into the biliary tract or pancreatic duct in avian hosts (Yamaguti, 1939). Also in the human host, *G. seoi* was found infected in one patient suffering from acute pancreatitis (Lee *et al.*, 1993) and two patients suffering from diabetes mellitus (Lee *et al.*, 1995b). For this reason, a strong relationship between *G. seoi* infection and pancreatic diseases was suggested (Lee *et al.*, 1995b). Hence, treatment and prevention of this infection should be very important.

Although *G. seoi* infection is well treated with praziquantel, the infection cycle is continued among the population of the endemic area due to their raw eating habit of oysters. Therefore,

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management of the disease by drug only is not so efficient. Recently, irradiation of foods is applied to control various kinds of human infecting pathogens. The present study was carried out to observe the effects of radiation on the infectivity of *G. seoi* metacercariae to C3H mice, and to assess the applicability of radiation for use in the control of gymnophalloidiasis.

MATERIALS AND METHODS

1. Irradiation of *G. seoi* metacercariae

The oysters infected with *G. seoi* metacercariae were collected from Aphae-do (Island), Shinan-gun, the known endemic area of *G. seoi* (Lee *et al.*, 1994). The experimental groups were divided into non-irradiated control, oyster-irradiation, and metacercaria-irradiation groups. In the oyster-irradiation group, irradiation was done on the oysters within their shell and the metacercariae were isolated from the oysters after irradiation. In the metacercaria-irradiation group, the metacercariae were isolated from oysters, and then irradiated.

Isolation of metacercariae was done as described by Lee *et al.* (1995a). Briefly, after opening the shell, the mantle surface of the oysters was carefully examined under stereomicroscopy whether it contained gymnophallid metacercariae. Pieces of oyster tissues containing the metacercariae were cut by scissors, and loaded on Baermann's apparatus. Two hours later, the metacercariae were collected from the sediments.

The metacercariae isolated or present *in situ* of oysters were irradiated on a petri-dish containing physiological saline. The source of gamma-ray was MK 1-68 Cs-irradiator (JL Sheperd and Associates Co., U.S.A.) in Cancer Research Center, Seoul National University. The radiation dose was determined as 50 Gy, 200 Gy, or 1,000 Gy.

2. Experimental infection of C3H mice and worm recovery

In a preliminary study, C3H mice were found to be a suitable experimental host in terms of the worm recovery rate and maturity of worms (unpublished data). Therefore, C3H mice, 20-25 g male or female, were used as the laboratory animal to infect *G. seoi* metacercariae.

One hundred metacercariae were orally fed to each mouse. Each group consisted of 5 mice. After infection, the mice were kept supplying normal diet and water. They were sacrificed 7 days after infection, and their small intestine was resected. Each of the small intestine was opened longitudinally for recovery of adult flukes. The opened small intestine was loaded on Baermann's apparatus, and freed adult flukes were collected from the sediment and counted. The worms were fixed in 10% formalin under coverslip pressure and stained with acetocarmine. The worm recovery rate and number of intrauterine eggs were compared among three groups, and statistically analyzed.

RESULTS

1. Control group

The mice infected with non-irradiated metacercariae of *G. seoi* revealed 12.8% worm recovery rate on average (Table 1). Among them, one mouse showed higher than 20% recovery rate, whereas another only 5% recovery rate, revealing a wide variation. The recovered worms actively moved. The average number of intrauterine eggs was 103.7 (Table 2). The reproductive organs such as the ovary, testes, and vitellaria were well developed.

2. Metacercaria-irradiation group

Mice of 50 Gy irradiated group revealed 7.7% worm recovery rate on average (Table 1). The rate was much lower than non-irradiated controls, but the difference was statistically not significant. The recovered worms actively moved. The number of intrauterine eggs was reduced compared to controls, and revealed statistically significant difference (Table 2). Developmental retardation of worms was not recognizable.

In 200 Gy irradiated group, the worm recovery rate was remarkably reduced to 0.6%, which was statistically significant ($p < 0.05$). From two mice of this group, no worms were recovered. The motiliy of a few recovered worms from three mice were quite active, but

Table 1. Worm recovery rates of *G. seoi* from mice in oyster-irradiation and metacercaria-irradiation groups

Irradiation dose[a] (Gy)	Worm recovery rate by groups	
	Metacercaria-irradiation (Avg. ± S.D.)	Oyster-irradiation (Avg. ± S.D.)
0 (control)	12.8 ± 7.0	12.8 ± 7.0
50	7.7 ± 4.9	5.6 ± 2.1[b]
200	0.6 ± 0.5[b]	2.2 ± 2.6[b]
1,000	0.0 ± 0.0[b]	0.0 ± 0.0[b]

[a]Each dose group consisted of 5 C3H mice for each scheme. [b]Significantly lower than the controls ($p < 0.05$).

Table 2. The number of intrauterine eggs in surviving *G. seoi* worms in oyster-irradiation and metacercaria-irradiation groups

Irradiation dose[a] (Gy)	No. of intrauterine eggs/worm by groups	
	Metacercaria-irradiation (Avg. ± S.D.)	Oyster-irradiation (Avg. ± S.D.)
0 (control)	103.7 ± 28.2	103.7 ± 28.2
50	39.9 ± 17.7[b]	63.1 ± 31.8[b]
200	45.0 ± 37.0[b]	52.0 ± 49.6[b]
1,000	—	—

[a]For each dose and each scheme 10 worms were observed. [b]Significantly lower than the controls ($p < 0.05$).

contained only 45.0 eggs on average. No worm was recovered from the 1,000 Gy irradiated group (Table 1).

3. Oyster-irradiation group

The worm recovery rate from C3H mice by each radiation dose was as follows; 5.6% with 50 Gy, 2.2% with 200 Gy, and 0% with 1,000 Gy (Table 1). The recovery rate with doses higher than 50 Gy was significantly reduced compared to controls ($p < 0.05$). The surviving worms, however, actively moved, and revealed no significant developmental anomalies except for smaller number of intrauterine eggs. The average number of eggs was 63.1 in 50 Gy, and 52.0 in 200 Gy groups, all of which were significantly lower than 103.7 of non-irradiated controls (Table 2).

DISCUSSION

International Atomic Energy Agency (IAEA) have considered irradiation as a control measure for food-borne parasitic infections (IAEA, 1989 & 1991), because radiation exerts an enormous influence on helminth parasites. However, radiation effects depend not only on the species and developmental status of helminths but also on the milieu of conditions around the environment. For example, metacercariae of *Clonorchis sinensis* encysted in cyprinoid fish were more resistant to radiation than isolated ones (Chai *et al.*, 1993). This phenomenon was explained due to an anoxic state of the muscle of dead fish. Since radiation breaks the DNA structure by forming hydroxy radicals (Hall, 1978), the presence of oxygen in the environment should enhance the effects of radiation.

However, in this study, the radiosensitivity of *G. seoi* metacercariae was not significantly different between the metacercaria- and oyster-irradiation groups. An explanation could be that the metacercariae of *G. seoi* were not located deeply in the oyster tissue, but simply attached on the mantle surface (Lee *et*

al., 1995a). The space between the oyster body and its shell is normally filled with body fluid which might be abundant of oxygen. Therefore, environmental conditions around the metacercariae in the two kinds of irradiation schemes should have been approximately the same, especially in the concentration of oxygen.

It is generally accepted that trematode larvae are fairly susceptible to radiation whilst nematode and cestode larvae are not. It is difficult to explain the reason properly. A speculation could be that there is marked difference in the velocity of cell division. Generally trematode larvae grow and mature rapidly in the body of their final hosts to become adults compared with nematode or cestode larvae, requiring rapid cell division. It may contribute to the higher susceptibility of trematode larvae to radiation than nematode or cestode larvae.

However, even among the trematodes radiosensitivity appeared variable by species of trematode larvae. In the case of *C. sinensis*, 50 Gy irradiation was required to control infectivity of metacercariae (Chai *et al.*, 1993), and 80 Gy was needed to reduce *Schistosoma mansoni* worm recovery to 1% after cercarial infection (Smithers, 1962). *Metagonimus yokogawai* metacercariae were slightly resistant to radiation and 200 Gy was required to lower the recovery rate to 0.5% (Chai *et al.*, 1995). But *G. seoi* revealed higher worm recovery than 3% even with 200 Gy, suggesting some resistance to radiation. The reason may be that, unlike *M. yokogawai* metacercariae which have poorly developed reproductive organs, *G. seoi* metacercariae have well developed reproductive organs, which could help them a little resistant to radiation.

The number of intrauterine eggs of a parasite is often used as a criterion for assessing the degree of worm development and maturity. In this study, significant reduction in the number of uterine eggs of surviving worms was recognized at 50 Gy. It means that low doses of radiation, although some worms survive, should be effective for control of transmission of gymnophalloidiasis. Similarly, in the case of *M. yokogawai*, 90% of surviving worms after irradiation with 50 Gy could not produce eggs (Chai *et al.*, 1995). Also they were very small in size and retarded in the growth of reproductive organs (Chai *et al.*, 1995).

In this study, the number of *G. seoi* metacercariae to infect C3H mice was determined as 100 per mouse. This infection dose was based on a result of our preliminary study (unpublished data). In that study, when 200-500 metacercariae were fed to a mouse, most of them were found naturally expelled from the host intestine probably due to a phenomenon suggestive of a crowding effect.

Baermann's apparatus was originally devised for recovery of nematodes, but it was also successfully applied in this study to isolate trematode metacercariae which had no cyst wall. Moreover, it was also convenient for recovery of adult flukes from the mouse intestine. The sediment from Baermann's apparatus revealed a clear field of vision with little foreign debris, and it could reflect the accurate worm recovery rate owing to easy worm recovery and little worm loss.

From this study, it was suggested that *G. seoi* metacercariae are a little resistant to radiation compared with other species of trematodes, but irradiation with 200-1,000 Gy was highly effective to control their infectivity to C3H mice. Many kinds of practical problems remain to be solved, however, irradiation of oysters could be considered as a measure for control of gymnophalloidiasis in endemic areas.

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=초록=

방사선 조사가 참굴큰입흡충 피낭유충의 C3H 마우스 내 생존 및 발육에 미치는 영향

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우리 나라 고유의 인체 기생 장흡충의 하나인 참굴큰입흡충(*Gymnophalloides seoi*) 피낭유충에 대한 방사선 조사가 실험적 종숙주인 C3H 마우스 감염 능력에 어떠한 영향을 미치는지 관찰하였다. 유행지에서 굴을 채집하여 비조사 대조군, 피낭유충 조사군 및 굴 조사군 등 3개 군으로 나누고, 각 군에서 얻은 피낭유충을 C3H 마우스 마리당 각각 100개씩 경구감염시킨 다음 7일째에 충체 회수율을 관찰하였다. 연구 결과, 피낭유충 조사군에서는 200 Gy 이상 조사시 대조군에 비해 유의하게 충체 회수율이 감소하였다. 생존 충체의 자궁 내 충란수는 50 Gy 이상 조사시 통계적으로 유의한 감소를 보였다. 굴 조사군에서는 50 Gy 이상 조사시 충체 회수율이 유의하게 감소하였으며, 50 Gy 이상 조사시 자궁내 충란수의 유의한 감소를 보였다. 이상의 결과를 종합할 때 굴에 200-1,000 Gy를 조사할 경우 참굴큰입흡충 피낭유충의 살멸 효과가 뚜렷하였으며, 따라서 이 흡충증 관리 방법의 하나로 이용할 수 있을 것으로 전망하였다.

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