

Effects of gamma-irradiation on the survival and development of *Metagonimus yokogawai* metacercariae in rats

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Abstract: To evaluate the feasibility of irradiation as a control measure for metagonimiasis, the metacercariae of *Metagonimus yokogawai* were irradiated with gamma ray, either after isolation from the sweetfish (*Plecoglossus altivelis*) or *in situ* of the fish, and their survival and development in rats were observed at 7 days post-infection. The radiation dose varied from 5 to 100 Gy for the metacercaria-irradiation group and from 5 to 500 Gy for fish-irradiation group. The results showed that the worm recovery rate from the irradiation groups decreased as the radiation dose was increased. Higher doses of radiation were required for the fish-irradiation group to obtain the same results as the metacercaria-irradiation group. The LD₅₀ of the metacercaria-irradiation group was 4.5 Gy, whereas that of the fish-irradiation group 6.2 Gy. A few number of worms which survived until 7 days in rats were severely retarded especially in the growth of their reproductive organs, i.e., complete or partial failure in the development of testes and formation of uterine eggs. The present study revealed that irradiation of sweetfish by 200 Gy is effective to control infectivity as well as development of *M. yokogawai* metacercariae in rats.

Key words: *Metagonimus yokogawai*, metacercariae, gamma irradiation, survival, development, recovery rate, rat

INTRODUCTION

Metagonimus yokogawai has been one of the most important human intestinal flukes in Korea. At present, endemic areas of *M. yokogawai* are scattered almost all over the large and small streams in eastern and

southern coastal areas, especially in the valley near the Somjin-gang (River) (Seo *et al.*, 1981; Chai and Lee, 1990). Human infection with this fluke is contracted by eating raw sweetfish (*Plecoglossus altivelis*) which harbour the metacercariae. Therefore, metagonimiasis could be prevented if the metacercariae in the fish are killed or inactivated.

Studies have been performed on the use of irradiation to kill or weaken infectivity of not only protozoa (Dubey *et al.*, 1986) but also helminth parasites (Hughes, 1962; Bickle *et al.*, 1979; Lee *et al.*, 1989). Most of the irradiated helminths were either significantly

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attenuated or killed, depending on the dose of radiation. Thus, irradiation of the infective stage larvae is applied as a measure for making live or attenuated vaccines for helminth infection, for example, schistosomiasis (David *et al.*, 1995).

The purpose of the present study is to observe the effect of gamma-irradiation on the survival and development of *M. yokogawai* in rats and ultimately to evaluate the usefulness of irradiation as a control measure for metagonimiasis.

MATERIALS AND METHODS

1. Collection of *M. yokogawai* metacercariae

Metacercariae of *M. yokogawai* were isolated from the muscle of the sweetfish, *Plecoglossus altivelis*, caught from the main stream of the Somjin-gang (River), Chollanam-do, Korea, by digestion technique with artificial gastric juice (pepsin 6 g and HCl 8 ml dissolved in 1 L distilled water) and collected under stereomicroscope. They were washed several times and stored in cold physiological saline until use.

2. Irradiation of isolated metacercariae of *M. yokogawai* (metacercaria-irradiation group)

One thousand metacercariae were placed on each petri dish (8 cm in diameter) containing 20 ml saline. The dishes were put on the rounding plate of a MK 1-68 ¹³⁷Cs gamma-irradiator (JL Sheperd and Associates Co.) and irradiated at the rate of 3.8 Gy/min from 40 cm distance. The radiation dose was 5, 20, 50 or 100 Gy. Non-irradiated metacercariae served as controls.

3. Irradiation of sweetfish (fish-irradiation group)

The sweetfish (15-20 cm in length) infected with the metacercariae of *M. yokogawai* was irradiated at the dose of 5, 50, 200 or 500 Gy, half dose on one side and another half on the other side to equalize the regions to be irradiated. After irradiation, the metacercariae were isolated from the digested material of the sweetfish and used for infection of rats. Non-

irradiated fish served as controls.

4. Experimental infection of rats

Each of albino rats (Harlan-Sprague-Dawley) purchased from the Laboratory Animal Center, Seoul National University, was given orally 500 irradiated or non-irradiated metacercariae through a gavage needle. For each dose group, 10 rats were used.

5. Recovery of *M. yokogawai* and morphological observation

The rats infected with the metacercariae were sacrificed by spinal shock one week after the infection. The small intestines were put on a petri dish containing 0.85% saline solution and opened longitudinally along the mesenteric border for recovery of the worms. The opened small intestines were soaked in cold saline and gently shaken to detach the worms. The freed worms were then counted using a stereomicroscope and the worm recovery rate was calculated.

The worms recovered were, by groups, fixed in 10% neutral formalin, stained with Semichon's acetocarmine, and observed under light microscope.

6. Statistical analysis

Chi-square or student t-test was done for statistical analysis of the results.

RESULTS

1. Morphology of the irradiated metacercariae

The metacercariae which were exposed to gamma irradiation showed no apparent morphological difference from non-irradiated controls by microscopic examination. The irradiated metacercariae, however, showed weaker movement within their cysts. The outlines of the suckers, excretory bladder and ceca were clearly discernible.

2. Recovery rate of *M. yokogawai* from the rats

1) Metacercaria-irradiation group

From the rats of the non-irradiated group infected with non-irradiated metacercariae, an average of 30.2% of the introduced worms was

recovered one week after the infection (Table 1). In contrast, the rates of worm recovery from the irradiation groups were significantly lower than those from the non-irradiated control group ($P < 0.001$). The rate was decreased as the radiation dose increased; 9.4% in 5 Gy group, 6.2% in 20 Gy group, 0.5% in 50 Gy, and 0.4% in 100 Gy group (Table 1). The LD_{50} by this scheme was calculated as 4.5 Gy, based on the regression equation, $\ln Y = -1.17 \ln X + 1.94$, where X is radiation dose (Gy) and Y is worm recovery rate (%).

2) Fish-irradiation group

In general, more worms were recovered from the fish-irradiation group than the metacercariae-irradiation group when a same dose of radiation was given. The recovery rates were 16.8% and 1.8%, in 5 Gy and 50 Gy groups, respectively (Table 2). Even by 200 Gy and 500 Gy, the recovery rates were still 0.5% and 0.1%, respectively. The LD_{50} by this scheme was calculated to be 6.2 Gy, based on the regression equation, $\ln Y = -1.07 \ln X + 2.03$, where X is radiation dose (Gy) and Y is worm recovery rate (%).

3. Morphological characteristics of the recovered worms

Gamma-irradiation of *M. yokogawai*

metacercariae caused morphological changes of the worms recovered from the experimentally infected rats, which included decrease in the body length and width, reduction of egg production, and malformation of reproductive organ (Table 3, Figs. 1-4).

The average size of worms recovered from the rats infected with the metacercariae irradiated with 5, 20, 50 or 100 Gy was $416 \times 206 \mu\text{m}$, $393 \times 205 \mu\text{m}$, $396 \times 208 \mu\text{m}$, and $369 \times 168 \mu\text{m}$, respectively (normal non-irradiated controls; $441 \times 211 \mu\text{m}$). Worms recovered from 100 Gy metacercaria-irradiation group showed absence of uterine eggs, and complete agenesis of testes. The vitelline follicles, ovary, seminal receptacle and seminal vesicle were difficult to observe. The excretory bladder was dilated remarkably.

The average size of worms recovered from fish-irradiation groups at the doses of 5, 50, 200 or 500 Gy, measured $408 \times 210 \mu\text{m}$, $380 \times 168 \mu\text{m}$, $331 \times 176 \mu\text{m}$, and $321 \times 171 \mu\text{m}$, respectively. The egg-containing rate of worms recovered from 50 Gy fish-irradiation group was 30%, higher than that of metacercariae-irradiation group (10%) and the eggs of both groups were immature. Worms irradiated with more than 200 Gy showed no eggs, complete agenesis of testes, and malformation of other

Table 1. Recovery rate of *M. yokogawai* from the rats of metacercaria-irradiation group

Irradiation dose (Gy)	No. of rats	Aver. No. of recovered worms (%)	Range of No. recovered worms
0	7	151(30.2)	95-223
5	10	47 (9.4)	8-113
20	10	31 (6.2)	3-91
50	10	3 (0.5)	0-14
100	9	2 (0.4)	0-5

Each rat was infected orally with 500 irradiated metacercariae.

Table 2. Recovery rate of *M. yokogawai* from the rats of fish-irradiation group

Irradiation dose (Gy)	No. of rats	Aver. No. of recovered worms (%)	Range of No. recovered worms
5	9	84(16.8)	5-226
50	10	9 (1.8)	0-32
200	10	3 (0.5)	0-14
500	10	1 (0.1)	0-4

Each rat was infected orally with 500 irradiated metacercariae.

Table 3. Morphological features of *M. yokogawai* recovered from the rats infected with normal (non-irradiated control group) or irradiated metacercariae (metacercaria-irradiation group or fish-irradiation group)

	Control group	Radiation dose (Gy)							
		Metacer.-irradiation group				Fish-irradiation group			
		5	20	50	100	5	50	200	500
Length (μm)	441	416	393	396	369	408	380	331	321
Width (μm)	211	206	205	208	168	210	168	176	171
% of worms containing eggs									
none	10	20	50	90	100	50	70	100	100
immature	0	60	30	10	0	20	30	0	0
mature	90	20	20	0	0	30	0	0	0
% of worms with testes									
no testis	0	0	20	80	25	0	0	90	100
one testis	0	20	20	0	55	0	10	10	0
two testes	100	80	60	20	10	100	90	0	0

In each dose group, 10 acetocarmine-stained *M. yokogawai* specimens were observed.

reproductive organs.

DISCUSSION

The effect of ionizing radiation on parasites depends on the dose of radiation, developmental and physiological status of worms, and other various physical parameters during or after the irradiation (Hall, 1978; Deborah *et al.*, 1987). In general, older worms are more radiation-resistant than younger ones (Ignatowicz and Boczek, 1984), and females are more susceptible than males (Ignatowicz and Boczek, 1984). When parasites are exposed to radiation, they can develop growth retardation, vacuolization of the interstitium, elevation of the tegument, malformation or underdevelopment of reproductive organs, failure of reproduction, and weakened pathogenicity in the host (Bickle, 1979).

In the present study, the effects of gamma irradiation on *M. yokogawai* metacercariae were remarkable; reduction of their survival rate in the rat host, growth retardation, malformation and underdevelopment of the reproductive organs, and failure of egg reproduction. Such morphological abnormality was recognized in the worms recovered from 20 Gy metacercaria-irradiation and from 50 Gy

fish-irradiation groups. The effects were radiation-dose dependent. All of the surviving worms were sexually immature by 100 Gy or more irradiation in metacercaria-irradiation group and by 200 Gy or more in fish-irradiation group.

More worms of *M. yokogawai* survived in the fish-irradiation group than in the metacercaria-irradiation group. In other words, the metacercariae irradiated *in situ* of the flesh of the sweetfish were less susceptible to radiation than those irradiated after isolation from the fish. In case of fish irradiation, the worm recovery rate was 1.8% by 50 Gy irradiation and 0.5% by 200 Gy (the same result as the metacercaria-irradiation group with 50 Gy). It was very difficult to suppress completely the infectivity of *M. yokogawai* metacercariae, and still a few worms (0.1%) were recovered even from 500 Gy fish-irradiation group.

Such difference in the radiation susceptibility of *M. yokogawai* metacercariae may be caused by differences of the milieu of conditions around the metacercariae. It is well known that gamma rays penetrate deeply into the animal tissue with little loss (Hall, 1978). Radiation is known to destroy DNA structures directly or by forming hydroxy radicals in the tissue, so there may be three times more cell damage in the oxygenated state than in the

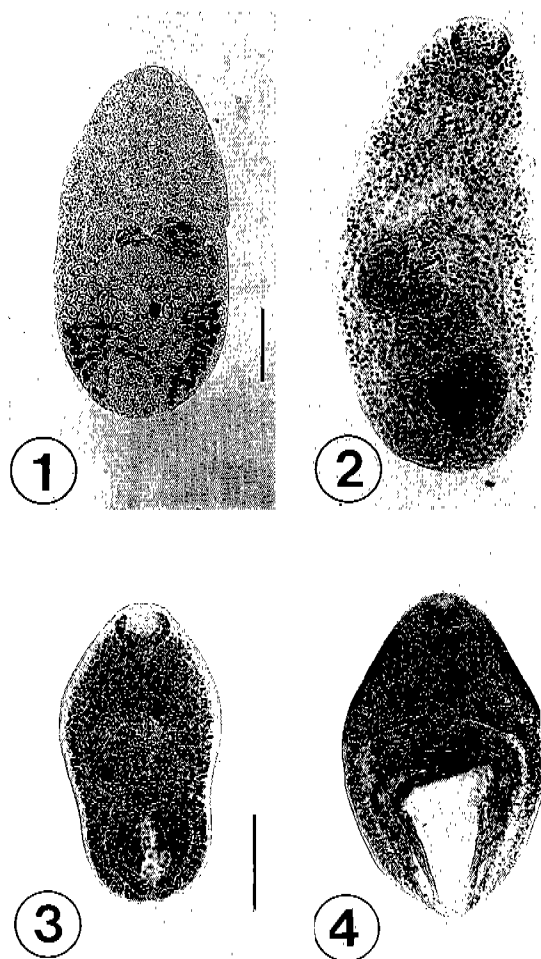


Fig. 1. A 7-day old adult worm of *M. yokogawai* recovered from a rat of non-irradiated control group showing its normal body contour, many intrauterine eggs, well-developed ovary, seminal vesicle, seminal receptacle, vitelline follicles, and two testes. Acetocarmine stained. **Fig. 2.** An age-matched specimen recovered from a rat infected with 100 Gy-irradiated metacercariae (metacercaria-irradiation group) showing no uterine eggs, no vitelline follicles, poorly developed ovary, and only one testis. Acetocarmine stained. **Fig. 3.** Another age-matched specimen recovered from a rat infected with 200 Gy-irradiated metacercariae (fish-irradiation group) which shows no uterine eggs, no ovary, no vitelline follicles, and two poorly developed testes. The excretory bladder slightly expanded. Acetocarmine stained. **Fig. 4.** Another specimen recovered from 200 Gy fish-irradiation group showing no eggs and no reproductive organs. The bladder is distended severely. Acetocarmine stained.

*Bar represents 100 micrometers.

anoxic one (Hall, 1978). Similar results were observed in the case of *Clonorchis sinensis* (Lee *et al.*, 1989), and it was suggested that the flesh of the dead fish could have been anoxic. Hence, there was less damage of cells of the worm, and the irradiated metacercariae in the flesh survived more (Lee *et al.*, 1989).

It was reported in arthropods that their sensitivity to radiation was greatest during the periods of rapid cell division, *i.e.*, embryogenesis, ecdysis, and gametogenesis (Entrekin *et al.*, 1987). Conversely, however, it is interesting to note that doses of 50-100 Gy caused increased oviposition and hatchability of a mite, *Tyroglyphus farinae* (Melville, 1958), and doses of 50 Gy increased the number of metaphase and anaphase meiotic figures in testicular cells of *Acarus siro* (Contine and Davis, 1969). Fifty Gy apparently stimulated population growth of this species through F₁ and F₂ generations, however, a significant long-range reduction occurred in F₄ and subsequent generations. Doses of 150, 250, and 350 Gy reduced population growth somewhat, and extreme population depression occurred by 500-600 Gy (Davis, 1972).

It was also reported that X-ray induced morphological abnormalities in the tapeworm, *Hymenolepis microstoma* (Djoen, 1965). It was shown, however, that newly formed segments did not suffer as much damage as those present at the time of irradiation. Apparently, radiation affected only a certain stage of cells, and highly resistant cells were present, which gave rise to the new strobila. Villella and Weinbren (1965) described the results of studies with *Schistosoma mansoni* in which gamma radiation at the dose between 20 and 25 Gy on the cercariae produced morphological changes, which included decrease in the body length, parenchymal vacuolization, cuticular swelling, and malformation of reproductive organs.

In this study, *M. yokogawai* appeared highly sensitive to radiation compared to other helminths. The LD₅₀ of *M. yokogawai* metacercariae was only 4.5 Gy. In case of *C. sinensis* metacercariae, it was 16.5 Gy (Lee *et al.*, 1989). *Paragonimus ohirai* metacercariae were sensitive to irradiation and were dead in the liver of the host after exposure to 20 Gy

(Ikeda and Tani, 1984). Unlike the above-mentioned trematodes, 1% of *Schistosoma* adults were still recovered after irradiation at a high dose; 80 Gy (Erickson, 1965), and migrating schistosomules became undetectable in the lungs only after irradiation at a considerably large dose, 200 Gy (Bickle *et al.*, 1979).

Irradiation against parasites has been applied for various other purposes. For example, mice immunized with irradiated *S. mansoni* cercariae became increasingly important as a model for anti-schistosome vaccine development (David *et al.*, 1995). Immunization with irradiated-attenuated sporozoites of *Plasmodium berghei* has been shown consistently to protect hosts against a sporozoite challenge by eliciting cellular and cytokine responses (Scheller *et al.*, 1995).

In conclusion, irradiation of *M. yokogawai* metacercariae, either *in situ* of the sweetfish or after isolation, was found to be able to control their infectivity to the final host. Therefore, irradiation of sweetfish with 50-200 Gy could be considered as a measure for prevention and control of metagonimiasis in endemic fields.

REFERENCES

- Bickle QD, Dobinson T, James ER (1979) The effects of gamma-irradiation on migration and survival of *Schistosoma mansoni* schistosomula in mice. *Parasitology* **79**: 223-230.
- Chai JY, Lee SH (1990) Intestinal trematodes of humans in Korea: *Metagonimus*, heterophyids and echinostomes. *Korean J Parasitol* **28** (suppl.): 103-122.
- Contine YM, R Davis (1969) Some effects of gamma radiation on the chromosomes of *Acarus siro* L. (Acarina: Acaridae). *J Georgia Entomol Soc* **4**: 149-152.
- David AD, Beverly LM, Fred AL (1995) Comparison of two strains of *Schistosoma mansoni* with respect to the sites and kinetics of immune elimination in irradiated cercaria-immunized mice. *J Parasitol* **81**(1): 43-47.
- Davis R (1972) Some effects of relative humidity and gamma radiation on population development in *Acarus siro* (Acarina: Acaridae). *J Georgia Entomol Soc* **7**: 57-63.
- Entrekin DL, Oliver JH Jr, Pound JM (1987) Effects of gamma radiation on development, sterility, fecundity, and sex ratio of *Dermanyssus gallinae* (Degeer) (Acari: Dermanyssidae). *J Parasitol* **73**(3): 549-554.
- Erickson DG (1965) The fate of gamma-irradiated *Schistosoma mansoni* cercariae in mice. *Am J Trop Med Hyg* **14**(4): 574-578.
- Djoen TB (1965) X-ray induced abnormalities and recovery in *Hymenolepis microstoma*. *J Parasitol* **51**(2): 42
- Dubey JP, Brake RJ, Murrell KD, Frayer R (1986) Effect of irradiation on the viability of *Toxoplasma gondii* cysts in tissues of mice and pigs. *Am J Vet Res* **47**(3): 518-522.
- Hall EJ (1978) Radiobiology for the Radiologist, 2nd ed. Harper & Row, Maryland.
- Hughes DL (1962) Reduction of the pathogenicity of *Fasciola hepatica* in mice by x-irradiation. *Nature* **193**: 1093-1094.
- Ignatowicz S, J Boczek (1984) Effects of gamma radiation on the fertility of *Tyrophagus putrescentiae* (Schrank) (Acarida: Acaridae). In Acarology VI-Vol. 1, DA Griffiths and CE Bowman (eds.) Ellis Horwood Limited, Chichester, England, 457-463.
- Ikeda T, Tani S (1984) The effects of X-irradiation of metacercariae on infection of rats with *Paragonimus ohirai*. *Jpn J Parasitol* **33**(5):377-384.
- Lee SH, Park YH, Sohn WM, *et al.* (1989) The effects of gamma irradiation on the survival and development of *Clonorchis sinensis* metacercariae. *Korean J Parasitol* **27**(3): 187-195.
- Melville C (1958) An apparent beneficial effect of gamma radiation on the flour mite. *Nature* **181**: 1403-1404.
- Scheller LF, Stump KC, Azad AF (1995) *Plasmodium berghei*: Production and quantitation of hepatic stages derived from irradiated sporozoites in rats and mice. *J Parasitol* **81**(1): 58-62.
- Seo BS, Cho SY, Lee SH *et al.* (1981) An epidemiologic study on clonorchiasis and metagonimiasis in riverside areas in Korea. *Korean J Parasitol* **19**(2): 137-150.
- Villella JB, Weinbren MP (1965) Abnormalities in adult *Schistosoma mansoni* developed from gamma-irradiated cercariae. *J Parasitol* **51**(2): 42.

=초록=

감마선 조사가 요코가와흡충의 흰쥐 내 생존 및 발육에 미치는 영향

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요코가와흡충증에 대한 관리책 모색의 일환으로 감마선 조사의 효용성을 관찰하고자 이 연구를 시행하였다. 은어 근육 내에 있는 요코가와흡충의 피낭유충(은어 조사군)과 근육으로부터 분리시킨 피낭유충(피낭유충 조사군)에 감마선을 조사하여 각 군별로 500개씩을 흰쥐에 감염시킨 후 7일째에 생존과 발육상황을 관찰하였다. 피낭유충 조사군의 조사량은 5 Gy, 20 Gy, 50 Gy 및 100 Gy 이었으며, 은어에 조사한량은 5 Gy, 50 Gy, 200 Gy 및 500 Gy로 하였다. 연구 결과, 조사량 증가에 따라 총체 회수율이 급격히 감소하는 양상을 보였으며, 은어 조사군의 경우 같은 조사량에서 피낭유충 조사군에 비하여 더 많은 총체가 회수되었다. 피낭유충 조사군의 LD₅₀은 4.5 Gy, 은어 조사군은 6.2 Gy로 계산되었다. 회수된 총체들의 형태학적 변화가 피낭유충 조사군은 100 Gy에서, 은어조사군은 200 Gy 이상에서 인정되었으며, 변화의 예로는 고환의 발육 부진 또는 소실, 자궁내 총란수 감소 또는 소실 등을 들 수 있었다. 이상의 결과로 볼 때, 은어에 200 Gy 정도를 조사한다면 요코가와흡충 피낭유충의 감염력을 소실시킬 수 있으므로 이 흡충증의 관리방법의 하나로 이용할 수 있을 것으로 기대된다.

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