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Effects of gamma-irradiation on the infectivity and chromosome aberration of *Clonorchis sinensis*

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Abstract: Effects of gamma irradiation on the worm survival and chromosomal aberration of *Clonorchis sinensis* were studied. The metacercariae irradiated with various amounts of gamma radiation (ranging from 5 Gy to 50 Gy) were fed to rats, and the effects were compared with those of non-irradiated controls. Recovery rates of adult worms in irradiated groups were reduced gradually as increasing of the irradiation doses. No worm was recovered from rats which were fed with 50 Gy irradiated metacercariae. The chromosome number was 2n = 56 in all worms from all experimental groups. However, the groups irradiated with 20 Gy, 25 Gy or 30 Gy showed variations in the chromosome number, depending on different cells in the same individual. Radiation doses used in this study did not appear to induce chromosome aberrations, however, irradiation with 30 Gy showed slightly reduced chromosome size.

Key words: *Clonorchis sinensis*, gamma rays, chromosomes

INTRODUCTION

Clonorchiasis is an important food-borne parasitic zoonosis in East Asia including Korea, Japan, China, Taiwan and northern part of Vietnam. People in endemic areas acquire infection by eating raw or undercooked freshwater fish. At present, clonorchiasis is recognized as one of the most important endemic diseases in Korea (Rim, 1990).

Clonorchiasis could be prevented if the metacercariae in the fish are killed or inactivated. Studies on the use of radiation to kill or weaken infectivity of not only protozoa but also helminth parasites have been carried out (Lee et al., 1989; Chai et al., 1995; Dubey et al., 1998), and most of the irradiated helminths are either significantly attenuated or killed depending on the dose of radiation. Recently, irradiation of foods such as fish has been attempted to control various kinds of human pathogens (Dela and Banzon, 1989; Steinhagen et al., 1998).

When parasites are exposed to radiation, they show the growth retardation, vacuolization of the interstitium, elevation of the integument, malformation or underdevelopment of reproductive organs and weakened pathogenicity to the host (Ikeda and Tani, 1984). The effects of ionizing radiation on parasites depend on the radiation dose, developmental and physiological status of worms, and other various physical parameters during or after the irradiation (Hall, 1978). Only a small proportion of *Schistosoma mansoni* cercariae which are

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irradiated with 23 Gy grow to adults, and the majority of females are sterile (Bickle et al., 1979). The migration activity of schistosomula is also weakened by irradiation (Bickle et al., 1979). Such effects of radiation have also been reported in other helminths (Barriga and Myser, 1987; Chai et al., 1995).

However, mechanisms of irradiation-induced chromosomal aberration of *Clonorchis sinensis* have not been studied yet. The purpose of the present study was to investigate the effects of irradiation of the metacercariae on the survival and change of the chromosomes of *C. sinensis*.

MATERIALS AND METHODS

Collection of *C. sinensis* metacercariae

Clonorchis sinensis metacercariae were obtained from artificially-digested material of top-mouth minnow, *Pseudorasbora parva*, collected from Gimhae in Gyeongsangnam-do, Korea.

Irradiation and experimental groups

Metacercariae were irradiated with a PICKER-9 gamma irradiator at the rate of 16.40 Gy/min from 41 cm distance. The doses of radiation employed were 5 Gy, 10 Gy, 15 Gy, 20 Gy, 25 Gy, 30 Gy and 50 Gy.

Experimental infection to rats and recovery of adult worms

Metacercariae were administered to rats (Sprague-Dowley) within 6 hr after irradiation. Three rats in each group were orally administered with either irradiated or non-irradiated 500 metacercariae per rat. At six weeks of post-infection, one rat from each of the 20 Gy, 25 Gy, 30 Gy and 50 Gy irradiated groups and the control group was killed. The remaining rats of irradiated and control groups were killed at eight weeks of post-infection. The number of worms recovered from livers was determined to obtain the recovery rate.

Cytogenetic study

Four adult worms from each group were used for cytogenetic examination. The chromosome in the gonadal tissues of the fluke was studied by simple cell cultivation method (Park et al., 2000). Morphological features of the chromosomes such as relative length and total lengths of the mitotic metaphase chromosomes were compared. The prepared slides were observed under an Olympus BX50 microscope with a 100X oil immersion objective. The nomenclature of the centromeric position for the classification of chromosomes, as proposed by Levan et al. (1964), was followed.

RESULTS

Recovery rate of worms

Adult worms obtained from rats which were fed with the irradiated metacercariae showed significant differences in the worm recovery rates among experimental groups (Table 1). In the control rats infected with non-irradiated metacercariae, 55.2% of worms was recovered at 6 and 8 weeks after the infection. The recovery rates of the irradiated groups were 23.6%, 25.0%, 21.4%, 15.6%, 7.8% and 3.4% with irradiation doses of 5 Gy, 10 Gy, 15 Gy, 20 Gy, 25 Gy and 30 Gy, respectively. Furthermore, no worms were

Table 1. Recovery rate of *Clonorchis sinensis* adult worms from rats fed with normal and irradiated metacercariae

Experimental groups (Gy)[a]	No. of rats used	Total No. metacercariae infected	No. of worms recovered		
			Total	Range	Averge
0[b]	3	1,500	828	244-296	276
5	3	1,500	354	120-136	118
10	3	1,500	375	109-142	125
15	3	1,500	321	96-123	107
20	3	1,500	234	62-89	78
25	3	1,500	117	14-57	39
30	3	1,500	51	13-22	17
50	3	1,500	0	0	0

[a]Irradiated dosage of gamma ray to metacercariae.

[b]Non-irradiated control group.

Table 2. Chromosome numbers of adult worms[a)] from rats fed with normal and irradiated *Clonorchis sinensis* metacercariae

Experimental groups (Gy)	No. of cells with diploid chromosome numbers					
	52	53	54	55	56	57
0	-	-	-	-	15	-
5	-	-	-	-	12	-
10	-	-	-	-	8	-
15	-	-	-	1	6	1
20	-	-	1	3	11	-
25	1	-	1	2	7	1
30	1	-	2	-	6	-

[a)]Four worms in each radiation dose were examined.

recovered from rats which were fed with 50 Gy irradiated metacercariae.

Chromosomal aberration in the worms from the irradiated groups

Mitotic chromosomes were observed in 78 cells from 24 individuals. In all of the metaphase figures of mitosis in *C. sinensis*, 56 (2n) chromosomes were recognized. Table 2 shows the chromosome numbers of adult worms from each experimental group. The groups irradiated with 20 Gy, 25 Gy or 30 Gy showed variations in chromosome numbers among different cells from the same individuals. Distinct 28 and 56 chromosomes were observed from non-irradiated controls and irradiated groups, being diploid numbers, respectively (Fig. 1). The metaphase chromosomes consisted of 8 pairs of large chromosomes and 20 pairs of small chromosomes. Chromosomes of adult worms in 5 Gy and 15 Gy irradiation groups (Fig. 1B & C) were not changed significantly when compared with those of non-irradiated controls (Fig. 1A). In comparison with the control group, the group irradiated with 30 Gy showed slightly reduced in chromosome size. A few chromosomes in the worms from 30 Gy irradiated group showed slight contraction in the chromatid, resembling a thread with a node (Fig. 1D). The mean total length of diploid complement was 138.49 ± 3.7 μm in control groups and 117.63 ± 5.3 μm in 30 Gy irradiated group.

DISCUSSION

The effects of radiation on trematodes were dependent on the irradiation dosage, preconditioning and physiological status of animals to irradiation (Villella and Weinbern, 1965). The percentage of metacercariae developed in control rats in this study was similar to other reports (Concepcion and Barriga, 1985; Lee et al., 1989). The groups which were fed with metacercariae exposed to 25 Gy and 30 Gy showed a significant ($p < 0.05$) reduction in the recovery rate compared to that of the control group. No worms were recovered from the 50 Gy irradiation group. *Clonorchis sinensis* in the present study appeared to be quite sensitive to radiation, compared with other helminths. *Paragonimus ohirai* metacercariae were also sensitive to irradiation and could not grow in the host after exposure to 20 Gy (Ikeda and Tani, 1984). A remarkable difference of radio-sensitivity was observed depending on species of parasites: for examples, trematodes are more sensitive to irradiation in general than nematodes or cestodes. However, the difference of radiosensitivity may also be seen in the same species at various stages of development, in which the frequency of cell division is different. In *Schistosoma mansoni*, spermatogenesis and oogenesis are suppressed by 23 Gy (Bickle et al., 1979). Oliver et al. (1972) have noted clumping of chromatin material after irradiation and recorded chromosomal bridges and rings. Radiation is well known to destroy DNA structures directly or by forming hydroxy radicals in the tissue. Therefore there may be three times more cell destruction in the oxygenated state than in the anoxic one (Hall, 1978). Variations in the chromosome number from 1 to 5 among different cells within the same individual were observed by irradiation with 20 Gy, 25 Gy and 30 Gy. Supernumerary chromosomes have generally been defined as those which have additional number to the karyotype and are non-homologous or homologous to chromosomes of the normal complement (White, 1973). Doses of up to 20 Gy of gamma irradiation in this study increased the number of supernumerary chromosomes in metaphase stage of *C. sinensis*.

The chromosome numbers and karyotypes of *C.*

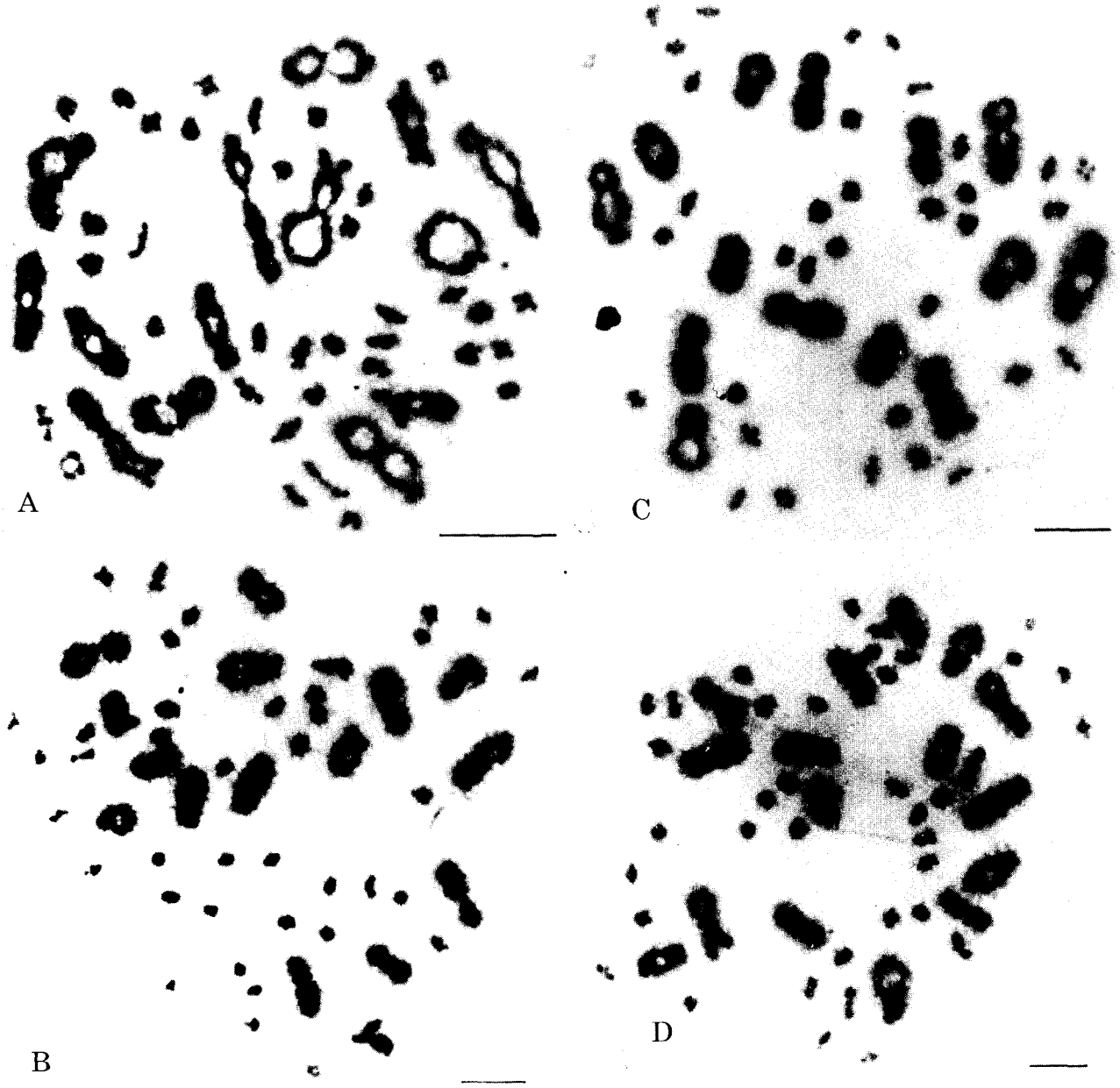

Fig. 1. Mitotic chromosomes of *Clonorchis sinensis*. Non-irradiated control group (A). 5 Gy irradiated group (B) and 15 Gy irradiated group (C), showing slightly reduced in chromosome size than control group. 30 Gy irradiated group (D), showing slightly contraction in the chromatid than 5 and 15 Gy irradiated group. Scale bar= 10 μm.

sinensis have been reported (Park et al., 2000). In this study, we observed the same chromosome number. Sasaki (1961) have been reported that highly contracted chromosomes tend to have their centromeres more median than less contracted ones in different cells, while longer chromosomes tend to contract more strongly on colchicine treatment than shorter ones in the same cell. In this chromosomal study, we made preparations under the same conditions, and in fact, this air-drying method produced more uniform and complete flattening of cells than could be achieved by the usual squash methods. Results of present study did not indicate any change in the number of chromosomes during spermatogenesis among the different radiation groups, but did show slightly reduced in chromosome size in the worms from 25 and 30 Gy irradiated groups. In general, the chromosomes gradually become shorter as the irradiation doses increased. Nonetheless, irradiation doses used in the

present study did not alter chromosome numbers of the survived parasites after gamma irradiation.

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