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EFFECTS OF GAMMA-RADIATION ON OVARIAN FOLLICLES

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In order to observe the morphological and endocrinological changes of the rat and mouse ovarian follicles by gamma-radiation, rats were whole-body irradiated with doses of 3.2 Gy and 8.0 Gy and mice with 2.9 Gy and 7.2 Gy. Sections of ovaria were examined by light microscopy. Concentrations of progesterone, testosterone, and estradiol in ovarian homogenate were determined by radioimmunoassay techniques. Gamma-radiation resulted in the increased percentage of atretic follicles in the groups killed on day 0, day 4, and day 8 after irradiation. The decrease in granulosa cell viability was found in animals killed on day 4 after irradiation. The finding of the high ratio of testosterone to estradiol compared to that of progesterone to testosterone suggests that aromatase activity — steroid biosynthesis from testosterone to estradiol — in granulosa cell could be affected by gamma-radiation.

Key words:
estradiol, mice, ovarian histology, progesterone, rats, testosterone

Radiation can change the characteristics of the cell nucleus and cytoplasm, as mammalian germ cells are very sensitive to ionizing radiation (1). According to *Spalding and co-workers* (2), preantral and Graafian follicles showed histological sensitivity in whole-body irradiated rats with doses of 7.5 Gy and 30 Gy. *Mole and Papworth* (3) stated that atretic follicles had appeared dominantly through the influence of high-dose radiation. Many of the qualitative observations have been confirmed by morphological studies (1). Radiation-induced changes in the production of hormones by mammalian gonads are a suitable index for assessing endocrinological effects of radiation on germ cells. There have been reports of the increase in rat serum corticosterone, prolactin, and thyrotrophin by microwave radiation (4), in rat progesterone by gamma-radiation (5), and in gravid rat testosterone by gamma-radiation (6).

By now, however, there has not been an endocrinological study to demonstrate functional changes in ovarian follicles and to complement the morphological observations. The present study demonstrates the morphological and endocrinological effects of gamma-radiation on rat and mouse ovary.

MATERIALS AND METHODS

Rats and mice used in this experiment were young, adult, virgin females in estrus, Sprague Dawley rats, 8-week-old and ICR mice, 5-week-old, as determined by the vaginal smear test. The rats had been bred at the Korea Advanced Institute of Science and Technology (KAIST). The animals were whole-body irradiated with gamma-rays (^{60}Co, dose rate: 0.5 Gy/min., Panoramic Irradiator, approximately 1000 Ci capacity, Atomic Energy of Canada Ltd.) in doses of 3.2 Gy (LD_{20}) and 8.0 Gy (LD_{50}) for rats and 2.9 Gy (LD_{20}) and 7.2 Gy (LD_{50}) for mice. The dose rate was determined by Fricke dosimeter (7). After irradiation, the animals were divided in groups consisting of 8 animals each. The animals were killed by cervical dislocation under diethyl-ether anaesthesia on day 0, day 4, and day 8 after irradiation.

The right ovaries were trimmed from surrounding tissue, homogenated and suspended in 500 μl gelatin phosphate buffered saline. Granulosa cell viability (8) was determined in a 1% nigrosin solution (Sigma Chem. Co.) by haemocytometer (Neubauer, Superior) with 100 μl of the above fluid. The left ovaries were cleaned and fixed in Bouin's solution for 24 to 48 hours. They were dehydrated in graded ethanol series, embedded in paraplast (Monoject), cut in sections thick 7 μm, stained with Harris haematoxylin and eosin, and every tenth section with oocytes was examined with a light microscope. Atretic follicles were determined by finding pyknosis in the follicular granulosa cells and the hypertropy of theca cell layer (9). Steroid hormones progesterone, testosterone, and estradiol were analysed by radioimmunoassay (10). Statistical significance among the groups was analysed using the Student's *t*-test.

RESULTS

The percentage of atretic follicles of the groups of rats and mice killed on day 4 after irradiation increased in relation to the groups killed on day 0. There were no further significant increases in the percentage of atretic follicles in the groups killed on day 8 (Table 1 and Table 2). The groups killed on day 4 manifested lower granulosa cell viability than did the control groups. It recovered to the level of the control group in animals killed on day 8. There were no significant differences in the percentage of atretic follicles between the animals that received the high- and the low-dose irradiation.

Table 1 *The percentage of atretic follicles and viable granulosa cells in irradiated rats.*

Day	Dose (Gy)	Atretic follicles (N=8)		Granulosa cell viability
		preantral	antral	
	0	44.6 ± 4.65	50.4 ± 3.03	62.5 ± 4.00
0	3.2	59.0 ± 6.93*	52.4 ± 9.77	57.1 ± 9.28
	8.0	65.9 ± 9.35*	66.7 ± 8.68	64.6 ± 9.66
4	3.2	70.7 ±10.6*	78.4 ±10.1*	46.7 ± 5.07*
	8.0	69.2 ±14.5*	80.0 ±14.7*	53.4 ±16.2
8	3.2	66.3 ±10.1*	68.7 ± 6.98*	71.4 ±10.3
	8.0	67.6 ±10.2*	76.0 ±11.9*	64.5 ±11.9

*P<0.05

Table 2 *The percentage of atretic follicles and viable granulosa cells in irradiated mice.*

Day	Dose (Gy)	Atretic follicles (N=8)		Granulosa cell viability
		preantral	antral	
	0	36.8 ± 5.35	40.1 ± 4.70	64.6 ± 9.34
0	2.9	40.3 ± 6.13	31.6 ± 5.24	71.6 ± 8.02
	7.2	38.6 ± 6.03	32.3 ± 7.00	56.9 ± 9.97
4	2.9	75.2 ± 10.8*	84.4 ± 5.56*	46.7 ± 6.19*
	7.2	84.6 ± 13.9*	80.0 ± 7.11*	59.5 ± 3.42
8	2.9	68.2 ± 9.65*	71.4 ± 6.10*	64.8 ± 6.41
	7.2	77.5 ± 11.2*	79.3 ± 7.54*	64.5 ± 11.3

*P<0.05

Progesterone concentration in rats was similar to the control level. All irradiated groups of mice showed higher progesterone concentrations than did the control group. Rat testosterone concentration did not change, but that of mice decreased in both irradiated groups of day 4 and day 8 at high doses. Estradiol levels in both rats and mice were significantly lower than in the control groups (Fig. 1 and Fig. 2).

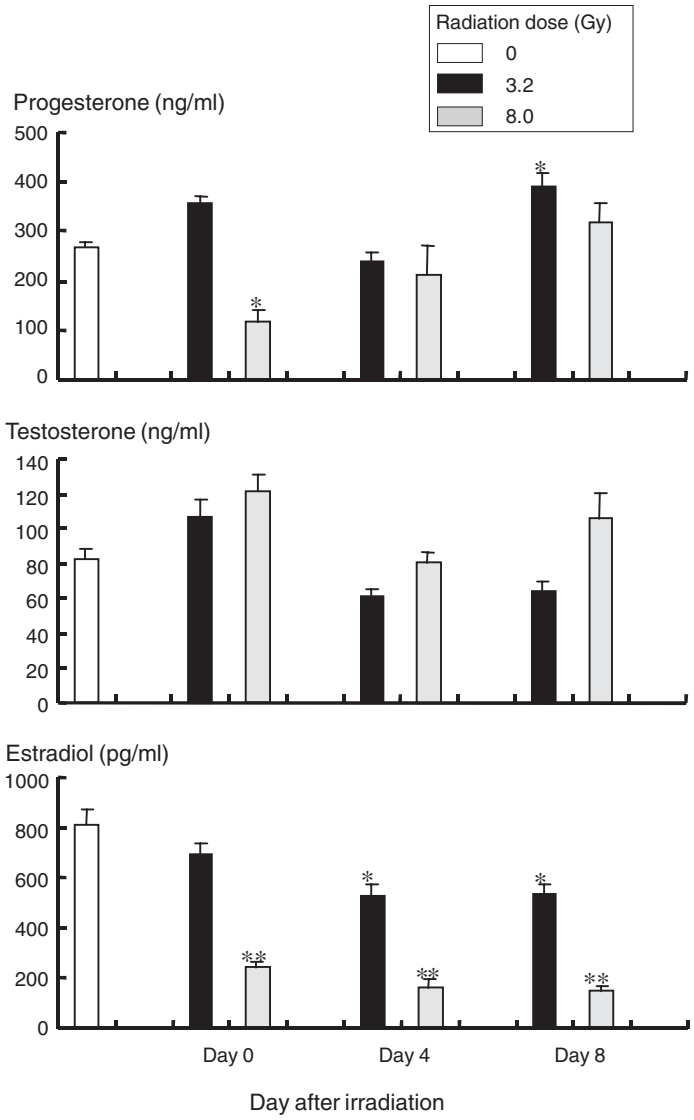

Fig. 1 Concentrations of progesterone, testosterone and estradiol in irradiated rat ovarian homogenates. Data represent means and SEM of 8 animals per group. Significantly different to the control (dose 0): *P<0.05; **P<0.01.

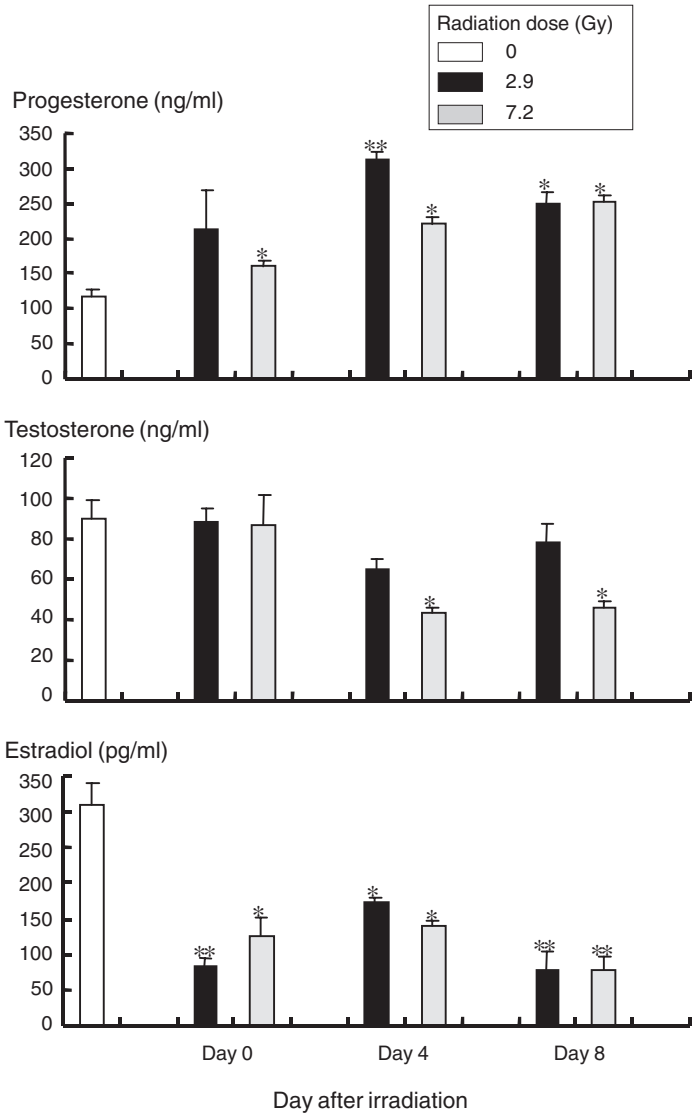

Fig. 2 Concentrations of progesterone, testosterone and estradiol in irradiated mouse ovarian homogenates. Data represent means and SEM of 8 animals per groups. Significantly different to the control (dose 0): *P<0.05; **P<0.01.

DISCUSSION

The lethal dose 50 of irradiation in this study was 8.33 Gy for rats and 7.20 Gy for mice, which is similar to the results of *Hobbs and McClellan* (11). It generally depends on the species, the radiation source (X-ray), dose and other irradiation conditions. Morphologically, the follicular atretic rate in most experimental groups were higher than in the control groups, and the number of oocytes in the sections after irradiation was smaller than in the control group. The lower granulosa cell viability in the groups killed on day 4 after irradiation grew to the control level in the groups killed on day 8. According to the report by *Dobson and Felton* (1), the higher percentage of atretic follicles seems to be a result of destruction of oocyte cells and the dysfunction of granulosa cells induced by radiation. Considerations about spontaneous atresia suggest that the theca cell and/or granulosa cell may be the starting points for the follicular atresia (9). The target size of radiation was larger in the ovarian follicle than in the granulosa cell but smaller than in the oocyte (12). The above suggests that the atretic process resulted from radiation in granulosa cells might proceed toward other follicular cells and oocytes.

Steroid hormones synthesized in ovary are distributed in the body by blood circulation (9). In contrast to progesterone and testosterone, estradiol concentration in the ovarian homogenates declined after irradiation. The ratio of progesterone to testosterone did not change in rats, but increased in mice in both groups. However, the concentration ratios of testosterone to estradiol were high in both rats and mice. This suggests that there might be an inactivation of hydroxy steroid dehydrogenase and/or isomerase in the theca cell in mice (13) and the blockade of aromatase activity in the granulosa cell by radiation in both rats and mice (14). To confirm this assumption, it is necessary to conduct further experiments with the coenzymes related to these enzymes. The number of luteinizing hormone receptors in atretic follicles, reported to be smaller than that in normal follicles, indicates that the decrease in luteinizing hormone receptors may have not caused the follicular atresia, but the sudden cessation of leuteinizing hormone secretion induced by radiation (15). The results do not clarify whether radiation directly affects the inactivation of enzymes involved in follicular cells or it leads to internucleosomal fragmentation and the consequent decrease in these enzymes.

We have unpublished evidence that gamma-radiation induces the breakdown of nucleosomal units. In treated animals**,** it appears that changes in steroidogenesis occurred somewhat earlier than in the ovarian morphology. This suggests that radiation could interrupt conversion of testosterone to estradiol in granulosa cells and, to a lesser extent, the conversion of progesterone to testosterone in theca cells.

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Sažetak

UČINCI GAMA-ZRAČENJA NA FOLIKULE JAJNIKA

U radu su procjenjivane strukturne i endokrinološke promjene u folikulima jajnika štakorica i mišica izazvane gama-zračenjem. Štakorice su bile izložene zračenju od 3,2 Gy ili 8,0 Gy, a mišice od 2,9 Gy ili 7,2 Gy. Životinje su usmrćene 0, 4, odnosno 8 dana nakon ozračenja. Rezovi debljine 7 µm pripremljeni su za mikroskopiranje. Koncentracije progesterona, testosterona i estradiola u homogenatu jajnika određene su specifičnim radioimunoesejem. Gama-zračenje uzrokovalo je povećanje broja atretičnih folikula u obje skupine životinja usmrćenih 4 odnosno 8 dana nakon ozračivanja. Gama-zračenje također je smanjilo životni vijek granuloza stanica u skupinama usmrćenim 4. dan nakon ozračivanja. Utvrđeno povećanje omjera testosterona prema estradiolu u usporedbi s omjerom progesterona prema testosteronu upućuje na to da gama-zračenje utječe na aktivnost aromataze u steroidnoj biosintezi testosterona u estradiol u granuloza stanicama.

Ključne riječi:
estradiol, histološki pregled, mišice, progesteron, štakorice, testosteron

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