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Effect of Gamma Radiation on Vascular Epithelial Growth Factor Receptors Expression in a Rat Kidney

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Abstracts

Ionizing radiation generates side effects on normal tissues during radiotherapy. In order to evaluate the impairment of renal region, F344 rats were given wholebody gamma irradiation. All groups received a single dose of 6.5 Gy. Two weeks after whole body irradiation, organs and sera were collected for measuring the induced damage. Hematological analysis was accomplished in plasma. Most of components (RBC, WBC, Hb, etc.) showed marked decreases. Body weights and organ indices of the irradiated group decreased significantly compared with those of the control. Expression of molecules related apoptotic death showed unusual pattern and expression of VEGF-receptors on irradiated kidney was elevated higher than the control level. Taken together, ionizing radiation caused the injury in angiogenesis and vascularization on normal tissue. Noteworthy, VEGF action to renewal impairments caused by radiation can affect the activity of the Bcl 2 family, and VEGF and its receptors give rise to inhibition of the radiation-induced programmed cell death.

1. Introduction

Ionizing radiation is an effective modality for the treatment of many tumors. But it also generates side effects on normal tissues. The most treatment to complete the radiotherapy is to combine cytotoxic chemotherapeutic agents with radiation. The cytotoxicity of chemotherapeutic agents, however, is not limited to tumor cells because treatments of tumors with these agents can result in significant normal tissue toxicity [1-3]. There are two kinds of drugs, antiangiogenesis and vascular targeting agents, related to combine with radiation [4]. These agents to improve the efficiency of cure in radiotherapy caused side effects on normal tissues, in addition, ionizing radiation can affect to angiogenesis and neovascularization on normal for itself. Therefore, the present study tests the effect of gamma irradiation to renal region and the expression of renewal angiogenesis-related growth factors after irradiation.

Biological process of wound healing, which occurs in three phases revascularization (inflammatory, proliferative, and maturation) is an important essential step in regulating this process. Blood vessels serve as carriers for various cells, cytokines, and growth factors that are needed for tissue repair. The formation of new blood vessels is a necessary event during embryogenesis, but it occurs rarely in the adult with few exceptions, such as in the female reproductive system and wound healing. Angiogenesis is controlled by a variety of mitogenic, chemotactic, and inhibitory peptide and lipid factors that act on invading endothelial and smooth muscle cells [5]. One of the most important angiogenic factors is the vascular endothelial growth factor (VEGF), a glycosylated protein of 46-48 kD composed of two disulphide linked subunits. The VEGF family consists of six members, five splicing forms of VEGF and the placenta-derived growth factor (PIGF). In normal, VEGF is expressed during embryogenesis and in a limited number of sites in adults. In disease states, VEGF can be detected in various tumor cells, the synovial pannus in rheumatoid arthritis, and in keratinocytes during wound healing. Five different VEGF isoforms, with 121, 145, 165, 189, and 106 amino acids, can be generated as a result of alternative splicing from the single VEGF gene. The VEGF molecules bind to receptors known as VEFGR-1 (FLT-1, fms-like tyrosine kinase 1), VEGFR-2 (KDR, kinase domain region/FLK-1, fetal liver kinase 1), VEGFR-2 (FLT-4), neurophilin-1, neurophilin-2, and heparan sulfate proteoglycans. The signaling tyrosine kinase receptors VEGFR-1 (FLT-1) bind VEGF121

and VEGF165, and VEGFR-2 (KDR/FLK-1) additionally VEGF145 (apart from certain VEGF-related peptides).The co-receptors neuropilin-1 and -2 selectively bind the 165-residue VEGF isoform [6-8].

2. Materials and methods

2.1 Animals and Irradiation

Fifteen, 4-week-old, 85-90 g male Fisher 344 rats were purchased from Daehan Biolink (Chungbuk, Korea). Rats were acclimated for at least 3 days before the experiment was started. They were kept in cages containing chip bedding, three rats per cage. All rat were maintained under the following conditions; temperature (23°C) and lighting (12 hr light: 12 hr dark) and allowed free access to food and water.

The fifteen rats were allocated randomly into three groups of five rats each. Irradiated groups were exposed to λ-irradiation using a ^{60}Co source with a total dose of 6.5 Gy, and a dose rate of 12.8 Gy/hr [9]. All rats were euthanized two weeks after irradiation. Immediately after death, blood was collected from the heart. The blood was left to clot at room temperature for 1 hr, centrifuged and serum was immediately frozen at -70°C until analysis.

2.2 RNA Isolation and Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from frozen tissue using a Trizol (Gibco BRL) reagent according to the manufacturer's instructions. The RNA concentration was determined by spectrophotometer. For a cDNA synthesis, 10 ug of the total RNA was taken and reverse transcribed to cDNA in a final volume of 40 l, using AMV transcriptase (Promega) and oligo(d)T15 primers (Promega) following the protocol provided by the enzyme supplier. The PCR was carried out with a PCR Thermal Cycler 480 (TaKaRa). Amplication was performed for 35 cycles under the following conditions: denaturation at 95℃ for 1 min, annealing at an optimal temperature for each primer [10-12], elongation at 72℃ for 1min, and after the last cycle an additional elongation step for 5 min at 72℃. The PCR products were loaded in 2% TBE-buffered agarose gel and analyzed after gel electrophoresis by EtBr-staining and UV light illumination. The intensities of each gene expression signal on the film were normalized

with the glyeraldehyde-3-phosphate dehydrogenase (GAPDH) internal control [10]. The intensities were determined by scanning laser densitometry.

2.3 Statistical Analysis

Statistical analysis was performed by Student's *t* test for a simple comparison of the each treatment group with the sham control group using Sigma Plot® software (Jandel Scientific, Germany). They are expressed as mean ± SEM.

3. Results and Discussions

Difference of increase of body weight between control and radiation groups was indicated in Figure 1. Radiation group showed 25% decrease compared with weight increase of control.

As shown in Table 1, the weight of liver, kidney, testis, and spleen decreased 8%, 5%, 32%, and 40%, respectively, compared with that of control. In case of liver and kidney, rate of weight decrease did not show distinguished differences as well as testis and spleen, a well-known radiosensitive organ.

Results of the hematological analysis of plasma from the experimental group are represented in Table 2. Values of RBC, WBC, platelet, HCT and HB significantly reduced in the irradiated rats, while those of MCV, MCH, and MCHC were slightly elevated in the irradiated group. In case of serum level of WBC, both of the groups show a decrease compared to the control. MCV, MCH and MCHC were not different from those of the control.

To evaluate the relation between radiation and neovascularization, mRNA expressions of apoptosis- or vascularization- related molecules were determined in kidney two weeks after wholebody irradiation. Expression of Bax and Bcl-2 in irradiated kidney showed usual pattern after irradiation, but mRNA expression of caspase-3, a key executioner of programmed cell death, did not increase than control level (Figure 2). These results indicated that activities of VEGF and its receptor inhibit activity of Bcl 2 family and apoptotic cell death [13]. Molecules related neovascularization showed remarkable jump compared with

those of control. It means that renewal including vascularization and angiogenesis take place in injury region caused by gamma radiation.

Taken together, ionizing radiation caused the injury in angiogenesis and vascularization on normal tissue. Noteworthy, VEGF action to renewal radiation-induced impairments can affect to activity of the Bcl 2 family and VEGF and its receptors give rise to inhibit the radiation-induced programmed cell death.

5. References

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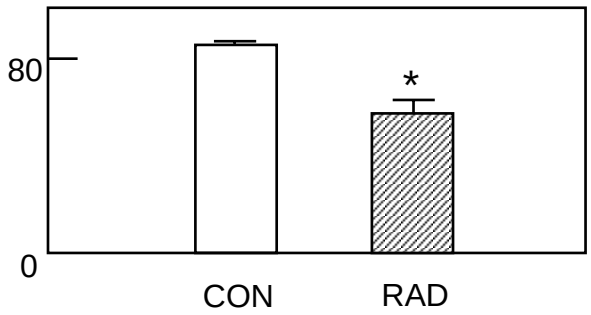

Figure 1. The rate of increase in the body weight two weeks after wholebody irradiation.

Abbreviatations; CON, control group; RAD, irradiated group. Asterisk, $p<0.05$.

Table 1. Organ weights (g) of experimental groups

	Body W	Liver W	Kidney W	Testis W	Spleen W
CON	144.6 ± 1.27	6.6 ± 0.4	0.64 ± 0.05	0.868 ± 0.05	0.42 ± 0.01
RAD	123.8 ± 5.81	6.1 ± 0.5	0.62 ± 0.03	0.598 ± 0.05	0.25 ± 0.03

The value represents the mean ± SD.

Table 2. The hematological results from plasma of the experimental groups†

	RBC	WBC	Platelet
CON	100 ± 0.2	100 ± 0.4	100 ± 6.5
RAD	60.1 ± 0.1**	11.5 ± 0.03**	27.4 ± 8.3**

	HCT	Hb	MCV	MCH	MCHC
CON	100 ± 1.6	100 ± 0.5	100 ± 0.8	100 ± 0.3	100 ± 0.3
RAD	63.1 ± 1.5*	64.5 ± 0.5*	103 ± 0.9	107 ± 0.5	102 ± 0.3

†, All values expressed as means ± SEM (n = 5 in each group).

* and ** indicate $p<0.05$ and $p<0.02$, respectively.

Abbreviations; CON, control group; IRR, irradiated group; RBC, red blood cell count; WBC, white blood cell count; HCT, hematocrit; HB, hemoglobin; MCV, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration.

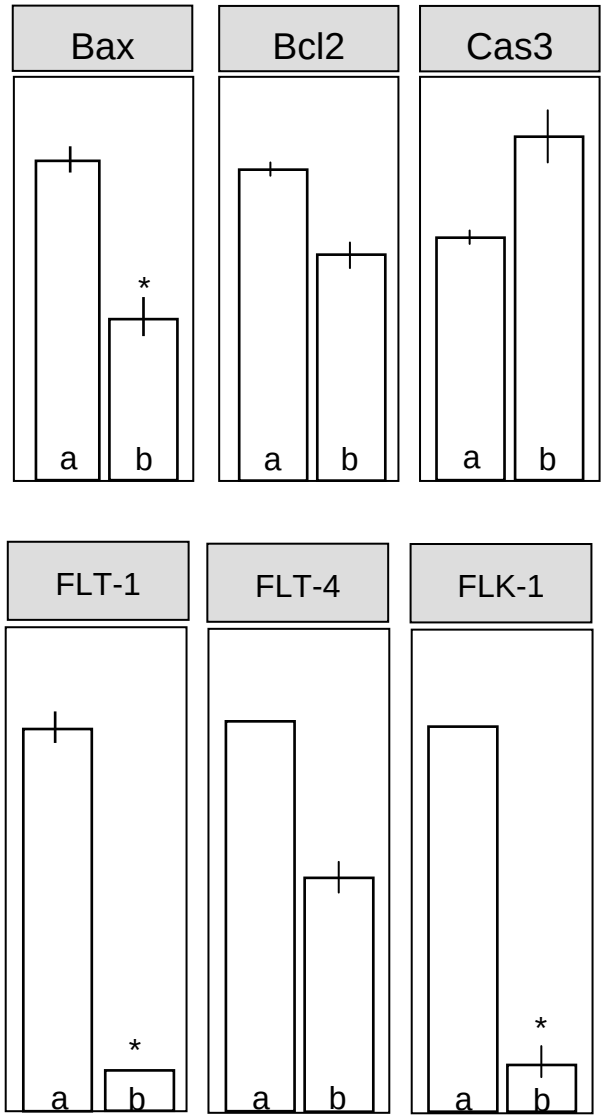

Figure 2. Semiquantitative expression of Bax, Bcl-2, Caspase-3, Flt-1, Flt-4, and Flk-1 relative to GAPDH mRNA expression, using a reverse transcription-polymerase chain reaction assay. Densitometric analysis was performed using a scanning laser densitometry. a and b represent irradiated and control group, respectively. Asterisk, $p<0.05$.