Relative Expression of Apoptotic and Vascular Epithelial Growth Factor Receptor Genes in Gamma-Irradiated Rat Kidney

Ji Hyang Kim[a,b], Ki-Jung Chun[a], Yong-Dal Yoon[b], Jin Kyu Kim[a]
a, Korea Korea Atomic Energy Research Institute, Daejeon 305-353, Korea, jhkim0924@intizen.com
b, Department of Life Science, Hanyang University, Seoul 133-791,

1. Introduction

Biological process of wound healing, which occurs in three phases of 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 [1]. 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 (PDGF). 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 an 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 [2].

Ionizing radiation can affect the angiogenesis and neovascularization on normal tissues in radiotherapy or by background radiation surrounding living beings. Kidney belongs to the urinary system and classified to the radio-resistant organ according to the previous studies. Therefore, the present study tested the effect of gamma irradiation and mercury chloride ($MgCl_2$) to the renal region by confirming the apoptosis and the expression of renewal receptors to signals for angiogenesis-related growth factors after irradiation.

2. Methods and Results

Fifteen, 4-week-old, 85-90 g male Fisher 344 rats were purchased from Daehan Biolink (Chungbuk, Korea). The fifteen rats were allocated randomly into three groups of five rats each. Irradiated groups were exposed to γ-irradiation using a ^{60}Co source with a total dose of 6.5 Gy, and a dose rate of 12.8 Gy/hr [3]. Mercury chloride ($HgCl_2$) was administered 1 mg/kg in drinking water. All the rats were euthanized two weeks after γ-radiation and mercury exposure.

2.1 Changes of Kidney Index after Irradiation

Two weeks after whole-body irradiation, difference of the increase of the body weight between the control and radiation groups was indicated in Figure 1. Radiation group showed about 32% decrease compared with the weight increase of the control. Kidney weight of the irradiated group showed an increased pattern because of reduction of the body weights.

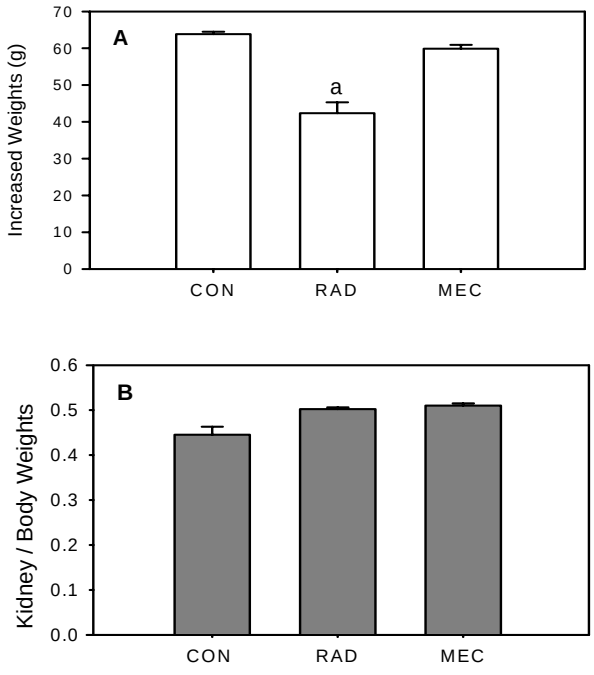

Figure 1. Changes of the body weights and kidney weights 2 weeks after whole-body irradiation and mercury exposure. a, $p<0.05$.

2.2 Expression of Bax, Bcl-2, Caspase-3, FLT-1, FLT-4, FLK-1 mRNA in the irradiated kidney

Total RNA was isolated from kidney of the experimental group using the Trizol reagent (GibcoBRL, Scotland, UK) according to the manufacturer's instructions. The strand cDNA was

synthesized by a reverse transcription of 3 μg total RNA using polyA(d)T random primers (Promega, USA) according to the manufacturer's suggested protocol. Expression levels of bcl-2, bax, caspase-3, FLT-1, and FLT-4 mRNA were measured by previous investigations [3].

In the irradiated group, caspase-3 showed a significant increased expression. But expression of FLK-1 and FLT-4 decreased significantly. In the mercury-exposed group, mRNA of FLK-1 was elevated highly compared with expression of the other group. The result indicated relation between the distribution of FLK-1 and target of mercury chloride (II). And it was distinguished from the irradiated group.

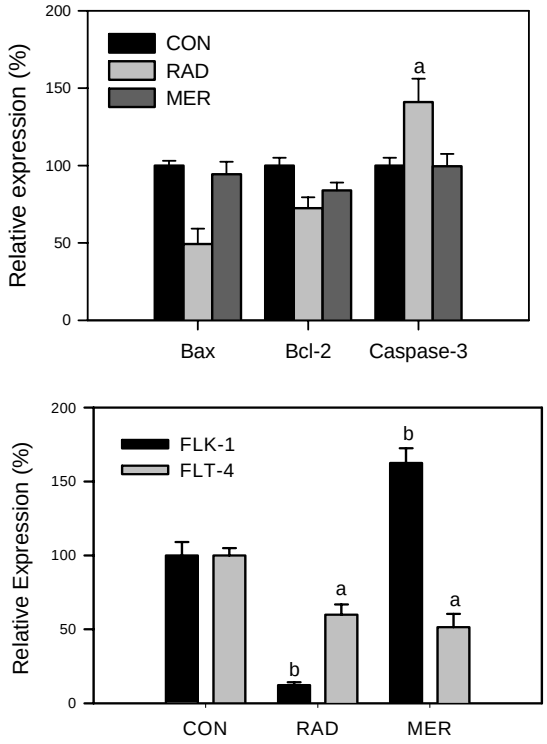

Figure 2. Relative expressions of the mRNA for Bax, Bcl-2, Caspase-3, FLK-1 and FLK-4 in irradiated or mercury-exposed male rats. The expression of the each mRNA was compared with β-actin. a, $p<0.05$ and b, $p<0.02$.

2.3 Determination of Apoptosis using TUNEL Assay

TUNEL staining was performed using the ApopTag kit (Oncor, Purchase, NY). Briefly, sections (4 μm) were deparaffinized in xylene, rehydrated in decreasing concentrations of ethanol, boiled in Citra (Biogenex, San Raman, CA) for 10 min, and digested in 0.5% pepsin for 60 min at 37℃, before endogenous peroxidase was blocked in 3% hydrogen peroxide. Dilutions (1:16) of terminal deoxynucleotidyl transferase (TdT) in reaction buffer (containing a fixed concentration of digoxigenin-labelled nucleotides) were applied to serial sections for 1 h at 37℃, before the slides were placed in Stop/Wash buffer for 10 min. Following washes, a prediluted anti-digoxigenin peroxidaseconjugated antibody was applied for 30 min. Apoptotic cells were detected after incubation in the 3,3'-diaminobenzidine (DAB) chromogen (DAKO, Carpinteria, CA) for approximately 3 min and slides were counterstained with Methyl Green (Sigma, St Louis, MO).

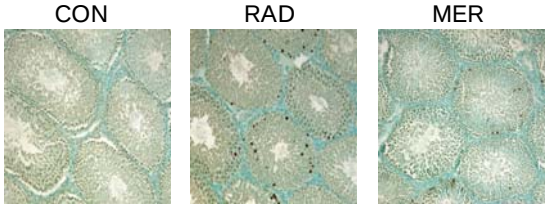

Figure 3. Paraffin sections were labeled by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) technique using the Apoptag kit and counterstained with methyl green.

By TUNEL assay, apoptotic occurrences compared the irradiated group with the mercury-exposed group. TdT-stained cells in the irradiated group were threefold occurrence. As testis was a radiosensitive organ, apoptosis by irradiation showed remarkable elevation.

2.4 Statistical Analysis

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

3. Conclusion

Ionizing radiation caused the damage with different action of mercury chloride (II) in the angiogenesis and vascularization on normal tissue, kidney. Noteworthy, VEGF action to renewal radiation-induced impairments can affect the activity of the Bcl 2 family and the VEGF and its receptors give rise to inhibition of the radiation-induced programmed cell death.

REFERENCES

[1] M. H. Maurer, W. K. C. Tripps, R. E. Feldmann, W. Kuschinsky, Expression of vascular endothelial growth factor and its receptors in rat neural stem cells, Neurosci Lett., Vol. 344, p. 165, 2003.

[2] G. Neufeld, T. Cohen, S. Gengrinovitch, Z. Poltorak, Vascular endothelial growth factor (VEGF) and its receptors, FASEB J., Vol. 13, p. 9, 1999.

[3] J.K. Kim, J.H. Kim, Y.D. Yoon, Evaluation of caffeine as a radioprotector in whole-body irradiated male mice, In Vivo, Vol. 17, p. 197, 2003.