# Radioresponse of human lymphocytes pretreated with boron and gadolinium as assessed by the comet assay

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**Background:** Boron and gadolinium are among the nuclides that hold a unique property of being a neutron capture therapy agent. Neutron beams have often a considerable portion of gamma rays with fast neutrons. Gamma rays, as beam contaminants, can cause considerable damage to normal tissues even if such tissues do contain high boron concentrations. **Materials and Methods:** The modification of radioresponse in human lymphocytes pretreated with boron or gadolinium compound was studied by assessing the DNA damage using single cell gel electrophoresis (SCGE), the comet assay. The lymphocytes from the human peripheral blood were irradiated with 0, 1, 2 and 4 Gy of gamma rays from a $^{60}$Co isotopic source with or without pretreatment of boron or gadolinium compound for 10 minutes at 4℃. Post-irradiation procedures included slide preparation, cell-lysing, unwinding and electrophoresis, neutralization, staining, and analytic steps, gel electrophoresis. **Results:** The results indicate that pretreatment with boron compound (50 nM or 250 nM of $^{10}$B) is effective in reducing the radiosensitivity of the lymphocyte DNA. Conversely, pretreatment with gadolinium compound (50 nM) led to a dose-dependent increase in the radiosensitivity, most prominently with a dose of 4 Gy *(P<0.001)*. Furthermore, when the lymphocytes were pretreated with a combined mixture (1:1) of boron (250 nM) and gadolinium (50 nM) compounds, the reduced radiosensitivity was also observed. **Iran. J. Radiat. Res., 2009; 7 (2): 63-68**

***Keywords:** Radiosensitivity, lymphocyte, DNA damage, boron, gadolinium, comet assay.*

## INTRODUCTION

The comet assay, commonly known as the single cell gel electrophoresis (SCGE), was first introduced by Östling and Johanson [1] and named after the appearance of 'Comet'. The comet assay is considered to be a sensitive methodology for monitoring of genotoxic effects [2,3]. Above all, the alkaline single-cell gel electrophoresis assay has been used as a powerful and sensitive technique for detecting different types of DNA damage, ranging from DNA single-strand breaks to alkali-labile lesions, in individual eukaryotic cells [4-6]. The comets, consisting of heads and tails, reflect the types of DNA damages and the degree of DNA strand breaks.

The clinical application of treating brain tumors with boron neutron capture therapy (BNCT) is very encouraging. Also, $^{157}$Gd is one of the nuclides that hold the unique property of being a neutron capture therapy agent [7]. For neutron capture therapy (NCT), neutron beams contain often a considerable portion of the gamma rays with fast neutrons having high energies in the range of several MeV [8,9]. Gamma rays, as one of the beam contaminants, can cause considerable damage to normal tissues even if such tissues do contain high $^{10}$B concentrations. The present study was designed to study the modifying effect of boron and gadolinium compounds on the biological efficacy of γ-rays in human lymphocytes using the comet assay to support the fundamental understanding of neutron capture therapy. Beside the neutron capture reactions, the treatment with the two chemicals can also give rise to radio-

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modifying effects in various biological systems irradiated with sparsely ionizing radiation. Such radioresponse modification in biological systems is a particularly interesting phenomenon from the radiobiological stance. In the present study, applying the comet assay, we examined the effect of boron and gadolinium compounds on γ-ray-induced lymphocyte DNA damage, a contaminant of the therapeutic neutron beams.

## MATERIALS AND METHODS

### Isolation of lymphocytes

Human peripheral blood samples were obtained from a healthy male donor (28 years old). Lymphocytes were isolated by mixing 100 µl of heparinized blood and 200 µl of RPMI 1640 (Sigma) medium with 10% foetal bovine serum (FBS) and kept on ice. Two hundreds microliters of Histopaque 1077 (Pharmacia) was underlaid 300 µl RPMI 1640 diluted blood. The blood cells were centrifuged at 400 X $g$ for 4 min at 4 $^o$C. The obtained ficoll layer was washed in 1 ml cold phosphate buffered saline (PBS, pH 7.4). Viability of cells after isolation, determined using the trypan blue exclusion was ≥98%.

### Chemical treatment and Irradiation

Isolated lymphocytes were treated with various concentrations (0~250 nM) of boron (Borax; $Na_2B_4O_7.10H_2O$ supplied by Hayashi Pure Chemical Co., Osaka) and gadolinium (Gd-DTPA; as Magnevist supplied by Schering, Berlin) compounds for 10 min at 4℃, and then irradiated at 4℃ with various doses (0, 1, 2 and 4 Gy) of gamma rays from a $^{60}$Co source (dose rate: 20.1 cGy/min., source strength: 150 TBq, Panoramic Irradiator, Atomic Energy of Canada Ltd.) at Korea Atomic Energy Research Institute (KAERI). Under the same condition of $^{60}$Co source, the normal isolated lymphocytes without chemical pretreatment were irradiated with the same range of doses.

### Comet assay

The comet assay was performed as described by Singh *et al.* [6] with minor modifications. All steps were carried out on ice to avoid repairing of damaged cell DNA and under a dim light to prevent the occurrence of additional DNA damage. Two hundred microliters of 1% normal melting point agarose (NMA) diluted in distilled water was added at 50℃ to fully frosted microscope slides, and kept for 10 min at room temperature to dry. The coverslips were removed. As a second layer, 100 µl of 0.75% low melting point agarose (LMA) was added together with 1-2 × 10$^5$ suspended cells and covered with a coverslip. The slides were kept cold for 10 min at 4 $^o$C. After removal of the coverslips, 100 µl of 0.75% LMA was added as a third layer and then the slides were again covered with coverslips, kept for 10 min at 4 $^o$C and removed the coverslips. The slides were immersed in a jar containing cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 10% DMSO were added fresh). The slides were kept at 4℃ for at least 1 hr. After the lysis, the slides were placed on a horizontal electrophoresis chamber. The unit was filled with a freshly made alkaline electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH 13.0) to a level of 0.25 cm above the slides. The cells were exposed to alkali for 20 min to allow for DNA unwinding and expression of alkali-labile sites. For the DNA electrophoresis an electric current of 25 V (0.75 V / cm) and 300 mA was applied for 20 min. After electrophoresis, the slides were placed horizontally and some drip of neutralization buffer (0.4 M Tris, pH 7.4) was added to remove the excess alkali. Finally, 50µl ethidium bromide (20 $\mu$g/ml) was added to each slide, covered with a coverslip, kept in a humidified box. DNA damage was identified using the image analyzing system Kinetics (Version 4.0). The images of 50 randomly selected cells (25 cells from each of two replicate slides) were analyzed from each group from a fluorescence microscope

(Olympus BX50) equipped with an excitation filter of 515~560 nm and a barrier filter of 590 nm.

### Statistical analysis

The significance of the difference was statistically evaluated by the non-parametric Mann-Whitney *U*-test (Instat, GraphPad software) in the effects between the experimental and the control groups.
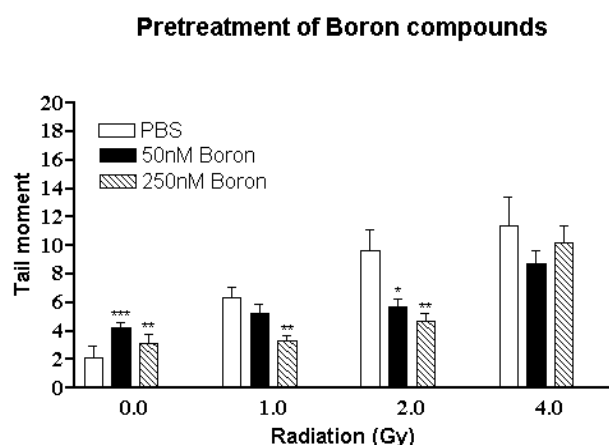
## RESULTS AND DISCUSSION

The results of this study showed the radiomodifying effects of boron and gadolinium compounds in human lymphocytes irradiated with gamma-rays using the comet assay.

### Effect of gamma-radiation

The lymphocytes pretreated only with PBS were exposed to gamma-radiation doses from 0 to 4 Gy. The tail moment value in the comet assay reflects the level of DNA damage. As can be seen in figure 1 and figure 2, the increases in the tail moments clearly indicate the dose-response relationship of cellular DNA damage with radiation dose.

### Effect of boron compound (Borax)

The results of this study indicated that

both boron and gadolinium compounds had genotoxic effects on human lymphocytes *in vitro* (figure 1 and figure 2). Borax is previously known as cytotoxic agent [10]. However we found that borax had genotoxic effects as well. The radiation-induced DNA damage in boron-preretreated cells is shown in figure 1. The tail moments were increased by the treatment of boron compound in non-irradiated cells. However, the pretreatment of the boron compound resulted in decreased tail moments after irradiation. The higher concentration (250 nM) of boron was more efficient in decreasing DNA damages than the lower concentration.

### Effect of gadolinium compound (Gd-DTPA)

The lymphocytes pretreated with 250 nM Gd-DTPA were found inappropriate for the comet assay because of its cytotoxicity. Thus the effects of 50 nM Gd-DTPA on human lymphocytes is shown in figure 2. The treatment of Gd-DTPA caused a significant increase in DNA damage not only in the non-irradiated control but also in the irradiated groups. The dose-response relationship of DNA damage was linear in the dose range 0 to 2 Gy. DNA damages in the Gd-DTPA pretreated cells were increased by more than 50 % at a dose of 4 Gy.
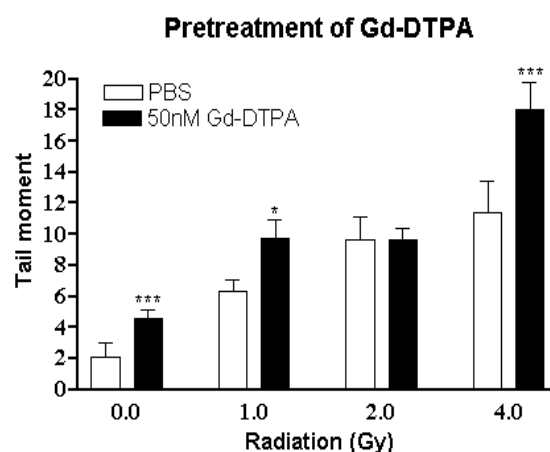
**Pretreatment of Boron compounds**



**Figure 1.** Effect of boron compound (Borax) on radiation induced DNA damage.
Bars indicate the standard error of the mean with duplicated measurements in each. Values significantly different from that of the control are given as: *P<0.05, **P<0.01, and ***P<0.001.
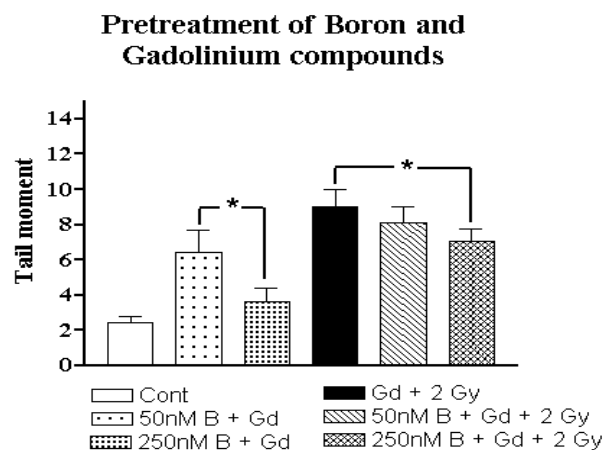
**Pretreatment of Gd-DTPA**



**Figure 2.** Effect of gadolinium compound (Gd-DTPA) on radiation-induced DNA damage
Bars indicate the standard error of the mean with duplicated measurements in each. Values significantly different from that of the control are given as: *P<0.05, and ***P<0.001.

### *Effect of the mixture of boron and gadolinium compounds (Borax and Gd-DTPA)*

The damage in DNA of lymphocytes pretreated with a mixture (1:1) of boron and gadolinium compounds was also evaluated by means of comparing the difference between the experimental groups (figure 3). The mixtures were the 50 or 250 nM of boron compound mixed with the same volume of 50 nM of gadolinium compound. The mixture caused a definite increase in the tail moment values in all the groups. The increase in the tail moments was somehow related with the concentration of boron compound in the mixture in both non-irradiated and irradiated groups. Higher concentration of boron compound in the mixture made a role in a decrease of DNA damage. This result also confirmed the decrease in radiosensitivity of lymphocyte DNA due to the pretreatment of boron compound. In addition, both type of the mixtures had the radiomodifying effects on the lymphocytes, by which the decrease in DNA damage appeared in a boron concentration-dependent manner.



**Figure 3.** Effect of the mixture of boron and gadolinium compounds on radiation-induced DNA damage. The concentration of gadolinium compound was limited to 50 nM. Bars indicate the standard error of the mean with duplicated measurements in each. Values significantly different between those of the experimental groups are given as: *P<0.05.

## CONCLUSION

Recently, the use of the comet assay, which has many advantages for genotoxicity studies, has been increasing [2, 11, 12]. It appears to be sensitive enough for the detection of DNA damage induced by a low-dose of radiation [3, 13]. It was also shown that DNA damage detected by SCGE assay is directly related to the chromosomal damage in following mitosis [14, 15]. Ionizing radiation is known to induce a variety of cellular responses, in particular, DNA damages, inducing predominantly single-strand breaks, double-strand breaks, alkali-labile sites, and oxidized purines and pyrimidines in animal and plant cells [16, 17].

The objective of the present experiment was to study the modification effect of boron and gadolinium compounds on γ-ray-induced DNA damage using the comet assay. The results indicate that the radiomodifying effect of sodium borate (Borax) is different from those of other boron compounds, such as sodium borocaptate-$^{10}$B (BSH) and boronophenylalanine-$^{10}$B (BPA) and so on [18]. Though this study revealed that the pretreatment of sodium borate caused a decrease in rediosensitivity of lymphocyte DNA to gamma rays, the detailed mechanisms of radiomodification by boron compounds remain to be further elucidated. Also of interest, DAC-1, one of boron neutron capture agents did not affect the biological efficacy of γ-rays [19].

Gd-DTPA is widely used to enhance the contrast of magnetic resonance imaging. And the element of gadolinium gathers a lot of attention since it has a high neutron capture cross section. The present study was encouraged by the possibility of the gadolinium compound as being an NCT agent. A high concentration of Gd-DTPA proved highly cytotoxic to the lymphocytes. The cells treated with 250 nM Gd-DTPA were not suitable for further evaluation. On the other hand, addition of 50 nM of the compound led to a significant increase in the DNA damage of lymphocytes in terms of tail moment (figure 2). The tail moment values of the lymphocytes treated with Gd-DTPA before γ-irradiation were higher than those of the cells irradiated with gamma-rays without any pretreatment. However, the

increase in the tail moment values was not dose-dependent. The increase in DNA damage seen after the addition of Gd-DTPA may indicate the production of hydroxyl radicals, as increasing of sister chromatid exchange (SCE) in the presence of Gd-DTPA [20, 21]. Figure 3 shows the effect of the mixture of Gd-DTPA and borax on the damage in lymphocyte DNA.

Of the two concentrations of the boron compound, the higher *one* was more effective to reduce radiation-induced damage. The result indicates that the boron compound plays a major role in reducing the DNA damages due to gamma-ray irradiation in a cellular level. However, it is very difficult to explain such a radioprotective action of the boron compound. The possible mechanisms of radioprotective action may be summarized as follows: 1) radical scavenging action, 2) induction of increased repair ability, 3) changes in oxygen tension (competition with oxygen), 4) protection against direct action of radiation. It is considered that the radiomodification effect comes from either the free radical scavenging action of the boron compound or the direct action of sodium-containing compounds.

A great potential of NCT lies in the fact that physiological differences between healthy and tumor cells can be utilized to enhance the delivered dose to tumor cells selectively. This is achieved by administering, prior to the radiation treatment, a boron or gadolinium compound that can selectively accumulate or be retained in the desired target cells. Subsequently, the organ containing the tumor is irradiated with neutrons. $^{10}$B and $^{157}$Gd, by virtue of their high neutron capture cross section, will give rise to two densely ionizing particles through the neutron capture reaction. For an effective BNCT, the $^{10}$B must be selectively delivered to and distributed in the target tumor cells. The tumor region is then irradiated with low-energy epithermal neutrons because thermal neutrons lack the capability of penetration into tissues. The low-energy neutron beams currently used for BNCT are produced using nuclear reactors even if neutron beams contain high-energy contaminants. It is apparent from our data that the boron compound could reduce the DNA damage induced by gamma-radiation, one of beam contaminants of therapeutic beams from a nuclear reactor. To our knowledge, the present study is the first demonstration of the modifying effect of boron and gadolinium compounds on the biological efficacy of gamma-rays in human lymphocytes by means of the comet assay. Therefore, the present results may support the fundamental understanding of neutron capture therapy. Although the mechanisms by which those compounds affect radiation responses are not clearly defined, possible explanations that are amenable to further study do exist.

## ACKNOWLEDGEMENT

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