DNA damage of lymphocytes in mice pretreated with boron compounds by neutron and ɣ -irradiation

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1. Introduction

The alkaline single-cell gel or comet assay is a sensitive, reliable, and rapid method for DNA double- and single-strand breaks, alkali-labile sites and delayed repair site detection, in eukaryotic individual cells(1). Given its overall characteristics, this method has been widely used over the past few years in the area of genetic toxicology and a number of investigators have used this version to evaluate in vitro and/or in vivo genotoxicity of several chemicals and radiation. From this reason, we evaluated the DNA damage in blood and splenic lymphocytes after neutron and gamma irradiation in C57BL/6 mice pretreated with boron compounds.

2. Methods and Results

2.1 Boron compounds and irradiation

Animals were used C57BL/6 mice, 20-25g body wt, 6 weeks of age. Boron compounds used two kinds such as BPA and BSH. BPA was administrated with 750 mg/kg body wt. by *i.p.* injection 3 hrs before irradiation and then BSH was administrated with 75mg/kg body wt. by tail vein injection 1hr before irradiation. Irradiation were exposed to neutron using at BNCT facility on Hanaro reactor and to γ-irradiation using a cobalt-60 source. Irradiation dose was 15Gy. Spleen were surgically excised on 3 days after irradiation and were carefully isolated the splenic lymphocytes. Also blood was collected at that time and then were carefully isolated the blood lymphocytes by centrifugation using Ficoll-histopaque gradient.

2.2 Single-cell gel electrophoresis(comet) assay

Seperated cells are mixed with 1% low-melting agarose and then placed on microscope slide coated with 1% normal agarose. The cells are lysed in a detergent solution(2.5M NaCl, 100mM EDTA-disodium, 10mM Tris, pH 10) for 1 hr and then the slides are put into an alkaline buffer(300mM NaOH, 1mM Na_2EDTA, pH>13) in a electrophoresis chamber for 20 mins, allowing the DNA unwinding, the electrophoresis for 20mins at 25V, 300mA is carried out, resulting in the migration of small pieces from the core of DNA, toward the electric field. After electrophoresis the slides are rinsed with neutralization buffer(0.4M Tris, pH 7.5) for 5 mins and cells are stained with a ethidium bromide(20μg/ml). The stained cells are observed by a fluorescence microscope(Olympus, Japan) and analyzed the value of the Olive tail moment(TM) and tail length by image analysis system software(Komet 4.0, Kinetic imaging, Ltd., Great Britain).

2.3. Olive TM and tail length of lymphocytes

DNA is considered to be the primary target for cell killing by ionizing radiation. Radiation produces many lesions including DNA single-and double-strand breaks and damage to the DNA bases and sugars(2, 3). We observed the DNA damage of the blood and splenic lymphocytes in irradiated mice pretreated with boron compounds by the comet assay. The values of the TM at the presence of the boron compounds in two experimental groups were almost the same compared to the irradiated control group, except for the splenic lymphocyte of the combination BPA and γ-ray irradiation (Figure 1, 2).

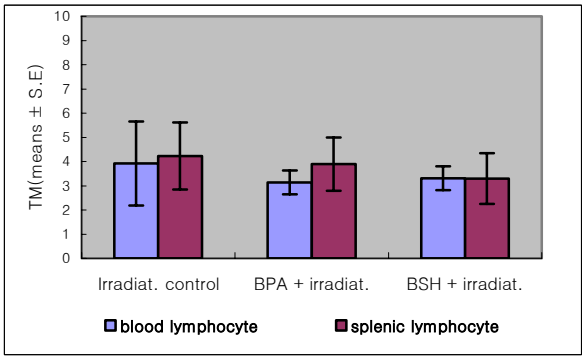

Figure 1. Olive TM value of blood and splenic lymphocyte with neutron irradiation in mice.

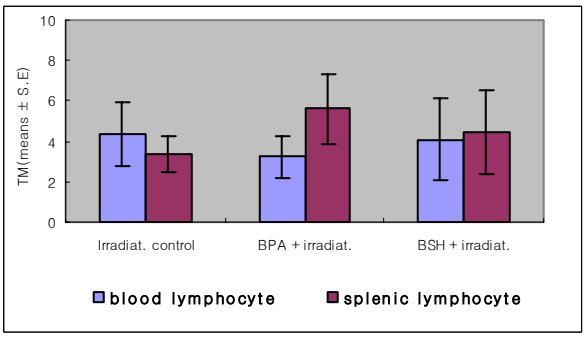

Figure 2. Olive TM value of blood and splenic lymphocyte with γ-ray irradiation in mice.

Table 1. Tail length of blood and splenic lymphocyte with neutron irradiation in mice.

Experimental Group	Tail Length	
	Blood lymphocyte	Splenic lymphocyte
Irradiat.	40.0 ± 6.8	42.3 ± 7.7
BPA + irradiat.	39.3 ± 7.4	34.3 ± 7.7
BSH + irradiat.	38.6 ± 4.7	35.0 ± 5.9

In case of the tail length in treatment of the boron compounds, the results were showed the similar compared to the irradiated control group(Table 1, 2). DNA in mice blood and splenic lymphocytes with neutron and γ-irradiation at the presence of the boron compounds have no damaged a little.

Table 2. Tail length of blood and splenic lymphocyte with γ-ray irradiation in mice.

Experimental Group	Tail Length	
	Blood lymphocyte	Splenic lymphocyte
Irradiat. control	52.3 ± 9.6	40.9 ± 6.6
BPA + irradiat.	53.5 ± 7.4	56.7 ± 6.4
BSH + irradiat.	52.6 ± 12.2	42.9 ± 7.7

3. Conclusion

The value of the TM and tail length of the blood and splenic lymphocytes in neutron and γ-irradiated mice pretreated with boron compounds showed almost the similar compared to the irradiated control group. These results show that pretreatment with boron compounds like BPA and BSH were no effective against the DNA damage of the blood and splenic lymphocytes in neutron and γ-irradiated mice.

References

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