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DNA damage of melanoma cells B16F0 treated with boron compounds after neutron irradiation

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1. Introduction

DNA damage can be assessed by the single cell gel electrophoresis(SCGE), alias, Comet assay; a fast, simple and sensitive technique. Within the last 20 years, the comet assay has been used to investigate primary DNA damage, such as double-strand breaks, single strand breaks, alkali-labile sites, incomplete repair sites and crosslingks(1, 2, 3). Therefore, comet assay has been applied in a great number of studies to investigate the early biological effects of DNA-damaging agents in environmental, occupational and pathological conditions or exposure to chemicals at the cellular level(4). From this reason, we investigated the DNA damage in melanoma cells B16F0 treated with boron compounds such as BPA and BSH after neutron irradiation *in vitro*.

2. Methods and Results

2.1 Cell culture

Melanoma cells (B16-F0) were grown in a monolayer in DMEM supplemented with 10% fetal bovine serum, 4mM L-glutamine, 1% penicillin (50 units/ml)-streptomycin (50 ㎍/ml), in an atmosphere of 5% CO_2 in a water-jacketed incubator at 37℃. Confluent cells were harvested with 0.25% (w/v) trypsin-0.02% (w/v) EDTA solution and centrifuged at 1000 X g for 3min. The cell viability was estimated by the trypan blue exclusion test(5).

2.2 Cell treatment and Irradiation

The harvested cells are dissolved in PBS and treated with 0.1, 1 and 10mg of BPA and BSH. Then the cells were exposed to neutrons at BNCT facility on Hanaro reactor. Irradiation doses were 10, 20 and 30 Gy.

2.3 Single-cell gel electrophoresis(comet) assay

The irradiated cells were embedded in 0.5% low-melting point agarose and spread on agarose-precoated microscope slides. Slides were immersed 1hr at 4℃ in freshly prepared cold lysing solution (2.5M NaCl, 100mM EDTA, 10mM Tris, 1% Triton X-100 and 10% DMSO added fresh). Subsequently, the cells were exposed to alkali buffer (1mM EDTA and 300mM NaOH, pH 13.4), at 4℃, for 20 min to allow DNA unwinding and expression of alkali-labile sites. In the same solution, electrophoresis was conducted at 4℃, for 20min, at 25V and 300mA. After electrophoresis, the solides were neutralized(0.4M Tris, pH 7.5), stained with 40 ㎕ EtBr(20 ㎍/ml), and analyzed in a fluorescence microscope.

2.4. Olive Tail Moment and Tail Extent Moment

We observed DNA damage of the melanoma cells treated with boron compounds such as BPA and BSH by the comet assay. The olive tail moment of the neutron irradiation and boron treatment group increased remarkably in comparison with that of the control group(non-irradiation and no boron treatment). The values were increased clearly when the contents of BPA, BSH and irradiation doses were higher (Figure 1,2).

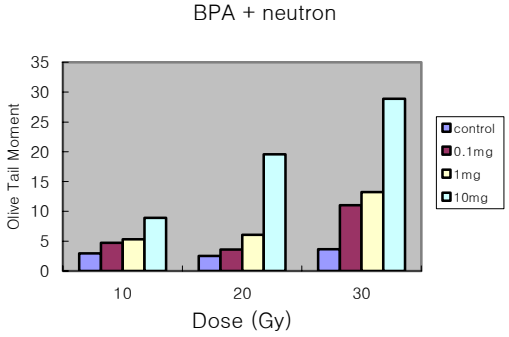

Figure 1. The olive tail moment of melanoma cell treated with BPA after neutrons; olive tail moment = (tail mean – head mean) x tail % DNA/100.

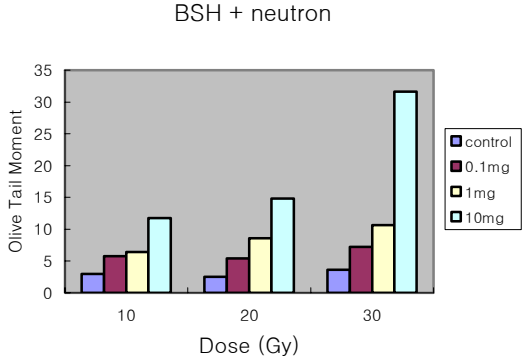

Figure 2. The olive tail moment of melanoma cell treated with BSH after neutrons.

BPA treatment (mg)	Tail Extent Moment		
	10 Gy	20 Gy	30 Gy
0	4.65 ± 2.94	3.73 ± 1.73	4.69 ± 2.4
0.1	7.57 ± 4.62	4.83 ± 2.96	19.17 ± 8.28
1	7.85 ± 4.81	8.64 ± 3.9	24.41 ± 7.41
10	12.72 ± 3.8	32.87 ± 7.27	51.98 ± 8.44

Table 1. The tail extent moment of melanoma cells treated with BPA after neutrons; tail extent moment = tail length x tail % DNA/100.

BSH treatment (mg)	Tail Extent Moment		
	10 Gy	20 Gy	30 Gy
0	4.65 ± 2.94	3.73 ± 1.73	4.69 ± 2.4
0.1	7.68 ± 3.49	8.55 ± 3.45	13.05 ± 4.95
1	10.69 ± 5.31	15.63 ± 6.64	18.33 ± 6.52
10	20.73 ± 8.28	21.15 ± 5.94	54.02 ± 10.11

Table 2. The tail extent moment of melanoma cells treated with BSH after neutrons.

In case of the tail extent moment with treatment of the boron compounds, the results were higher compared to the control group(Table 1,2). By the comet assay, DNA damage of melanoma cell treated with boron compounds and neutron irradiation can be clearly detected *in vitro*.

3. Conclusion

The present experiment was carried out to indicate that the DNA damage according to neutron dose and boron compounds contents. In the group treated with BPA and BSH, DNA damage exhibited higher than that of the control group showing the linearity according to the neutron irradiation doses and boron contents. These results mean that boron compounds such as BPA and BSH were effective against the DNA damage of melanoma cells by neutrons *in vitro*.

References

[1] R.R. Tice, E. Ahurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu, Y.F. Sasaki, Single-cell gel/Comet assay: guidelines for in vitro and in vivo genetic toxicology testing, Environ. Mol. Mutat., Vol.35, p.206, 2000.
[2] A. Hartmann, E. Agurell, C. Beevers, S. Brender-Schwaab, B. Burlinson, P. Clay, A. R. Collins, A. Smith, G. Speit, V. Thyband, R.R. Tice, Recommendations for conducting the in vivo alkaline Comet assay, Mutagenesis, Vol.18, p.45, 2003.
[3] A. R. Collins, The Comet assay for DNA damage and repair: principles, applications, and limitations, Mol. Biotechnol., Vol.26, p.249, 2004.
[4] G. Speit, A. Hartmann, The Comet assay: a sensitive genotoxicity test for the detection of DNA damage, Methods Mol. Biol., Vol.291, p.85, 2005.
[5] Lord-Fontaine S, Agostinelli E, Przybytkowski E, Averill-Bates D.A. Amine oxidase, spermine, and hyperthermia induce cytotoxicity in P-glycoprotein overexpressing multidrug resistant Chinese hamster ovary cells. Biochem Cell Biol., Vol.79, p.1, 2001.