# A study by transmission electron microscopy on human glioblastoma cells after BNCT

Ki-Jung Chun, Woo-Jung Kim, Hyun Woo Oh*
*Korea Atomic research Institute, Deajeon 305-353, Korea,kjchun@kaeri.re.kr*
** Korea Research Ihstitute of Bioscience and Biotechnology*

## 1. Introduction

Irradiation of the stable isotope $^{10}B$ with biologically low effective thermal neutrons is followed by a transmutation of $^{10}B$ to $^{7}Li$ and an emission of high energy alpha particles which can lethally damage cell organelles and their molecules. This principle of cell damage was applied to the development of a new approach for selective destruction of a tumor cell, referred to as the boron neutron capture therapy (BNCT) [1, 2]. This can cause cell damage to DNA, cell membranes, or other cell organelles, apoptosis and can finally induce a cell death [3]. Apoptosis is characterized by specific morphological changes, including a chromatin condensation, nuclear and cytoplasmic fragmentation, and so on.

In this study we used a transmission electron microscopy (TEM) for a detection of the morphological change in glioblastoma cells pretreated with BPA after a neutron irradiation.

## 2. Methods and Results

### *2.1 Cell culture and boron compounds treamtent*

The human glioblastoma cells were grown in a monolayer in RPMI-1640 supplemented with 10% fetal bovine serum, 4mM L-glutamine, 1% penicillin (50 units/ml)-streptomycin (50 $\mu$g/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 [4]. A BPA (500ul of 100mg/ml) was added to 3-day-old cultures at each dose of the culture media.

### *2.2 Neutron irradiation*

The cells were cultured on plastic strips and sampled at 1 hr after a boron compound administration. The harvested cells were dissolved in PBS and exposed to neutrons at the BNCT facility in the Hanaro reactor. Irradiation doses were 30 Gy.

### *2.3. Transmission Electron Microscopy*

Cell structure of the glioblastoma cells were observed by a transmission electron microscopy. The cells were fixed in 2.5% paraformaldehyde-glutaraldehyde mixture buffered with 0.1M phosphate (pH 7.2) for 2 hours, postfixed in 1% osmium tetroxide in the same buffer for 1 hour, dehydrated in graded ethanol and propylene oxide, and embedded in Epon-812. Ultra-thin sections, made by ULTRACUT E (Leica, Austria) ultramicrotome, were stained with uranyl acetate and lead citrate and examined under CM 20 (Philips, Netherlands) electron microscope.

In the results, morphological changes of glioblastoma cells were observed, Figs. 1, 2, 3 and 4. In the cells only treated with BPA, nuclear among cell structure was characterized by a chromatins condensation within an intact nuclear membrane (Fig 2). And cells treated with BPA after a neutron induced considerable chromatins condensation and a destruction of the nuclear membrane when compared to the BPA treated cells (Fig 3 and 4). Morphological changes in the 30 Gy irradiation cells were more when compared to the 10 Gy irradiation cells.

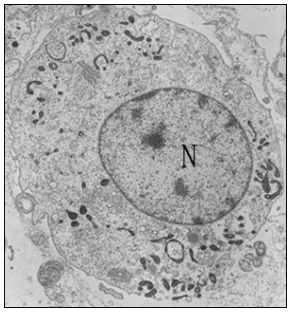


Figure 1. Electron microscopic photograph of control condition of glioblastoma cells.

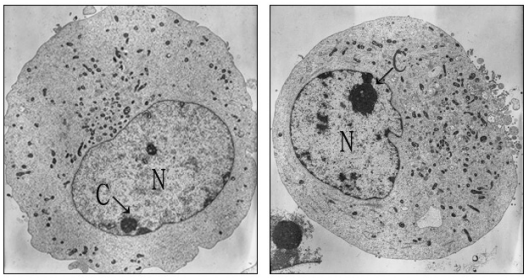


Figure 2. Electron microscopic photograph of glioblastoma cells treated with BPA. C: chromatin, N: nuclear

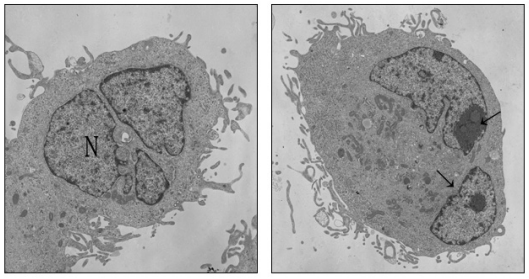


Figure 3. Electron microscopic photograph of glioblastoma cells pretreated with BPA after 10 Gy neutron irradiation

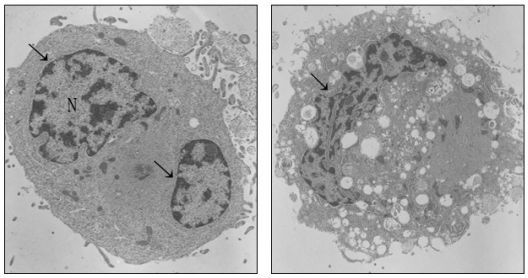


Figure 4. Electron microscopic photograph of glioblastoma cells pretreated with BPA after 30 Gy neutron irradiation

### 3. Conclusion

The present study carried out a transmission electron microscopy (TEM) for the detection of morphological changes in glioblastoma cells pretreated with BPA after a neutron irradiation. Nuclear structure of the cells treated with BPA were not changed showing a chromatin condensation when compared to the control cell, however, cells treated with BPA after a neutron irradiation were changed a lot when compared to the control and BPA treated cell. This result means that BPA + neutron can increase the toxicity in the nuclear structure of a cell by apoptosis. TEM was very effective for a detection of the morphological changes in the cell biological study of the BNCT.

### References

[1] V. Mareš, D. krajči, V. Lisá, The subcellular targets of mercaptoborate(BSH), a carrier of $^{10}B$ for neutron capture therapy (BNCT) of brain tumors, Physiol. Res., Vol.52, pp629-635, 2003.

[2] M. Neumann, U. Kunz. H. Lehmann, D. Gabel, Determination of the subcellular distribution of mercaptoundecahydro-closo-dodecaborate (BSH) in human globlastoma multiforme by electron microscopy, J. Neuro Onc., Vol.57, pp.97-104, 2002.

[3] R. Micbael, G. F.J.M. Vrensen, J. Van Marle, L. Ban, P.G. Söderberg, Apotposis in the rat lens after in vivo threshold dose ultraviolet irradiation., Invest. Ophthalmol. & Vis. Sci.Vol.39, pp.2681-2687, 1998.

[4] 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.