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Differential Responses of Several Chemicals including Boron-Enriched Compounds in Gamma Ray-Exposed Gliomas

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1. Introduction

Despite many developments in neurosurgical and pharmacological techniques, a malignant glioma is one of the most deadly cancers and to date lacks an effective treatment in humans [1].

Boron neutron capture therapy (BNCT) has been regarded as a promising treatment for the malignant gliomas [2]. BNCT is a two step radiotherapy in which a selective radiation effects on tumor cells is achieved by first targeting the tumor with ^{10}B and then exposing it to low energy neutrons. The stable ^{10}B isotope has a large cross-sectional area for the capture of thermal neutrons. The reaction yields intensively ionizing particles, $^{4}He^{2+}$ (α particles) and recoiling $^{7}Li^{3+}$ nuclei. These highly cytotoxic nuclear fragments destroyed the tumor cells. For the successful BNCT, high amount boron molecules must be carried into the target tumor cells selectively.

Borocaptate sodium (BSH) is a low molecular weight substance originally tested for the BNCT [3]. Clinical trials using BSH for the BNCT of brain tumors reported significant outcomes with long-term survivals in patients with malignant gliomas [4]. Boronated amino acid analogs, *p*-boronophenylalanine (BPA), are used in the BNCT for the treatment of malignant melanomas [5]. Recently, BPA has been reported to be preferentially taken up by glioma cells [6]. These boron-enriched compounds should be kept low to minimize the damage to normal tissues. Therefore, the preliminary study for the enhancement of selective delivery of boron atoms to tumor cells has been reported. Also, several investigators proposed abilities of boron compounds as a radioprotector against gamma ray-exposed condition. The present study tested the comparative effect of boron compounds and known radioprotectant agents in the gamma-irradiated gliomas.

2. Methods and Results

2.1 Materials and methods

Boron-enriched borocaptate sodium (^{10}B-enriched BSH, FW 209.76) and D,L-*p*-^{10}boronophenylalanine (BPA, FW 209.01) were purchased from the Czech Chemicals (www.ryscor.com). Caffeine and melatonin were obtained from Sigma Chemicals (St. Louis, MO). BSH and caffeine were dissolved in a Dulbecco's phosphate-buffered saline (DPBS). BPA and melatonin were dissolved in trifluoroacetic acids and ethanol, respectively.

The present investigation used the U373MG cell line. The glioma cells were propagated in RPMI-1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Sigma, St. Louis, MO), 100 U/ml of penicillin, and 100 μg/ml of strepromycin (Gibco) in a humidified 5% CO_2 atmosphere at 37℃ in 75 mm^2 flasks (Falcon, Becton Dickinson, NJ).

Human astroglioma cells, U373MG, were treated with various concentrations of each compound before irradiation. The irradiated groups were exposed to γ-irradiation using a ^{60}Co source with a total dose of 10 Gy, and a dose rate of 30 Gy/hr [7]. After irradiation, the cells were incubated for an additional 6 hr and 24 hr under the same culture conditions. The cells were then washed 3 times with DPBS and replaced by a compound-free medium. To determine the respiratory rate in the experimental group, cells were incubated in the presence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoli-um bromide (MTT; Sigma). The MTT assay [8] was based on the conversion of tetrazolium salt into an insoluble formazan product by various dehydrogenases in mitochondria inside cells. After additional incubation, formazan crystals in the experimental group were dissolved in 100 μl DMSO and then the absorbance was measured at 570 nm by using a Microplate Reader (MultiskanEX, Thermo Labsystems).

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).

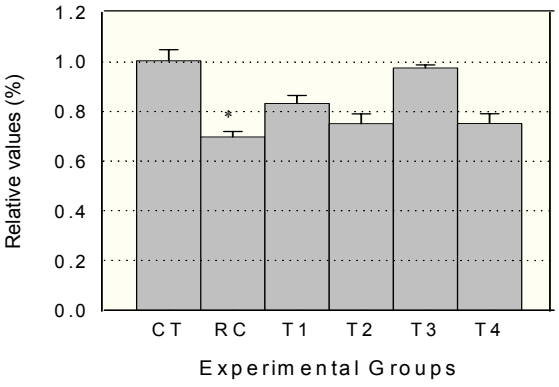

Figure 1. Effects of melatonin (T1), caffeine (T2), BPA (T3), and BSH (T4) 6 hr after irradiation (10 Gy) in the human malignant gliomas. *, $p<0.05$.

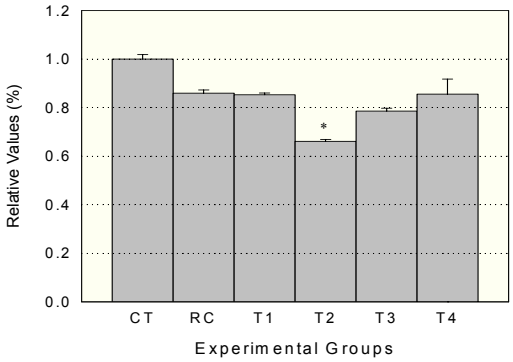

Figure 2. Changes of mitochondrial function 24 hr after irradiation (10Gy) in the human malignant gliomas. *, $p<0.05$.

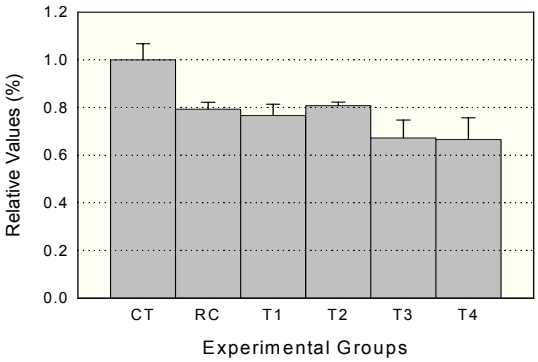

Figure 3. Changes of mitochondrial function 24 hr after irradiation (20 Gy) in the human malignant gliomas.

The radioprotective effects of boron compound were compared to those of melatonin and caffeine in synchronous experiments after gamma irradiation. Figure 1 showed changes of mitochondrial function 6 hr after gamma irradiation. The group treated with melatonin (2 mM) showed increasing pattern compared with the irradiated control group (RC group). In case of the BPA (0.5 mg/ml) treated group, the rate of respiratory maintained the level of the control group. However, the level of mitochondrial respiratory 24 hr after irradiation was similar to the values of the irradiated group (Figure 2). A significant difference between the irradiated groups was not observed as shown in Figure 3.

It is difficult to describe the radioprotective effects of the boron compounds such as BPA and BSH based on simple results. However, differences in Figure 1 and 3 showed effects of the treated compound depending on optimal dose and condition in the same kind cells. Moreover, the medium for culture after irradiation was one including the treated compound. These compounds may affect the renewal of the irradiated gliomas.

In conclusion, the boron compounds showed a temporary protection immediately after gamma irradiation. As time goes by, the boron compounds had the inhibitory effect on the renewal of the irradiated malignant gliomas.

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