Expanded view

Boron neutron capture therapy as a novel modality of radiotherapy for oral cancer: Principle and antitumor effect

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A B S T R A C T
Radiotherapy is essential for the treatment of oral cancer, especially in advanced cases. There has been marked progress in this field due to the prevalence of intensity-modified radiation therapy and introduction of particle radiotherapy using protons and carbon-ions. However, these treatments are still non-selective. Boron neutron capture therapy (BNCT) is a unique modality in which neutron beams destroy only boron compound-bearing tumor cells while leaving the surrounding normal tissues intact. Thus, BNCT is a selective form of radiotherapy, if high tumor/normal tissue ratio in boron concentration could be achieved. The principle of BNCT, and the basic study of the mechanism by which BNCT exerts antitumor effects using oral squamous cell carcinoma (SCC) cells and oral SCC xenografts in mice are described.

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1. Introduction

Oral cancers account for 30% of head and neck cancers and treatment is dependent on the stage of the disease [1]. In stages I and II, either surgery or therapy with external or internal irradiation is recommended. In stages III and IV, radiochemotherapy is recommended as a first step. Thereafter, remaining tumors can be removed by surgery [2]. In advanced cases, however, surgical treatment causes severe cosmetic and functional disturbance, lowering quality of life. Radiotherapy is usually applied to tumors alone or in combination with chemotherapy [3]. Indeed, the best studied concurrent chemoradiotherapy regimen involves radiation and cisplatin, and is well-established as a standard of care in the management of patients with unresectable head and neck cancer, and oropharyngeal cancer and post-operative patients with positive margins, extracapsular nodal extension, lymphovascular invasion, and perineural invasion (Fig. 1) [2,4]. It was also reported that cetuximab, an IgG1 monoclonal antibody against the ligand-binding domain of epidermal growth factor, enhanced the cytotoxic effects of radiation in squamous cell carcinoma (SCC) [5,6]. Thus, radiotherapy is essential for the treatment of oral cancer.

The radiotherapeutic management of oral cancer has changed significantly over the past 10 years, as intensity-modulated radiation therapy (IMRT) has become the de facto standard treatment [7,8]. In contrast to conventional radiotherapy, particle therapy using carbon-ions or protons is also gaining importance worldwide [9,10]. Compared with photons such as X-rays and gamma rays, the in-depth dose distribution of particles allows a more accurate administration, resulting in an increased therapeutic ratio (Fig. 2). Nevertheless, it remains unclear, mainly because of the absence of randomized trials, whether particle therapy is superior to radiotherapy with photons in cases of head and neck cancer.

In spite of these developments, in principle, radiotherapy is still non-selective. Although the effect is dependent on the dose, most
incorporate 10 B with 5–10 Y.

Fig. 1. Treatment plan for oral cancer with surgery, radiation and chemotherapy. In stages I and II, either surgery or treatment with external or internal irradiation is recommended. In stages III and IV, radiochemotherapy is recommended as the first step. Thereafter, remaining tumors can be removed by surgery. Usually, radiotherapy is applied to tumors singly or in combination with chemotherapy.

Fig. 2. Types of radiation. X-rays, gamma rays, protons, carbon-ions and neutrons are used for cancer therapy. LET, linear energy transfer.

cells exposed to radiation are damaged in a similar fashion. In this respect, boron neutron capture therapy (BNCT) is unique in its selectivity in appropriate conditions [11–13]. Oral cancers can be accessed relatively easily. This makes oral cancer a candidate for treatment by BNCT [14–17]. In this paper, we review the introduction of the principle of BNCT and the mechanism by which BNCT exerts antitumor effects on oral SCC.

2. Principles of BNCT

The first theoretical account of the biological effects and therapeutic possibilities of BNCT was published by Locher [18]. BNCT is a two-component modality, in which boron-10 (10B)-enriched compounds such as boronophenylalanine (BPA) and borocaptate sodium (BSH) are administered, prior to irradiation with a thermal neutron beam. 10B absorbs the neutrons and releases two linear energy transfer particles, an α(4He) particle and a lithium (7Li) nucleus (Fig. 3). These products from the 10B(n, α)7Li reaction have path lengths in water of 5–10 μm. This suggests that the effectiveness of BNCT depends on the maintenance of relatively high concentrations of 10B in the tumor compared with the surrounding normal tissues, and that BNCT potentially targets neoplastic tissue selectively [11–13]. Thermal neutrons are responsible for the boron capture reaction, but epithermal neutrons are now more generally used to improve the depth-dose profile. It should be also stated that boron captures thermal neutrons to produce two high linear energy transfer (LET) particles, i.e. α(4He) particle and lithium (7Li) nucleus. However, they are also captured non-selectively by tissue nitrogen and hydrogen to produce high LET protons and low LET gamma rays which are by definition not tumor specific.

Thus, it is essential to elevate the intracellular boron concentration in tumor cells, while maintaining low levels of 10B in normal tissue. This results in a high tumor:normal tissue (T/N) ratio. Another important factor is the use of nuclear reactors, because the source of neutrons is limited to neutron beams derived from nuclear reactors. Thus, the treatment can be done only in certain countries and areas. Reactor-derived neutrons are classified according to their energies as thermal (En < 0.5 eV), epithermal (0.5 eV < En < 10 keV), or fast (En > 10 keV). Thermal neutrons are the most important for BNCT as they initiate the 10B(N, α)7Li capture reaction. Because they have a limited depth of penetration, epithermal neutrons, which lose energy and fall into the thermal range as they penetrate tissues, are now preferred for clinical therapy. Several nuclear reactors with good neutron beam quality have been developed. These include the Massachusetts Institute of Technology reactor (MITR) in the USA, the clinical reactor at Studsvik
Medical AB in Sweden, the FiR1 clinical reactor in Helsinki, Finland, the R2-0 High Flux Reactor at Petten in the Netherlands, the LVR-15 reactor at the Nuclear Research Institute in Rez, Czech Republic, the Kyoto University Research reactor in Kumatori, Japan, JRR4 at the Japan Atomic Energy Research Institute, and the RA-6 CNEA reactor in Bariloche, Argentina [19]. However, MITR is no longer used. Studsvik Medical has been decommissioned and Petten no longer has a license to treat patients. Bariloche in Argentina is also not used for patients now. Other nuclear reactors available for BNCT research include TRIGA mark II reactor at University of Mainz in Germany, the TRIGA MarkII reactor of the University of Pavia in Italy, RA-3 reactor at Buenos Aires in Argentina, Tsing Hua Open-pool reactor (THOR) in Taiwan, and the Xi’an Pulsed Reactor (XAPR) in China [20–24].

3. BNCT in oral SCC cell cultures

The mechanism of radiotherapy is dependent on the type of LET. In low LET, the major effect is DNA damage, whereas high LET acts directly to cut the DNA strand. Although conventional radiation is effective against a variety of cancers, high LET radiation such as carbon-ion irradiation and BNCT offers several potential advantages.

Fig. 4. Surviving fraction of human oral SCC cells after in vitro boron neutron capture therapy (BNCT). Oral SCC cells were incubated with boronophenylalanine (BPA) at a 10B concentration of 50 ppm for 2 h to allow the incorporation of 10B. Thereafter, they were exposed to a neutron beam. Similarly, cells were exposed to a neutron beam alone or gamma ray. Thereafter, the colony-forming ability of the irradiated cells was examined and the surviving fraction was determined. The result indicates that BPA-mediated BNCT is more effective than neutron alone and gamma ray.

Fig. 5. Effect of boron neutron capture therapy (BNCT) on the cell cycle. SAS cells were treated with BNCT or gamma rays at a physical dose of 6 Gy and then subjected to flow cytometric analysis. From an analysis of DNA histograms, the percentages of cells in the sub-G1, G0/G1, S, and G2/M phases were evaluated. The cell cycle of the BNCT-treated cells was arrested at the G1 and G2 checkpoints, and sub-G1 cells showing apoptotic cell death appeared.

Fig. 6. Schematic presentation of cell-cycle arrest by boron neutron capture therapy (BNCT). Immunoblot analysis of BNCT-treated oral squamous cell carcinoma cells revealed the phosphorylation of p53, up-regulation of p21, and down-regulation of Rb at 6 h after BNCT. Twelve hours after BNCT, the up-regulation of Wee1, phosphorylation of cdc2, and up-regulation of cyclin B1 were observed. Cleavage of PARP occurred at 6 h after BNCT. This indicates the arrest at the G1 and G2 checkpoints and apoptosis associated with G1 arrest in BNCT-treated cells.
advantages over X irradiation [25], including a reduction in the oxygen enhancement ratio with a proportionately greater killing effect on hypoxic cells, less variation in cell cycle-related radiosensitivity, and less capability of repair of radiation injury. Thus, high LET radiation has a higher relative biologic effectiveness, compared with X irradiation and gamma irradiation (Fig. 2).

In BNCT, one important point to be clarified is the incorporation of $^{10}$B into cells when BPA are used as $^{10}$B compounds. We incubated oral SCC cells with BPA at a concentration of 50 ppm for 2 h. The cells were washed to remove unincorporated compound and then exposed to thermal neutrons. The proportion of cells capable of forming colonies decreased exponentially, depending on the physical dose. The survival fraction in cells treated with BPA-mediated BNCT was lower than those of neutrons alone and gamma ray [26] (Fig. 4), indicating that BPA potentiated the effect of neutron beam on cell survival. It can be stated that the BPA-mediated BNCT shows stronger biological effect on cell survival than low LET gamma X-ray.

Cell death is categorized into two types, intermediate death and mitotic; the intermediate cell death being apoptosis. Gamma irradiation induces DNA damage and leads to cell-cycle arrest which in eukaryotic cells protects the integrity of the genome. Arrest at G1 permits repairs for replication, whereas arrest at G2 permits repair of the genome prior to its mitotic segregation [27]. Furthermore, a signal transduction pathway that communicates signals from DNA damage to the cell-cycle machinery involves the activation of many gene products including p53, p21, 14-3-3σ, ataxia telangiectasia mutated, checkpoint kinase 1, Wee1, cell division cycle 25 (cdc25), and cell division cycle 2 (cdc 2) to arrest the cell cycle, thus providing cells with the critical time needed to repair damaged DNA [28–34]. The effects of BPA-mediated BNCT on the cell cycle of human oral SCC cells were examined at a physical dose of 6 Gy. In the flow-cytometric analysis of BNCT-treated cells, the cell cycle was arrested at the G1 and G2 checkpoints, and sub-G1 cells showing apoptotic cell death appeared (Fig. 5). Immunoblot analysis revealed the phosphorylation of p53, up-regulation of p21, and down-regulation of Rb at 6 h after BNCT. Twelve hours after BNCT, the up-regulation of Wee1, phosphorylation of cdc2, and up-regulation of cyclin B1 were observed. Cleavage of poly(ADP-ribose) polymerase (PARP), a marker of apoptosis, occurred from 6 h after BNCT [26,35]. These results indicate that the early inhibitory effect

![Fig. 7. Effect of boron neutron capture therapy (BNCT) on the growth of tumors. Group 1 (△), 2 (●) and 3 (▲) animals received a boronophenylalanine (BPA) injection and then neutron irradiation 1, 2, or 3 h later, respectively. The physical dose was 11.32 Gy. Group 4 (□) and 5 (◇) animals received either BPA or neutron irradiation alone. The tumor volume was markedly suppressed in animals that received BNCT.](image)

![Fig. 8. Immunohistochemical staining of 5-bromo-2′-deoxyuridine (BrdU)-incorporated tumor cells and tumor blood vessels. SAS tumor-bearing mice were treated by boron neutron capture therapy (BNCT) at a dose of 11.32 Gy. One day later, tumor tissues were examined for the incorporation of BrdU and the expression of a marker of blood vessels, von Willebrand factor (vWF). The number of BrdU-positive tumor cells (arrows) was markedly reduced after BNCT (A) as compared with the unirradiated control (B). Disintegration of tumor blood vessels (arrows) was observed in BNCT-treated tumors (C), but not in unirradiated control tumors (D). Bars, 25 μm in A and B, and 50 μm in C and D.](image)
of BNCT on the growth of human oral SCC cells can be ascribed to arrest at the G1 and G2 checkpoints and apoptosis associated with G1 arrest (Fig. 6).

4. BNCT in oral cancer models

There are two animal models for BNCT; the hamster cheek pouch model and the mouse oral SCC xenograft model. In the hamster model, a chemical carcinogen, 9,10-dimethyl-1,2-benzanthracene (DMBA), is applied repeatedly to cheek pouches to generate papillomatous SCCs. Using this model, the effects of BNCT on oral carcinogenesis and growing tumors have been studied in Argentina [22,36–38]. At a dose of 300 mg/kg, BPA delivers 36.9 ± 17.5 ppm of boron to tumor tissue with T/N and tumor/blood ratios of 2.4 and 3.2, respectively. The total tumor dose was estimated to be 14.9 Gy-Eq and BNCT led to a complete remission by 15 days post treatment in 78% of tumors and partial remission in an additional 13% of tumors with virtually no damage to normal tissue. In the oral SCC xenograft model, we injected BPA at a dose of 250 mg/kg intraperitoneally [39]. Two hours after the injection, the 10B concentration in tumor was 16 ± 2 ppm and the T/N ratio was 4.2. Tumors were then given neutron irradiation 1, 2, and 3 h later at a physical dose of 11.32 Gy. In control animals that received BPA alone, the tumors continued to grow and were 1100% of the initial volume 21 days after the start of the experiment. Tumor growth in BNCT-treated animals was significantly inhibited, irrespective of the interval between the BPA injection and neutron irradiation. There was a significant decrease as compared with the BPA only group [40,41] (Fig. 7). All untreated animals died within 50 days of the start of the experiment. Tumors in BNCT-treated groups 1 (BPA 1 h) and 3 (BPA 3 h) decreased markedly, but showed regrowth after a period, and the animals had died by days 83 and 96, respectively. In contrast, those in group 2 animals (BPA 2 h) regressed completely and the mice survived the experimental period of 120 days, indicating the importance of the interval between the BPA injection and neutron irradiation and the 10B concentration during neutron irradiation. It is concluded that BPA is the most reliable compound, because of the selective incorporation into tumor cells and that BPA-mediated BNCT can cure oral SCC. The T/N ratios in chemically induced hamster model and xenograft model were 2.4:1 and 4.2:1. The difference will clearly influence the responses.

When tumors treated by BNCT were subjected to immunohistochemical staining, BNCT reduced the incorporation of 5-bromo–2′-deoxyuridine (BrdU) into tumor cells, and induced the enlargement and vacuolation of tumor cells. The disintegration of blood vessels and dense inflammatory cell infiltration were also observed in the stroma of the tumor, but not surrounding normal tissues [40] (Fig. 8). Thus, the disintegration of blood vessels in tumor stroma may contribute to tumor remission by BNCT.

5. Conclusion

BNCT is a novel form of radiotherapy. Accumulating data have shown the effectiveness for patients who had been treated with a full dose of conventional radiotherapy previously, because of selectivity of BNCT. However, this property is dependent on the tumor/normal tissue ratio in boron concentration attained. The selectivity is still partial. Basic research in vitro and in vivo revealed the concentration of 10B and physical dose required, the efficiency of high LET particles produced by born neutron reaction, and associated vascular alterations in the tumors. These findings must be taken into consideration when the BNCT is used as an initial therapy for oral cancer alone and in combination with conventional therapy.

Conflict of interest

The authors state that they have no conflicts of interest.

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