Applicability of combining the vascular-targeting agent ZD6126 with boron neutron capture therapy

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The aim of this study was to evaluate the antitumor efficacy of the vascular-targeting agent ZD6126 (N-acetylcolchinol-O-phosphate) in the rodent SCC VII carcinoma model, in combination with boron neutron capture therapy (BNCT).
A tubulin-binding agent with selective tumor vascular targeting activity, causing disruption of the cytoskeleton of proliferating endothelial cells that results in endothelial cell shape changes and leads to thrombus formation and a consequent secondary cascade of ischemic tumor cell death.

It might cause entrapment of $^{10}$B-carrier in tumors.
Materials and Methods

1. $^{10}$B pharmacokinetic study

Sodium borocaptate-$^{10}$B (BSH, 125 mg/kg, i.p.) or l-para-boronophenylalanine-$^{10}$B (BPA, 250 mg/kg, i.p.) was injected into SCC VII tumor-bearing mice, and 15 minutes later ZD6126 (100 mg/kg, i.p.) was administered. Then the $^{10}$B concentrations in tumors, blood, muscles, skins and lives were measured by prompt $\gamma$-ray spectrometry.
2. *Thermal neutron beam exposure experiment*

SCC VII tumor-bearing mice were initially given 5-bromo-2’-deoxyuridine (BrdU) to label all proliferating (P) cells in the tumors, followed by treatment with BSH or BPA combined with ZD6126 according to the same protocol as the $^{10}$B pharmacokinetic analyses.

To obtain similar intratumor $^{10}$B concentrations during neutron exposure, thermal neutron beam irradiation was initiated 30 minutes after injection of BSH only, 90 minutes after BSH + ZD6126 administration, 120 min after the injection of BPA only, and 180 minutes after BPA + ZD6126 administration.
Mice bearing the tumors received 5-bromo-2’-deoxyuridine (BrdU) continuously to label all proliferating (P) cells in the tumors.

The tumor-bearing mice received thermal neutron exposure.

After the treatments, the tumors were excised, minced, and trypsinized to obtain single tumor cell suspensions.

3. The Method for Selective Detection of the Response of Intratumor Quiescent Cell Populations

P and Q cells in solid tumors

Cell loss

P: Proliferating fraction
Q: Quiescent fraction
Continuous labeling with BrdU
Immunofluorescence staining for BrdU to detect BrdU-labeled tumor cells

The tumor cell suspensions thus obtained were incubated with a cytokinesis blocker (cytochalasin-B), and the micronucleus (MN) frequency in cells without BrdU labeling \[Q\] was determined using immunofluorescence staining for BrdU.

Tumor cells from the tumors that were not pretreated with BrdU.

The MN and frequency in total \((P + Q)\) tumor cells were determined from the tumors that were not pretreated with BrdU.

Colony forming assay was also carried out using *in vivo-in vitro* assay method.
Micronucleus assay

**Arrows:**
- BrdU-unlabeled (**Quiescent**) binuclear tumor cell with a micronucleus (colored with nuclear staining (PI, red) only)

**Arrowheads:**
- BrdU-labeled (**Proliferating**) binuclear tumor cell with a micronucleus (colored with immunofluorescence staining (FITC, green) and nuclear staining (PI, red))
The MN frequency in BrdU-unlabeled cells (= Q cells at treatment) could be examined by counting the micronuclei in the binuclear cells that showed only red fluorescence. The MN frequency was defined as the ratio of the number of micronuclei in the binuclear cells to the total number of binuclear cells observed.

The ratios obtained in tumors not pretreated with BrdU indicated the MN frequency at all phases in the total (P + Q) tumor cell populations.
Tumor

Boron-10 concentration (µg/g)

Minutes after BSH or BPA administration

- BSH only
- BSH + ZD6126
- BPA only
- BPA + ZD6126
Boron-10 concentration (µg/g)

Minutes after BSH or BPA administration

(a) Blood

(b) Muscle

(c) Liver

(d) Skin

BSH only

BSH + ZD6126

BPA only

BPA + ZD6126
Results-1

1. Combination with ZD6126 greatly increased the $^{10}$B concentrations in tumors after 60 min following BSH injection and after 120 min following BPA injection.

2. The concentrations of $^{10}$B from BSH in normal tissues were also raised by combination with ZD6126, although not so clearly as those in tumors.

3. Combination with ZD6126 had almost no effect on the concentrations of $^{10}$B from BPA in normal tissues.
Normalized micronucleus frequency

Physical absorbed radiation dose (Gy)

Total cells
Q cells

- Neutrons only
- ZD6126+Neutrons
- BSH+Neutrons
- BSH+ZD6126+Neutrons

- Neutrons only
- ZD6126+Neutrons
- BPA+Neutrons
- BPA+ZD6126+Neutrons
<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Total tumor cells</th>
<th>Quiescent cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>&lt;Surviving fraction&gt;</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>1.25 (1.2-1.3)</td>
<td>-----</td>
</tr>
<tr>
<td>0.05</td>
<td>1.25 (1.2-1.3)</td>
<td>-----</td>
</tr>
<tr>
<td>BPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>1.1 (1.0-1.2)</td>
<td>-----</td>
</tr>
<tr>
<td>0.05</td>
<td>1.1 (1.0-1.2)</td>
<td>-----</td>
</tr>
<tr>
<td><strong>&lt;Micronucleus frequency&gt;</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>1.35 (1.3-1.4)</td>
<td>-----</td>
</tr>
<tr>
<td>0.2</td>
<td>1.3 (1.25-1.35)</td>
<td>1.45 (1.4-1.5)</td>
</tr>
<tr>
<td>0.1</td>
<td>1.3 (1.25-1.35)</td>
<td>1.45 (1.4-1.5)</td>
</tr>
<tr>
<td>BPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>1.25 (1.2-1.3)</td>
<td>-----</td>
</tr>
<tr>
<td>0.2</td>
<td>1.2 (1.15-1.25)</td>
<td>1.3 (1.25-1.35)</td>
</tr>
<tr>
<td>0.1</td>
<td>1.15 (1.1-1.2)</td>
<td>1.3 (1.25-1.35)</td>
</tr>
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</table>
Dose modification factors for quiescent cells relative to the total tumor cell populations

<table>
<thead>
<tr>
<th>Normalized MN frequency</th>
<th>ZD6126 (-)</th>
<th>ZD6126 (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No ^10^B-carrier</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>1.15 (1.1-1.2)</td>
<td>1.1 (1.0-1.2)</td>
</tr>
<tr>
<td>0.1</td>
<td>1.15 (1.1-1.2)</td>
<td>1.1 (1.0-1.2)</td>
</tr>
<tr>
<td><strong>BSH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>1.75 (1.65-1.85)</td>
<td>1.7 (1.55-1.85)</td>
</tr>
<tr>
<td>0.1</td>
<td>1.8 (1.7-1.9)</td>
<td>1.65 (1.55-1.75)</td>
</tr>
<tr>
<td><strong>BPA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>3.1 (2.9-3.3)</td>
<td>3.0 (2.8-3.2)</td>
</tr>
<tr>
<td>0.1</td>
<td>3.2 (3.0-3.4)</td>
<td>2.9 (2.7-3.1)</td>
</tr>
</tbody>
</table>
1. The clonogenic surviving fractions of total tumor cells and the MN frequencies of both total and Q tumor cells were reduced and increased by combination with ZD6126, respectively, whether BSH or BPA was employed.

2. However, the degrees of these changes in the clonogenic surviving fractions and the MN frequencies were more obviously observed in tumors from BSH-injected mice than from BPA-injected mice, and in Q tumor cells than in total tumor cells regardless of the employed $^{10}$B-carrier.
Conclusion

1. Combination with ZD6126 was regarded as more promising in BSH-BNCT than BPA-BNCT, and more effective for enhancing the sensitivity of the Q tumor cells than that of the total tumor cells.

2. This resulted in the decrease in the extended difference in the sensitivity between the total and Q tumor cells caused by the use of $^{10}$B-carrier for BNCT.
Thank you so much indeed for your very close attention.