Impact of lymphatic chemotherapy targeting metastatic lymph nodes in patients with tongue cancer (cT3N2bM0) using intra-arterial chemotherapy

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Abstract

Introduction
We report that our novel drug-delivery system is feasible for lymphatic chemotherapy targeting sentinel lymph nodes (SLNs) in patients with cT3N0M0 tongue cancer with occult metastasis in SLNs. Neck metastasis is a significant prognostic factor of tongue cancer. It is, therefore, imperative that intra-arterial chemotherapy is performed to preserve organs and to control neck metastasis when treating tongue cancer.

Objective
Evaluate lymphatic chemotherapy targeting neck metastases in patients with tongue cancer (cT3N2bM0) using intra-arterial chemotherapy.

Methods
Seven patients with tongue cancer (cT3N2bM0) were treated by intra-arterial chemotherapy as neoadjuvant chemotherapy. After a week of chemotherapy, patients underwent surgical treatment. Intra-arterial chemotherapy was administered at 75 mg/m2 of cis-diaminedichloroplatinum-II (CDDP) two times weekly. At the beginning of surgery, 5 mg of indocyanine green (ICG) was administered to the lingual artery. SLNs were detected using ICG fluorescence imaging and a conventional radioactivity method. The effect of lymphatic chemotherapy was evaluated by apoptosis using Trevigen’s apoptosis detection kit.

Results
The mean CDDP concentrations in the metastasis and non-SLNs were 2.35 μg/g and 1.08 μg/g, respectively (p = 0.034). Of 27 metastatic nodes, 24 (89%) were identified by ICG fluorescence imaging; however, only 18 (67%) were identified by the conventional method (p = 0.043).

Of 22 measurable metastatic nodes, eight responded (partial response) and 14 did not respond (stable disease). Apoptosis was detected in all metastatic nodes.

Conclusion
CDDP concentrations in metastatic nodes were significantly higher than those in non-SLNs. This novel drug-delivery system is feasible for lymphatic chemotherapy targeting metastatic nodes in patients with cT3N2bM0 tongue cancer.

Introduction
The sentinel lymph node (SLN) is defined as the lymph node that firstly receives lymphatic drainage from the primary cancer. SLN is considered to be the first possible micrometastatic site via lymphatic drainage from the primary cancer. Thus, the pathological status of SLN can predict the status of all regional lymph nodes. This SLN concept is well-established in the treatment of patients with several types of solid carcinomas, such as melanoma and breast cancer. The SLN concept has been extended to many other solid tumours, including head and neck cancers. These techniques can be applied for lymphatic chemotherapy targeting SLN by preventing various surgical complications and improving prognosis. In this study, we consider a newly developed lymphatic chemotherapy procedure targeting SLN using intra-arterial chemotherapy for tongue cancer to improve prognosis and preserve significant organs.

Objective
Evaluate lymphatic chemotherapy targeting neck metastases in patients with tongue cancer (cT3N2bM0) using intra-arterial chemotherapy and evaluate the measurement of cis-diaminedichloroplatinum-II (CDDP) concentrations in metastatic lymph nodes and non-SLNs.

Methods and patients
Seven patients with tongue cancer (cT3N2bM0) were treated by intra-arterial chemotherapy as neoadjuvant chemotherapy from September 2011 to February 2012. After a week of chemotherapy, surgical treatment including partial resection of the tongue and neck dissection was performed. Intra-arterial chemotherapy was administered at 75 mg/m2 of CDDP two times weekly. Computed tomography (CT)-angiography confirmed that the areas of tongue cancer were stained and that lymph nodes were not stained via arteries (Figure 1). Five milligrams of indocyanine green (ICG) was administered via a catheter positioned in the lingual artery at the beginning of the surgery (Figure 2). SLNs were detected by ICG fluorescence imaging (Photodynamic Eye, Hamamatsu...
Photonics) and non-SLNs were detected in two submandibular lymph nodes located near the tongue cancer. These were monitored as controls. To measure CDDP concentrations, 0.1 g of each of the SLNs and the two non-SLNs were resected and the rest of each of the SLNs were examined intra-operatively by means of a routine frozen pathological examination. A conventional method of identifying SLNs using radioactive injection was also performed the day before surgery. The patient characteristics are shown in Table 1.

The effect of lymphatic chemotherapy was histologically evaluated by apoptosis using Trevigen’s Tumour TACS™ in situ Apoptosis Detection Kit. This kit was specifically designed for the detection of DNA fragmentation in tumour tissues. Apoptotic cells were identified using the terminal deoxynucleotidyl transferase (TDT) mediated deoxyuridine triphosphate biotin nick-end labelling (TUNEL) method. Dewaxed and rehydrated specimens were incubated in 40 μg/mL of proteinase K for 1 h at 37°C and were treated with 3% H₂O₂ in methanol for 30 min at room temperature. After adding equilibration buffer for 5 min at room temperature, TDT enzyme was pipetted onto the sections and incubated at 37°C for 2 h. The reaction was stopped by incubating the sections in stop buffer for 30 min at 37°C. Antidigoxigenin peroxidase was added to the slides, followed by incubation for 30 min at 37°C. Slides were stained with diaminobenzine for 10 min and counterstained with haematoxylin.

Measurable metastatic lymph nodes (n = 22) were evaluated according to the guideline. The definitions of the criteria (response evaluation criteria in solid tumors, RECIST) involved determining the objective tumour response for target lesions. The criteria takes into account the measurement of the longest diameter only for all target

**Figure 1:** Magnetic resonance imaging (MRI) before treatment and lymphoscintigraphy of tongue cancer cT3N2bM0. (a) MRI showed multiple metastatic lymph nodes in levels I, II and III. (b) Frontal view of lymphoscintigraphy. Due to ‘shine-through’ and obstruction of afferent lymphatic canals, only a few metastatic lymph nodes were indicated by lymphoscintigraphy.

**Figure 2:** Computed tomography (CT)-angiography infusing the lingual artery. (a) Lateral view of lingual artery angiography indicated that the tongue cancer invaded the base of the tongue and the lingual artery was stenosed in distal. (b) Tongue cancer with indocyanine green (ICG) fluorescence imaging was injected via the right-side lingual artery with the left-side lingual artery compressed by the operator’s finger. (c) CT-angiography confirmed that the stained tongue cancer invaded the base of the tongue. However, there was no staining in any metastatic lymph node indicated by arrows.
Human Ethics Review Committee of Juntendo University. The difference between the CDDP concentrations of the two groups was tested using Student’s t-test; p values < 0.05 were considered to indicate significance.

Results
Detection of SLNs was clearly demonstrated by ICG fluorescence imaging (Figures 3 and 4). The mean number of SLNs was 5.1 (4–8). ICG fluorescence imaging showed a greater number of SLNs in intra-arterial infusion than seen when injecting the radiocolloid intra-tumour (mean 3.3). SLNs detected by ICG fluorescence imaging included all SLNs detected by the conventional radioisotope method.

Histopathological examination was performed for 35 SLNs and 188 non-SLNs (Table 1). All seven patients with histopathologically verified metastasis in their SLNs demonstrated positive results in ICG fluorescence imaging. Of 27 metastatic lymph nodes, only 3 (11%) were not identified within each SLN basin. Of 27 metastatic lymph nodes, 9 (33%) were also not identified by means of conventional methods.

The mean CDDP concentrations of metastatic lymph nodes and non-SLNs were 2.35 μg/g and 1.08 μg/g, respectively. The CDDP concentration of metastatic lymph nodes was the sum of the longest diameter of target lesions; and stable disease (SD)—neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

An informed consent was obtained from the patient prior to treatment and this study was approved by the

Table 1  Patient characteristics

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>M/F</th>
<th>TNM</th>
<th>SLNs by RI</th>
<th>SLNs by ICG</th>
<th>Non-SLNs</th>
<th>FN by RI</th>
<th>FN by ICG</th>
<th>No. of MLN</th>
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<tr>
<td>1</td>
<td>61</td>
<td>M</td>
<td>cT3N2bM0</td>
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<td>63</td>
<td>M</td>
<td>cT3N2bM0</td>
<td>5</td>
<td>5</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>F</td>
<td>cT3N2bM0</td>
<td>5</td>
<td>8</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>4</td>
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<tr>
<td>4</td>
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<td>4</td>
<td>5</td>
<td>51</td>
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<td>2</td>
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<td>26</td>
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<td>26.9</td>
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<td>0.11</td>
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</table>

FN, false negative; ICG, indocyanine green; LN, lymph node; MLN, metastatic lymph nodes; RI, radiocolloid; SLN, sentinel lymph node

Figure 3: Intra-operative navigation surgery using indocyanine green (ICG) fluorescence imaging. Numbers 1–5 indicate metastatic lymph nodes. (a, b) Represent level I lymph nodes and the same lymph nodes by ICG fluorescence imaging, respectively. (c, d) Represent level II and III lymph nodes and the same lymph nodes by ICG fluorescence imaging, respectively.
significantly higher than that of non-SLNs (p = 0.034).

Of measurable 22 metastatic lymph nodes based on preoperative CT, eight lymph nodes responded PR and 14 did not respond up to PR (SD) according to RECIST. In the result, four patients were evaluated as PR and three patients were evaluated as SD. This result is shown in Table 2.

Vast metastatic cancer was almost diminished and resulted in scar tissue. Apoptosis was detected in all 27 metastatic lymph nodes (Figure 4). Many lymph node sizes of metastasis were not as shrunk as the baseline; however, a pathological effect was achieved.

No haematological complications were caused by intra-arterial chemotherapy. All patients are alive, with no evidence of disease and are able to consume food as they were able to before surgery.

### Table 2

<table>
<thead>
<tr>
<th>Case</th>
<th>Metastatic level</th>
<th>Pretreatment size</th>
<th>Post-treatment size</th>
<th>Regression rate (%)</th>
<th>Result</th>
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<td>Ib</td>
<td>20 × 18 / 15 × 17 / 20 × 21 / 35 × 25</td>
<td>10 × 9 / 10 × 5 / 13 × 10 / 25 × 20</td>
<td>37.50</td>
<td>PR</td>
</tr>
<tr>
<td>2</td>
<td>Ib</td>
<td>18 × 15 / 10 × 10</td>
<td>15 × 13 / 9 × 8</td>
<td>15</td>
<td>SD</td>
</tr>
<tr>
<td>3</td>
<td>Ib</td>
<td>20 × 15 / 20 × 13 / 15 × 13</td>
<td>11 × 12 / 14 × 10 / 14 × 11</td>
<td>49.40</td>
<td>PR</td>
</tr>
<tr>
<td>4</td>
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<td>22 × 21 / 18 × 16 / 10 × 10 / 11 × 8</td>
<td>15 × 17 / 13 × 12 / 6 × 5 / 10 × 5</td>
<td>24.60</td>
<td>SD</td>
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<tr>
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<td>III</td>
<td>7 × 8 / 10 × 9 / 10 × 11 / 6 × 5</td>
<td>7 × 5 / 8 × 7 / 10 × 8 / 5 × 4</td>
<td>11.80</td>
<td>SD</td>
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<tr>
<td>6</td>
<td>II</td>
<td>22 × 15 / 12 × 10</td>
<td>15 × 13 / 8 × 6</td>
<td>33.40</td>
<td>PR</td>
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<tr>
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<td>Ia</td>
<td>18 × 12 / 18 × 13 / 10 × 9</td>
<td>12 × 9 / 12 × 8 / 6 × 5</td>
<td>39.20</td>
<td>PR</td>
</tr>
</tbody>
</table>

Recently, we have demonstrated that intra-arterial chemotherapy for the treatment of primary tongue cancer also resulted in lymphatic chemotherapy that aided in the control of the subclinical metastatic tumour in SLNs. The schema of lymphatic chemotherapy is illustrated in Figure 5. This schema shows that CDDP administered to the primary tongue cancer selectively moves to SLNs including metastatic lymph nodes via lymphatic canals. CDDP is accumulated in the metastatic lymph nodes and results in a high CDDP concentration in the metastatic lymph nodes.

In our preliminary study, all SLNs were detected by ICG fluorescence imaging infused via the lingual artery in cT3N0M0 tongue cancer patients. However, of 27 metastatic lymph nodes, 24 (89%) were detected by ICG fluorescence imaging infused via the lingual artery in seven cT3N2bM0 tongue cancer patients. The number of SLNs including metastatic lymph nodes resulting from intra-arterial infusion was greater than that seen by means of a conventional injection into the tumour. This is because ICG was administered to the lingual artery and ICG spread throughout half of the tongue (Figure 2). ICG moved via lymphatic canals from half of the tongue, including the tongue cancer. Even in micrometastatic SLNs, an afferent lymphatic was sometimes occluded by micrometastatic cancer based on sentinel navigation or CT lymphography. In the current study, we also did not detect 9 (33%) metastatic lymph nodes by conventional methods because of occlusion of afferent lymphatics from the tongue cancer (Figure 6). However, the lymph node contained CDDP concentration as high as 4.65 μg/g. This was because each lymph node has several afferent lymphatics and ICG or CDDP could move to metastatic lymph nodes via several other afferent lymphatics in the case of intra-arterial infusion. CDDP was released into the tumour. This is because ICG was administered to the lingual artery and ICG spread throughout half of the tongue (Figure 2). ICG moved via lymphatic canals from half of the tongue, including the tongue cancer. Even in micrometastatic SLNs, an afferent lymphatic was sometimes occluded by micrometastatic cancer based on sentinel navigation or CT lymphography. In the current study, we also did not detect 9 (33%) metastatic lymph nodes by conventional methods because of occlusion of afferent lymphatics from the tongue cancer (Figure 6). However, the lymph node contained CDDP concentration as high as 4.65 μg/g. This was because each lymph node has several afferent lymphatics and ICG or CDDP could move to metastatic lymph nodes via several other afferent lymphatics in the case of intra-arterial infusion. CDDP was released into the tumour.

### Discussion

Chemoradiation therapy has significantly enhanced the preservation of important organs in the treatment of head and neck cancer. However, because of severe mucositis and low sensitivity to chemotherapy, tongue cancer is not treated by chemoradiation as often as other sites of head and neck cancer. CDDP is the most promising drug for the treatment of advanced tongue cancer (Figure 6). However, the dairy protein concentration in the metastatic lymph nodes and results in a high CDDP concentration in the metastatic lymph nodes.

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### Competing interests

None declared.

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Figure 4: Pathological findings of metastatic lymph nodes after chemotherapy. (a) Most of the metastatic cancer resulted in scar tissue; however, small cancer nests were residual (low-power magnification). (b) Cancer cells appeared to survive within small cancer nests (high-power magnification). (c) Many apoptotic bodies were detected within small cancer nests (triphsosphate biotin nick-end labelling technique.) Arrows indicate apoptotic cells.

Figure 5: The schema of lymphatic chemotherapy using intra-arterial chemotherapy. Figure used upon obtaining permission from BioMed Central (Yokoyama et al.)

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The CDDP concentrations in metastatic lymph nodes were significantly higher than those in non-SLNs. This supports the idea that a new minimally invasive multimodal drug-delivery system is feasible.

Further investigations in the near future may lead to the development of a new minimally invasive multimodal therapy targeting both the primary tumor and lymph node metastases.

Conclusion

The CDDP concentrations in metastatic lymph nodes were significantly higher than those in non-SLNs. This novel drug-delivery system is feasible for lymphatic chemotherapy targeting metastatic nodes in patients with tongue cancer cT3N2bM0.

Abbreviations list

CDDP, cis-diaminedichloroplatinum-II; CR, complete response; CT, computed tomography; ICG, indocyanine green; MRI, magnetic resonance imaging; PD, progressive disease; PR, partial response; RECIST, response evaluation criteria in solid tumors; SD, stable disease; SLN, sentinel lymph node; TDT, terminal deoxynucleotidyl transferase; TUNEL, triphosphate biotin nick-end labelling.

Acknowledgement

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References


Figure 6: The schema of increasing cis-diaminedichloroplatinum-II (CDDP) concentration of metastatic lymph nodes. Despite occlusion of afferent lymphatics from the tongue cancer, each lymph node has several afferent lymphatics, and indocyanine green or CDDP could move to metastatic lymph nodes via several other afferent lymphatics in the case of intra-arterial infusion. (1) Occluded afferent lymphatics (red arrow). (2–4) Open afferent lymphatics (black arrows).


