Apoptosis in Cervical Cancer after Balloon-occluded Arterial Infusion of Anticancer Drugs

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Abstract. Background: This study was designed to investigate the relationship between apoptosis and Bcl-2 and Bax expressions in uterine cervical cancer after balloon-occluded arterial infusion (BOAI). Materials and Methods: Twenty-four specimens were obtained before and after BOAI. The occurrence of apoptosis was examined with molecular biochemical techniques. The expressions of Bcl-2 and Bax proteins were investigated by immunohistochemical staining. Results: Labelling of DNA in situ indicated that apoptotic cells were sporadically seen before BOAI (6.1±1.9). Apoptotic cells apparently increased at 5 days (25.1±6.4) after BOAI. The autoradiographic analysis revealed that the DNA ladder was identified at 5 days after BOAI. Although Bcl-2 immuno-reactivity was faintly detected, the expression of Bax increased at 3 days (49.4±10.4%) after BOAI. Conclusion: The results indicated that treatment with BOAI resulted in transient increases of apoptosis in cervical cancer in association with the increased expression of Bax.

Most patients with locally advanced carcinoma of the uterine cervix are currently treated with radiotherapy and/or chemotherapy. Several studies have shown that chemotherapy improved the survival rate in patients with cervical cancer (1, 2), and platinum-based chemotherapy has recently proved to be an effective therapy for this disease. Previous reports have demonstrated that the arterial infusion of anticancer drugs is more effective in reducing the local tumor size than systemic chemotherapy (3, 4). Balloon-occluded arterial infusion (BOAI) is a method for the local administration of anticancer drugs to temporarily occlude the feeding artery of malignant tumors with a balloon catheter and to infuse anticancer drugs into the artery distal to the balloon. The drugs are kept at high concentrations in the cancer-affected uterus by the interruption of the arterial blood flow, so that the blood in the uterus is almost completely replaced by anticancer drugs. After BOAI, the patients are treated with surgical or radiation therapy. We previously reported that BOAI might improve the prognosis of advanced cervical carcinoma of the uterus (5).

In female reproductive organs, it has been demonstrated that the occurrence of apoptosis and the expressions of Bcl-2 and Bax are detected in the normal uterine endometrium (6, 7). There is now considerable interest in apoptosis in oncology research (8). We have previously reported that apoptosis appeared to occur in carcinomas of the cervix and of the uterine endometrium (9-11). It is commonly accepted that apoptosis is an important process, not only for untreated neoplastic cell death, but also for cell death induced by radiotherapy and chemotherapy (8). During the radiotherapy of patients with squamous cell carcinoma of the uterine cervix, the transient occurrence of apoptosis was found to closely correlate with the expression of Bax, but not with Bcl-2 expression (12). Although some investigators demonstrated that apoptosis increased in cervical carcinoma after chemotherapy, these groups estimated apoptotic changes only before and 3 to 4 weeks after chemotherapy (13, 14). However, it has been indicated that the apoptosis induced by anticancer drugs in vitro reached a peak within 48 hours after treatment (15, 16). The current study was designed, therefore, to investigate the occurrence of apoptosis after chemotherapy by molecular biochemical techniques, as well as the expressions of Bcl-2 and Bax proteins by immunohistochemical staining.

Materials and Methods

Patients. The cervical tissues were obtained by punch biopsy from 5 patients, 61-88 years of age, with squamous cell carcinoma of Stage Ib and IIb disease who had received BOAI at an affiliate hospital of Wakayama Medical University (Wakayama, Japan) from 1998 to 2000. The clinical stage was based on the criteria of the
In situ analysis of DNA fragmentation in histological sections. Analysis of the apoptotic fragmentation of DNA. Isolation and analysis of the apoptotic fragmentation of DNA.

Results

Analysis of the apoptotic fragmentation of DNA. Immunohistochemical analyses of Bcl-2 and Bax proteins. In situ analysis of DNA fragmentation in histological sections.

Figure 1. Apoptotic fragmentation of DNA before and after BOAI. Arrows indicate the point at which the gels were cut for quantitative analysis of high and low molecular weight DNA. Autoradiogram from patient 3.
**Immunohistochemical staining of Bcl-2.** Immunostaining specific for Bcl-2 was located in the cytoplasm of the cells. Bcl-2 immunoreactivity was faintly detected in 3 out of 5 cases before BOAI (Figure 2C). After BOAI, the expression of Bcl-2 slightly increased in 4 out of 5 patients (Figure 2D), but these changes did not differ significantly. The localization of Bcl-2 was 1.5±0.5% before BOAI, 3.6±1.8% on Day 1, 4.2±1.7% on Day 3, 3.4±1.2% on Day 5 and 2.3±1.0 on Day 7. The details of the Bcl-2 immunoreactivity for each patient are provided in Table III.

**Immunohistochemical staining of Bax.** Immunostaining specific for Bax was also cytoplasmic and immunoreactivity was detected in all 5 cases before BOAI (Figure 2E). The expression of Bax increased on Day 1, and strong positive localization for Bax was observed on Days 3, 5 and 7 (Figure 2F). The peak expression of Bax was identified on Day 3 (49.4±10.4%), which was significantly increased compared with the values before BOAI (8.1±2.1%) and one day after BOAI (17.6±0.7). The accumulation of Bax was 36.7±9.9% on Day 5 and 40.5±12.1 on Day 7, neither value being statistically significant compared to values before BOAI. The details of Bax localization are given in Table IV.

**Discussion**

Apoptosis is an important process for the cell death induced by chemotherapy (8) and those anticancer drugs which induce apoptosis in vitro reached a peak within 48 hours after treatment and then slowly declined (15, 16). Our autoradiographic analysis revealed that the ladder pattern characteristic of the apoptotic cleavage of DNA was obtained on Days 3 and 5 after treatment. The labelling index of DNA in situ significantly increased in the cancer cells on Day 5 compared with that before BOAI. In addition, the results of the analysis in situ and the electrophoretic analysis demonstrated the transient occurrence and time dependency of the chemotherapy-induced apoptosis in patients with locally advanced cervical carcinoma after BOAI.

While the overexpression of the Bcl-2 protein blocked apoptosis, Bax has been shown to induce the apoptosis inhibited by Bcl-2 in several tissues (18, 19). It has been reported that transient increases of apoptosis are associated with an increased expression of Bax in invasive squamous cell carcinoma of the uterine cervix (12, 20). Recent studies showed that irradiation induced the expression of Bax in vitro without increased occurrence of Bcl-2 (21, 22). In the current study, while the immunoreactivity for Bcl-2 did not increase after BOAI, increased immunoreactivity for Bax was observed on Days 3, 5 and 7. It is possible that the increased expression of Bax induced by BOAI may be correlated with the transient increases of apoptosis.

BOAI allows for the efficient administration of anticancer drugs to the focal site and is effective in reducing the local tumor size. We have reported the use of BOAI before radiotherapy (5). The 5-year survival rate of stage III patients with BOAI was significantly higher than that of stage III patients without BOAI. BOAI also

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### Table I. Apoptotic cleavage of DNA into small fragments before and after BOAI.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>0 day</th>
<th>1 day</th>
<th>3 day</th>
<th>5 day</th>
<th>7 day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ladder (%)</td>
<td>Ladder (%)</td>
<td>Ladder (%)</td>
<td>Ladder (%)</td>
<td>Ladder (%)</td>
</tr>
<tr>
<td>1</td>
<td>(−) 000</td>
<td>(+) 146</td>
<td>(+) 351</td>
<td>(+) 526</td>
<td>(+) 102</td>
</tr>
<tr>
<td>2</td>
<td>(−) 000</td>
<td>(±) 173</td>
<td>(+) 333</td>
<td>(+) 566</td>
<td>(+) 293</td>
</tr>
<tr>
<td>3</td>
<td>(−) 000</td>
<td>(±) 218</td>
<td>(+) 268</td>
<td>(+) 365</td>
<td>(+) 259</td>
</tr>
</tbody>
</table>

**BOAI:** balloon-occluded arterial infusion.

Ladder: the occurrence of DNA laddering.

−: negative; ±: faint; +: strong.

%: radioactivity of the small fragments of DNA in each sample was divided by the value before BOAI, arbitrarily designated as 100%.

### Table II. Results of the apoptotic index before and after BOAI.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Before</th>
<th>1 day</th>
<th>3 day</th>
<th>5 day</th>
<th>7 day</th>
</tr>
</thead>
<tbody>
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<td>7.7</td>
<td>20.6</td>
<td>27.7</td>
<td>38.3</td>
<td>25.0</td>
</tr>
<tr>
<td>3</td>
<td>9.7</td>
<td>14.3</td>
<td>35.7</td>
<td>34.3</td>
<td>11.3</td>
</tr>
<tr>
<td>4</td>
<td>10.0</td>
<td>5.0</td>
<td>2.0</td>
<td>5.0</td>
<td>8.0</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>10.0</td>
<td>N.A.</td>
<td>15.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**BOAI:** balloon-occluded arterial infusion.

Apoptotic index: the number of apoptotic cells / the total number of cells) x 100; N.A.: not available because fixed samples were not stored. Each apoptotic index represents the mean value from cells in three different microscopic fields per specimen.
reduced the side-effects caused by the anticancer drugs. Recently, neoadjuvant chemotherapy before surgery has been used for patients with advanced disease. After BOAI, the patients are treated with surgical or radiation therapy. In the current study, 3 patients (patients 1, 2 and 3) with stage IIIb were treated with radiotherapy and the others (patients 4 and 5) with stage IIb underwent surgery. The post-operative stage based on the criteria of the International Union Against Cancer was diagnosed a pT1a, pN0, pM0 for patient 4 and pT0, pN0, pM0 for

Figure 2. In situ 3’ end labelling of DNA and immunohistochemistry of Bcl-2 and Bax in the sections before and after BOAI. Photomicrographs A and B show results of 3’ end labelling of DNA. Positive cells are indicated by the blue color of the nuclei. Before BOAI, apoptotic cells were seen sparsely (A). Dense labelling was detected in a large fraction of tumor cells on Day 5 (B). The accumulation of Bcl-2 protein (brown coloration of the cytoplasm) is shown in photomicrographs C and D. Bcl-2 immunoreactivity was not seen before BOAI (C) or on Day 5 (D). The results in E and F demonstrate Bax expression (brown color). Immunoreactivity specific for Bax was observed sporadically before BOAI (E). Most cells showed intense positive staining for Bax on Day 5 (F). Each photomicrograph is from a serial section from patient 3. Magnification, X200.
patient 5. Only one patient (patient 1) died of the primary disease at 7 months after BOAI, while the other 4 patients have survived without recurrence. Patient 1 received radiotherapy after BOAI. We have already demonstrated that the occurrence of apoptosis during radiotherapy may be a useful predictor of response to radiotherapy and prognosis (12). The occurrence of apoptosis in patient 1 was less than that of another 2 patients (patients 2 and 3) during radiotherapy.

By using molecular biochemical techniques, transient increases in apoptosis and Bax expression after BOAI were demonstrated. However, the Bcl-2 expression did not change significantly after BOAI. Our results suggest that chemotherapy-induced apoptosis may be related more closely to increased Bax expression than to that of Bcl-2.

Acknowledgements

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References


