Intrapleural Hyperthermic Perfusion With Chemotherapy Increases Apoptosis in Malignant Pleuritis

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Background. Previously, we reported on the effectiveness of intrapleural hyperthermic perfusion with chemotherapy, a new treatment we developed for patients with malignant pleuritis. The present study analyzes the mechanism of the effectiveness of this therapy by examining the induction ratio of apoptosis among tumor cells following the perfusion treatment.

Methods. This study included 11 consecutive patients with primary pulmonary adenocarcinoma and accompanying pleural seedlings and pleural effusions containing tumor cells but without distant metastasis. All patients underwent surgical resection of the primary lesion and then received sequential perfusion treatment. Tumor cells collected from the effusion both before and again at 24 hours following the perfusion treatment were subsequently examined using an immunocytochemical stain to determine apoptosis among tumor cells. The percentage of positively stained cells was expressed as the apoptotic index. We compared the survival rate of these 11 patients with the survival rate of a second group of 11 patients with malignant pleuritis who underwent surgical resection of the primary lesion but who did not receive the perfusion treatment (control group).

Results. The ratio of spontaneous apoptosis of untreated tumor cells was 2.8% ± 2.0%. Following the perfusion, apoptosis among tumor cells was 25.2% ± 4.6%, clearly a significant increase. While the median survival time for patients receiving the perfusion treatment was 20 months, the median survival time for the control group was 6 months.

Conclusions. In patients with malignant pleuritis, intrapleural hyperthermic perfusion with chemotherapy induced potent apoptosis of tumor cells in the pleural cavity and also improved the survival rate of these patients as compared with patients who did not receive the perfusion treatment.


A diagnosis of malignant pleuritis, including intrapleural disseminated lesions and malignant pleural effusions in advanced lung cancer, is indicative of a poor prognosis for patients even though they may have undergone a pleurectomy or the local administration of a sclerosing agent [1]. As a new therapeutic option for patients with malignant pleuritis, our surgical department developed intrapleural hyperthermic perfusion with chemotherapy (IPHC) using hyperthermic perfusion. The effectiveness of this new treatment was reported previously [2]. IPHC, which includes the administration of Cis-platinum (CDDP), resulted in enhanced anti-tumor effects [2]. The present study analyzes the mechanism of the effectiveness of IPHC by examining the induction ratio of apoptosis among tumor cells following IPHC treatment.

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Table 1. Characteristics of Patients With Malignant Pleuritis With or Without IPHC

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IPHC (n = 11)</th>
<th>Control (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender: male/female</td>
<td>8/3</td>
<td>9/2</td>
</tr>
<tr>
<td>Age: mean (range)</td>
<td>63.9 (33–78)</td>
<td>65.4 (41–79)</td>
</tr>
<tr>
<td>Pleura: seeding/effusion</td>
<td>11/11</td>
<td>11/11</td>
</tr>
<tr>
<td>T-factor: T4</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>N-factor: N0/N1/N2</td>
<td>7/2/2</td>
<td>8/1/2</td>
</tr>
<tr>
<td>M-factor: M0</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Primary organ: lung</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Pathological diagnosis: adenocarcinoma</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Resection of primary lesion</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Systemic chemotherapy</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

IPHC = intrapleural hyperthermic perfusion with chemotherapy.

The patients of this control group were selected retrospectively on the basis of clinical factors before the introduction of IPHC. To avoid statistical bias with regard to patient survival rates, patients selected for the control group had similar clinical characteristics as the IPHC group, including age, pleural seeding, pleural effusion, TNM factors, primary organ, pathological diagnosis, resection of primary lesion, and systemic chemotherapy. Clinical characteristics of both groups are presented in Table 1.

Before treatment, signed informed consent forms concerning treatment with IPHC and apoptosis assays were obtained in all cases. The Institutional Review Board of Miyazaki Medical College approved this research using the apoptosis assay.

Methods

As previously reported [2], with IPHC therapy the thoracic cavity is irrigated for 2 hours with a 43% saline solution (3000 mL) containing 200 mg/m² of CDDP. After surgical resection of the primary lesion and with the patient under general anesthesia, two thoracic drainage tubes are placed in the thoracic cavity and are connected to a specially devised circuit (modified CRPH-3000C; Mera Ltd, Tokyo, Japan). The CDDP solution is perfused through the circuit while the temperature is increased. All of the fluid in the thoracic cavity is removed at the end of the perfusion. Tumor cells collected from the effusion both before IPHC and again 24 hours following IPHC are subsequently examined using an immunocytochemical stain to determine apoptosis among tumor cells.

Immuno-cytochemical Studies (Apoptosis Assay)

The ApopTag detection kit (Oncor, Gaithersburg, MD) was used to detect apoptosis. ApopTag labels apoptotic cells in situ by modifying genomic DNA. Samples of tumor cells removed from pleural effusions are collected and processed using the ApopTag detection kit according to the manufacturer’s instructions. In brief, tumor cells in the effusion are fixed in 1% paraformaldehyde in PBS for 10 minutes and are dried on a microscope slide. After the slides are rinsed with PBS, they are incubated in a reaction mixture containing terminal transferase and digoxigenin dUTP at 37°C for 1 hour. After the slides are washed, antidigoxigenin antibody coupled to horseradish peroxidase is added, and the tissue slides are incubated for 30 minutes at room temperature. Following another rinsing with PBS, 3,3′-diaminobenzidine tetrachloride (DAKO, Carpinteria, CA) is added for 10 minutes at 37°C. The slides are then examined under light microscopy to detect DNA fragmentation in the nucleosome of apoptotic cells.

Apopotic Index

Both positively stained cells and negatively stained cells per ten fields at a magnification of x400 were randomly counted, and the average count for each patient was recorded. The mean ± standard error percentage of positively stained cells was expressed as the apoptotic index (A.I.).

Survival Rate of Patients

Survival duration was calculated from the date of perfusion (IPHC group) and the date of surgical resection of the primary lesion (control group) to the date of the last known follow-up or death. The probability of survival was computed according to the Kaplan-Meier method.

Statistical Analysis

The significance between the A.I. of both pre- and post-IPHC was evaluated using the Student’s t-test. The statistical significance of the difference between the survival curves was determined using the log rank test. A p value of <0.05 was considered significant. All statistical methods were performed using the Statistical Analysis Software (SPSS, version 6.1J for the Macintosh NT; SPSS Institute Inc., Cary, NC).

Results

Immuno-cytochemical Studies (Apoptosis Assay)

ApopTag was used to stain tumor cells collected both pre- and post-IPHC. Tumor cells collected pre-IPHC stained negative, showing no tumor cell death except for spontaneous apoptosis (Fig 1A). Tumor cells collected post-IPHC, however, stained positive, indicating tumor cell death (Fig 1B).

Apopotic Index

The pre-IPHC spontaneous apoptotic index was 2.8% ± 2.0%. The post-IPHC apoptotic index was 25.2% ± 4.6%, clearly a significant increase (Fig 2).

Survival Rate of Patients

Survival rates were calculated using the Kaplan-Meier method. As shown in Figure 3, although the median survival time for patients receiving IPHC was 20 months, the median survival time for the 11 patients who did not receive IPHC (control group) was 6 months, a significant decline (p < 0.05).
Comment

Malignant pleuritis with intrapleural disseminated lesions and malignant pleural effusions has long been considered an advanced stage of cancer. The most recent TNM classification designates malignant pleuritis as a T4 Stage IIIB disease with an associated 6% to 8% 5-year survival [3]. More recent studies [4–6] have reported that the survival rate of patients with malignant pleuritis and a classification of Stage IIIB disease is more similar to the survival rate of patients without malignant pleuritis and a Stage IIIB classification. Based on this information, it appears that the treatment for lung cancer patients with malignant pleuritis should be approached in a similar manner as the treatment for patients with Stage IV disease; ie, malignant pleuritis should be treated primarily with chemotherapy [7].

Based on our experimental study on the anti-tumor effect of regional hyperthermia [8, 9], our surgical department developed a new therapy for patients with malignant pleuritis associated with advanced lung cancer. IPHC, a hyperthermic perfusion technique used in combination with the administration of CDDP, results in enhanced antitumor effects. This technique provides a potent tumorcidal effect and has demonstrated clinical effectiveness by improving the prognosis for lung cancer patients with malignant pleuritis [2]. Although the basic mechanism of hyperthermic cell injury is still poorly understood, many studies have shown that programmed cell death or apoptosis can be markedly induced using hyperthermic treatment in several neoplastic cells both in vivo and in vitro [10–12]. Apoptosis, the major mode of programmed cell death after exposure to external stresses such as radiation, hyperthermia, and some chemotherapeutic agents [13, 14], is controlled by multiple signaling and effectors and a number of complicated biochemical reactions [15, 16].

It is well known that hyperthermia enhances not only the cytotoxic effect but also enhances apoptosis in murine and man neoplastic cells [17]. CDDP, the most widely used chemotherapeutic agent for treating lung cancer, has also been shown to induce apoptosis in malignant cells [18]. Therefore, treatment-induced tumor apoptosis should be considered an important therapeutic target in hyperthermic perfusion with chemotherapy. The precise mechanism of apoptosis induction by both hyperthermia and CDDP, however, has not been determined. Similarly, the mechanism of the effectiveness of IPHC has not yet been clarified. In an effort to better understand how IPHC works, we examined the induction ratio of apopto-

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Fig 1. (A) In situ apoptosis detection using ApopTag in specimens obtained pre-IPHC. Most tumor cells were negatively stained (original magnification ×400). (B) In situ apoptosis detection using ApopTag in specimens obtained post-IPHC. Irregularly shaped DNA breakage has occurred and positively stained (dark brown) nuclei of tumor cells demonstrate apoptosis (original magnification ×400); the arrow indicates apoptosis. (IPHC = intrapleural hyperthermic perfusion with chemotherapy.)

Fig 2. Differences in the apoptotic index (A. I.) (mean ± standard error percentage). Asterisk indicates p < 0.001 versus pre-IPHC. (IPHC = intrapleural hyperthermic perfusion with chemotherapy.)

Fig 3. Survival rate of IPHC group and control group. Although the median survival time of the IPHC group was 20 months, the median survival time of the control group was 6 months, a significant difference (p < 0.05). (IPHC = intrapleural hyperthermic perfusion with chemotherapy.)
sis among tumor cells using an apoptosis assay (ApopTag detection kit) that modifies genomic DNA using terminal deoxynucleotidyl transferase and then labels apoptotic cells using antidigoxigenin antibodies and immunoperoxidase staining. In apoptosis, DNA fragmentation is usually associated with ultra structural changes in cellular morphology [19]. With an untreated tumor, the rate of spontaneous apoptosis is reported to be about 2 to 5% of tumor cells at any one time [20, 21]. Our study demonstrated remarkable apoptosis among tumor cells following IPHC (25.2% ± 4.6%) as compared with the A.I. recorded before IPHC (2.8% ± 2.0%).

The survival rate for patients with malignant pleural effusions and intrapleural disseminated lesions is poor, even for those who have undergone tumor resections or exploratory thoracotomies. The median survival time of these patients is 6 to 9 months [4], a rate similar to that of patients with Stage IV disease. The survival rate of our control group (patients with malignant pleuritis but without IPHC) demonstrated a median survival time of 6 months. The IPHC group, however, demonstrated a significantly improved median survival time of 20 months as calculated by the Kaplan-Meier method. In the absence of randomized studies, we could not determine the significant prolonged survival rate of IPHC patients as compared with the control group.

Although we could not provide evidence of a direct relationship between apoptosis of tumor cells and patient survival in this study, we hypothesize that increased apoptosis of tumor cells may contribute to a better prognosis for these patients.

Further studies on the effect of IPHC including degeneration, necrosis, and other immuno-cytochemical assays are needed, and we plan to compare the effect of hyperthermia and of CDDP separately and in combination on the degree of tumoricidal effect in vitro.

In conclusion, in patients with malignant pleuritis, intrapleural hyperthermic perfusion with chemotherapy induced potent apoptosis of tumor cells in the pleural cavity and also improved the survival rate of these patients as compared with the patients who did not receive the perfusion treatment.

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References


INVITED COMMENTARY

Hyperthermia has a long history in the treatment of various forms of cancer dating back to Coley’s work first published in 1893. The literature is replete with examples that demonstrate that hyperthermia is selectively lethal to various subsets of cancers. Currently, a major area of interest in cancer research is the selective induction of