Original Article

Induction of Apoptosis by Intrapleural Perfusion Hyperthermo-Chemotherapy for Malignant Pleural Mesothelioma

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Purpose: Despite extensive clinical research, no effective therapy for advanced malignant pleural mesothelioma has been established. In this study, we induced apoptosis in patients with this disease, using intrapleural perfusion hyperthermo-chemotherapy, a new procedure developed in our surgical department. We then measured the tumorcidal effect.

Material and Methods: Our study included 6 consecutive patients with malignant pleural mesothelioma (stage III: 5; stage IV: 1). Because of the advanced stage of the disease, none of the patients underwent tumor resection or pleurectomy. All patients, however, received perfusion treatment. Tumor cells collected from pleural effusions pre- and at 0, 24, and 48 h postperfusion were examined using an immunocytochemical stain to determine apoptosis. The percentage of positively stained cells was expressed as the apoptotic index.

Results: Preperfusion, the apoptotic index was 3.8% ± 2.0%, indicating spontaneous apoptosis of untreated tumor cells. Postperfusion, the apoptotic index at 0, 24, and 48 h was 22.8% ± 5.15%, 63.8% ± 8.2%, and 47.8% ± 6.9%, respectively. The patients had a median survival time of 30 months. No patient morbidity was associated with the perfusion treatment.

Conclusion: In patients with malignant pleural mesothelioma, intrapleural perfusion hyperthermo-chemotherapy induced potent apoptosis of tumor cells, increasing immediately postperfusion and peaking at 24 h. (Ann Thorac Cardiovasc Surg 2008; 14: 161–165)

Key words: malignant pleural mesothelioma, apoptosis, hyperthermia, chemotherapy

Introduction

Malignant pleural mesothelioma (MPM), an extremely aggressive thoracic malignancy, is associated with a poor prognosis and a survival time of <12 months from the onset of symptoms.[1] Although treatments for MPM include surgical resection, chemotherapy, radiotherapy, or a combination of these approaches, Alberts et al. reported that the disease was not affected by these therapeutic maneuvers.[2] At present, multimodality therapies are being studied.[3] Previously, we reported on the effectiveness of intrapleural perfusion hyperthermo-chemotherapy (IPHC) for patients with pleuritis carcinomatosa.[4,5] IPHC, which includes the administration of cis-platinum (CDDP), resulted in enhanced antitumor effects and prolonged survival rates.[4,5] Because of the small number of MPM patients in our present study, we focused this investigation on an analysis of apoptosis induction rather than on the prognostic value of IPHC for MPM patients.

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Patients and Methods

Patient profiles

This study included 6 consecutive patients (3 males, 3

females) with MPM and accompanying pleural effusions who underwent IPHC from October 2000 to March 2007 in our surgical department. Patient ages ranged from 60 to 75 years with a mean age of 67. Clinical characteristics are summarized in Table 1. A diagnosis of MPM was confirmed by pleural biopsy. Clinical staging by the International Mesothelioma Interest Group indicated 5 patients with stage III disease and 1 with stage IV. Pathological studies established 5 epithelial and 1 biphasic disease types. Because of the advanced stage of MPM, none of the patients underwent surgical resection or pleurectomy. Following IPHC, however, 3 patients received adjuvant chemotherapy (irinotecan + epirubicin/gemcitabine + carboplatinum).

Prior to IPHC treatment and apoptosis assay, signed informed consent forms were obtained in all cases. The Institutional Review Board of Miyazaki Medical College approved this research.

Methods

With patients under general anesthesia, we employed video-assisted thoracoscopic surgery to examine the pleural cavity and to perform a tumor biopsy without tumor resection or pleurectomy. Two thoracic drainage tubes were then placed in the pleural cavity and connected to a specially devised circuit (modified CRPH-3000C, MERA, Ltd.). Prior to perfusion, tumor cells were collected from the pleural effusion. Following IPHC treatment guidelines,[4,5] the thoracic cavity was irrigated for 2 h with a 43°C saline solution (3,000 mL) containing 200 mg/m^2 of CDDP. At the end of the perfusion, all fluid in the thoracic cavity had been removed. To determine apoptosis, tumor cells were collected from pleural effusions at 0, 24, and 48 h post-IPHC and subsequently examined using the apoptosis assay.

Immunocytochemical studies (apoptosis assay)

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

Apoptotic index (A.I.)

Both positively and negatively stained cells per 10 fields at a magnification of 400¥ were randomly counted, and the average count for each effusion/patient was recorded at the time of pre-IPHC and at 0, 24, and 48 h post-IPHC. The mean ± standard error percentage of positively stained cells was expressed as the A.I.

Survival rate of patients

Survival duration was calculated from the date of IPHC 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 pre- and post-IPHC was evaluated using the Student's *t*-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, USA, 1996).

Results

Immunocytochemical studies (apoptosis assay)

As seen in Fig. 1A, pre-IPHC tumor cells stained negative, showing no tumor cell death except for spontaneous apoptosis. Tumor cells collected at 0, 24, and 48 h post-IPHC, however, stained positive, indicating tumor cell death (Fig. 1, B, C, and D, respectively).

A.I.

Figure 2 compares the A.I. of pre-and post-IPHC apoptosis. The A.I. for untreated tumor cells, indicating spontaneous apoptosis, was 3.8% ± 2.0%. The A.I for tumor cells at 0, 24, and 48 h post-IPHC, however, was 22.8% ± 5.1%, 63.8% ± 8.2%, and 47.8% ± 6.9%, respectively, clearly a significant increase with a peak at 24 h.

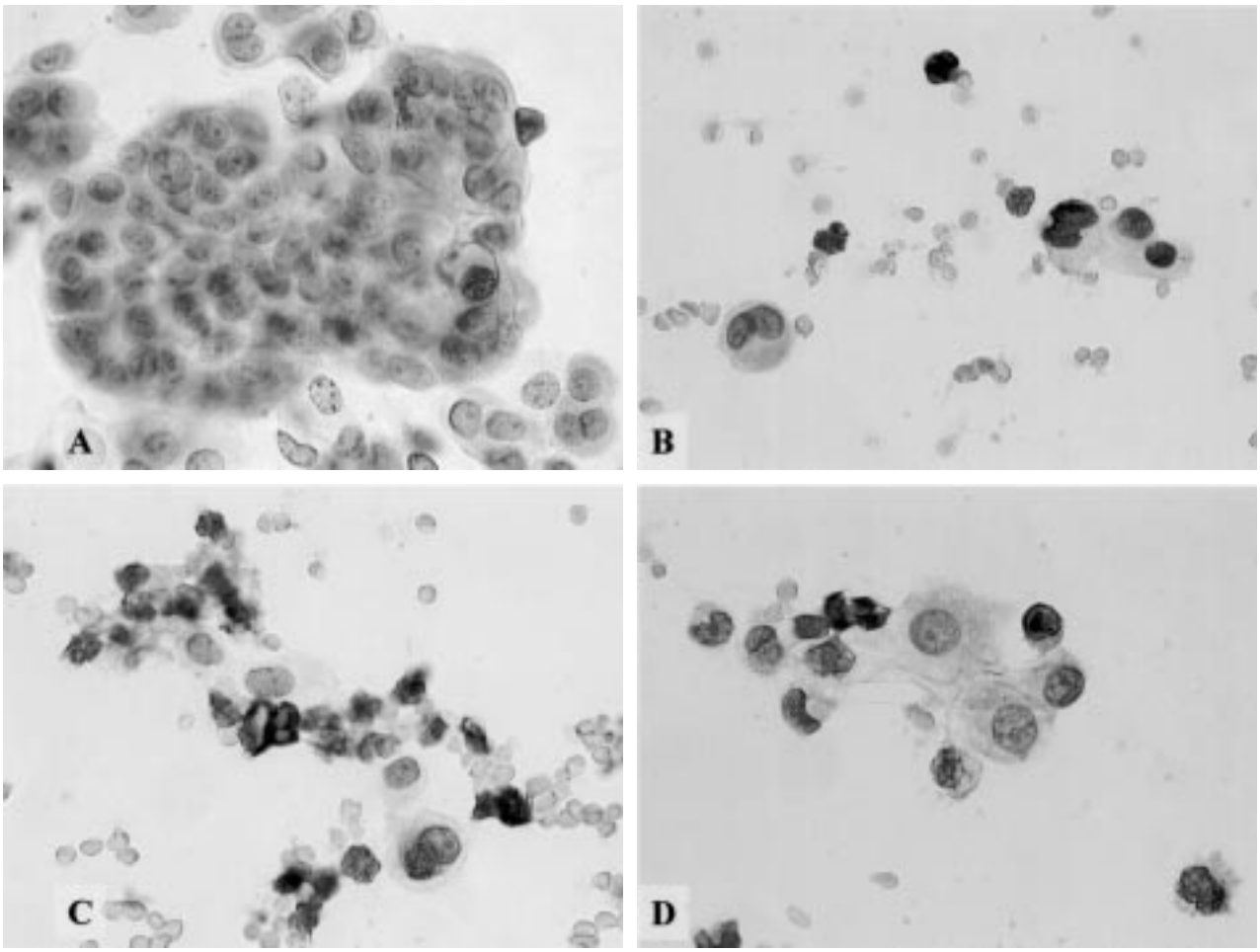

Fig. 1. **A**: *In situ* apoptosis detection using ApopTag™ in specimens obtained pre-IPHC. Most tumor cells that formed clusters were negatively stained (100×). **B–D**: *In situ* apoptosis detection using ApopTag™ in specimens obtained post-IPHC at 0, 24, and 48 h, respectively. Irregularly shaped DNA breakage has occurred, and positively stained nuclei (red brown) of tumor cells indicate apoptosis (100×). IPHC, intrapleural perfusion hyperthermo-chemotherapy.

Survival rate of patients

As shown in Fig. 3, the median survival time (MST) for the 6 MPM patients who received IPHC was 30 months. There was no patient morbidity associated with IPHC treatment.

Discussion

Despite extensive clinical research, no effective therapy for advanced MPM has been established. Without treatment, the MST for MPM patients is <12 months.[6] Although some clinical trials have shown that surgical resection may reduce the symptoms, the MST remains poor at 8–11 months.[2,7] At present, multimodality therapies including surgical resection, chemotherapy, radiotherapy, or a combination of these approaches are being studied.[3] Weder et al.[8] reported on patients with potentially resec-

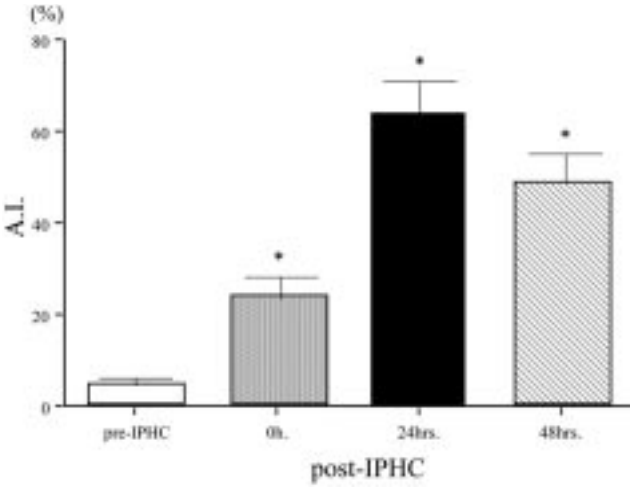

Fig. 2. Differences in the A.I. (mean ± standard error percentage). Asterisk indicates $p < 0.001$ vs. pre-IPHC. A.I., apoptotic index; IPHC, Intrapleural perfusion hyperthermo-chemotherapy.

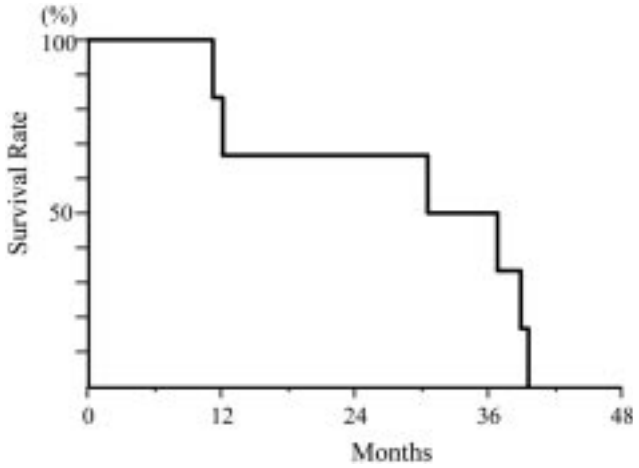

Fig. 3. Survival rate of MPM patients who underwent IPHC (n = 6). Median survival time was 30 months. MPM, malignant pleural mesothelioma; IPHC, intrapleural perfusion hyperthermo-chemotherapy.

Table 1. Characteristics of patients with MPM who underwent IPHC

Case	Age (years)/gender	Stage (IMIG)	Pathology	Adjuvant chemotherapy (cycles)	Prognosis (months)
1	71/male	III (T3N0)	Epithelial	3	39 (dead)
2	71/male	III (T3N0)	Epithelial	0	39 (dead)
3	74/female	III (T3N2)	Epithelial	0	11 (dead)
4	60/male	III (T3N0)	Biphasic	0	30 (dead)
5	65/female	III (T3N0)	Epithelial	7	36 (dead)
6	75/female	IV (T4N2)	Epithelial	7	12 (dead)

MPM, malignant pleural mesothelioma; IPHC, intrapleural perfusion hyperthermo-chemotherapy; IMIG, international mesothelioma interest group; adjuvant chemotherapy, irinotecan + epirubicin/gemcitabine + carboplatinum.

table MPM who had undergone induction chemotherapy using CDDP and gemcitabine followed by extra-pleural pneumonectomy. For these patients, the MST was 23 months. Yoshino et al.[9] investigated select patients with resectable MPM who underwent hemithorax radiotherapy with gemcitabine/vinorelbine/CDDP, achieving an MST of 22 months. Other trials for MPM involve antiangiogenic therapy[10] and photodynamic therapy (PDT).[11,12] The treatment effect of PDT after surgery is superficial, which is similar to IPHC, mostly because of the limited depth of light absorption in tissue surfaces and body cavities after surgical debulking procedures.

Several researchers have investigated the use of hyperthermic techniques in the treatment of mesothelioma. Sugarbaker et al.[13] used hyperthermic intracavitary chemotherapy after cytoreduction to enhance locoregional control for peritoneal mesothelioma. One recent study[14] investigated the feasibility of intraoperative hyperthermic CDDP lavage after pleurectomy/decortication in MPM. This treatment, however, resulted in 18 months of MST with several morbidities. Another study[15] reported that IPHC was applied for stage I MPM after extrapleural pneumonectomy. This seems to be a theoretical trial on the point of view of combined cytoreductive treatment.

Based on our own previous experimental studies on the antitumor effect of regional hyperthermia,[16,17] our surgical department developed a new treatment for patients with malignant pleuritis associated with advanced lung cancer. IPHC, a hyperthermic perfusion technique used in combination with the administration of CDDP, induces a potent tumorcidal effect with demonstrated clinical effectiveness in improving the prognosis for lung cancer patients with malignant pleuritis.[4,5] CDDP, an alkylating antineoplastic agent enters the cell through diffusion and has been shown to cause tumor cell damage through DNA binding. Systemic treatment with CDDP, however, is often limited by adverse side effects that manifest at a higher dose. The combination of hyperthermia and CDDP may act synergistically for two important reasons: the increased temperature facilitates entry of CDDP into the tumor cell, and it also results in an increased metabolic rate, magnifying the effect of CDDP. In an earlier study,[4] we selected regional perfusion as the method of delivery of a lethal dose of CDDP directly to the target tumor, sparing adjacent tissue. Since IPHC delivers constant exposure to both CDDP and hyperthermia, the drug

and thermal dose can be delivered directly to tumors in the pleura. Because MPM disease is also located primarily in the pleura, we hypothesized that IPHC would be indicated in the treatment of pleural tumors associated with MPM disease.

In another study,[5] we used the apoptosis assay, a commercially available and established method, to detect changes in genomic DNA, a hallmark of apoptosis. This technique presented us with a semiquantitative means of measuring the effectiveness of IPHC and confirmed the feasibility of the IPHC technique. In our present study with MPM patients, we achieved a potent regional-hyperthermia induction of apoptosis as measured using the apoptosis assay. The A.I. of 63.8% at 24 h post-IPHC for MPM patients was higher than the 25.2%[5] achieved in our patients with adenocarcinoma, suggesting that MPM could be more sensitive than lung carcinoma to IPHC. Although we could not ascertain the prognostic value of IPHC for MPM patients in this study because of the small number, our patients had an average MST of 30 months, despite the advanced stage of their disease. In conclusion, IPHC induced potent apoptosis of tumor cells in MPM patients, increasing immediately post-IPHC and peaking at 24 h. We hypothesized that increased apoptosis of tumor cells may contribute to a better prognosis for patients with MPM and that IPHC may constitute a multimodality therapy for these patients. The efficacy of this therapy for MPM remains to be confirmed in further studies involving a larger subject population.

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