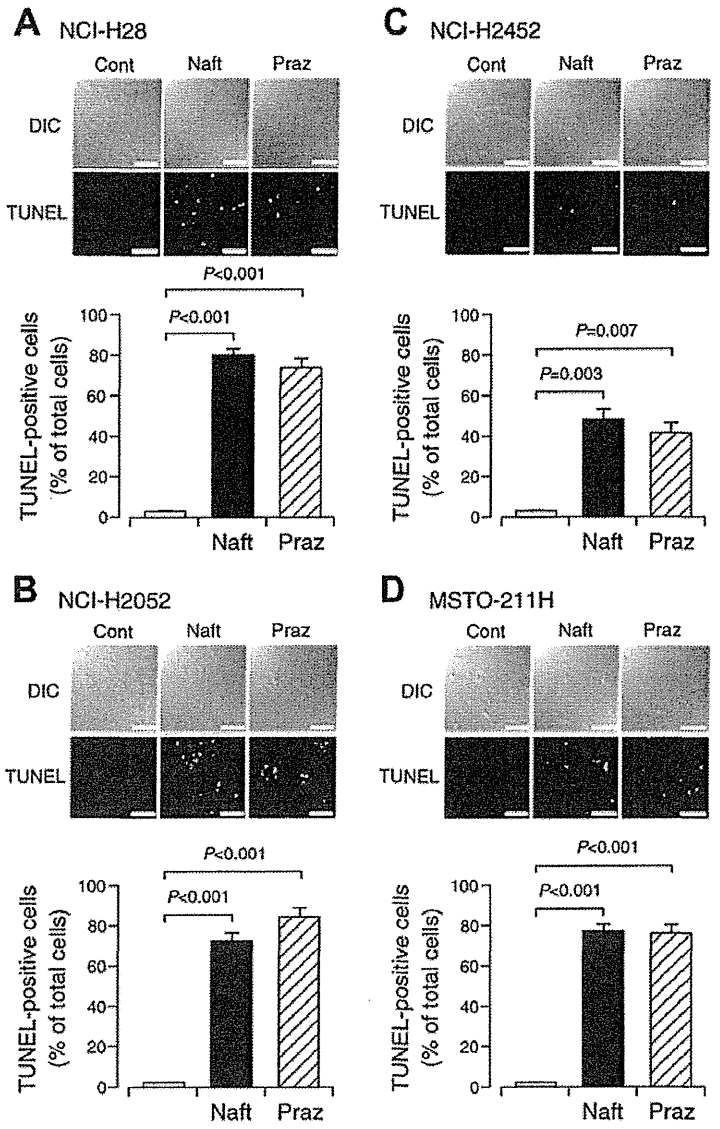

Figure 2. *Terminal deoxynucleotidyl transferase-mediated dUTP nick- end labeling (TUNEL) staining. TUNEL staining was carried out on NCI-H28 (A), NCI-H2052 (B), NCI-H2452 (C), and MSTO-211H cells (D) untreated (Cont) and treated with naftopidil (Naft) (100 μM) or prazosin (Praz) (100 μM) for 12 and 24 h, respectively. DIC, Differential interference contrast. Bars=100 μm. TUNEL-positive cells were counted in an area (0.4 mm × 0.4 mm) selected randomly. In the graphs, each column represents the mean (±SEM) percentage of TUNEL-positive cells relative to total cells (n=4 independent experiments). p-Values were defined from unpaired t-test.*

increased (Figure 5A-D). This indicates that malignant mesothelioma cell apoptosis is not induced by blocking α_{1D}-adrenoceptor; in other words, naftopidil- and prazosin-induced apoptosis of malignant mesothelioma cells is not due to α_1-adrenoceptor blocking action.

Naftopidil activates caspase-3/-8 in malignant mesothelioma cells. For all the malignant mesothelioma cell lines examined here, naftopidil significantly activated caspase-3 and -8, but otherwise did not activate caspase-9 except for NCI-H28 cells (Figure 6A-D). Prazosin also activated caspase-3 and -8 without affecting caspase-9 activity except for MSTO-211H cells (Figure 6E-H). These results imply that naftopidil and prazosin activate caspase-8 and the effector caspase-3, thereby inducing apoptosis of malignant mesothelioma cells. The results also suggest that naftopidil and prazosin could still activate caspase-9 followed by caspase-3 for certain types of malignant mesothelioma cell.

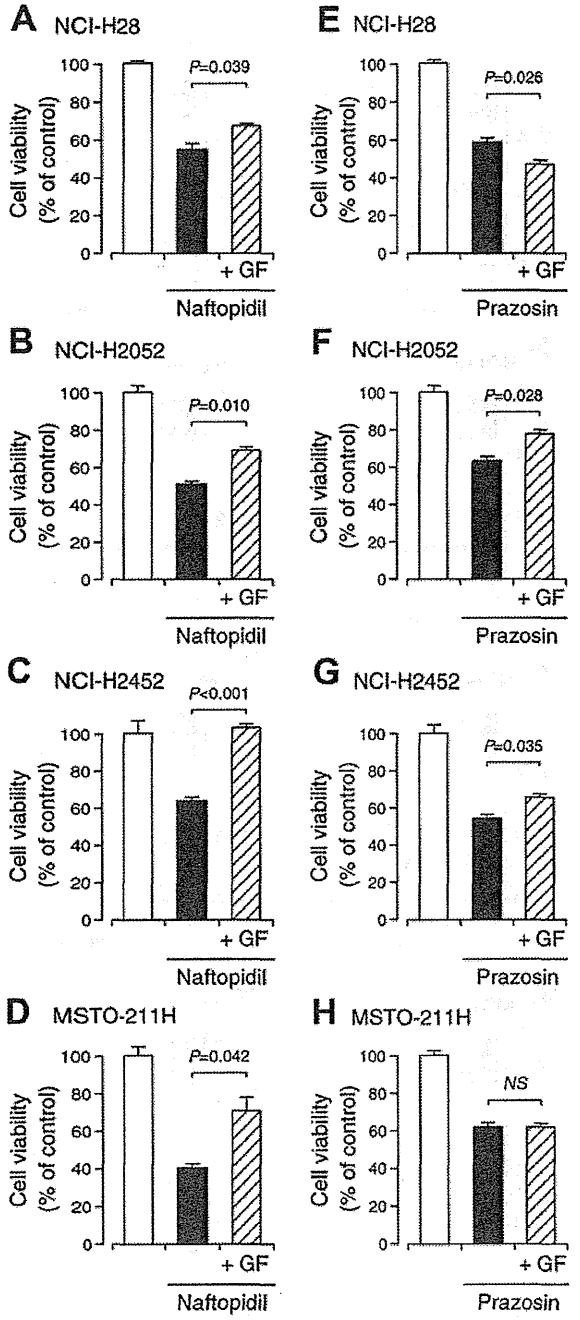

Figure 3. *The effect of protein kinase-C inhibitor GF109203X on cell death induced by naftopidil or prazosin. NCI-H28 (A, E), NCI-H2052 (B, F), NCI-H2452 (C, G), and MSTO-211H cells (D, H) were treated with naftopidil (50 μM) or prazosin (50 μM) for 24 h in the presence and absence of GF109203X (GF) (100 nM), and then 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was carried out. In the graphs, each column represents the mean (±SEM) percentage of control cell viability (MTT intensities of cells untreated with naftopidil or prazosin in the absence of the inhibitor) (n=4 independent experiments). p-Values were defined from unpaired t-test. NS, Not significant.*

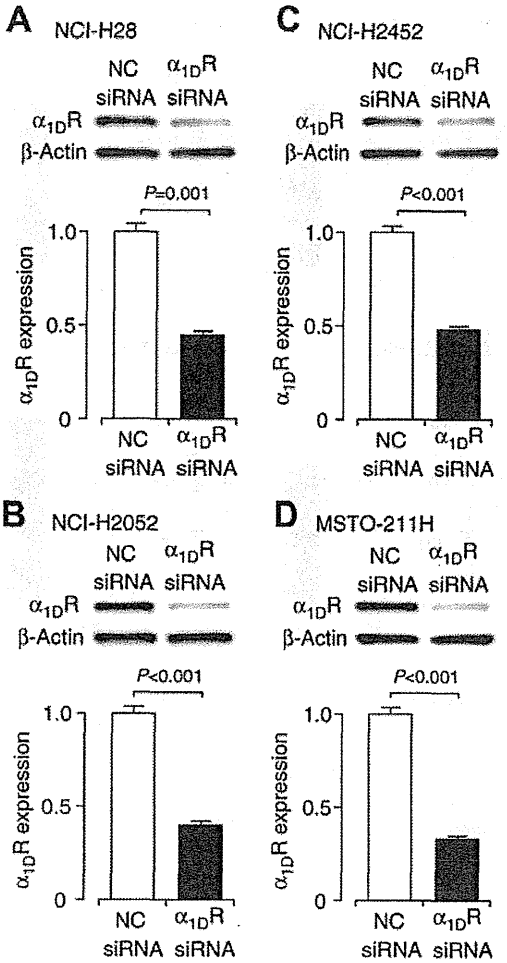

Figure 4. *α_{1D} Adrenoceptor knock-down. Western blotting for NCI-H28 (A), NCI-H2052 (B), NCI-H2452 (C), and MSTO-211H cells (D) transfected with negative control (NC) siRNA or α_{1DR} siRNA 48 h after transfection. Signal intensities for α_{1D}-adrenoceptor protein were normalized by those for β-actin. In the graphs, each column represents the mean (±SEM) α_{1D}-adrenoceptor protein intensity (n=4 independent experiments). p-Values were defined from unpaired t-test.*

Discussion

Naftopidil, an inhibitor of α_{1A}- and α_{1D}-adrenoceptors, has been clinically used for the treatment of benign prostate hyperplasia and hypertension (26). Interestingly, recent evidence has shown that naftopidil exerts an antitumor action on prostate cancer cells (27, 28). In the present study, naftopidil induced apoptosis in the human malignant mesothelioma cell lines NCI-H28 and NCI-H2052 sarcomatoid cells, NCI-H2452 epithelioid cells, and MSTO-211H biphasic cells. A similar effect was obtained with prazosin, another α_1-adrenoceptor blocker. α_1-

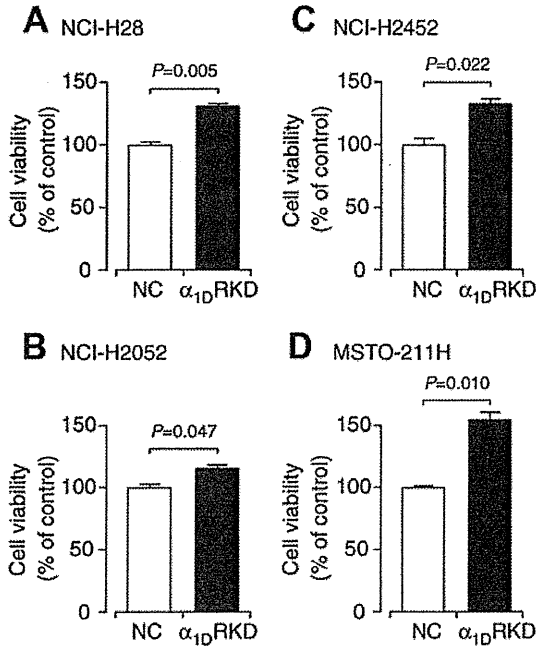

Figure 5. *The effect of α_{1D}-adrenoceptor knock-down on cell viability. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay for NCI-H28 (A), NCI-H2052 (B), NCI-H2452 (C), and MSTO-211H cells (D) transfected with negative control siRNA (NC) or α_{1DR} siRNA (α_{1DR} KD) 48 h after transfection (n=4 independent experiments). In the graphs, each column represents the mean (±SEM) percentage of control cell viability (MTT intensities for cells transfected with NC siRNA at 48 h after transfection)(n=4 independent experiments). p-Values were defined from unpaired t-test.*

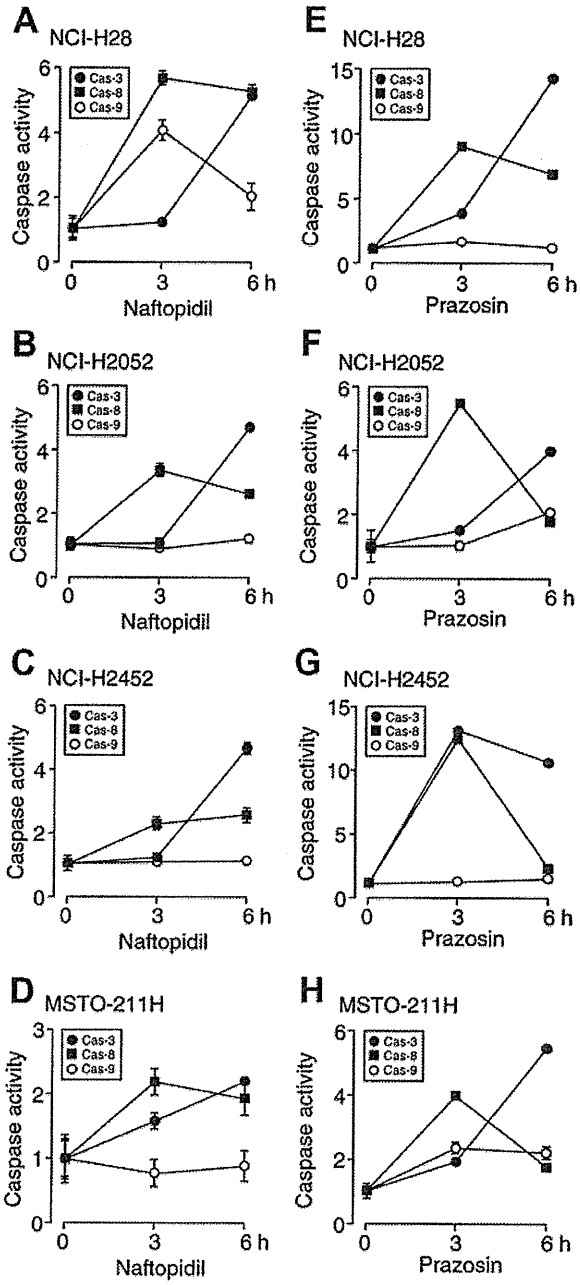

Figure 6. *Activities of caspase-3, -8, and -9. NCI-H28 (A, E), NCI-H2052 (B, F), NCI-H2452 (C, G), and MSTO-211H cells (D, H) were treated with naftopidil (100 μM) or prazosin (100 μM) for 3-6 h, and then activities of caspase-3, -8, and -9 were enzymatically assayed. In the graphs, each point represents the mean (±SEM) ratio to basal caspase activities (before treatment with naftopidil or prazosin) (n=4 independent experiments).*

Adrenoceptor blockers such as naftopidil and prazosin, thus, might exert an antitumor action on malignant mesothelioma cells.

α_1-Adrenoceptor linked to $G_{q/11}$ protein engages PKC activation. Naftopidil and prazosin, therefore, should inhibit PKC following α_1-adrenoceptor blocking. Surprisingly, naftopidil- and prazosin-induced cell death of all the malignant mesothelioma cell lines and some cell lines, respectively, was attenuated by the PKC inhibitor GF109203X. In addition, malignant mesothelioma cell death was not induced by knocking-down the α_{1D}-adrenoceptor; conversely, cell proliferation was promoted. Taken together, these results show that naftopidil and prazosin are likely to induce apoptosis of malignant mesothelioma cells by a mechanism independent of α_1-adrenoceptor blocking. In support of this, α_1-adrenoceptor antagonists have been shown to modulate differentiation and death of human erythroleukemia cells, regardless of α_1-adrenoceptor blocking (32).

Naftopidil and prazosin activated caspase-3 and -8 in all the investigated malignant mesothelioma cell lines. This indicates that they activate caspase-8 and the effector caspase-3 to induce apoptosis of malignant mesothelioma cells. Naftopidil and prazosin also activated caspase-9 for some

malignant mesothelioma cell lines. This suggests that naftopidil and prazosin could still ultimately activate caspase-3. Caspase-8 is recognized as being activated through death receptors such as tumor necrosis factor receptor-1 (TNFR1), FAS/apoptosis antigen-1 (APO1)/CD95, death receptor-3 (DR3)/APO3/WSL-1/lymphocyte-associated receptor of death (LARD)/TRAMP, DR4/TNF-related apoptosis-inducing ligand receptor-1 (TRAIL-R1), DR5/TRAIL-R2/TNF-related apoptosis-inducing ligand receptor inducer of cell killing-2 (TRICK2)/KILLER, and DR6 (33). FAS, activated by FASL, recruits the adaptor protein FAS-associated protein with death domain (FADD) to aggregate procaspase-8, which cleaves to initiate the active form of caspase-8 (34). TNFR1, activated by TNF-α, alternatively, forms a complex of TNFR1-associated death domain (TRADD)/receptor interacting protein-1 (RIP1)/FADD/procaspase-8 to activate caspase-8 (35). In contrast, caspase-9 is activated in concert with mitochondrial damage, allowing cytochrome c efflux from the mitochondria into the cytosol, to form an apoptosome complex with apoptotic protease activating factor 1 (APAF-1) or dATP (36-38). How naftopidil or prazosin activates caspase-8 or caspase-9 in malignant mesothelioma cells remains to be explored. To address this question, we are currently carrying out further experiments.

Conclusion

The results of the present study show that naftopidil and prazosin have the potential to induce apoptosis of malignant mesothelioma cells by activating caspase-8 and the effector caspase-3, regardless of α_1-adrenoceptor blocking. Naftopidil, which has been permitted for clinical use, may have a future role in treatment of human malignant mesothelioma.

Conflicts of Interest

None of the Authors have any potential conflicts of interest.

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A Feasibility Study of Induction Pemetrexed Plus Cisplatin Followed by Pleurectomy/Decortication Aimed at Macroscopic Complete Resection for Malignant Pleural Mesothelioma

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A prospective multi-institutional study has been initiated in Japan to evaluate the feasibility of induction chemotherapy using pemetrexed plus cisplatin, followed by pleurectomy/decortication aimed at macroscopic complete resection in patients with resectable malignant pleural mesothelioma. The study was initiated on September 2012, for which 24 patients will be recruited over a period of 2 years. The primary endpoint is the macroscopic complete resection rate, regardless of the surgical technique employed (i.e. pleurectomy/decortication or extrapleural pneumonectomy). The secondary endpoints are the pleurectomy/decortication rate, macroscopic complete resection rate by pleurectomy/decortication, pulmonary function at 3 months after surgery, adverse events, treatment-related mortality, response rate to chemotherapy and 3-year overall survival rate.

Key words: extrapleural pneumonectomy – induction chemotherapy – malignant pleural mesothelioma – macroscopic complete resection

INTRODUCTION

Malignant pleural mesothelioma (MPM) is an extremely poor-prognosis malignant tumor caused by asbestos exposure. The number of cases of this tumor in Japan is expected to rise in the future (1–3). MPM is very difficult to cure. While extrapleural pneumonectomy (EPP) is performed with radical intent, the outcome is not very good in patients treated with surgery alone (4). The current standard for possible cures for this disease has shifted to a multidisciplinary approach combining induction chemotherapy with cisplatin and pemetrexed followed by EPP and radiation therapy (trimodality therapy).

In recent years, another operative method, known as pleurectomy/decortication (P/D), has come into the spotlight. EPP is a very invasive surgery and shows cardiorespiratory depression and high rates of mortality and complications. P/D is less invasive than EPP. As of yet, it is not apparent which risk-benefit ratio of P/D and EPP is better as a part of multimodality therapy. It has been reported that the survival rate of P/D is higher than or equal to that of EPP (5–8). The possible reasons for this are as follows:

(1) The perioperative mortality rate of P/D is lower than that of EPP.
(2) Patients who had P/D receive better treatment than those who received EPP at the time of recurrence.

Postoperative quality of life is maintained to a larger extent in those patients who have undergone P/D rather than EPP (9). The results of major clinical trials for trimodality therapy, including EPP, have been reported by cancer study groups in North America, the University of Toronto and Europe (10–12). In all clinical trials, only around 50% of patients completed trimodality therapy, thus suggesting that trimodality therapy, including EPP, poses major difficulties even at some of the world's most experienced and top-ranking facilities. In addition, both a high complication rate and a number of treatment-related deaths were reported in a Japanese multi-institutional clinical trial for trimodality therapy conducted in 2008. Considering this, the survival benefits of this therapy reported from clinical trials in Europe and the USA are not high. Therefore, the risk-benefit ratio of this treatment is not satisfiable.

There is no good evidence of multimodality therapy involving P/D. However, the benefit of adding induction chemotherapy to P/D may be speculated in the light of that for EPP (13–15). The study protocol is a clinical trial to evaluate induction chemotherapy with pemetrexed plus cisplatin followed by P/D aimed at macroscopic complete resection (MCR) for resectable MPM (16). The study protocol was approved by the protocol review committee and activated on 12 October 2012. The study has been registered at the UMIN Clinical Trials Registry as UMIN000009092 (http://www.umin.ac.jp/ctr/index.htm).

PROTOCOL DIGEST OF THE STUDY

Purpose

The aim of this study is to evaluate the feasibility of multimodality therapy for resectable MPM, comprised induction chemotherapy using pemetrexed plus cisplatine (PC) followed by P/D aimed at MCR.

Study Setting

This is a multi-institutional, single-arm study.

Study Method

Figure 1 shows a flow chart of the study.

Endpoints

The primary endpoint is MCR rate regardless of the surgical technique employed (i.e. P/D or extrapleural pneumonectomy). MCR is defined as the surgical removal of all gross tumor tissue (16,17). Secondary endpoints are as follows: (i) P/D rate, (ii) MCR rate by P/D, (iii) pulmonary function

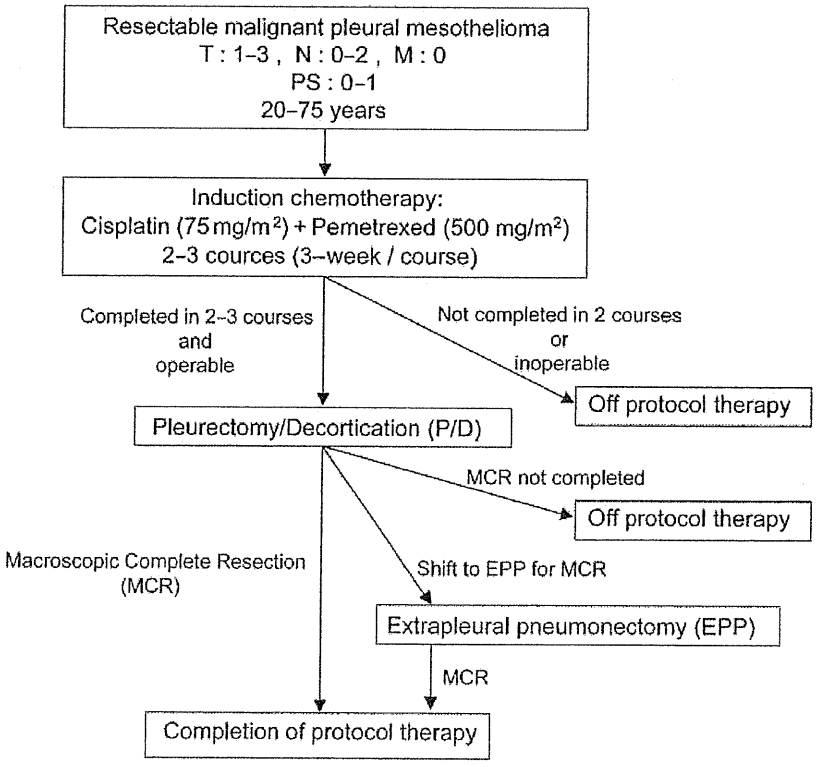

Figure 1. Flow chart of the study.

at 3 months after surgery, (iv) incidence of treatment-related adverse events according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 guidelines (18), (v) treatment-related mortality, (vi) response rate for induction chemotherapy evaluated by a modified version of the Response Evaluation Criteria in Solid Tumors [modified RECIST (19)], (vii) 3-year overall survival rate in all eligible patients with MCR.

Eligibility/Inclusion Criteria

Patients are eligible for the trial if they have a histologically confirmed diagnosis of MPM, including all subtypes and clinical T1–3, N0–2, M0 disease considered to be resectable. Other requirements are as follows: no prior treatment with chemotherapy, surgery or radiation therapy (RT) for the disease; age between 20 and 75 years; Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; a predicted postoperative forced expiratory volume of >1000 ml in 1 s; adequate bone marrow, hepatic, renal, cardiac and respiratory functions; a life expectancy of >12 weeks; and written informed consent.

Exclusion Criteria

Patients are excluded if they meet any of the following criteria: serious systemic complications including poorly controlled diabetes or hypertension, active infectious diseases, interstitial pneumonia or lung fibrosis; simultaneous or metachronous (within 5 years) double cancers; serious drug allergy or hypersensitivity to any drugs; pregnancy or breast-feeding; Grade 2 or greater peripheral neuropathy at registration; or considered as clinically inappropriate for registration.

TREATMENT METHODS

Induction Chemotherapy

Induction chemotherapy consists of three cycles of pemetrexed at 500 mg/m^2 followed by cisplatin 75 mg/m^2 on Day 1, given every 21 days. Folic acid (0.5 mg per daily oral administration) and vitamin B12 (1 mg intramuscularly every 9 weeks) are administered a week before the first dose of chemotherapy and continue to be administered throughout the induction chemotherapy. Dose adjustments of chemotherapy are required for renal and nonhematologic toxicity as well as hematologic effects. Dose delays of up to 42 days are permitted for recovery from drug toxicity. Tumor response is assessed through computed tomography (CT) following the completion of induction chemotherapy using unidimensional measurement of the pleural thickness perpendicular to the chest wall or mediastinum and modified RECIST criteria.

Pleurectomy/Decortication and Extrapleural Pneumonectomy

All patients undergo P/D or EPP within 42 days of the last dose of induction chemotherapy unless there is deterioration of organ functions that would make the surgery intolerable. P/D complies with the definition of the International Association for the Study of Lung Cancer (IASLC) staging committee and the International Mesothelioma Interest Group (IMIG). The above report does not prescribe whether P/D mandates the removal of a part of the pleura without macroscopic disease. Therefore, in this study, it is stipulated that P/D requires mandatory removal of all the parietal pleura and removal of all the area of the visceral pleura with macroscopic disease. If it is necessary to achieve MCR, P/D permits resecting either of the diaphragm, pericardium, chest wall and lung parenchyma. EPP is defined as an en-bloc resection of the entire pleura, lung, ipsilateral diaphragm and pericardium (20). Also, while it is impossible to achieve MCR through P/D, EPP is performed in cases where operators deem that MCR can be achieved through EPP. If lymph node metastasis is confirmed by pathological examination, excision of this is also a prerequisite for MCR. Mediastinal nodal dissection is recommended in all patients having either P/D or EPP.

Study Design and Statistical Methods

The primary analysis of this study was to estimate the MCR rate and 95% confidence interval (CI). If the lower limit of the 95% CI exceeds 0.5, the protocol treatment will be considered feasible. Thus, 24 patients were planned to be enrolled onto this study, with planned accrual of 2 years and follow-up of 3 years after the accrual completion. This sample size was considered sufficient to estimate 95% confidence intervals for the true MCR rate within a width of ± 0.2, when the true MCR rate is expected to be 70%.

Study Monitoring

The Data and Safety Monitoring Committee (DSMC) will make independent recommendations to investigators regarding the continuation, termination or modification of the trial. Protocol compliance, safety and study progress will also be monitored by the DSMC.

Participating Institutions

A total of 24 institutions in Japan with certified specialists in oncology and surgery will participate in this trial.

Funding

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Conflict of interest statement

None declared.

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Prognostic significance of metabolic response by positron emission tomography after neoadjuvant chemotherapy for resectable malignant pleural mesothelioma

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Background: To select optimal candidates for extrapleural pneumonectomy (EPP), we retrospectively evaluated the usefulness of metabolic response by fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT) after neoadjuvant chemotherapy to predict prognosis for patients with resectable malignant pleural mesothelioma (MPM) who underwent EPP in a multicenter study.

Patients and methods: We carried out high-resolution CT (HRCT) and FDG-PET/CT before and after neoadjuvant platinum-based chemotherapy on 50 patients with clinical T1–3 N0-2 M0 MPM who underwent EPP ± postoperative hemithoracic radiotherapy. A decrease of ≥30% in the tumor maximum standardized uptake value (SUV_{max}) was defined as a metabolic responder. The radiologic response using the modified RECIST or metabolic response and surgical results were analyzed.

Results: The median overall survival (OS) from diagnosis was 20.5 months. Metabolic responders significantly correlated to OS with median OS for metabolic responders not reached *versus* 18.7 months for non-responders. No correlation was observed between OS and radiologic response with median OS for radiologic responders and non-responders. Based on the multivariate Cox analyses, decreased SUV_{max} and epithelioid subtype were significantly

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independent factors for OS.

Conclusions: The metabolic response after neoadjuvant chemotherapy is an independent prognostic factor for patients with resectable MPM. Patients with metabolic responder or epithelioid subtype may be good candidates for EPP.

Key words: extrapleural pneumonectomy, malignant pleural mesothelioma, metabolic response, neoadjuvant chemotherapy, positron emission tomography

introduction

Malignant pleural mesothelioma (MPM) is an uncommon tumor characterized by locally aggressive behavior which leads to a fatal prognosis, but its incidence has increased in recent times within industrialized nations including Japan [1–4]. The optimal management of resectable MPM continues to be an ongoing topic of debate with general agreement in regard to poor disease control by any single-modality therapy [5]. The current standard for possible cure of the disease has shifted to a multidisciplinary approach combining extrapleural pneumonectomy (EPP) with chemotherapy and/or radiotherapy. Because of the difficulty in administering adjuvant chemotherapy after EPP, we conducted trimodality therapy with neoadjuvant chemotherapy followed by EPP and hemithoracic radiotherapy [6]. Several studies have been reported on trimodality therapy, such as chemotherapy followed by EPP and radiotherapy for resectable T1–3 N0-2 M0 MPM, in which the median survival from the start of chemotherapy or from registration ranged from 14.0 to 25.5 months [7–11]. The MARS feasibility trial, which failed to meet the primary end-point of assigning 50 patients to EPP or non-EPP after induction chemotherapy within 1 year, suggested that EPP within the trimodality therapy offers no benefit and possibly harms the patients, with median survival from randomization of 14.4 months for the EPP group and 19.5 months for the non-EPP group [12]. Currently, the role of neoadjuvant chemotherapy followed by EPP for resectable MPM is controversial, and we need the selection criteria for those patients most likely to benefit from EPP after neoadjuvant chemotherapy. In unresectable MPM, early response evaluation by 18F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) rather than by computed tomography (CT) is a promising method to predict the patient survival outcome [13]. To our knowledge, there are no data available using FDG-PET/CT in regard to metabolic response after neoadjuvant chemotherapy for resectable MPM. The purpose of this multicenter study was to evaluate the usefulness of metabolic response by FDG-PET/CT after neoadjuvant chemotherapy in predicting prognosis for patients with resectable MPM who underwent EPP.

patients and methods

We enrolled 73 patients with clinical T1–3 N0-2 M0 malignant pleural mesothelioma who were scheduled for multimodality therapy comprising neoadjuvant platinum-based chemotherapy followed by EPP and postoperative hemithoracic radiotherapy. Patients were staged according to the system developed by the International Mesothelioma Interest Group [14] at two institutions (Hyogo College of Medicine and Hiroshima University) between 1 January 2004 and 31 December 2011. Among these patients, we excluded those who had not undergone EPP ($n = 11$) and those for whom the FDG-PET/CT data before or after neoadjuvant chemotherapy were lacking ($n = 12$). Ultimately, 50 patients were enrolled in this retrospective study (Figure 1). We obtained appropriate approval for this multicenter study from the Institutional Review Board of each institution, which waived the requirement for informed consent from individual patients for this retrospective review from a prospective database.

Patients were eligible for trimodality therapy if they had a histologically confirmed diagnosis of MPM, including all subtypes and clinical T1–3 N0-2 M0 disease considered to be completely resectable; no prior treatment with chemotherapy, surgery, or radiotherapy for the disease; Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; a predicted postoperative forced expiratory volume in 1 s of >1000 ml; and adequate bone marrow, hepatic, renal, cardiac and respiratory function. Patients were staged with high-resolution CT (HRCT) scanning of the chest and abdomen, brain magnetic resonance imaging or CT, and FDG-PET/CT.

Neoadjuvant chemotherapy consisted of three or four cycles of cisplatin-based chemotherapy followed by HRCT and FDG-PET/CT restaging. Cisplatin (Nippon Kayaku Co., Ltd., Tokyo, Japan) plus pemetrexed was predominantly used for neoadjuvant chemotherapy. A surgery was carried out 3–6 weeks after the end of chemotherapy.

EPP was defined as an en bloc resection of the lung, pleura, pericardium, and diaphragm without entering the pleural cavity. Partial or no removal of the pericardium or diaphragm was sometimes carried out for a parietal pleural tumor separable from the pericardium or diaphragm. Previous biopsy sites were removed with limited chest wall resection.

Adjuvant hemithoracic radiotherapy was carried out within 12 weeks of surgery. Patients received three-dimensional conformal radiotherapy using a linear accelerator for 6–20 MV photon energies. A total dose of 54 Gy was delivered in 30 fractions of 1.8 Gy/day. The target volume included the hemithorax and chest wall incisions.

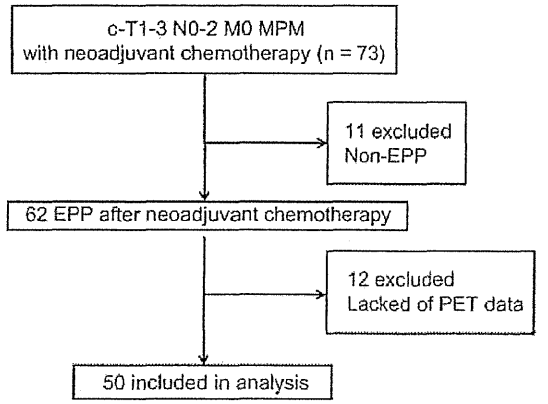

Figure 1. Flow chart of patients in the study.

Postoperatively, all patients underwent a physical examination every 3 months, and CT of the chest and abdomen every 6 months.

HRCT

HRCT was carried out at baseline before neoadjuvant chemotherapy, and repeated after neoadjuvant chemotherapy. Chest images were obtained using 16-row multidetector CT independent of subsequent FDG-PET/CT examinations. High-resolution images of tumors were acquired using the following parameters: 120 kVp, 200 mA, section thickness 1–2 mm, pixel resolution 512 × 512, scanning time 0.5–1.0 s, and a high spatial reconstruction algorithm with a 20 cm field of view and mediastinal (level, 40 HU; width, 400 HU) window setting.

FDG-PET/CT

FDG-PET/CT imaging was carried out at baseline before neoadjuvant chemotherapy, and repeated after neoadjuvant chemotherapy. Patients fasted for at least 4 h before being intravenously injected with 3.7 MBq/kg of FDG, and they then rested for about 1 h before being scanned. Blood glucose was measured before tracer injection to ensure a level of <150 mg/dl, and patients with blood glucose ≥150 mg/dl during FDG-PET/CT image acquisition were excluded. All the patients were assessed using an integrated FDG-PET/CT scanner, either Discovery ST16 (GE Healthcare) or GEMINI GXL (Philips Medical Systems). An unenhanced CT image of a 2–4 mm thick section that matched the PET images was obtained from the head to the pelvic floor of each patient using a standard protocol. Immediately after CT, PET covered the identical axial field of view (2–4 min per table position depending on the condition of the patient and scanner performance). Both PET and CT studies proceeded with normal tidal breathing. All PET images were reconstructed using an iterative algorithm with CT-derived attenuation correction using Fourier rebinning followed by ordered-subset expectation maximization. A maximum standardized uptake value (SUV_{max}) was established by drawing regions of interest (ROI) around the primary tumor on attenuation-corrected FDG-PET images and calculated using the dedicated software of the PET/CT scanner based on the following formula: $SUV_{max} = [C(\mu C_i/ml)/ID(\mu C_i)]/w$, where C is defined as activity at a pixel within the tissue identified by ROI and ID is defined as the injected dose/kg body weight (w). We adopted SUV_{max} in the present analysis because it is less variable than the mean SUV in terms of measurements [15].

response evaluation

Radiologic response was assessed by HRCT after completion of neoadjuvant chemotherapy using unidimensional measurement of pleural thickness perpendicular to the chest wall or mediastinum and modified RECIST criteria [16].

The metabolic response on FDG-PET/CT was measured after completion of neoadjuvant chemotherapy adjusted to modified RESICT criteria [16]. Complete resolution of FDG uptake within the tumor volume so that it was indistinguishable from the surrounding normal tissue was considered as a complete response (CR). A partial response (PR) was defined as a reduction of ≥30% in tumor FDG uptake. An increase in tumor SUV_{max} of ≥20% within the ROI defined on the baseline scan, or the appearance of new FDG uptake in another region, was classified as progressive disease (PD). Stable disease (SD) was classified as an increase in tumor SUV_{max} of <20% or a decrease of <30%.

statistical analysis

Data are presented as numbers (%) or mean ± standard deviation unless otherwise stated. Overall survival (OS) was calculated as the time (in days) from diagnostic biopsy until death from any cause; patients who were alive on the date of the most recent follow-up were censored on that date. The duration of OS was analyzed using the Kaplan–Meier method. The differences in OS were assessed using the log-rank test. To assess the potential independent effects of decreased SUV_{max} on OS, we carried out multivariate analyses using the Cox proportional hazards model using variables with a P value of <0.1 in the univariate analyses; $P < 0.05$ was considered statistically significant. Data were statistically analyzed using SPSS software (version 10.5, SPSS Inc., Chicago, IL).

Table 1. Patient characteristics

All cohort patients ($n = 50$)	
Age (year)	61.6 ± 7.6
Sex	
Male	45 (90%)
Female	5 (10%)
Side	
Left	26 (52%)
Right	24 (48%)
Clinical stage	
I	17 (34%)
II	16 (32%)
III	17 (34%)
Histology	
Epithelioid	38 (76%)
Biphasic	10 (20%)
Sarcomatoid	2 (4%)

results

The characteristics of the 50 study patients are summarized in Table 1. The mean follow-up period after diagnosis was 18.6 ± 13.5 months. The chemotherapy regimen was cisplatin plus pemetrexed in 47 (94%) patients and cisplatin, CPT-11, and doxorubicin (adriamycin) (Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan) in 3 (6%) patients. Patients received three to four cycles of neoadjuvant chemotherapy (3.3 ± 1.1 cycles).

According to modified RECIST criteria, 20 (40%) patients had an objective response [1 CR and 19 PR], 28 (56%) were classified as SD and 2 (4%) had PD (Table 2).

FDG-PET/CT measurement identified 14 (28%) responders (1 CR and 13 PR), 20 (40%) patients with SD, and 16 (32%) patients with PD.

Table 2. Discrepancies between the radiologic and metabolic responses

	Metabolic response				Total
	CR	PR	SD	PD	
Radiologic response					
CR		1			1 (2%)
PR	1	9	4	5	19 (38%)
SD		3	16	9	28 (56%)
PD				2	2 (4%)
Total	1 (2%)	13 (26%)	20 (40%)	16 (32%)	50

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

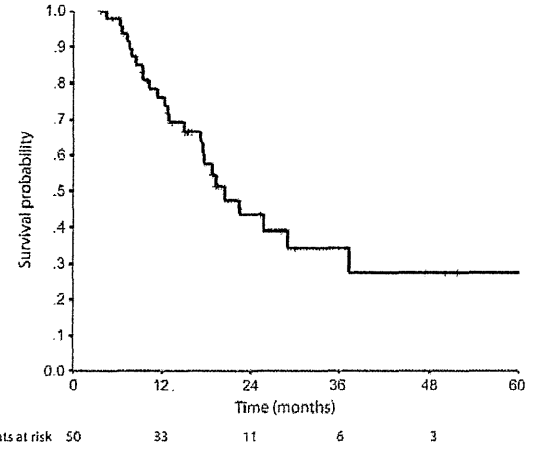

Figure 2. Cumulative overall survival (OS) of all 50 patients (median, 20.5 months; 95% CI, 15.0–26.0 months).

All 50 patients underwent EPP. A complete macroscopic resection of the tumor (R0 or R1 resection) was achieved in 48 (96%) patients. Two (4%) patients had gross residual disease (R2 resection) remaining on the chest wall. One patient died after surgery (aortic hemorrhage), with a postoperative 30-day mortality rate of 2.0%. Major complications occurred in 16 (32%) patients including diaphragmatic hernia ($n = 4$), bronchopleural fistula ($n = 3$), intrathoracic hemorrhage ($n = 2$), empyema ($n = 2$), respiratory failure ($n = 2$), chylothorax ($n = 1$), cardiac hernia ($n = 1$), and heart failure ($n = 1$).

A total of 29 (58%) patients completed adjuvant hemithoracic radiation after EPP.

The median OS for all 50 patients was 20.5 months [95% confidence interval (CI), 15.0–26.0 months], with a 3-year OS rate of 34.2% from diagnosis (Figure 2).

No correlation was observed between OS and radiologic response with median OS for radiologic responders of 25.7 months ($n = 20$; 95% CI, 14.5–37.0 months; 3-year OS rate,

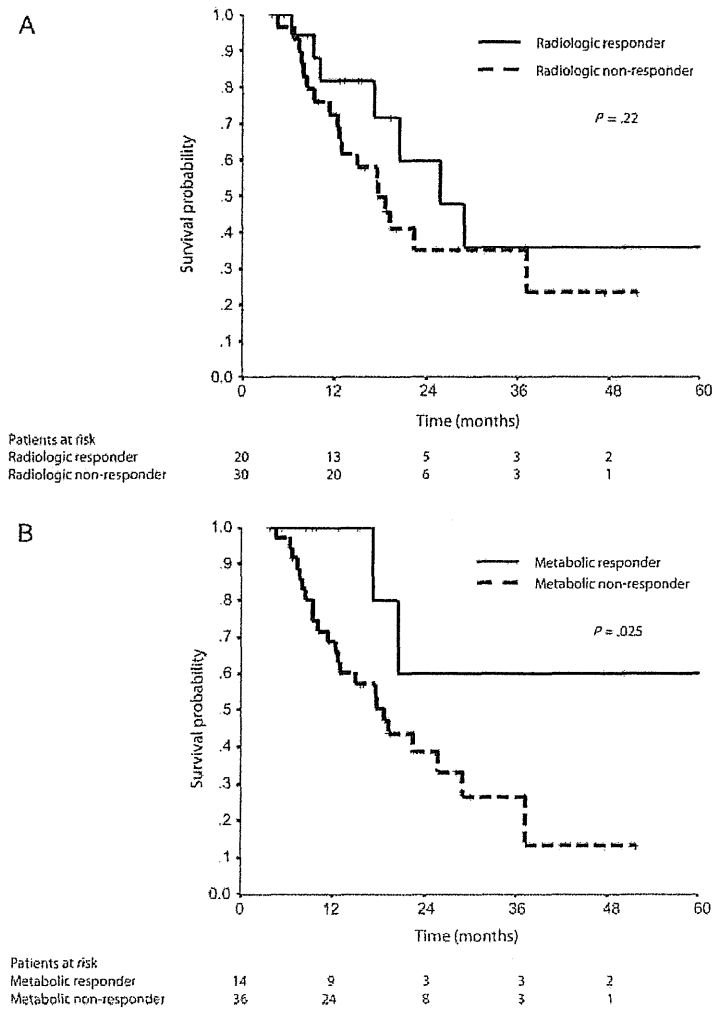

Figure 3. Overall survival (OS) by (A) radiologic response and (B) metabolic response. No significant difference was observed between the radiologic responders and the non-responders ($P = 0.22$); a significant difference was observed between the metabolic responders and the non-responders ($P = 0.025$).

Table 3. Univariate and multivariate analyses of overall survival (OS)

	HR	95% CI	*P* value
Univariate analyses			
Age	1.05	0.98–1.12	0.13
Sex: male	0.74	0.22–2.49	0.63
Side: right	0.71	0.31–1.61	0.41
Histology: epithelioid	0.43	0.17–1.11	0.08
c-Stage: III	1.53	0.67–3.51	0.31
Tumor shrinkage (ratio)	0.34	0.98–1.20	0.09
Decrease of SUV_{max} (ratio)	0.82	0.70–0.97	0.02
Multivariate analyses			
Model 1			
Histology: epithelioid	0.39	0.12–1.22	0.11
Tumor shrinkage (ratio)	0.46	0.14–1.54	0.21
Model 2			
Histology: epithelioid	0.36	0.14–0.97	0.04
Decrease of SUV_{max} (ratio)	0.80	0.67–0.95	0.01

SUV_{max}, maximum standardized uptake value; HR, hazard ratio; CI, confidence interval.

35.8%) versus 17.7 months ($n = 30$; 95% CI, 12.8–22.6 months; 3-year OS rate, 35.1%) for non-responders ($P = 0.22$, Figure 3A). In contrast, metabolic responders significantly correlated to OS with median OS for metabolic responders not reached ($n = 14$; 3-year OS rate, 60.0%) versus 18.7 months ($n = 36$; 95% CI, 13.3–24.2 months; 3-year OS rate, 26.5%) for non-responders ($P = 0.025$, Figure 3B). The OS of patients with epithelioid subtype ($n = 38$; median OS, 22.4 months; 95% CI, 13.7–31.1 months; 3-year OS rate, 39.4%) was better than for those with non-epithelioid subtype ($n = 12$; median OS, 14.9 months; 95% CI, 9.4–20.4 months; 3-year OS rate, 0%, $P = 0.072$).

Univariate analysis of the OS in all patients included the variables such as age, sex, location (side), histology, clinical stage, tumor shrinkage by HRCT, and SUV_{max} decrease by FDG-PET/CT (Table 3). Epithelioid histology, tumor shrinkage, and SUV_{max} decrease were potentially associated with a long OS ($P < 0.1$). Multivariate analysis that included histology and tumor shrinkage (model 1), or histology and SUV decrease (model 2) showed that epithelioid histology [hazard ratio (HR) 0.36; 95% CI, 0.14–0.97; $P = 0.04$] and SUV_{max} decrease (HR 0.80; 95% CI, 0.67–0.95; $P = 0.01$) were independent prognostic factors for OS (Table 3).

discussion

The median OS from diagnosis for all cohort patients in this study was 20.5 months, which is similar to the reported range of 18.0–27.5 months for subsets of patients undergoing EPP after neoadjuvant chemotherapy [7–12]. In the MARS trial, the median OS of the non-EPP arm from the start of treatment was about 23.1 months [12]. The median OS of EPP patients for unselected resectable MPM is not always better than that of non-EPP patients. In a systematic review of EPP outcomes, the 30-day mortality rates ranged from 0% to 11.8%, and the perioperative morbidity rates ranged from 22% to 82% [17]. Although the 30-day mortality and morbidity rates were both relatively low (2% and 32%, respectively) in the current study, the median OS was not satisfactory. To avoid futile surgery with high mortality and morbidity, we need the criteria for selection of patients with potentially resectable MPM who may benefit from EPP.

The current study demonstrated that decreased SUV_{max} as evaluated by FDG-PET/CT after neoadjuvant chemotherapy is an independent prognostic factor for patients with resectable MPM who underwent EPP. In patients with MPM who underwent EPP, decrease in SUV_{max} was more useful in predicting prognosis than tumor shrinkage on HRCT by modified RECIST. Radiographically, MPM is difficult to evaluate, given the nonradical and inconsistent pattern of growth and response to treatment. Although the RECIST criteria have been developed and have become widely accepted and used in clinical trials, the application of these criteria in MPM could be variably interpreted by different investigators, and this may lead to unsatisfactory results [18]. Modified RECIST has now been developed to avoid difficult and ambiguous situations concerning the interpretation in clinical trials, and this successfully distinguished between the responders and the non-responders in regard to survival parameters [16]. However, measuring the tumor thickness of resectable MPM is sometimes more difficult than that of unresectable bulky MPM because the thickness of resectable MPM tumors is often subcentimeter. We failed to distinguish between the responders and the non-responders in regard to OS using the modified RECIST in this study. In unresectable MPM, it was reported that a significant correlation between the metabolic response as evaluated by PET assessment after two cycles of pemetrexed-based chemotherapy and the patient outcome as measured by time to progression (TTP) was observed, whereas the radiologic response assessed by CT scanning was not predictive of improved TTP [13]. The findings of our study in regard to resectable MPM are consistent with those of the above study on unresectable MPM [13]. This is the first paper to describe the significance of metabolic response for resectable MPM using FDG-PET/CT.

The cut-off value of SUV_{max} decrease in defining the metabolic response is important in clinical practice. Although a 20%–25% decrease in tumor FDG uptake is a widely accepted definition in patients undergoing palliative chemotherapy, higher threshold values seem more appropriate for neoadjuvant therapy [19]. Therefore, at the cut-off value of 30% for metabolic response used in this study, there was a significant difference in OS between the metabolic responders and the non-responders. Regardless of the cut-off value, SUV_{max} decrease as a continuous variable was an independent prognostic factor for OS, which indicates the importance of metabolic response in predicting the survival in patients with resectable MPM who underwent EPP. Considering the satisfactory 3-year OS rate of 60% for metabolic responders, a cut-off value of 30% seems to be suitable to define metabolic responders who may benefit from EPP.

There were some discrepancies between the radiologic response and the metabolic response. Nine (18%) radiologic responders were defined as metabolic non-responders, whereas three (6%) radiologic non-responders were defined as

metabolic responders. Overall, 14 (28%) metabolic responders may have been good candidates for EPP.

Epithelioid subtype is well known as a prognostic factor in patients with MPM [20–22]. In our analysis, it was an independent factor for OS, and patients with this subtype had a better prognosis than those with the non-epithelioid subtype.

The limitations of our study include the relatively small number of patients, the retrospective nature of the study, and no comparison with pleurectomy/decortication (P/D) or nonsurgical patients. One of the controversies surrounding the treatment of resectable MPM is whether EPP is more effective than less extensive operations such as P/D [20, 23]. The survival of metabolic responders who underwent P/D or who did not undergo surgery is unknown in our study. Randomization to surgery versus no surgery after induction chemotherapy is very difficult in patients with resectable MPM [12].

One of the limitations of this multicenter study in regard to the use of PET is the wide variation in SUV among institutions. Many factors such as preparation procedures, scan acquisition, image reconstruction, and data analysis can affect the SUV. Using the metabolic response, which is the ratio of change in SUV_{max}, one can minimize the effect of SUV_{max} discrepancies among institutions.

In conclusion, the epithelioid subtype and metabolic response after neoadjuvant chemotherapy are both independent prognostic factors for patients with resectable MPM who underwent EPP. Patients with a metabolic response or epithelioid subtype may be suitable candidates for EPP after neoadjuvant chemotherapy. Further confirmation in a large cohort is required.

disclosure

The authors have declared no conflicts of interest.

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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

Circulating Tumor Cells (CTCs) in Malignant Pleural Mesothelioma (MPM)

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ABSTRACT

Purpose. To investigate the diagnostic and prognostic value of circulating tumor cells (CTCs), a potential surrogate of micrometastasis, in malignant pleural mesothelioma (MPM).

Methods. We prospectively evaluated CTCs in 7.5 mL of peripheral blood sampled from patients with a suspicion of MPM. A semiautomated system was used to capture CTCs with an antibody against the epithelial cell adhesion molecule.

Results. Of 136 eligible patients, 32 were finally diagnosed with nonmalignant diseases (NM), and 104 had MPM. CTCs were detected in 32.7 % (34 of 104) of MPM patients but in only 9.4 % (3 of 32) of NM patients ($P = 0.011$). The CTC count was significantly higher in MPM patients than in NM patients ($P = 0.007$), and a receiver operating characteristic (ROC) curve analysis showed an insufficient capability of the CTC test in discrimination between MPM and NM, with an area under ROC curve of 0.623 (95 % confidence interval, 0.523–0.723; $P = 0.036$). Among MPM patients, CTCs were more frequently detected in patients with epithelioid subtype (39.7 %, 31 of 78) than in those with nonepithelioid subtypes (11.5 %, 3 of 26; $P = 0.016$). Positive CTCs (CTC count ≥ 1) were a significant factor to predict a poor prognosis among epithelioid patients (median overall survival, 22.3 months for positive CTCs vs. 12.6 months for negative CTCs; $P = 0.004$) and not in nonepithelioid patients ($P = 0.649$). A multivariate analysis showed that positive CTCs were a significant and independent factor to predict a poor prognosis (hazard ratio, 2.904; 95 % confidence interval, 1.530–5.511; $P = 0.001$) for epithelioid MPM patients.

Conclusions. CTC was a promising marker in diagnosis and prediction of prognosis in MPM, especially in epithelioid MPM.

Malignant pleural mesothelioma (MPM) is a highly aggressive malignant tumor of the pleura associated with asbestos exposure.[1–3] The gold standard for the diagnosis is histologic examination, which usually needs invasive procedures such as core-needle biopsy or video-assisted thoracoscopic biopsy.[3,4] Such invasive procedures are not feasible for mass screening for an asbestos-exposed high-risk population or may not be performed for patients with poor performance status (PS). Accordingly, it is clinically important to develop and establish noninvasive diagnostic procedures to accurately predict and/or exclude the diagnosis of MPM. A number of noninvasive markers have emerged and have been evaluated, in accordance with recent advances in the understanding of molecular and

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biological characteristics of MPM.[5,6] Among them, serum soluble mesothelin-related protein is the most promising diagnostic marker in discrimination of MPM from nonmalignant (NM) diseases or from other malignant diseases.[5] However, the use of soluble mesothelin-related protein in daily clinical practice is not recommended, because no prospective validation study has confirmed the diagnostic performance.[4,5,7] MPM is a uniformly fatal disease with a median survival time of 4–12 months, because there is no established treatment modality for the cure.[2,8–10] In addition to the lack of effective treatment options, a lack of useful clinical indicators predicting prognosis and/or response to treatment may contribute to the poor prognosis.[5]

Circulating tumor cells (CTCs) are tumor cells that have shed from a primary tumor and circulate in the peripheral blood. Recent experimental and clinical studies have shown that CTCs can be detected not only in late-stage malignant tumors with apparent distant metastases, but also in early-stage diseases, and that the CTC test can be a potentially useful clinical marker in the diagnosis of and decision-making for malignant tumors.[11] The CellSearch system (Veridex LLC, Raritan, NJ, USA), a semiautomated system for quantitative evaluation of CTCs, has been recently developed. In it, CTCs are immunomagnetically captured with an antibody against the epithelial cell adhesion molecule (EpCAM).[12] The most important advantage of the CellSearch system is reproducibility across different laboratories, which is validated by a prospective multicenter study in metastatic breast cancer.[13] On the basis of accumulating data supporting the accuracy and precision in evaluating CTCs, the CTC test using the CellSearch system has been approved in the United States by the Food and Drug Administration for monitoring blood from metastatic breast and colon cancer patients.[14–16] In addition, several clinical studies have shown that the CTC test is potentially useful in the diagnosis and therapy of other malignant tumors, such as prostate cancer.[17–20] In MPM, however, no previous study has been reported about the incidence of CTCs, and its clinical significance remains unknown. Thus, in the present study, we prospectively examined the diagnostic performance and prognostic value of the CTC test in MPM.

METHODS

Study Design

Patients who presented at the Hyogo College of Medicine Hospital to receive a pleural biopsy with a suspicion of MPM on computed tomography (CT) and positron emission tomography scanning were eligible. All patients provided written informed consent before enrollment.

A 7.5-mL peripheral blood sample from each patient was used for the CTC test. Complete clinical data including history, physical examination, and laboratory and radiographic studies were also collected. For all patients, pleural biopsy was performed, and a final pathologic diagnosis was established. For patients with MPM, whole-body CT as well as brain CT or magnetic resonance imaging were routinely conducted to evaluate tumor progression. Clinical (c-) stage was determined according to the current tumor-node-metastasis (TNM) classification as determined by the International Mesothelioma Interest Group.[21] This study was approved by the Institutional Review Board of Hyogo College of Medicine.

Evaluation of CTCs

Blood samples drawn into the CellSave tube containing cell preservatives (Veridex LLC, Raritan, NJ, USA) were maintained at room temperature and were processed within 72 h after collection. CTCs were isolated from peripheral blood by using the CellSearch system (Veridex LLC), and the number of CTCs was determined according to the manufacturer's protocol.[12] In brief, epithelial cells that were captured using ferroparticles coupled to an anti-Ep-CAM antibody were separated in a magnetic field, and the enriched samples were then stained with 4′,6-diamidino-2-phenylindole (DAPI) and anti-cytokeratin–phycoerythrin. Contaminated white blood cells were excluded by negative selection for CD45. Stained cells were then analyzed on a florescent microscope by using the Cell Track Analyzer II (Veridex LLC). The criteria for each cell to be defined as a CTC were as follows: round to oval morphology, a visible DAPI-positive nucleus, positive cytokeratin staining in the cytoplasm, and negative staining for CD45. All evaluations were performed by two authors (K.Y. and F.T.; both completed the Cell Interpretation Proficiency Assessment managed by the Veridex LLC for identification of CTCs) independently without knowledge of the clinical characteristics of patients.

Statistics

Counts were compared by the Chi-square test. Continuous data were compared by using Student's t test if the distribution of samples was normal or by using nonparametric tests (Mann–Whitney U-test for comparison between 2 groups and Kruskal–Wallis test for comparison among 3 or more groups) if the sample distribution was asymmetrical.

The diagnostic performance of CTCs was assessed by constructing a receiver operating characteristic (ROC) curve and was evaluated by calculating the area under each ROC curve (AUC-ROC).[22] An AUC-ROC = 1 denotes

perfect discrimination of a test, whereas an AUC-ROC equal to 0.5 denotes complete lack of discrimination of a test. *P* values were calculated for the difference between each AUC-ROC and 0.5 (completely useless test).

Survival curves were generated by using the Kaplan–Meier method, and the differences were assessed by the log-rank test. Cox's regression model was used for a multivariate analysis of prognostic factors.

For each test, two-sided *P* values less than 0.05 were considered statistically significant. All statistical manipulations were performed by using the SPSS for Windows software system (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient Characteristics

From September 2007 to July 2010, 139 consecutive patients were enrolled in the study. Pleural biopsy was performed and final pathologic diagnosis was established in all patients; 3 patients were excluded because the biopsy specimen showed pleural disseminated adenocarcinoma of the lung. Of 136 eligible patients, 32 were finally diagnosed pathologically with NM pleural diseases, and the other 104 had MPM. Most MPM patients presented with advanced disease, and most had the epithelioid subtype (Fig. 1; Table 1 in Appendix).

The incidence of patients with good PS (Eastern Cooperative Oncology Group 0 or 1) was significantly higher in NM disease; there was no difference in any other patient characteristic. Most patients had a history of asbestos exposure (Table 1 in Appendix).

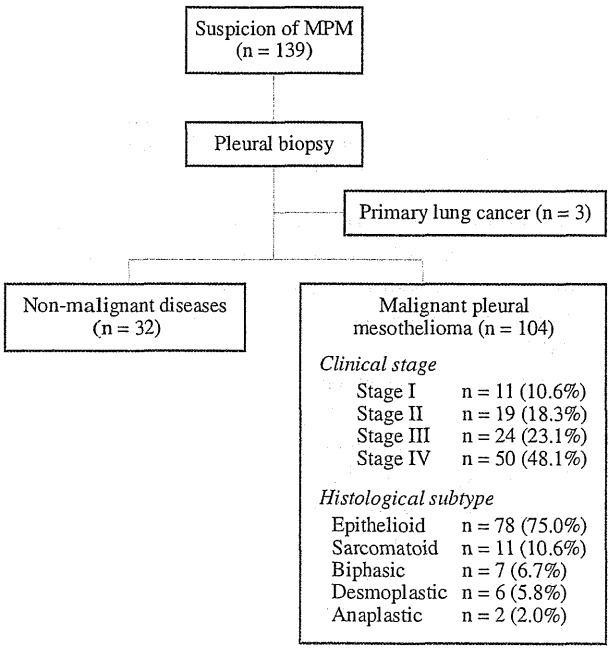

FIG. 1 Flowchart of diagnosis of patients enrolled in the study from September 2007 through July 2010

Chemotherapy using pemetrexed with or without platinum agents (cisplatin or carboplatin) was performed in 80 (76.9 %) of 104 MPM patients; after chemotherapy, extrapleural pneumonectomy was performed in only 6 patients (6.7 %).

CTCs in MPM and NM Patients

CTCs were identified in the peripheral blood of 3 (9.4 %) NM patients, and the CTC count was 1 in all 3 patients. CTCs were identified in the peripheral blood of 34 (32.7 %) patients with MPM, and MPM patients had a significantly higher CTC count than patients with NM (Fig. 2a).

The AUC-ROC for CTC count for discrimination between primary MPM and NM patients was 0.623, and the difference from 0.5 reached statistical significance ($P = 0.036$; Fig. 2b). When a cut-off point for the diagnosis of MPM was 1, that is, patients with 1 or more CTCs were judged as MPM patients, the sensitivity and specificity were 32.7 and 90.6 %, respectively.

CTCs in MPM Patients

CTCs were more frequently detected and the CTC count was also higher in patients with epithelioid-subtype MPM (Fig. 3a), as well as in advanced-stage patients (Fig. 3b). Univariate analyses revealed that poor PS, nonepithelioid histology, advanced clinical stage, and no chemotherapy were significantly associated with a poor prognosis (Table 2 in Appendix).

CTC-negative patients seemed to have a better overall survival than CTC-positive patients, but the difference did not reach statistical significance (median overall survival, 12.7 and 7.6 months, respectively; $P = 0.160$; Fig. 4a). However, when analyzed only in epithelioid patients, the CTC test provided a statistically significant prognostic value (median overall survival, 22.3 and 12.6 months, respectively; $P = 0.004$; Fig. 4b), and a multivariate analysis also indicated that positive CTCs were a significant and independent factor to predict a poor prognosis (hazard ratio, 2.904; 95 % confidence interval, 1.530–5.511; $P = 0.001$; Table 4 in Appendix).

DISCUSSION

The present study is the first clinical study on CTCs in MPM. First, we assessed the diagnostic value of the CTC test in patients with a suspicion of having MPM and

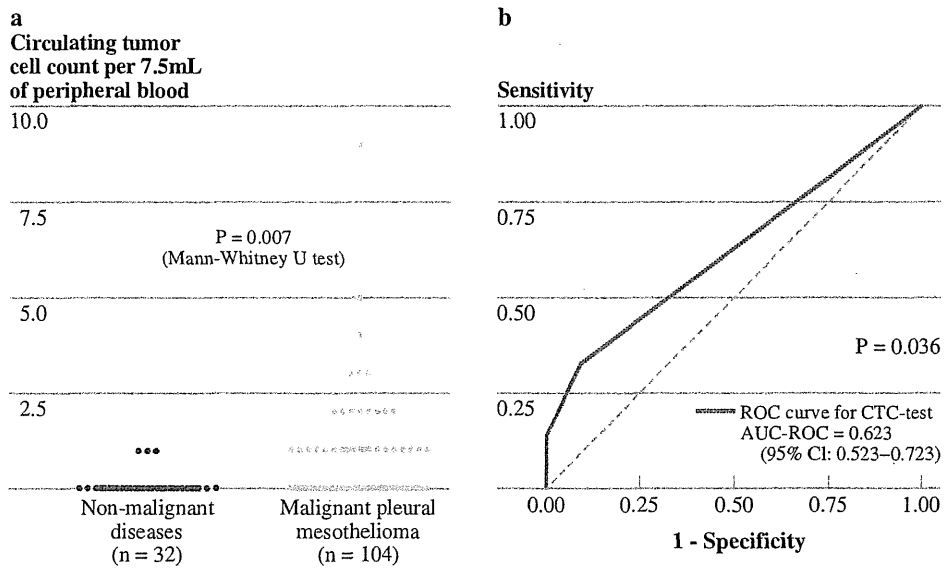

FIG. 2 **a** Distribution of CTC count in patients with NM diseases and in patients with MPM, **b** ROC curves for the CTC test to discriminate malignant pleural mesothelioma from nonmalignant diseases. The AUC-ROC is calculated, and the *P* value for testing the significance of difference from 0.5 (lack of discrimination) is indicated. The sensitivity and specificity of the CTC test at the cut-off value of CTC = 1 were 32.7 and 90.6 %, respectively

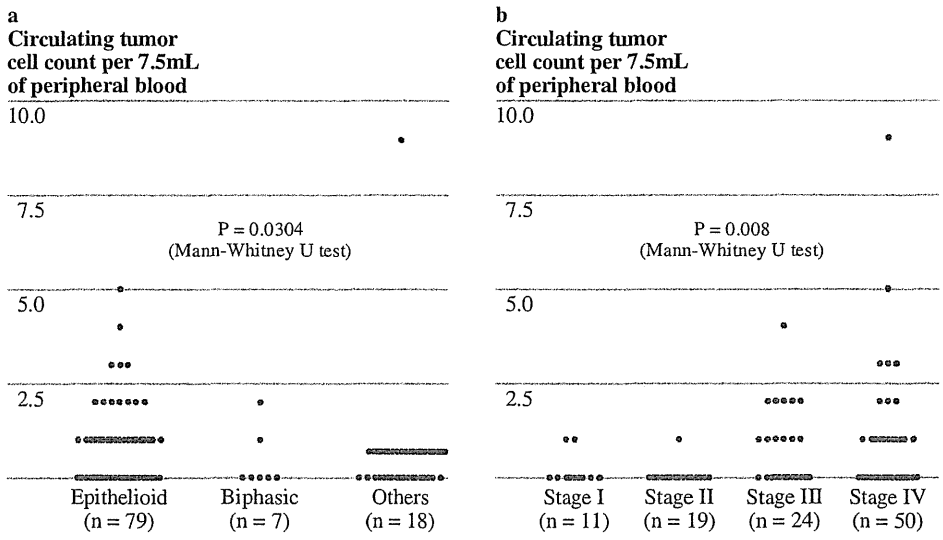

FIG. 3 **a** Distribution of CTC count in patients with MPM according to histologic subtype, **b** distribution of CTC count in patients with MPM according to clinical stage

showed that the CTC count was significantly higher in MPM patients than in NM patients. The ROC curve analysis showed that the CTC test provided a significant diagnostic capability in discrimination of MPM from NM diseases with an AUC-ROC of 0.623 ($P = 0.036$), thus suggesting that the CTC test is a promising noninvasive marker in the diagnosis of MPM. When the cut-off value of CTC-count was 1, the sensitivity and specificity were 32.7 and 90.6 %, respectively, and the positive predictive value and negative predictive value were 91.9 and 29.3 %, respectively. Alternatively, when a cut-off point of 2 was adopted, the specificity reached 100 % with no false-positive case, but the sensitivity decreased to 13.5 %. These results have indicated that the CTC test is characterized by low sensitivity and negative predictive value as well as high specificity and positive predictive value. Accordingly, the CTC test may be useful not for MPM screening, but for selection of patients who should receive invasive examinations such as video-assisted thoracoscopic biopsy after screening with more sensitive tools such as SMRP test.

Here, attention should be paid to the fact that some patients (9.4 %, 3 of 32 patients) were finally diagnosed with NM diseases, whereas one tumor cell was identified in the peripheral blood. These results are similar as those in a previous study showing that up to 3 CTCs were detected in a small subset of healthy volunteers or NM patients.[12] Such "false-positive" cases may be classified into "true" false-positive cases or "false" false-positive cases. A "true" false-positive result, indicating that patients without any malignant tumor are judged to have a malignant tumor according to the CTC test, can be brought about by several

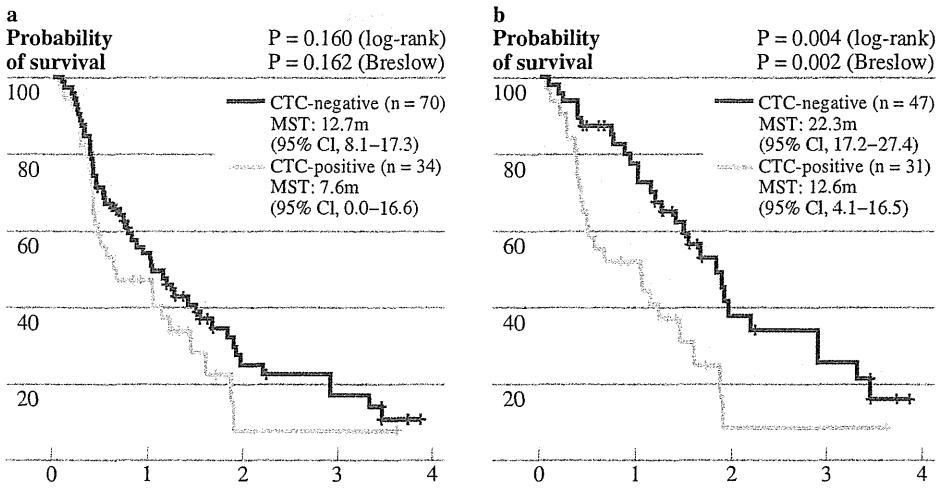

FIG. 4 **a** Survival curves according to CTC count in all MPM patients, **b** survival curves according to CTC count in epithelioid-type MPM patients. *MST* mean survival time

factors: contamination of epithelial cells in blood samples due to a variety of technical issues such as inappropriate blood sampling, false-positive staining of contaminated nonepithelial cells for cytokeratin/DAPI during sample processing, or inappropriate judgment in identification of CTCs by researchers. A "false" false-positive result can occur when detected CTCs originate from a clinically undetectable malignant tumor. An individual reason for 3 false-positive cases in the present study remains unclear, and careful long-term follow-up to watch for the development of malignant tumors should be performed. In fact, in one of the 3 false-positive cases, MPM had developed 2 years after initial pleural biopsy, which might indicate that "positive CTC" at the time of initial pleural biopsy was not "false-positive" but "true-positive."

The most important issues of the CTC test in the diagnosis of MPM were the low sensitivity (32.7 %) and the low negative predictive power (29.3 %). The CellSearch system used in the present study is the only commercially available detection system for detection and identification of CTCs, and the accuracy and reproducibility have been established. However, many previous studies have shown that the most critical issue in the use of the CellSearch system is its low sensitivity for detecting CTCs in other malignant tumors.[17,19] Recently, a novel microfluidic platform for detecting CTCs (the CTC chip) has been developed. This CTC chip consists of an array of 78,000 microposts coated with anti-EpCAM antibodies, and CTCs are captured by interaction of these cells with the EpCAM-coated microposts under laminar flow conditions. The CTC chip may provide a higher sensitivity in identification of CTCs, because a pilot study showed that CTCs were detected in most blood samples taken from patients with a variety of malignant tumors, including lung, prostate, pancreatic, breast, and colon cancer.[23] The most important reason for the low sensitivity in detecting CTCs in MPM was that the CellSearch system can principally capture tumor cells expressing EpCAM. Most malignant tumors of epithelial origin express EpCAM, and such tumor cells circulating in the blood can be captured with the CellSearch system. However, mesothelioma, originating from mesothelium that does not express EpCAM, may not express EpCAM. In fact, our preliminary study of immunohistochemical staining with an anti-EpCAM antibody showed that only 11 of 21 MPM tumors showed positive EpCAM expression and that EpCAM expression was exclusively observed in epithelioid-type MPM.[24] These results explain the reason for low sensitivity in detection of CTCs in MPM patients, especially in patients with nonepithelioid subtypes, in the present study. Thus, to improve the sensitivity, it is essential to develop novel systems of EpCAM-independent detection of CTCs.

Next, we showed a trend of increase in CTC count along with tumor progression. However, even in stage IV diseases, CTCs were detected in only 19 (38 %) of 50 MPM patients; when analyzed according to histologic subtypes, CTCs were detected in 48.6 % (18 of 37) of epithelioid cases, but in only 7.7 % (1 of 13) of nonepithelioid cases. These results also indicate that the CellSearch system provided an insufficient sensitivity in detection of CTCs in MPM, especially in nonepithelioid subtypes.

Finally, we showed that positive CTCs were a significant factor to predict a poor prognosis in epithelioid MPM, but not in nonepithelioid MPM. There was no difference in mode of therapy, which may influence prognosis between CTC-negative and CTC-positive patients; in fact,