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Better Clinical Efficiency of TILs for Malignant Pleural Effusion and Ascites than Cisplatin Through Intrapleural and Intraperitoneal Infusion

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Abstract. *Background/Aim: To evaluate the clinical efficiency of tumor-infiltrating lymphocytes (TILs) compared to cisplatin for malignant pleural effusion and ascites through intrapleural and intraperitoneal infusion. Patients and Methods: Thirteen patients with malignant pleural effusion and ascites were divided into a TIL-treated group and a cisplatin-treated group. Patients were given TILs or cisplatin, through intrapleural and intraperitoneal infusion respectively, after drainage of the malignant serous effusion by thoracentesis or abdominocentesis. Results: The overall response rate and disease control rate of the TIL-treated group (33.33% and 83.33%) were higher than that of the cisplatin-treated group (28.57% and 71.43%). The progression-free survival for the TIL-treated group was significantly longer (p=0.002) and better than that of the cisplatin-treated group (66.67% vs. 28.57%). Quality of life apparently improved in the TIL-treated group and was clearly higher than that in the cisplatin-treated group. Conclusion: The use of TILs has a better clinical efficiency for malignant pleural effusion and ascites than cisplatin through intrapleural and intraperitoneal infusion without severe adverse effects.*

Malignant pleural effusion (MPE) and ascites result from pathological accumulation of fluid in the serous cavity and are normally associated with malignancy. They are the most common and severe complications of advanced malignancy and the first sign of cancer cell recurrency (1). The incidence of malignant serous effusion (MSE) in patients with advanced cancer is approximately 50%, and it is the main factor which compromises a patient's life expectancy (between 4 to 9 months) and quality of life (QOL) (2). Many researchers believe that the occurrence of malignant effusion is an indicator of poor prognosis (3). Generally, adenocarcinoma is the most common histological cancer type responsible for MSE, including lung cancer (37%), breast cancer (25%), lymphoma (10%), ovarian cancer (5%), stomach cancer (2%), unknown primary (7%), and other causes (14%) (4). The exact mechanism underlying the development of MSE is not entirely understood (5). It may arise from tumor involvement of the visceral pleura, direct extension from neighboring structures, or hematogenous or lymphatic spread to parietal pleura.

At present, chemotherapy (intracavitary administration) is the main treatment for MSE and ascites. There are many kinds of chemotherapeutic drugs for thoracic or abdominal cavity perfusion. Among them, cisplatin has been administered most frequently (6). However, standard chemotherapy is not efficient due to its low specificity for cancer cells, severe side-effects and low rate of concentration in tumor tissue.

The process of infusion of autologous immune cells back into a patient with tumour, after activation and amplification to a specific number *in vitro*, is called adoptive T-cell therapy (7) and is used in the treatment of tumours. Adoptive T-cell therapy using tumor-infiltrating lymphocytes (TILs) has become a promising method for therapy of metastatic melanoma.TILs are cell clusters with antigen effect resulting from tumorigenesis. They were first found and separated from tumor-bearing mice in 1989 (8). TILs largely comprise T-lymphocytes, B-lymphocytes and natural killer lymphocytes; among them, $CD8^+$ T-lymphocytes exert mainly anticancer activity (9). Many studies revealed that the

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number or proportion of TILs is associated with prognosis of various types of cancer (10, 11), especially melanoma. A retrospective study including 633 patients with invasive melanoma found that the absence of TILs was a reproducible parameter that was able to predict sentinel lymph node biopsy positivity (12). A study by Tran *et al*. revealed that TIL infusion obtained objective response in 51% of patients with metastatic melanoma (13). This rate was significantly higher than that in patients given interleukin-2 (IL2) or dacarbazine (12% and 15%, respectively).

In this retrospective study, we aimed to evaluate the efficiency of TILs for MPE and ascites through intrapleural and intraperitoneal infusion compared to that of cisplatin.

Patients and Methods

Patients. This study was approved by the Committee Board of YantaiYuhuangding Hospital (Shandong, China) and informed consent was thoroughly read, understood and signed by all participants.

Inclusion criteria: i). Advanced malignancies were diagnosed by histological or pathological examination. ii). MSE was confirmed by imaging examination and malignant tumor cells were quantified in the serous effusion. iii). No anticancer drugs or hardener had been injected by intrapleural or intraperitoneal infusion within 1 month before the study. iv). Patients were aged between 18 and 50 years at the time of recruitment and the estimated remaining survival time was longer than 3 months. v). Test results for blood cell count, heart rate, hepatic and renal functions were within normal ranges. vi). Previous chemotherapy had been discontinued for more than 4 weeks prior to the study.

Exclusion criteria: History of allergy to biological agents; current treatment with chemotherapy; major organ dysfunctions; pregnancy or breast feeding; infections; history of refractory psychiatric diseases. Patients were withdrawn based on the following conditions: request of the patient; patient non-compliance; grade III/IV side-effects related to TIL infusion; disease progression.

Twenty-seven patients with relapsed solid tumor and MSE as the first sign of recurrence were enrolled in the study from May 2014 to June 2016. Characteristics of the patients are summarized in Table I.

Table I. *Characteristics of patients*.

Characteristic	TILs, n=6	Cisplatin, n=7
Median age (years)	67	64
Gender, n (%)		
Male	2 (33.33)	3 (42.86)
Female	4 (66.67)	4 (57.14)
Hydrops type, n (%)		
Hydrothorax	3 (50.00)	4 (57.14))
Ascites, n (%)	3 (50.00)	3 (42.86)
Clinical stage, n (%)	IV	IV
Tumor type, n (%)		
NSCLC	3 (50.00)	3 (42.86)
Colorectal	1 (16.67)	2 (28.57)
Gastric	1 (16.67)	1 (14.29)
Cholangiocarcinoma	1 (16.67)	1 (14.29)
ECOG PS, n (%)		
<3	4 (66.67)	5 (71.43)
≥3	2 (33.33)	2 (28.57)

TIL, Tumor-infiltrating lymphocytes; NSCLC, non-small cell lung cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Status.

Table II. *Overall remission rate (ORR) and disease control rate (DCR) of the tumor-infiltrating lymphocyte (TIL)-treated group compared to the cisplatin-treated group*.

Group	TILs (n=6)	Cisplatin (n=7)
Clinical efficiency, n (%)		
CR	0	0
PR	2 (33.33)	2 (28.57)
SD	3 (50.00)	3 (42.86)
PD	1 (16.67)	2 (28.57)
ORR, n (%)	2 (33.33)	2 (28.57)
DCR, n (%)	5 (83.33)	5 (71.43)

CR, Complete remission; PR, partial remission; SD, stable disease; PD, progression disease.

Separation and culture of TILs. Approximately 500-1000 ml MSE was extracted under aseptic conditions, and centrifuged (1258 × *g*) for 5 min after heparin resistance coagulation. Discontinuous density gradient method was used to isolate mononuclear cells. After removal of tumor cells by the wall sticking method, TILs were separated from the suspension. The cell density was adjusted to 1×10^6 /ml and inoculated into a culture flask with the RetroNectin (Takara, Kusatsu, Shiga, Japan) (13) package and culture solution, with phytohemagglutinin, 2-mercaptoethanol, monoclonal antibody of CD3 without serum, was added. At the same time, IL2 (6000 IU/ml) (Abcam, Cambridge, UK) was added for activation and proliferation of TILs. Subculture was carried out every 2-3 days based on the velocity of cell growth and cell density was maintained at $0.5\text{-}2.0\times10^6$/ml. Antigens specific to targeted cancer type were added on day 13. TILs were harvested on the 14th day after lymphocyte subset and endotoxin examination to ensure the absence of bacteria, fungi, mycoplasma or endotoxin. Cell density of at least 1×10^{10}/l, and cell viability of 80%, immunophenotype of CD3, CD4, CD8, CD16, CD56, CD19, CD25, CD71 and human leukocyte antigen D (HLA-DR)-related were checked before administration.

Treatment protocol. After drainage of the MSE by thoracentesis or abdominocentesis, patients received intrapleural or intraperitoneal administration of either cisplatin (60 mg) or TILs (100 ml). Patients were asked to turn over every 15 min to encourage full access of the medicine to the celom. Both groups were treated with systematic chemotherapy, *i.e*. 500 mg/m^2 pemetrexed and 75 mg/m^2 cisplatin on day 1 for four cycles repeated every 21 days for lung adenocarcinoma. Supportive treatments included liver and stomach protection and anti-emetic administration before infusion.

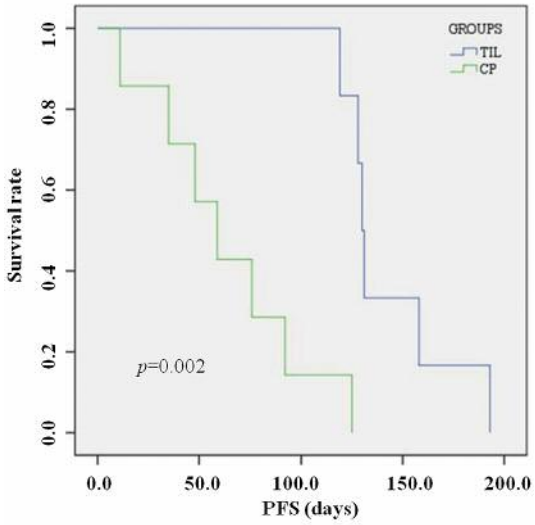

Figure 1. *Survival curves for progression-free survival (PFS) of patients treated with tumor-infiltrating lymphocytes (TIL) and those treated with cisplatin (CP). Patients treated with TILs survived significantly longer compared to those treated with cisplatin.*

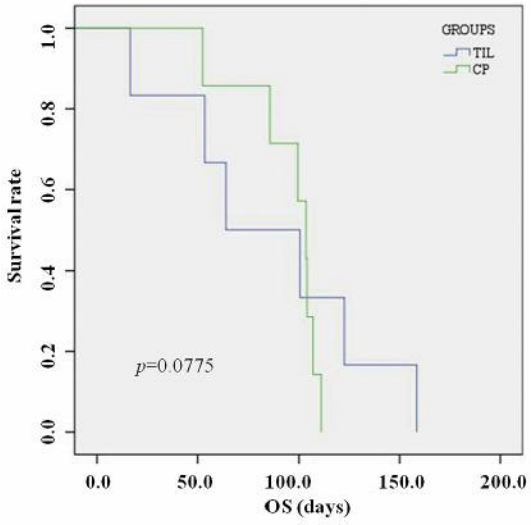

Figure 2. *Survival curves for overall survival (OS) of patients treated with tumor-infiltrating lymphocytes (TIL) and those treated with cisplatin (CP). No significant difference in OS was found between the TIL-treated group and the cisplatin-treated group.*

Dexamethasone (5 mg) and xylocaine (5 ml) were given before or after infusion to relieve side-effects. Type-B ultrasonography was used to evaluate the treatment efficacy at the end of each treatment course every 3 weeks.

Evaluation of efficacy. Complete remission (CR): accumulated effusion disappeared and remained stable for at least 4 weeks. Partial remission (PR): accumulated effusion decreased by 50%, associated with improved symptoms with no increased accumulation of fluid, and remained stable for at least 4 weeks. Stable disease (SD): less than 50% of MSE disappeared, or there was no noticeable change of symptoms. Progressive disease (PD): effusion increased with worsening of symptoms. Overall remission rate (ORR) was the sum of CR and PR. The disease control rate (DCR) was calculated by taking the sum of CR, PR and SD. Progression-free survival (PFS) refers to the period from the end of treatment to MSE increase. Overall survival (OS) refers to the period from the end of treatment to the end of follow-up or the death of the patient.

Adverse reactions were evaluated by the Common Toxicity Evaluation Criteria according to the National Cancer Institute (14). Patient QOL was assessed by KPS post-treatment as: Apparently improved: increase in KPS by ≥2; improved: increase in KPS by ≥10; stable: no obvious change in KPS; reduced: KPS decline of ≤10. Toxicity was classified grade 0-4 according to the WHO toxicity grading criteria (15). All cases were followed-up until June 2016.

Results

ORR and DCR were higher in the TIL-treated group compared with those of the cisplatin-treated group. The ORR and DCR of the TIL-treated group (33.33% and 83.33%) were higher than that of the cisplatin-treated group (28.57% and 71.43%) (Table II). However, no complete remission occurred in either the TIL-treated group or the cisplatin-treated group.

PFS was longer for the TIL-treated group compared with the cisplatin-treated group. The mean PFS of the TIL-treated group was 143.17 days [95% confidence interval (CI)=122.0-165.3 days], and that of the cisplatin group was 63.712 days (95% CI=35.7-91.7 days). The PFS for the TIL-treated group was significantly longer than that of the cisplatin-treated group (χ^2=9.249, p=0.002). Survival curves for PFS are shown in Figure 1.

OS did not differ between the two treatment groups. The mean OS of the TIL-treated group was 371.83 days (95% CI=289.70-453.96 days), and that of the cisplatin group was 389.43 days (95% CI = 359.37-419.49 days). There was no significant difference in OS between the TIL-treated group and the cisplatin-treated group (χ^2=0.085, p=0.775). Survival curves for OS are shown in Figure 2.

Serum carcinoembryonic antigen (CEA) differ before and after treatment in either group. CEA did not significantly change after TIL or cisplatin (Table III) infusion.

Quality of life. Patient QOL based on the change of KPS score following treatment was evaluated. The rate of apparent improvement in the TIL-treated group was

Table III. *Carcinoembryonic antigen (CEA) before and after intraperitoneal or intrathoracic infusion with tumor-infiltrating lymphocytes (TILs) or cisplatin. CEA levels did not alter significantly with treatment in either group.*

Group	Time point	Mean CEA (95% CI)	*p*-Value†
TILs	Pre-therapy	72.61 (−110.69-111,16)	0.873
	Post-therapy	72.37 (−111.08-111.55)	
Cisplatin	Pre-therapy	453.43 (−1160.51-449,16)	0.749
	Post-therapy	572.31 (−1275.08-563.99)	

CI, Confidence interval. †Difference between pre- and post-therapy values, Significance accepted at $p<0.05$.

Table IV. *Evaluation of quality of life.*

Group	Apparently improved, n (%)	Improved or stable, n (%)	Reduced, n (%)
TILs (n=6)	4 (66.67)	2 (33.33)	0
Cisplatin (n=7)	2 (28.57)	4 (57.14)	1 (14.29)

TILs, Tumor-infiltrating lymphocytes.

evidently higher than that of the cisplatin-treated group (66.67% *vs*. 28.57%, respectively). No correlation in the rate of improved or stable QOL was found between the two groups. No patient in the TIL-treated group developed a worse QOL. However, QOL of one case in the cisplatin-treated group became worse due to side-effects (Table IV).

Adverse effects. There were no other side-effects apart from fever (37.5-38.7˚C) in the TIL-treated group; only one patient was given indometacin suppositories to reduce their temperature, others achieved normal body temperature after physical cooling. However, myelosuppression (71.429%), nausea or vomiting (57.143%) and diarrhea (28.571%) occurred in the cisplatin-treated group.

Discussion

In approximately half of all patients with advanced cancer an association with MSE has been identified, and life expectancy is closely linked to the severity of MSE (1). MSE is one of the main causes of death among patients with advanced malignancy. The mechanism of the formation of MSE includes direct invasion of the serosa by tumor cells; increased permeability of the pleuroperitoneal capillary wall due to tumor-associated inflammation; blockage of the blood and lymphatic capillary or lymphatic circulation of the lymph nodes in the parietal *lamina serosa* by tumor embolus. Ideally, inhibition of any one of these mechanisms can effectively control MSE (16). In this study, both ORR and DCR of the TIL-treated group were higher than that of the cisplatin-treated group. The mean PFS of the TIL-treated group was twice as long as that of the cisplatin-treated group.

The reasons for these results may be closely related to different anticancer mechanisms between TILs and cisplatin. Cisplatin kills tumor cells through damaging the function of DNA, inhibiting its duplication (17). On the other hand, TIL infusion exerts a series of events to achieve an anticancer effect: $CD8^+$ T-lymphocytes can kill tumor cells directly after stimulation of T-cell receptors and CD28 as the main effective cell type; T-cell subsets can induce cancer cell apoptosis by Fas on the surface of T-lymphocytes combining with FasL located on the surface of cancer cells; T-cell subsets can induce the formation of polyperforin 'channels' located on the surface of cancer cells with the assistance of Ca^{2+}. The expression of cytokines such as interferon and IL2 associated with a cell-mediated immune response can be enhanced by TIL infusions (18). Finally, cytolysis or apoptosis of tumor cells occurs with synergistic effect between T-cells and granzyme.

The mean OS of the TIL-treated group (371.831 days) was similar to that of the cisplatin-treated group (389.43 days) ($p=0.775$). The differences in CEA levels, before and after TIL or cisplatin intraperitoneal or intrathoracic infusion, were not significant. It was recognized that these results could be due to the fact that treatment itself was topical rather than through a systemic regimen.

MSE can lead to chest distress, suffocation, cough, dyspnea, abdominal distension and abdominal pain, which can severely affect the patient's QOL. Effective control of MSE is critical in improving QOL of patients (19). Our study showed a relative improvement in QOL after treatment in both the TIL-treated and cisplatin-treated groups. This could be due to their effect in controlling MSE, however, the rate of apparent improvement in the TIL-treated group was higher than that in the cisplatin-treated group (66.67% *vs*. 28.57%). No sign of a lowered QOL was seen in the TIL-treated group but the QOL of one case in the cisplatin-treated group became worse due to side-effects. Therefore, QOL can be increased after treatment for controlling MSE with intrapleural and intraperitoneal infusion of TILs being better than cisplatin in increasing QOL.

Myelosuppression, nausea or vomiting and diarrhea are common adverse effects of platinum-containing therapeutics including cisplatin, which also occurred in the cisplatin-treated group in this study. However, in the TIL-treated group, there were no other side-effects beside fever (37.5-38.7˚C), with most patients recovering normal body temperature after physical cooling without medication. Therefore, the use of TILs is a relatively safe therapeutic method superior to cisplatin.

All in all, intrapleural and intraperitoneal infusion of TILs provides better clinical efficiency in managing MPE and ascites compared to cisplatin and has minimal adverse effects on the patient's QOL.

Conflicts of Interest

The Authors declare that they have no conflict of interest in regard to this study.

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