

## **Clinical observation of verapamil combined with antitumor drugs in treating malignant pleural effusion.**

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### **Abstract**

The aim of this study was to observe the clinical efficacy and side effects of verapamil combined with antitumor drugs *via* Pleural Catheter Perfusion (PCC). From December 2004 to December 2014, a total of 79 patients with Malignant Pleural Effusion (MPE) were randomly divided into the control group (C, n=37) and the treatment group (T, n=42). Group C was perfused with interleukin-2, 5-fluorouracil, and cisplatin, and group T was perfused with verapamil, interleukin-2, 5-fluorouracil, and cisplatin. The post-treatment clinical efficacy, side effects, median survival time, and overall survival were evaluated and compared between the two groups. After the treatment, the efficacy rate (CR+PR) of group C was 51.35%, and that of group T was 80.95%. There was significant difference between the two groups ( $P<0.05$ ); the median survival time of group C was 4 months, and that of group T was 13 months. There was significant difference between the two groups ( $P<0.01$ ). Grades I~II side effects appeared in group T after the treatment, but released in short period, and showed no significant difference with group C ( $P>0.05$ ). No significant side effect in the cardiovascular system appeared. This study can improve the clinical treatment of MPE and prolong the survival time.

**Keywords:** Malignant pleural effusion, Verapamil, P-glycoprotein, Multidrug resistance, Chemotherapy.

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### **Introduction**

Malignant Pleural Effusion (MPE) often appears in late stages of malignant solid tumors, develops rapidly, can seriously affect patients' life quality, or even threaten their lives [1]. Current treatment methods targeting malignant tumors, which fail chemotherapy or re-occur after treatments and associate with MPE, include thoracentesis, Indwelling Pleural Catheter (IPC), intravenous chemotherapy, pleurodesis, surgical treatment, targeted therapy, etc., but their clinical efficacies are rare [2,3], and the average survival time is only about 3-5 months [4,5]; furthermore, patients are prone to occurring cough, chest tightness, dyspnea, weight loss, or other symptoms, thus living in poor conditions [6].

At present, Indwelling Pleural Catheter (IPC) is more commonly used in clinics, which is safer and more thorough, and can direct inject antineoplastic drugs for the treatment of MPE, but its efficacy is poor [7-9]. The main reason for its poor efficacy is related to tumor cells' tolerance to chemotherapeutic drugs [10], so exploring effective treatment methods and drugs against MPE is a clinical emergent problem.

We have achieved good clinical results in our previous studies through applying innovative abdominal indwelling for the administration of verapamil combined with chemotherapeutic drugs in treating MPE [11]. In addition, changing the administration route of verapamil combined with chemotherapeutic drugs in treating liver cancer [12], colorectal cancer [13], gastric cancer [14], or lung cancer [15] also achieves good clinical efficacies, and no side effect in the cardiovascular system have been observed.

Based on our previous studies, we investigated the clinical efficacy, side effects, and survival time of applying verapamil combined with chemotherapeutic drugs for MPE through PCC.

### **Materials and Methods**

#### **General information**

From December 2004 to December 2014, a total of 79 patients with non-encapsulated MPE were treated in our department, who were all applied standardized chemotherapy while failed or recurred. All the patients voluntarily attended the research, including 41 males and 38 females, aging 25~85 years. The average age of group C was  $56.62 \pm 1.733$  (n=37), and the

average age of group T was  $56.67 \pm 1.546$  ( $n=42$ ). There was no significant difference in the age between the two groups ( $P=0.9845$ ,  $P>0.05$ ). Total cases of the two groups: 39 cases of Non-Small Cell Lung Cancer (NSCLC), 11 cases of Breast Cancer (BC), 16 cases of Esophageal Cancer (EC), 9 cases of Gastric Cancer (GC), and 4 cases of Colorectal Cancer (CC). Group C: 16 cases of NSCLC, 6 cases of BC, 10 cases of EC, 3 cases of GC, and 2 cases of CC. Group T: 23 cases of NSCLC, 5 cases of BC, 6 cases of EC, 6 cases of GC, and 2 cases of CC (Table 1). All the patients or their family members had signed the informed consent; this study was approved by our hospital ethics committee. The follow-up ended until December 31, 2016.

### **Inclusion criteria**

(1): Clearly diagnosed as malignant tumor by pathological evidence, and the exfoliative cytology of thoracic-abdominal water revealed tumor cells associated with MPE or clinically diagnosed as MPE [16]; (2) Karnofsky (KPS) Score  $\geq 50$  points; (3) heart rate  $>60$  beats/min while without Electrocardiogram (ECG) block; (4) expected survival time  $>3$  months; (5) aging 25-85 y old; (6) voluntarily accepted the treatment protocol and signed the informed consent.

### **Exclusion criteria**

(1) Female patients in pregnancy or in lactation; (2) with mental issue or retardation; (3) with acute infection; (4) with central nervous system symptoms; (5) with allergies; (6)  $WBC < 4.0 \times 10^9/L$ ,  $BPC < 10.0 \times 10^9/L$ , and  $Hgb < 60$  g/L; (7) with blood clotting disorders; (8) cannot strictly implement the treatment protocol; (9) with intolerable severe adverse reactions, or incomplete information and cannot perform adverse reaction assessment and efficacy evaluation; (10) with contraindications to cisplatin and 5-fluorouracil; (11) with small cell lung cancer, lymphoma, breast cancer while treated for the first time, and ovarian cancer.

### **Treatment**

After ultrasound positioning, one 14 G central venous catheter (Beijing Tiandihexie Tech Co., Ltd., Beijing, China) was placed into the chest using the seldinger technology [17], and its outer end was fixed on the chest wall with one drainage bag, which was closed with a heparin cap after the surgery. On the first day, the drained effusion was about 800~1000 ml, and on the second day, the drainage within 2 h should be  $<100$  ml after the pleural effusion was intermittently drained or obstruction factors such as catheter blockage were excluded; after confirmed by ultrasound or chest X-ray, each patient was performed catheterization for drug perfusion. Group C was injected with 3 M units of interleukin-2 (1M units/ampule, Shanghai Hua Xin High Biotechnology Co., Ltd., Shanghai, China), fluorouracil (0.25 g/ampule, Tianjin KingYork Group Co., Ltd.), and cisplatin (30 mg/ampule, Jiangsu Hanson Pharma Co., Ltd., Jiangsu, China). If the drainage amount was greater than 200 ml 24 h later, 3 M units of interleukin-2 and fluorouracil were re-injected; group T was injected with 15 mg

of verapamil injection (5 mg/ampule, Shanghai Hemu Pharmaceutical Co., Ltd., Shanghai, China), 3 M units of interleukin-2, fluorouracil, and cisplatin, followed by reperfusion 10 mg of verapamil. If the drainage amount was greater than 200 ml 24 h later, verapamil, 3M units of interleukin-2, and 5-fluorouracil were reperfusion; each patient was instructed laying in the lateral position (left or right) and coughing or breathing using a little force so as to facilitate the drug to contact with the parietal and visceral pleura.

The chemotherapeutic drugs (cisplatin+5-fluorouracil) referred to the diagnostic criteria of malignant tumors issued by the Chinese Society of Clinical Oncology (CSCO) [18]. Methods: 1) Preoperative routine intravenous infusion: 100 ml of 0.9% NS+5 mg of dexamethasone, intravenous infusion 100 ml of 0.9% NS+8~16 mg of ondansetron; 2) the dosages of the chemotherapeutic drugs (cisplatin+5-fluorouracil) were calculated referring to each patient's body weight and body surface area, combining with the general situations of each patient's heart, liver, and kidney function before the treatment. Medication and sequence of group C: 10 ml of 0.9% NS+3 M units of interleukin-2; 50 ml of 0.9% NS+60~120 mg of cisplatin; 100 ml of 0.9% NS+0.75~1.0 g of 5-fluorouracil; if the drainage amount was greater than 200 ml 24 h later, the patient was injected 10 ml of 0.9% NS+3 M units of interleukin-2; 100 ml of 0.9% NS+0.75-1.0 g of 5-fluorouracil; medication and sequence of group T: 50 ml of 0.9% NS+15 mg of verapamil; 10 ml of 0.9% NS+60-120 mg of cisplatin; 100 ml of 0.9% NS+0.75-1.0 g of 5-fluorouracil; 50 ml of 0.9% NS +10 mg of verapamil; if the drainage amount was greater than 200 ml 24 h later, the patient was injected 50 ml of 0.9% NS +15 mg of verapamil; 10 ml of 0.9% NS+3 M units of interleukin-2; 100 ml of 0.9% NS+10 mg of verapamil. The two groups were performed conventional symptomatic treatment after the treatment.

### **Clinical observation items**

The blood routine, liver and kidney functions, and ECG were examined before and after the treatment. Each patient was performed chest X-ray, ultrasound, and Computed Tomography (CT) to evaluate the clinical efficacy, adverse effects, sign and symptom changes, KPS score, and body weight before the treatment ( $T_0$ ), as well as on the 30<sup>th</sup> ( $T_1$ ) and 60<sup>th</sup> d ( $T_2$ ) after the treatment. Each patient's survival time was also followed up. The patients were evaluated the drug toxicity (grades 0-IV) according to the classification criteria of NCI-CTC 3.0 [19]. Meanwhile, the heart rate and blood pressure changes were monitored before and after the perfusion of verapamil, and the patients' cardiac functions were monitored periodically.

### **Efficacy determination**

According to the efficacy evaluation criteria of unmeasured lesions issued by World Health Organization (WHO) [20], Complete Remission (CR): pleural effusion disappeared for at least 4 w; Partial Remission (PR): pleural effusion was decreased by 50% or more, and maintained for at least 4 w; No Response (NR): pleural effusion was decreased or even

increased. Progressive (PD): pleural effusion was increased. CR and PR were recorded as valid, and NR and PD were recorded as invalid.

**Determination of clinical benefits**

Each patient’s clinical benefit was determined according to the conditions of analgesic consumption, KPS, and body weight [21]. 1: The condition with the amount of analgesic consumption reduced by >50% and maintained for 4 w or longer was defined as clinical benefit-positive; the condition with the amount of analgesic consumption increased was defined as clinical benefit-negative; any other outcomes were defined as clinical benefit-stable. 2: The condition with the KPS score increased by >20 points and maintained for 4 w or longer was defined as clinical benefit-positive; the condition with the KPS score decreased was defined as clinical benefit-negative; any other outcomes were defined as clinical benefit-stable. 3: The condition with the body weight increased by >7% and maintained for 4 w or longer was defined as clinical benefit-positive; the condition with the body weight decreased was defined as clinical benefit-negative; any other outcomes were defined as clinical benefit-stable. Among these three parameters, the patient with at least one parameter positive while no parameter negative can be defined as clinical benefit.

**Assessment of survival and median survival time**

The 79 patients in the two groups were followed up *via* phone until December 31, 2016.

**Evaluation criteria of toxicity**

The toxicities were evaluated according to the criteria of anticancer drug toxicities issued by the NCI-CTC (grades 0-IV) [19]. The classification of cardiac function was evaluated according to the criteria of New York Association (NYHA, grades I-IV) [22].

**Statistical analysis**

The data were analysed using the graphpad prism 6; the measurement data were compared using the two independent-sample t test and expressed as the mean ± standard deviation ( $\bar{x} \pm s$ ); the intergroup comparison used the unpaired t-test, with  $P < 0.05$  considered as statistical significance.

**Results**

**Efficacy determination**

After the treatment, group C exhibited 2 cases of CR and 17 cases of PR, with the effective rate (CR+PR) as 51.35%; group T exhibited 6 cases of CR and 29 cases of PR, with the effective rate (CR+PR) as 80.95%; the difference between the two groups was significant ( $P < 0.05$ ) (Table 2).

**Clinical benefit and survival time**

The clinical benefit rates of the KPS score, body weight, and analgesic dosage in group C were 27.02% (10/37), 16.21% (6/37), and 37.50% (3/8), respectively, and those in group T were 83.33% (35/42), 52.38% (22/42), and 66.66% (6/9), respectively, showing significant differences than group C after perfused with verapamil combined with chemotherapeutic drugs ( $P < 0.01$ ) (Table 3). The median survival time of group C was four months, and that of group T was 13 months, showing significant difference between the two groups ( $P < 0.01$ ), indicating that verapamil combined with chemotherapeutic drugs significantly prolonged the overall survival time and median survival time of the patients with MPE (Figure 1).

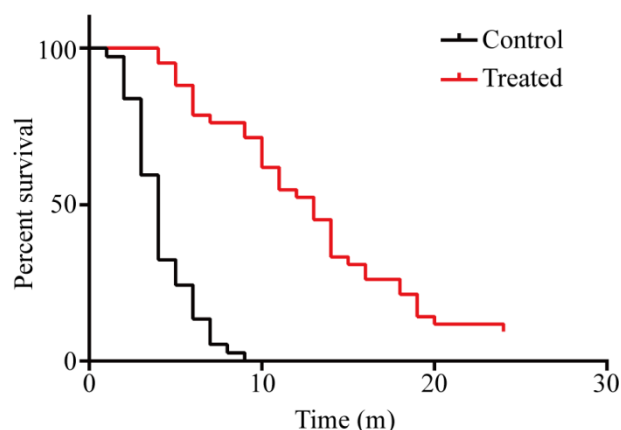


Figure 1. Analysis of survival time.

**Side effects**

The patients in group T exhibited grades I and II side effects and were relieved shortly (Table 4), including 17 cases with leukopenia (40.47%), 23 cases with nausea and vomiting (54.76%), 10 cases with diarrhea (23.80%), 6 cases with alopecia (14.28%), 19 cases with fever (45.23%), and 9 cases with liver damage (21.42%), showing no significant difference than group C. No significant change of vital signs (respiration, pulse, or blood pressure) in group T was found before and after the application of verapamil, nor ECG showed any significant difference (Tables 5 and 6).

Table 1. Distribution of patients’ data.

	NSCLC	BC	EC	GC	CC	Sum
T	23	5	6	6	2	42
C	16	6	10	3	2	37

Table 2. Efficacy determination.

	CR	PR	SD	PD
C (n=37)	2	17	11	7
T (n=42)	6	28	7	1

Note: P&lt;0.05.

**Table 3.** Determination of clinical benefits.

	Clinical benefit- positive of group C	Clinical benefit- positive of group T
KPS score	10	35
Body weight change	6	22
Analgesic dosage	3	6

Note: P<0.05.

**Table 4.** Side effects.

Side effect	C					T				
	0	1	2	3	4	0	1	2	3	4
Leukopenia	21	9	6	1	0	24	11	6	1	0
Hemoglobin reduction	25	7	5	0	0	23	14	5	0	0
Thrombocytopenia	30	3	4	0	0	33	5	4	0	0
Liver damage	31	4	2	0	0	33	5	4	0	0
Renal damage	30	5	1	1	0	34	5	2	1	0
Bleeding	37	0	0	0	0	42	0	0	0	0
Fever	20	14	3	0	0	23	15	4	0	0
Rash	34	3	0	0	0	39	3	0	0	0
Vomiting	17	13	6	1	0	19	15	7	1	0
Diarrhea	30	6	1	0	0	32	7	3	0	0
Acrotia	11	21	5	0	0	12	24	6	0	0
Alopecia	32	4	1	0	0	36	5	1	0	0
Peripheral neurotoxicity	34	3	0	0	0	38	4	0	0	0
Arrhythmia	37	0	0	0	0	42	0	0	0	0
Blood pressure increase	33	4	0	0	0	38	5	0	0	0

Note: P>0.05.

**Table 5.** Changes of respiration, pulse, and blood pressure in group T (n=42).

Observation item	Respiration (times/min)	BP (mmHg)	Pulse (beats/ min)
5 min before treatment	19 ± 2	122 ± 16/72 ± 10	73 ± 14
30 min after treatment	19 ± 3	115 ± 11/70 ± 14	70 ± 17
60 min after treatment	19 ± 2	115 ± 12/71 ± 13	72 ± 15

Note: P>0.05.

**Table 6.** ECG changes in Group T before and after the application of verapamil (n=42).

Observation item	P-R interphase (s)	Q-T interphase (s)	QRS (s)
5 min before treatment	0.16 ± 0.02	0.38 ± 0.05	0.08 ± 0.01
30 min after treatment	0.16 ± 0.03	0.38 ± 0.05	0.08 ± 0.02
60 min after treatment	0.16 ± 0.02	0.38 ± 0.03	0.08 ± 0.01

Note: P>0.05.

## Discussion

MPE is a common complication in advanced tumors, and can often seriously affect the patients' respiratory and circulatory functions after multiple standardized anti-tumor drug treatments, thus greatly reducing the patients' quality of life [23]. The current treatment against MPE mainly uses closed thoracic drainage and injects antitumor drugs or biological agents, but the effects of such treatments are still poor and prone to occurring encapsulated or multilocularpleural effusion [24,25], and the reason may be related to the internal, acquired Multidrug Resistance (MDR) existing inside the tumor cells within pleural effusion and the reduction of anti-tumor drug intake [7].

MDR is one of the main reasons that can limit the clinical effects of chemotherapy against malignant tumors [26,27]. As the main mechanism of MDR, P-glycoprotein (P-gp) can promote the hydrolyzation of ATP, which therefore produces ADP, releases energy, combines with the intracellular chemotherapeutic drugs *via* the participation of Ca<sup>2+</sup>, and then "pumps" them out of the cells, thus resulting in the intracellular drug concentrations to be decreased, reducing the toxic effects of the drugs against the tumors, and leading to the MDR [28,29].

Verapamil is an antagonist of Ca<sup>2+</sup> channel, and studies have found that it can inhibit the expression of *MDR-1* gene and the synthesis of P-gp, thereby increasing the chemotherapeutic drug concentrations in tumor cells and overcoming the resistance inside tumor cells [30]. Certain studies have shown that 6~10 μmol/L verapamil can completely inhibit the P-gp activity [31], thereby reversing the MDR of malignant cells and increasing the sensitivity of tumor cells to chemotherapeutic drugs. However, when the *in vivo* serum concentration of verapamil reaches 1~2 μmol/L, such side effects as heart rate and blood pressure decreases or atrioventricular block may appear. Some researchers clinically applied verapamil *via* venous administration while failed to reverse the MDR of tumor cells [32,33], which limited the venous application of verapamil against malignant tumors.

We previously perfused verapamil *via* canine hepatic artery, and the drug concentration in local tissues can reach 50 to 10 times than that in the blood, but no heart rate decrease, blood pressure decrease, or atrioventricular block can be observed [34]. Based on such results, we innovatively applied verapamil combined with chemotherapeutic drugs *via* trans-peritoneal catheter and achieved good clinical results [11]. In addition,

changing the administration ways of verapamil combined with chemotherapeutic drugs in treating liver cancer, colorectal cancer, gastric cancer, lung cancer, or other malignant tumors also have achieved good clinical efficacies, and no related cardiovascular side effect has been observed [12-15].

This clinical study is based on the above studies, and continued the treatment of verapamil combined with chemotherapeutic drugs against MPE *via* PCC. The 79 MPE patients were randomly divided into the control group (37 patients) and the treatment group (42 patients), while those with small cell lung cancer, lymphoma, breast cancer while treated for the first time, and ovarian cancer were not included into the study. After one-course treatment, group C exhibited 2 cases of CR and 17 cases of PR, with the effective rate (CR+PR) as 51.35%; group T exhibited 6 cases of CR and 29 cases of PR, with the effective rate (CR+PR) as 80.95%; the difference in the treatment efficacy between the two groups was significant ( $P<0.05$ ). In group C, from the KPS score, weight, analgesic dosage, the rates of clinical benefit were 27.02% (10/37), 16.21% (6/37), 37.50% (3/8); in group T, the patients got more benefit with intrapleural perfusion of chemotherapy and verapamil, from the KPS score, weight, analgesic dosage, the rates of clinical benefit were 83.33% (35/42), 52.38% (22/42), 66.66% (6/9), with significant difference ( $P<0.01$ ). The median survival time of group C was 4 months, and that of group T was 13 months, and the overall survival time between the two groups showed significant difference ( $P<0.01$ ). The patients in group T exhibited grades I and II side effects and were relieved shortly (Table 4), including 17 cases with leukopenia (40.47%), 23 cases with nausea and vomiting (54.76%), 10 cases with diarrhea (23.80%), 6 cases with alopecia (14.28%), 19 cases with fever (45.23%), and 9 cases with liver damage (21.42%), showing no significant difference than group C. The changes between groups C and T of respiration, pulse, blood pressure and ECG before and after treatment were not significantly different.

The results show that the application of verapamil combined with chemotherapeutic drugs *via* PCC can improve the efficacy, prolong the median survival time, and improve the prognosis and quality of life in MPE patients, while not increase the side effects. The effects are exact, the method is simple, and the operations are safe and repeatable. We will further expand the sample size and perform further follow-up so as to observe and judge the long-term clinical efficacy of this method. Moreover, we will investigate the metabolic rules of verapamil in MPE based on such clinical studies, thus providing more theoretical basis for the thoracic cavity perfusion therapy of verapamil combined with chemotherapeutic drugs.

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## Conflicts of Interest

The authors declare no conflict of interest.

## Author Contributions

Yabei Liu, Yingwei Zhu, Gaofei Fan, and Jin Huang contribute the same effects to this study, designed the clinical research, developed and implemented the specific treatment programs, analysed the data, and prepared the manuscript; Chengtao Weng, Qiaohong Duan, Tengyue Zhang, and Lili Tang provided the cases, images, and literature retrieval; Pingsheng Fan proposed the main frame of this study and guided the implementation of the treatment programs.

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