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Abscopal by Cytotoxic Drugs and Penicillin Intratumoral Injection Prolonged the Survival by Triggering High Cytokines and Autologous Antibodies of TAA

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1. Abstract

1.1. Aim: Comparing the patients treated with cytotoxic drugs and penicillin intratumoral injection (CDPI) to patients with cytotoxic drugs intratumoral injection (CDI), the abscopal effect associated with changes in cytokines and autologous antibodies of Tumor-Associated Antigen (TAA) were investigated.

1.2. Method: CDPI contains an oxidant, cytotoxic drugs and penicillin. CDI contains same drugs without penicillin. Pancreatic cancer (20 cases) treated with CDPI; another group (9 cases) treated with CDI. Patients' cytokines and serum of autologous antibodies of tumour-related antigens were examined and compared.

1.3. Results: The one-year survival rate of the CDPI reached 52.63% compared with the CDI in 11.11% with a statistically significant difference ($P<0.05$). A significant difference in the analysis of the cytokines; the hapten (CDPI) showed an increasing level of IFN-gamma and IL-4, and the non-hapten (CDI) showed a rising level of IL-12. The patient without prior chemotherapy showed only a difference in the level of the autologous antibody of Zeta ($P\leqq0.05$) before and after CDPI therapy; however, there is a considerable difference in IMP1 level before and after CDPI and CDI therapy for patients with prior chemotherapy ($P<0.05$), in which CDI with increasing level of IMP1 and CDPI with growing HCC1 level when compared with the control ($P<0.05$). There was a difference in TAA antibodies such as RalA, Zeta, p16 ($P<0.05$) between the prior chemotherapy with and without prior chemotherapy.

1.4. Conclusion: CDPI may offer an ideal intratumoral approach for chemical de-bulking of advanced pancreatic tumours. Penicillin plays a vital role in prolonging patients' survival time.

2. Introduction

The abscopal effect is a hypothesis for treating metastatic cancer. In this case, untreated and tumour shrinkage coincided with the scope of local treatment in 1950 [1]. This effect is of great significance for doctors to understand the mechanism and how to use it in cancer treatment. Since 1953, more than half a century, the agency

that caused this phenomenon is still unknown. Mole mentioned an overview in the phrase abstract. However, promising new research on the reappearance and immune response to the system is called absolute effect, which may eventually help us understand the key to metastatic disease. In radiation therapy programs, distant tumour regression is still a rare event. The number of cases is increasing, especially since clinical immune checkpoint inhibitors are presented. Therefore, there is a growing interest in the systematic use of the therapeutic potential of the systemic response stimulated by radiotherapy.

The present review briefly describes the history of radiotherapy-induced abscopal effects and local irradiation's activation of systemic antitumor immune responses [2]. We must understand the mechanism of abscopal impact more deeply. We need to know how to apply the abscopal effect to distant tumours in patients through local drugs instead of local radiation, especially for potential viewing Metastasis of missing tumour cells. Theoretically, this suggests that tumour necrosis induced by chemical drugs in CDI, which is not natural necrosis of the tumour or ionizing radiation necrosis, can also cause immune responses against tumour cells. It is reported that CDI can induce an excellent anti-tumour immune response in animal models and humans [3-4], which provides the possibility of suppressing tumour recurrence and metastasis, such as the abscopal effect. Human tumours are on the rise, which indicates that the human body is tolerant to its tumour antigens. This means that the immune system no longer monitors the tumour antigen, lowering the immune response to tumour cells. This is why, in daily clinical practice, we rarely witness this abscopal impact when the tumour is growing.

As a result of their low immunogenicity, high tolerance, or immune escape, most tumour antigens have difficulty triggering an immune response (immunological tolerance). It has been demonstrated that combining hapten with tumour antigen results in a more potent tumour antigen that can trigger the body's immune response and raise the likelihood of an abscopal effect, which could be critical in regulating the patient's undetectable metastasis. The term "hapten" was coined by Landsteiner and Jacobs [5] and is derived from the Greek word "hapten", which means "fasten". A hapten is low molecular weight (<1000 Dalton) chemical substance that must be combined with a carrier molecule to have antigenicity [6]. Haptenization often occurs when a chemical substance interacts with a protein. After intratumor injection, haptens can easily interact with proteins such as tumour antigens [7].

Our published data show that CDPI provides an ideal percutaneous intra-tumoral approach for the chemical de-bulking of advanced pancreatic cancer and advanced hepatocellular carcinoma, and hapten plays a vital role in prolonging the survival rate of patients [8-9]. Approved data confirm that CDPI can prolong survival time and use penicillin as a hapten to enhance tumour antigens' immunogenicity. A brief discussion on the abscopal effect is the clinical effectiveness of hapten-enhanced chemotherapy and neutral tumour antigens. The relationship between the new selection of modified haptens and TAA cytokines or autoantibodies and their survival rates. Therefore, we take the serum of patients with pancreatic cancer as an example to detect the residues of cytokines and TAA autoantibodies before and after CDPI and CDI treatment and analyze the differences and their relationship with a survival rate the clinical practice of abscopal effect in more detail.

3. Method

3.1. Patient selection

The selected patients were diagnosed with at least one solid pancreatic cancer tumour with a diameter of at least 1.5 cm, which was confirmed to be malignant by CT imaging, biopsy, and pathological examination. The pancreatic cancer patients studied have failed conventional therapies and have locally advanced and metastatic tumours that are not operatable. The study was conducted in China from November 2011 to August 2015, with 32 cases. All patients signed the informed consent form divided into CDPI and CDI treatment groups. The Baofa hospital's Ethics Committee with external members approved the study. Following all of the study in this hospital with this methods was going to over 15 years as off label use (EC approval letter No. TMBFZLLY002). All procedures performed were following the Declaration of Helsinki [26]. At the end of the follow-up, 19 patients remained, and both the CDPI group (n = 20) and the CDI group (n = 9) had a response and survival data. The baseline characteristics of the two groups of patients were well balanced, and the difference was not statistically significant (Table 1) (P>0.05).

Table 1: Patient Baseline Characteristics

		CDPI	CDI	Control group
		N	N	N
Enrolled patients		20	9	4
Sex	Male	12	3	2
	Female	8	6	2
Age rang		46-82（63.55±9.47）	44-74（62.56±10.68）	50-70（62.25±8.81）
KPS		50-80（66.50±8.13）	50-70（65.56±7.26）	40-70（55.00±11.91）
Diabetes		6	2	0

Cigarette smoking	6		3		2	
Alcohol intake	6		3		2	
Stage of disease						
Stage Ⅰ	0		1		0	
Stage Ⅱ	3		0		0	
Stage Ⅲ	6		3		2	
Stage Ⅵ	11		5		2	
Cytological diagnosed Cancer	15		5		0	
Tumor size	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
<4cm	6	9	1	3	3	no
4–5cm	6	6	4	2	0	no
>5cm	7	4	4	4	1	no
Previous treatment						
Prior chemotherapy	1		1		1	
Prior adjuvant therapy	10		3		3	
Disease status						
Locally advanced	10		6		4	
Metastatic disease	6		3		4	

3.2. CDPI and CDI indications and contraindications

The following contraindications preclude some patients from participating in the trial. The contraindications to treatment with CDPI and CDI are poor performance status (Kanowski status, ≤40%), nutritional disorders, high serum total bilirubin levels [> 3 mg/dL (51.3 μmol/L)] and renal Failure [serum creatinine level> 2 mg/dl (176.8μmol/L)]. Cardiovascular or respiratory failure is another exclusion criterion for this operation since it is not partial or complete thrombosis of the main portal vein. Patients suffering from pancreatitis, intestinal obstruction and other serious infections are not allowed to receive this treatment like pancreatitis, intestinal obstruction and other serious infections.

3.3. Preparation of the agents

As the pancreatic tissue is fragile, bleeding is a concern for injections, limiting its application. Fine-needle biopsy is performed in clinical practice, and it is used to diagnose and evaluate the treatment of pancreatic organs, which requires a fine needle with a sharp tip. At the same time, 25 gauge of spinal needles and inflators (inflation device, 30 atm/bar)) were purchased. The CDPI and CDI solutions are freshly prepared at the clinical site before each injection. CDPI contains oxidative agents that oxidize tumour stromal tissue, cytotoxic drugs (Cytarabine Hydrochloride (Ara-C) or Adriamycin Hydrochloride (Dox)), and penicillin (used as hapten that binds the antigen to enhance Antigenicity. CDI contains a clinically approved oxidant with cytotoxic drugs (Ara-C or Dox) without penicillin (both medicines are saturated in concentration) (8-9).

3.4. Treatment design

Cardiopulmonary function and peripheral complete blood count are frequently examined to rule out the need for a liver and/or pancreatic puncture, as well as any potential complications. Blood samples were collected before and after treatment to analyze T cell function. Before CDPI treatment, patients must fast for 14 hours to avoid side effects and infections. To control the pain during the treatment, 50 mg of morphine was injected intramuscularly at least 30 minutes before the treatment. The skin was cleaned, and local anaesthesia was applied to the injection area. The spinal needle was inserted into the tumour under the guidance of CT. After insertion, the core was removed from the needle (connected to the inflator used as a high-pressure syringe), and then the injection was performed (Figure 1). Ultrasound or CT guidance is used to scan and monitor the density changes at the points or areas of interest in pancreatic tumours. They monitored the changes in CT values around the edge of the tumor carefully to ensure that the drug is completely distributed to the edge of the tumor. The biopsy samples and blood of patients were collected before and after treatment to study cytokine and autologous tumour-associated antigen and (TAA) antibody evaluation. The average time of this process is about 30-45 minutes. The injection volume was calculated based on the diameter of the tumour ((Dt) x 2 for tumours of 1-5 cm, and (Dt) x 1.5 for tumours of 6 cm or larger). Each therapy is based on this calculation to deliver a sufficient dose to the tumour [8-9].

The size of the tumour (tumour mass) is closely re-examined by CT Scanning once per week, and the treatment was repeated every week for 3 weeks. There is a total of 3 treatments, including the initial treatment as a treatment cycle of CDPI and CDI. If the size of the tumour becomes unstable or smaller after re-examination after 8-9 weeks, additional treatment should be given; if the tumour size of other organs (such as the liver or abdomen) determined by CT or ultrasound is greater than 2 cm, the distant tumour will receive the same treatment as the primary pancreatic tumour. The patient is closely monitored 2 days after treatment to determine whether it is necessary to evaluate or treat significant systemic or

local adverse reactions.

3.5. Assessment

The treatment response to solid tumours was evaluated according to the evaluation criteria of EROTC (European Organization for Research and Treatment of Cancer) and RECIST (NCI, the United States and Canada) [10] in October 1998. All case report forms (CRF) were filled out by the attending physicians. In every hospital, all physicians were trained in standard procedures.

3.6. Statistical analysis

The statistical analysis is carried out by experts from the medical school. Overall survival (OS) was defined as the duration from the date of first treatment (not the date of diagnosis) to the date of death based on the Kaplan-Meier method. The chi-square test was applied to calculate the efficiency comparison, and SPSS 17.0 statistical software was used for statistical analysis. A P value of <0.05 was considered statistically significant.

4. Cytokine Detection and Analysis in Pancreatic Cancer Sera

4.1. Cytokine detection procedure

Perform cytokine detection to simultaneously identify 507 cytokines in the serum of pancreatic cancer patients and healthy controls. The nonparametric Mann-Whitney U test compared the control group, pretreatment patients and posttreatment patients pairwise. Fold changes greater than or equal to 1.5 or less than/similar to 1/1.5 were considered significant. The receiver operating characteristic curve was applied to evaluate the model's performance. Leave-one-out cross-validation was used to estimate the prediction error [11].

4.2. Antibody detection analysis

Enzyme-linked immunosorbent assay (ELISA). 14 Purified recombinant proteins were diluted in phosphate-buffered saline (PBS) to final concentrations of 0.125 -1.0 ug/ml and coat it in a 96-well microliter plate (100ul/well) overnight at 4°C. Incubate the 1:200 diluted serum in antigen-coated wells (100ul/well) at room temperature (RT) for 90 minutes, and horseradish peroxidase-conjugated goat anti-human IgG in 1:3,000 dilution was used as 2nd antibody. 2,2'-azidobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) substrate with 100ul/well hydrogen peroxide was added to each plate's well and left at room temperature (RT), which incubated in the dark for 10-15 minutes. The optical density (OD) value of each well was read at 405nm on a microplate reader (Thermo Fisher Scientific) in the shortest possible time to reduce the variation among plates. In Addition, 8 frozen human serum samples and 2 blanks control with 1% BSA in PBST were set up on each 96-well plate for normalization of OD value from different plates and the adjustment of background in every single plate for the Enzyme-linked immunosorbent assay (ELISA) [12-13].

4.3. Result

Clinical benefits of patients with pancreatic cancer: (Tables 2 and 3) show that the clinical response rate of the CDPI group and CDI group were 30% and 44.44% (P>0.05), and the similarity of the clinical benefit rates were 95.00% and 88.89% (P>0.05). The median survival time was 11.81 months and 5.64 months (P>0.05), in which the difference was not statistically significant. The 6-month survival rates were 73.68% (CDPI) and 44.44% (CDI), respectively (P>0.05). The one-year survival rate of the CDPI group reached 52.63%, while that of the CDI group was only 11.11%, the difference was statistically significant (P <0.05). This shows that CDPI treatment of pancreatic cancer could prolong the life of patients. In contrast, CDI cannot prolong the life of patients, and the reason is that CDPI not only de-bulk pancreatic cancer but also induces the systemic immunogenicity of TAA (Fig.1).

Table 2: Comparison of therapeutic effect between CDPI and CDI groups

Groups	N	CR	PR	SD	PD	Response rate (%)	Benefit rate (%)	*P*
CDPI	20	0	6	13	1	30	95.00	>0.05
CDPI	9	0	4	4	1	44.44	88.89	

Table 3: Comparison of the survival time between the UMIPIC and ITCT groups

Groups	n	Mean survival / month	Median survival / month	log-rank		6-month survival rate /%	Chi square	*p*	12-month survival rate /%	Chi square	*p*
				Chi square	*P*						
UMIPIC	19	11.81	12.27	0.16	>0.05	73.68	2.27	>0.05	52.63	4.41	0.035
ITCT	9	5.64	4.73			44.44			11.11		

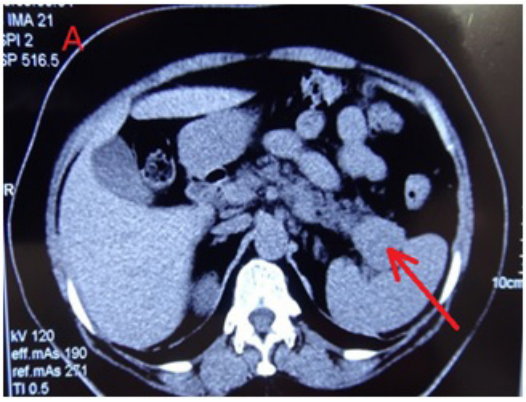


Figure 1A: One of these patient was diagnosed as pancreatic cancer at tail of pancreatic organ (Arrow show a tumor mass at tail of pancreas before any treatment)

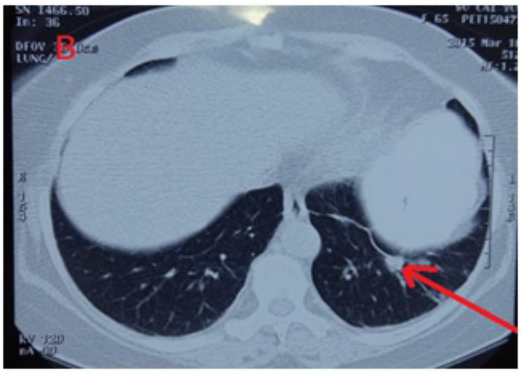


Figure 1B: This patient already had a metastasis at left lung before any treatment (Arrow show a small mass and clinical diagnoses as a metastasis without PET/CT and pathological proved).

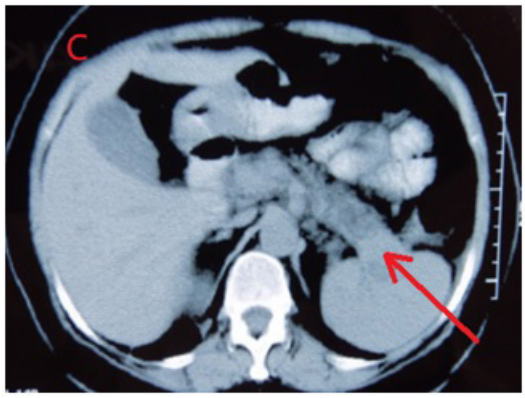


Figure 1C: After CDPI treatment tow months, the tumor at tail of pancreas was shrink and stable for a long time of two years. It showed that CDPI treatment can control pancreatic cancer for a long time not grow.

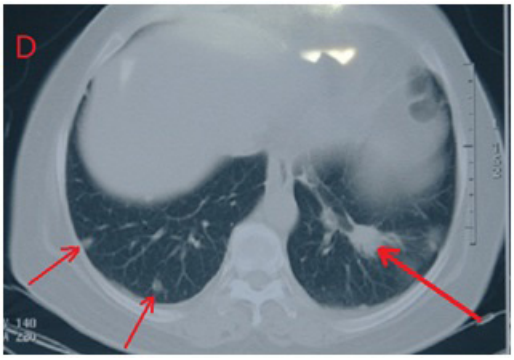


Figure 1D: Two years late, it was found that metastasis in left lung start to grow and old small mass was bigger than two years ago in the right lung. It indicated that CDPI therapy can not only control primary mass of pancreatic cancer but also can control small metastasis grow slowly for a two years by abscapale effect which induced by CDPI to whole body immunological systemic therapy. In the general, patient with pancreatic cancer with lung metastasis, there is few to live over one year, but she live over two years in tumor stable status after CDPI therapy, it indicated that CDPI could induce the immunologial response like cytokines and autologous antibodies in the research (left two arrows shows two of small mass as new metastasis and right arrow shows a small bigger mass as a old metastasis without PET/CT and pathological proved).

4.4. Analysis of cytokines in patients with pancreatic cancer

By comparing the general cytokine level between all patients with and without hapten after treatment. (Table 4) shows that the levels of IFN-γ and IL-4 in the hapten (CDPI) group increased, and the levels of IL-12 in the non-hapten (CDI) group increased. In the previous chemotherapy-free group, the levels of IL-6, IL-10, IFN-γ, IL-4, and IL-17 in CDPI were higher than those in the CDI group ($P < 0.05$); in patients who had received chemotherapy, IFN-γ, CCL3, IL-13, Collagen IVα1, TIMP-1 and other cytokines have lower response levels ($P > 0.05$). There are still significant differences compared with the control group. By comparing with the hapten (CDPI) group with or without prior chemotherapy, the results showed that the levels of CXCL8 and IFN-γ were increased in patients in the chemotherapy group ($P < 0.05$), while the levels of adiponectin, IL-13, Resistin, Collagen IV alpha 1 and TIMP-1 was Increased in the chemotherapy group ($P < 0.05$); the reason may be due to prior chemotherapy may inhibit or destroy some of the patient's immune function, and therefore have different responses to CDPI treatment.

Table 4: Analysis of Cytokines after and before of CDPI & CDI therapy and control group

Comparison between the Hapten, non-hepten and control group						
Name of Cytokines	Hapten (CDPI) N=20	Non-Hapten (CDI) N=8	Control N=3	$P_{CDPI\ V/S\ CDI}$	$P_{CDPI\ V/S\ Control}$	$P_{CDI\ V/S\ Control}$
IFN-gamma	94.89±42.5↑	83.96±9.72	46.04±8.67	0.467	0.034	0.125
IL-12 p70	219.94±32.35↑	220.12±32.27↑	177.92±24.29	0.989	0.042	0.06
IL-4	273.47±76.67↑↑	196.45±95.33	168.12±51.5	0.030	0.043	0.607
Comparison between patients without prior chemotherapy						
	Hapten (CDPI) N=11	Non Hapten (CDI) N=6	Control N=3	$P_{CDPI\ V/S\ CDI}$	$P_{CDPI\ V/S\ Control}$	$P_{CDI\ V/S\ Control}$
IL-6	10.95±3.8↑↑	7.18±1.67	5.6±0.63	0.027	0.016	0.474
IL-10	10.3±2.61↑	9.47±1.78	6.46±3.06	0.511	0.028	0.102
IFN-gamma	114.82±48.05↑	86.52±9.58	46.04±8.67	0.154	0.012	0.144
CCL3	1334.11±547.68	1509.48±371.88	775.27±309.07	0.479	0.090	0.044
IL-4	273.8±69.53↑	207.02±89.72	168.12±51.5	0.095	0.043	0.469
IL-17A	28.56±7.36↑	23.07±3.16	21.61±0.75	0.141	0.032	0.692
Comparison between patients with prior chemotherapy						
	Hapten (CDPI) N=9	Non Hapten (CDI) N=2	Control N=3	$P_{CDPI\ V/S\ CDI}$	$P_{CDPI\ V/S\ Control}$	$P_{CDI\ V/S\ Control}$
IFN-gamma	70.53±13.82	76.29±6.74	46.04±8.67↓	0.568	0.014	0.023
CCL3	1824.22±521.7	1856.82±190.66	775.27±309.07↓	0.931	0.006	0.028
IL-13	16377.38±2872.51	16677.32±4080.45	10971.67±564.42↓	0.892	0.013	0.044
Conllagen IV alpha 1	1419.67±810.72	2135.63±893.25	519.16±130.28↓	0.244	0.097	0.037
TIMP-1	108680.37±12295.43	117774.75±7485.08	59912±6552.02↓	0.316	0.000	0.000
Comparison between patient with and without prior chemotherapy CDPI group						
	No Prior chemotherapy N=11		Prior chemotherapy N=9		P	
CXCL8	332.67±289.52↑		63.33±44.59		0.012	
IFN-gamma	114.82±48.05↑		70.53±13.82		0.013	
Adiponectin	2355446.61±965403.62		3116080.08±372628.73		0.039	
IL-13	11384.59±4737.35		16377.38±2872.51↑		0.013	
Resistin	112550.05±96647.63		11653.5±11619.57↑		0.006	
Conllagen IV alpha 1	753.47±422.63		1419.67±810.72↑		0.029	
TIMP-1	64367.68±26321.01		108680.37±12295.43↑		0.000	

4.5. Analysis of autologous Antibody of TAA in pancreatic cancer patients

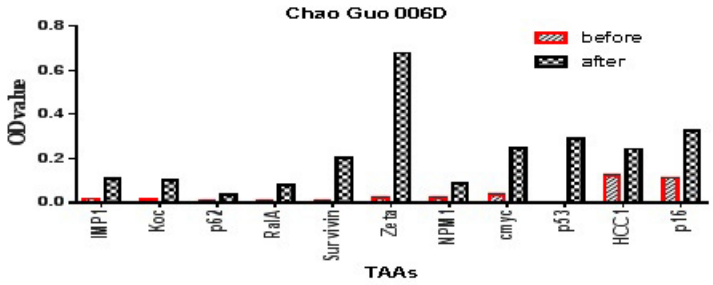
In general comparison, (Table 5) compares TAA autoantibodies between patients with hapten (CDPI) treatment group and non-hapten (CDI) treatment group. For the patients without prior chemotherapy, the level of the test genes has no statistically significant difference between hapten (CDPI) and non-hapten (CDI) groups (P>0.05). At the same time, there is a considerable difference in Zeta (P≦0.05) level between the two groups.

There was a significant difference in IMP1 levels between CDPI and CDI treatment groups (P <0.05). The IMP1 level increased in the CDI group, and the HCC1 level increased in the CDPI compared with the control group (P <0.05). For the CDPI treatment group, there were significant differences in TAA antibodies (such as RalA, Zeta, p16) between the groups with and without prior chemotherapy (P <0.05), there is an increase in RalA, Zeta, and p16 autoantibodies in the non-prior chemotherapy group; it may due to prior chemotherapy which may cause damage or inhibit some of the patient's immunological function and therefore have a different response to CDPI or CDI therapy (Fig. 2).

Table 5: Comparison of autolougus antibodies of TAA after and before of CDPI & CDI therapy and control group

Comparison between the Hapten, non-hepten and control group						
Name of Genes	Hapten (CDPI) N=20	Non Hapten (CDI) N=9	Control N=4	$P_{CDPI\ VS\ CDI}$	$P_{CDPI\ VS\ Control}$	$P_{CDI\ VS\ Control}$
IMP1	0.111±0.047	0.193±0.194	0.098±0.019	0.064	0.818	0.145
Koc	0.074±0.060	0.182±0.326	0.058±0.023	0.731	0.752	0.641
p62	0.029±0.025	0.025±0.019	0.032±0.028	0.618	0.820	0.589
RalA	0.058±0.039	0.047±0.028	0.059±0.023	0.429	0.975	0.576
Survivin	0.057±0.067	0.073±0.057	0.051±0.026	0.533	0.858	0.563
Zeta	0.080±0.054	0.203±0.239	0.050±0.027	0.416	0.343	0.254
NPM1	0.072±0.038	0.103±0.089	0.046±0.027	0.172	0.393	0.095
cmyc	0.285±0.736	0.173±0.129	0.165±0.093	0.641	0.713	0.981
p53	0.251±0.733	0.164±0.158	0.133±0.131	0.716	0.717	0.931
HCC1	0.128±0.056	0.168±0.067	0.180±0.036	0.098	0.113	0.733
p16	0.108±0.080	0.171±0.131	0.124±0.057	0.112	0.768	0.418
Comparison between patients without Prior chemotherapy before and after of CDPI and CDI Therapy						
	Hapten (CDPI)N=11	Non Hapten (CDI)N=7	Control N=4	$P_{CDPI\ VS\ CDI}$	$P_{CDPI\ VS\ Control}$	$P_{CDI\ VS\ Control}$
Zeta	0.108±0.043	0.253±0.250↑	0.050±0.027	0.05	0.503	0.037
Comparison between patient with prior chemotherapy before and after CDPI and CDI Therapy						
	Hapten (CDPI)N=9	Non Hapten (CDI)N=2	Control N=4	$P_{CDPI\ VS\ CDI}$	$P_{CDPI\ VS\ Control}$	$P_{CDI\ VS\ Control}$
IMP1	0.107±0.056	0.197±0.004↑	0.098±0.019	0.031	0.748	0.031
NPM1	0.055±0.017	0.085±0.002	0.046±0.027	0.074	0.425	0.037
HCC1	0.103±0.052	0.071±0.009	0.180±0.036	0.322	0.042	0.018
Comparison between patient with and without prior chemotherapy after CDPI Therapy						
	Non Prior Chemotherapy N=11		Prior Chemotherapy N=9		P	
RalA	0.074±0.035↑		0.039±0.035		0.038	
Zeta	0.108±0.043↑		0.047±0.047		0.007	
p16	0.166±0.061↑		0.037±0.018		0.000	

Notes：33 patients (hapten:20，no-hapten:9，control:4)



Date	treatment	sera ID	IMP1	Koc	p62	RalA	Survivin	Zeta	NPM1	cmyc	p53	HCC1	p16
2016.4.19	0	15	0.0185	0.0139	0.0081	0.0122	0.0076	0.0237	0.0209	0.0347	0.0019	0.1214	0.1119
2016.6.1	1	16	0.1085	0.0995	0.0374	0.0801	0.2041	0.6782	0.0878	0.2483	0.2882	0 .2423	0.3281

Figure 2: Example of autolougus antibodies form patient's blood was analysis before and after of therapy by Enzyme-linked immunosorbent assay (ELISA) (See above the table and chart).

5. Discussion

Pancreatic cancer is aggressive cancer characterized by a clear tendency to invasive and distant metastasize. Less than 20% of patients with pancreatic cancer can surgically remove resectable, borderline pancreatic cancer, and complications related to surgery are prevalent. Pancreatic cancer is also associated with a high concentration of multidrug resistance genes, so advanced pancreatic cancer can develop resistance to conventional treatment options, leading to suboptimal treatment effects [14]. Therefore, alternative drug delivery routes may be critical to achieving this clinical treatment goal to minimise toxicity and maximise therapeutic efficacy. Among all drug delivery routes, the percutaneous intra-tumoral way in our study combined with hapten cytotoxic drugs is considered a new option. Among these unresectable patients, the most tremendous potential is prolonging their survival and improving their quality of life. Pancreatic cancer Patient [15]. This is achieved by increasing drug concentration at the tumour site while minimizing systemic drug exposure and systemic toxicity [16].

In this clinical study, CDPI is the same as UMIPIC, and It is a patent combination [8-9] of treatment methods for solid tumours. In this clinic, it is explored in personalized doses according to tumour size, and at the same time, it uses patient-specific in vivo modification. The tumour antigens of human patients can be used as the specific response of auto-vaccine to the tumour. These regimens are the personalized, freshly prepared compound solutions containing oxidants, cytotoxic drugs and haptens. Each ingredient plays a vital role in the treatment. The drug combination in CDPI and CDI can penetrate the entire tumour matrix, even into tumour cells, and with the help of oxidants, it can be released continuously in the tumour for a long time [8-9, 17-18]. Coagulation can effectively transform the extracellular matrix (EM) and alter the morphology and biochemical components of the tumour, such as collagen, elastic fibres, reticular fibres, fibronectin, proteoglycans, hyaluronic acid, and other macromolecules, resulting in a soft semi-solid tumour [8-9]. When inflammation occurs, it also destroys the environmental conditions in which tumour cells grow. This may be due to the inflammatory response caused by coagulation or interaction with malignant cells and the high concentration of locally injected cytotoxic drugs [3,19].

The creation of an in situ vaccine library in tumours due to tumour-specific antigens is another attractive factor in the process of intratumoral chemotherapy [19]. In addition, CDPI induces vaccine-like effects in tumours and enhances system immunity by adding haptens [14]. When various autologous tumour antigens are released from tumour coagulation, cell death may trigger T cell response and induce effective immunity. These cell deaths are called "good deaths" [17, 20-21] because immunologic modulators (i.e., small molecule haptens embedded in denatured tumours) promote an in-vivo self-vaccination in the body thus triggering a weak immune response. Our clinical data and animal studies have shown that the immune response significantly improves after CDPI treatment, especially CD4 + T and B cell immunity (Tables 4 and 5) [18].

Because of the optimistic survival advantage of CDPI containing dual drugs and penicillin compared with CDI without penicillin (Table 3), the one-year survival time was significantly improved (52.63% vs 11.11%, $P < 0.01$), indicating that CDPI fully extended survival time. It may be due to long-term immune memory and more effective antitumor response by constitutive releasing of antigens in CDPI composed of penicillin and cytokines and TAA autologous antibodies related to the abscopal effect. Our published data show that inflammatory tumour cells attract different lymphocytes, including APCs, macrophages and DCs and then activate B cells and those that react with tumour-associated antigens (such as mesothelin tumour antigens, DNA, RNA, and other cell lysates) [22-25].

It was first discovered that TAA autoantibodies play a role in antitumor growth in this study. In earlier studies, there are many reports about TAA autologous antibodies that are only related to the diagnosis of cancer epidemiology [12], and there have never been reports related to cancer treatment. Oncogenes and tumour suppressing genes play an essential role in carcinogenicity. Their gene products are tumour-associated antigens (TAA) that can induce autoantibodies. Serum levels are reported to be related to cancer epidemiology. We discovered that the levels of several autoantibodies associated with hapten in therapy, such as HCC1, RalA, Zeta, and p16 genes, are related to survival time after heptane (CDPI) treatment and that the status of prior chemotherapy showed that less prior chemotherapy would respond to a higher degree of TAA autoantibodies. We don't know how TAA autoantibodies compete with cancer cells or how they get to cancer cells right now. If it works, it must penetrate the nucleus of cancer cells. Then it can play a role in fighting cancer by either inhibiting or destroying their gene products. Therefore, we need to conduct in-depth research further to understand the details of TAA autoantibodies in cancer treatment.

6. Conclusion

In conclusion, CDPI is A novel eclectic approach for treating pancreatic cancer. It is not only a de-bulking or chemical surgery for large tumour masses, but it also has the abscopal effect of generating systemic immunotherapy, including T and B cell function, which can synergistically eliminate leftover tumour cells throughout the body to protect against recurrence and metastasis. It offers the prospect of more precise tailoring treatments that may lead to better responses, especially in patients with advanced pancreatic cancer who are inoperable or drug-resistant. In the in-depth study of TAA autoantibodies, we need to clarify how they penetrate cancer cells, work with the cancer cell nucleus, and their relationship with the complement system reaction.

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