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ORIGINAL RESEARCH

No Significant Effect of Daikenchuto (TJ-100) on Peritoneal IL-9 and IFN-γ Levels After Pancreaticoduodenectomy

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Aim and Background: TJ-100 is a traditional Japanese medicine that affects inflammation and gastrointestinal motility, and is used as a preventive and treatment for paralytic ileus. This study aims at determining the effect of TJ-100 on the peritoneal levels of IFN-γ/IL-9, cytokines related to ileus, after pancreaticoduodenectomy (PD) in a clinical setting.

Methods: This was a subsidiary study of the clinical trial investigating the effect of TJ-100 on postoperative bowel function. Ascites was collected from 180 patients using an abdominal drainage tube on postoperative day 1 and 3 after PD (POD 1 or POD 3) and used to measure 27 cytokines. We performed univariate and multivariate analyses using several perioperative variables and administration of TJ-100/placebo to determine the effect of TJ-100 on the levels of IFN-γ and IL-9.

Results: Peritoneal levels of IL-9 and IFN-γ decreased between POD 1 and 3 (Wilcoxon signed-rank test $p<0.001$). Multivariate analysis was performed after univariate analysis to select the variables and patients with a body mass index of ≥ 22 kg/m^2, older age, use of epidural anesthesia, and longer surgery correlated with the levels of IL-9 and IFN-γ. However, we could not detect a correlation between the use of TJ-100 and cytokine levels in ascites either on POD 1 or 3.

Conclusion: TJ-100 did not affect peritoneal IL-9 and IFN-γ levels after PD. This was in accordance with published clinical findings showing no improvement in bowel function after PD and TJ-100 treatment.

Keywords: TNF, Kampo, Daikenchuto, herbal medicine, pancreatic cancer

Introduction

Cytokines regulate immune response and organ regeneration.[1–3] Owing to this, cytokines and their receptors have gained importance as novel targets for disease control and/or treatment.[4–6] Cytokine levels are investigated as potential biomarkers for postoperative complications, such as surgical site infection, anastomotic leakage,[7–9] and postoperative ileus.[10]

TJ-100 is a traditional Japanese herbal medicine that is used to treat conditions like paralytic ileus. The protective effect of TJ-100 on paralytic ileus after pancreaticoduodenectomy (PD) has been investigated by a multicenter randomized clinical trial. However, routine use of TJ-100 did not show any clinical benefits during the perioperative period.[11] Nevertheless, TJ-100 regulated the serum levels of interleukin (IL)-4, IL-9, IL-10, PDGF-BB, and TNF-α in patients without severe inflammation.[12] This suggests that TJ-100 exerts its effects under specific conditions that should be further studied in the future.

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In this study, we have measured peritoneal cytokine levels after PD to determine the effects of TJ-100. Since there is a correlation between IFN-γ and IL-9 levels and paralytic ileus, peristalsis, and bowel inflammation,[13–18] we focused our study on IFN-γ and IL-9.

Methods

Trial Design and Patient Recruitment

The protocol employed in the main clinical trial has been previously described [10]. Patients undergoing PD for periampullary tumor or pancreas head tumors were assessed for eligibilities and enrolled. Patients were randomized according to four clinical variables that influence postoperative bowel movement. Five grams of TJ-100 (TJ-100 Group) or Placebo (Placebo Group) were administered three times per day 3 days before surgery until postoperative day (POD) 14. TJ-100 (alternative name, Daikenchuto) is composed of Japanese pepper (Zanthoxylum fruit), processed ginger (Zingiberis Siccatum Rhizoma), and ginseng (Ginseng radix).[19]

Ascites was collected for a subsidiary study on POD 1 and 3 using an abdominal drainage tube. The samples were stored at lower than −70°C. Cytokine levels were measured by LSI Medience Corporation (Tokyo, Japan) using the Human Cytokine 27-plex assay (Bio-Rad Laboratories, California) and Bio-Plex 200 System (Bio-Rad Laboratories).

The institutional review board (IRB) of each participating institute approved the trial protocol after initial approval from the IRB of Wakayama Medical University (ID:1089). This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Patients provided written informed consent before randomization and intervention. Additional written informed consent was obtained for cytokine analysis. The study was registered at the UMIN Clinical Trial Registry (No. 000007975) and at ClinicalTrials.gov (No. NCT01607307).

Statistical Analysis

Values under the lower measurement limit were assigned the lower limit instead of discarding the data. The changes in the peritoneal levels of IL-9 and IFN-γ between samples from POD 1-3 and TJ-100/Placebo groups were assessed by the Wilcoxon signed-rank test.

The effect of TJ-100 on the peritoneal cytokine levels was studied using regression analysis. After log-transformation of the peritoneal IL-9 and IFN-γ levels, univariate regression analysis was performed to assess the correlation between the cytokines and variables. Variables used included the amount of TJ-100, age, gender, preoperative pathology, preoperative levels of gamma-glutamyl transpeptidase, estimated blood loss, length of surgery, presence of neoadjuvant chemotherapy, tumor stage, body mass index (BMI), use of epidural anesthesia, degree of lymph node dissection, presence of pancreatic fistula, and presence of abdominal abscess. Subsequently, we performed a multivariable regression analysis to determine the correlation between cytokine levels and use of TJ-100 and other factors chosen via univariate analysis with p-value <0.1. p-Value <0.05 was considered statistically significant.

Results

Patients

Drainage fluid was collected from 180 patients. The TJ-100 and Placebo group comprised 91 and 89 patients, respectively. We have previously summarized the clinical variables of these patients.[12] The tumor characteristics, operation variables, and patient characteristics were similar between the two groups. However, the mean age of the patients was slightly higher in the TJ-100 group than that of the Placebo group (64.9±8.0 vs 65.1±11.4, p=0.004, *t*-test).

Changes in Peritoneal IFN-γ and IL-9 Between Day 1 and 3

The median level of peritoneal IL-9 on POD 1 was 26.8 pg/mL (range: 10.2–4855.6 pg/mL) following which it decreased to 17.7 pg/mL (range: 10.2–2686.1 pg/mL) on POD 3 (Wilcoxon signed-rank test, p<0.001; Figure 1). The median level of peritoneal IFN-γ on POD 1 was 270.5 pg/mL (range: 19.0–1862.7 pg/mL) that decreased to 140.0 pg/mL (range: 6.5–1823.6 pg/mL) on POD 3 (Wilcoxon signed-rank test, p<0.001).

Comparison of Cytokine Levels Between TJ-100 and Placebo Groups

There were no significant differences between peritoneal IL-9 and IFN-γ levels between the TJ-100 and Placebo groups through POD 1-3 (Table 1). Cytokine levels differed widely among individuals. The levels of peritoneal IL-9 in the TJ-100 group on POD 1 ranged between 10.2 and 4855.6 pg/mL, while that in the Placebo Group on POD 1 ranged between 10.2 and 738.2 pg/mL.

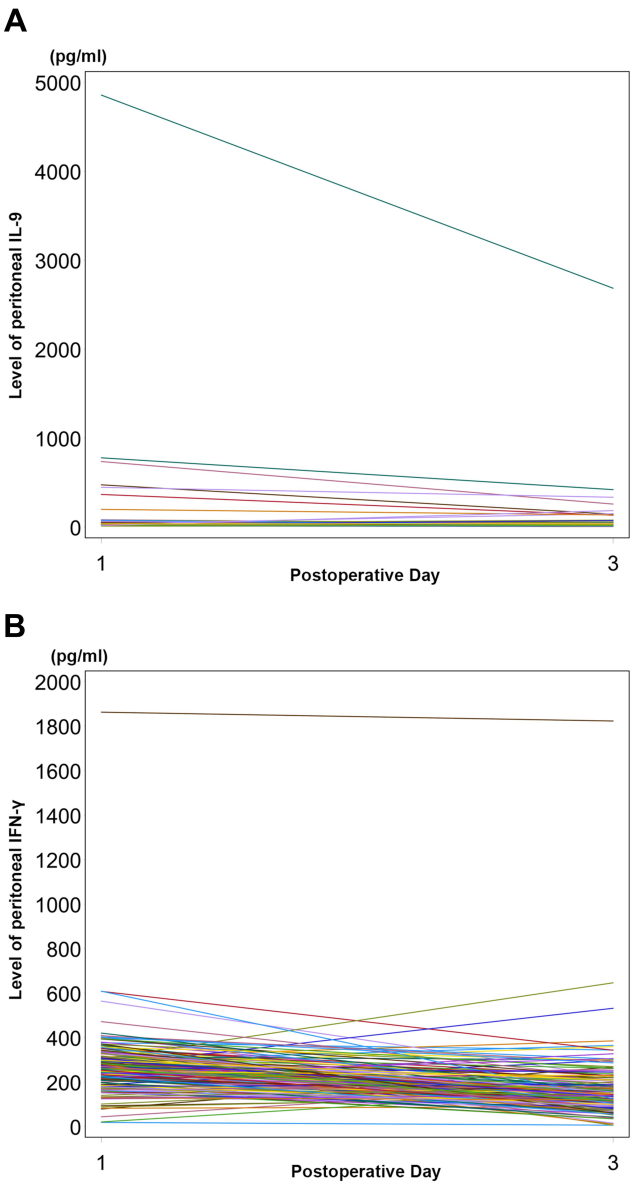

Figure 1 Peritoneal IL-9 and IFN-γ levels in patients between postoperative days 1 and 3. IL-9 (**A**) and IFN-γ (**B**) levels decreased between POD 1 and 3 in individual measurements. The peritoneal IL-9 levels accumulated in a narrow range (**A**), while variety of the levels were observed in IFN-γ. The patient having a high IL-9 level was different from the patient having high IFN-γ level. These two patients did not have sever postoperative complications or significant preoperative clinical features related to inflammation.

Univariate and Multivariable Analyses for Regulatory Factors for IFN-γ and IL-9 Levels

Univariate regression analysis helped identify the factors influencing the peritoneal IL-9 through POD 1-3 (Table 2). On POD 1, patients younger than 65 years showed lower IL-9 levels than that in the other categories. Longer surgical time positively correlated with IL-9 levels. On POD 3, patients with higher BMI had lower IL-9 levels as compared to that in the patients from other categories. Other variables, including gender, preoperative pathology, preoperative level of gamma-glutamyl transpeptidase, presence of neoadjuvant chemotherapy, tumor stage, degree of lymph node dissection, presence of pancreatic fistula, and presence of abdominal abscess, did not correlate with the IL-9 levels through POD 1-3. Multivariable regression analysis suggested that patients with a BMI between 22 and <25 kg/m^2 exhibited lower levels of IL-9 on POD 3.

Based on the univariate regression analysis of IFN-γ levels (Table 3), patients between 65 and <70 years correlated with higher levels of IFN-γ on POD 1. Patients with surgical time >480 min also showed higher levels of IFN-γ ranging POD 1-3. The use of epidural anesthesia was associated with decreased levels of IFN-γ on POD 3. Using the multivariable analysis, longer surgical times also correlated positively with higher levels of IFN-γ between POD 1 and 3; the use of epidural anesthesia correlated with decreased levels of IFN-γ on POD 3.

However, we found no correlation between the use of TJ-100 and peritoneal levels of IL-9 or IFN-γ on POD 1 or 3 (Tables 2 and 3).

Discussion

In animal models, TJ-100 suppresses the expression of IFN-γ in target organs, thereby reducing bacterial translocation[17] or inflammation of the large bowel.[18] Suppressed IFN-γ is associated with postoperative ileus.[15] It has also been shown that IL-9 levels enhance intestinal muscle contractility.[10,13] Therefore, we focused on these two cytokines despite the availability of the remaining 25 cytokines. We could not demonstrate that TJ-100 altered peritoneal cytokine levels after PD. The strong intervention with PD may be attributed to the discrepancy between the data from the animal models and this study. PD involves the manipulation of multiple organs, such as pancreas, stomach, small intestine, and gall bladder. We speculate that the intense stress associated with surgery may have masked the effects of TJ-100.

In this study, we found that the use of epidural anesthesia suppressed IFN-γ levels on POD 3. Previous reports have shown that the decreased expression of IFN-γ in target organs is related to reduced postoperative paralytic ileus.[15] Thus, the use of epidural anesthesia may have reduced paralytic ileus. However, our previous clinical study demonstrated that the use of epidural anesthesia is an independent risk factor for paralytic ileus after PD.[11]

Table 1 Peritoneal Levels of IL-9 and IFN-γ Within the TJ-100 and Placebo Groups

	TJ-100 (n=91)			Placebo (n=89)			p-value
	n	Median (pg/mL)	[Min–Max] (pg/mL)	n	Median (pg/mL)	[Min–Max] (pg/mL)	
Day 1							
IL-9	91	27.6	[10.2–4855.6]	88	25.0	[10.2–738.2]	0.31
IFN-γ	91	274.8	[19–1862.7]	88	267.7	[20.6–608.2]	0.26
Day 3							
IL-9	91	17.3	[10.2–2686.1]	89	18.4	[10.2–336.7]	0.74
IFN-γ	91	140.1	[6.5–1823.6]	89	134.6	[6.5–646.1]	0.67

Table 2 Univariate and Multivariate Regression Analyses for IL-9 Levels After Log Transformation

			Univariate		Multivariate	
		n	Coefficient	p	Coefficient [95% CI]	p
Log IL-9, POD 1						
Use of TJ-100	No	88	Reference		Reference	
	Yes	91	0.09	0.43	0.060 [−0.168, 0.289]	0.60
Age (years)	<65	53	Reference		Reference	
	65<70	50	0.279	0.06	0.260 [−0.030, 0.551]	0.08
	70<75	35	0.146	0.38	0.149 [−0.176, 0.473]	0.46
	75≤	41	0.108	0.50	0.098 [−0.215, 0.409]	0.54
BMI (kg/m^2)	<20	43	Reference			
	20<22	51	−0.157	0.32		
	22<25	52	−0.012	0.94		
	25≤	33	−0.092	0.60		
Surgical time (min)	<360	60	Reference		Reference	
	360<420	42	−0.156	0.30	−0.152 [−0.45, 0.147]	0.32
	420<480	35	0.264	0.10	0.248 [−0.072, 0.568]	0.13
	480≤	42	0.128	0.40	0.102 [−0.198, 0.401]	0.51
Log IL-9, POD 3						
Use of TJ-100	No	89	Reference		Reference	
	Yes	91	0.005	0.97	−0.031 [−0.26, 0.199]	0.79
Age (years)	<65	54	Reference			
	65–70	50	0.105	0.50		
	70–75	35	−0.050	0.77		
	75≤	41	0.098	0.55		
BMI (kg/m^2)	<20	43	Reference		Reference	
	20<22	51	−0.226	0.16	−0.234 [−0.547, 0.079]	0.14
	22<25	53	−0.322	0.05	−0.317 [−0.628, −0.007]	0.05
	25≤	33	−0.210	0.25	−0.332 [−0.694, 0.03]	0.07
Surgical time* (min)	<360	60	Reference		Reference	
	360<420	42	−0.100	0.53	−0.111 [−0.416, 0.195]	0.48
	420<480	36	0.265	0.11	0.304 [−0.023, 0.63]	0.07
	480≤	42	0.187	0.23	0.205 [−0.105, 0.515]	0.19

Notes: *Surgical time for Log IL-9 of POD 3 was a component of the multivariate analysis although univariate analysis showed that the p-value for category 420<480 min was 0.11. We observed a significant effect of surgical time on POD 1 IL-9 levels and POD 1/3 IFN-γ levels, and thus, included surgical time as a variable for the multivariate analysis of POD 3 IL-9 levels.
Abbreviation: BMI, body mass index.

Table 3 Univariate and Multivariate Regression Analyses for IFN-γ Levels After Log Transformation

Log IFN-γ, POD 1						
Use of TJ-100	No Yes	88 91	Reference 0.070	 0.32	Reference 0.05 [−0.09, 0.19]	 0.48
Age (years)	<65 65<70 70<75 75≤	53 50 35 41	Reference 0.205 0.102 0.160	 0.03 0.32 0.10	Reference 0.194 [0.015, 0.372] 0.089 [−0.11, 0.288] 0.185 [−0.007, 0.376]	 0.03 0.38 0.06
Epidural anesthesia	No Yes	53 126	Reference −0.057	 0.464		
Surgical time (min)	<360 360<420 420<480 480≤	60 42 35 42	Reference 0.117 0.139 0.274	 0.21 0.16 <0.01	Reference 0.140 [−0.044, 0.323] 0.140 [−0.056, 0.337] 0.274 [0.09, 0.458]	 0.14 0.16 <0.01
Log IFN-γ, POD 3						
Use of TJ-100	No Yes	89 91	Reference 0.064	 0.53	Reference 0.035 [−0.162, 0.232]	 0.73
Age (years)	<65 65<70 70<75 75≤	54 50 35 41	 0.046 −0.093 −0.062	 0.73 0.53 0.66		
Epidural anesthesia	No Yes	54 126	Reference −0.184	 0.09	Reference −0.255 [−0.475, −0.034]	 0.02
Surgical time (min)	>360 360–420 420–480 <480	60 42 36 42	Reference 0.161 0.091 0.241	 0.23 0.52 0.08	Reference 0.218 [−0.049, 0.484] 0.132 [−0.148, 0.413] 0.320 [0.048, 0.592]	 0.11 0.36 0.02

The discrepancy between the present findings and expected results from polished studies (increased IFN-γ in patients with epidural anesthesia) may suggest that paralytic ileus is influenced by multiple factors: IFN-γ levels may not be able to predict the occurrence of paralytic ileus after PD or control paralytic ileus.

This study has limitations, one of which is the lack of the cytokine measurement before surgery. Although collecting ascites before surgery is impractical in our setting, the data would allow us to understand the transition of the cytokine levels more clearly.

In conclusion, the present study did not demonstrate the effect of TJ-100 on peritoneal IL-9 and IFN-γ after PD. Thus, these cytokines may not be promising biomarkers or therapeutic targets of paralytic ileus.

Data Sharing Statement

All available data are described within this manuscript.

Ethics Approval and Consent to Participate

All patients provided written informed consent prior to study inclusion. The institutional review board of each participating institute approved the trial protocol.

Patient Consent for Publication

All patients provided written informed consent.

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Disclosure

Yasuhiro Kodera reports personal fees from Taiho Pharma, personal fees from Chugai Pharma, personal fees from Lilly Japan, personal fees from Johnson &

Johnson, personal fees from Takeda, personal fees from Yakult, personal fees from Otsuka, personal fees from Ono Pharma, personal fees from Covidien, personal fees from MSD, outside the submitted work. The authors declare that they have no other competing interests for this work.

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