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Original Article

Regional arterial infusion with 5-fluorouracil attenuates severe acute pancreatitis in a rat model

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Abstract: Administration of 5-fluorouracil (5-FU) has been demonstrated to be beneficial in treating severe acute pancreatitis (SAP). However, drugs administered by intravenous injection (IVI) have difficulty to reach the pancreatic tissue. In this study, we aimed to assess the effects of 5-FU administered with regional arterial infusion (RAI) on severe acute pancreatitis (SAP) in rats. Rats were randomly divided into 3 groups: the SAP group as a control group, the RAI group (5-FU infused through an arterial catheter), and the IVI group (5-FU infused through a left femoral venous catheter). Pancreatitis was induced by retrograde injection of 5% sodium taurocholate (1 ml/kg) into the main pancreatic duct. The pathologic changes in the pancreas were observed and scored. Levels of amylase, lipase, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and interleukin-10 (IL-10) in the serum were measured at 12 h and 24 h following the induction of SAP. RAI with 5-FU remarkably alleviated histological injury of the pancreas, decreased serum amylase and lipase, and down-regulated serum TNF-α, IL-1, and the ratio of IL-6/IL-10 sodium taurocholate-induced SAP. This study showed that regional arterial infusion with 5-FU increased the drug concentration locally in the pancreas and attenuated the severity of SAP.

Keywords: Regional arterial infusion, cytokines, 5-fluorouracil, severe acute pancreatitis

Introduction

Severe acute pancreatitis (SAP) remains a clinical challenge and is still associated with significant morbidity, mortality and hospitalization costs despite of recent advances in management. The mortality of SAP has not been substantially reduced over the past two decades [1, 2].

5-Fluorouracil (5-FU) has been used to treat acute pancreatitis since the 1970s [3, 4]. 5-FU, a typical anti-metabolic drug, can entirely suppress the synthesis of DNA which controls the proliferation of acinar cells and RNA which manages the protein synthesis. Several studies in experimental pancreatitis have shown promising results using 5-FU treatment, including effects on amylase, trypsin and survival rates [3, 5]. Chen et al. confirmed that 5-Fu can minimize the abnormal immune cytokine response and relieve the pathophysiological disorders associated with experimental acute pancreatitis [6]. Clinical studies have corroborated that treatment with 5-FU can reduce the mortality and hospitalization time [7, 8]. The administration of 5-FU has been considered a popular adjuvant therapy for acute pancreatitis in China [9], due to its significant beneficial effects and affordable price. However, 5-FU, as a cytotoxic drug used for treating a nonmalignant disease, is not yet fully accepted in the western world. Therefore, we performed an animal study to test the efficacy and safety of 5-FU on SAP.

Continuous regional arterial infusion (CRAI) is a drug delivery system that dramatically increases the drug concentration in the pancreas with minimal toxic effects [5]. Previous clinical and basic studies have demonstrated the possible therapeutic efficacy of CRAI for SAP [10]. Compared with an intravenous infusion route, CRAI significantly shortens the duration of elevated urine amylase and the duration of

abdominal pain, decreases the incidence of complications and overall mortality, shortens the duration of hospitalization, and increases the curative rate [11]. However, a study by Hamada et al. [12], showed that CRAI was ineffective in reducing the in-hospital mortality rate in acute pancreatitis patients and was associated with a longer hospitalization time and higher costs. In such a case, a rigorous evidence-based assessment of animal experiments using of RAI for SAP is necessary and urgent. Therefore, in our study we assessed the efficacy and safety of RAI with 5-fluorouracil (5-FU) in the treatment of SAP in rats.

Materials and methods

Ethics statement

The Institutional Animal Committee of Wenzhou Medical University, Wenzhou, China, approved the protocol for the animal experiment (Permit Number: wydw2010-0027). All animals received care in accordance with the 'Guide for the Care and Use of Laboratory Animals'. All surgeries were performed under chloral hydrate anesthesia.

Materials

The following materials were used: sodium taurocholate (Sigma, St. Louis, MO, USA); 5-fluorouracil injection (King York Amino Acid Co. Ltd, Tianjin, China); PE-10 catheter (AniLab Software and Instruments Co. Ltd, Ningbo, China); plasma amylase activity test kit (Nanjing Jiancheng Biotechnology Research Institute, Nanjing, China); and rat ELISA kit for detecting IL-1, IL-6, IL-10, and TNF-α (Westang Biotechnology Co. Ltd, Shanghai, China).

Animals and groups

The Laboratory Animal Center of Wenzhou Medical University (Wenzhou, China) supplied 36 healthy male Sprague-Dawley (SD) rats (weighing 250-300 g). The rats were maintained under specific pathogen free (SPF) conditions and the temperature was kept at 20-22°C. A 12 h light-dark cycle was maintained, and all the rats were fed standard laboratory chow and were given water ad libitum. All experiments were performed on animals after 12 h of fasting with free access to water.

Thirty-six rats were randomly divided into 3 groups: the SAP group was the control group (n=12), the RAI group (5-FU infused through an arterial catheter) (n=12), and the IVI group (5-FU infused through a left femoral venous catheter) (n=12). In all the three groups, SAP was induced through the retrograde intraductal infusion of 5% sodium taurocholate. After pancreatitis was induced, regional arterial infusion was performed using physiological saline for the SAP group, regional arterial infusion (RAI) was performed using 5-FU (40 mg/kg) for the RAI group and for the IVI group, the left gastric artery was ligated and an intravenous injection (IVI) with 5-FU (40 mg/kg) was performed through the left femoral vein (n=12). Every group was divided into 2 subgroups, namely, 12 h and 24 h subgroup (n=6).

Induction of severe acute pancreatitis and regional arterial infusion procedure

All procedures were performed in sterile conditions. The experimental animals were anesthetized through an intraperitoneal injection of 3 ml/kg of a 10% chloral hydrate solution. According to Aho et al. [13], SAP was induced through a retrograde injection of 5% sodium taurocholate (1 ml/kg) into the main pancreatic duct using a microinjection pump at a speed of 0.1 ml/min. After the model of SAP was established, the dorsal side of the stomach was clearly exposed. The celiac axis was identified giving off three branches, i.e., the splenic artery, the left gastric artery and the common hepatic artery (**Figure 1**).

After the left gastric artery was explored, the distal site was ligated with 4-0 silk to engorge the artery. A PE-10 catheter (inner diameter 0.25 mm, outer diameter 0.5 mm) with a sharp tip was carefully inserted through the left gastric artery in a retrograde direction to the celiac axis. It was necessary to ascertain an accurate position of the tip of the catheter. In our experiments, we found a lymph node around the celiac axis (**Figure 1e**), which helped to access the accurate position of the celiac axis. The location of the catheter tip was confirmed through the injection of a methylene blue solution (0.1 ml), which stained the whole pancreas. When the catheter was well placed, 40 mg/kg of 5-FU (this dosage is equal to 10-15 mg/kg in humans based on body surface) was administered through this artery at a speed of 0.1 ml/min in

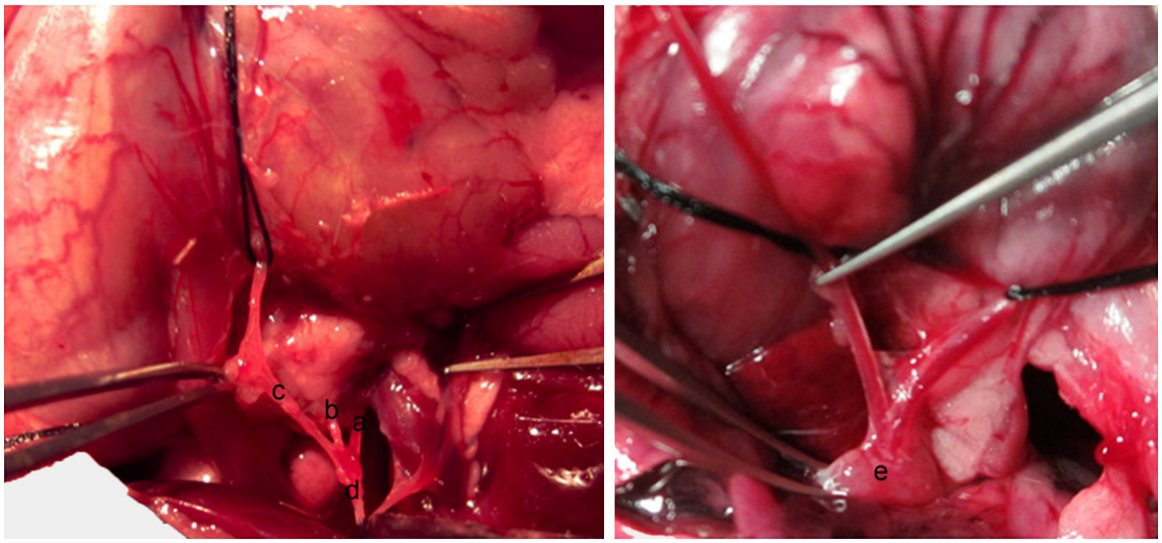

Figure 1. The dissection of the celiac axis and its branches, and the procedure for arterial cannulation. The celiac axis (a) gives off three branches counterclockwise, i.e., the splenic artery (b), the left gastric artery (c), and the common hepatic artery (d). With a catheter inserted from the left gastric artery towards the celiac axis, the accurate position of the catheter tip was detected using microscopic forceps. When the catheter reached the accurate position (e), 0.1 ml of a methylene blue solution was injected to confirm the optimal catheter placement.

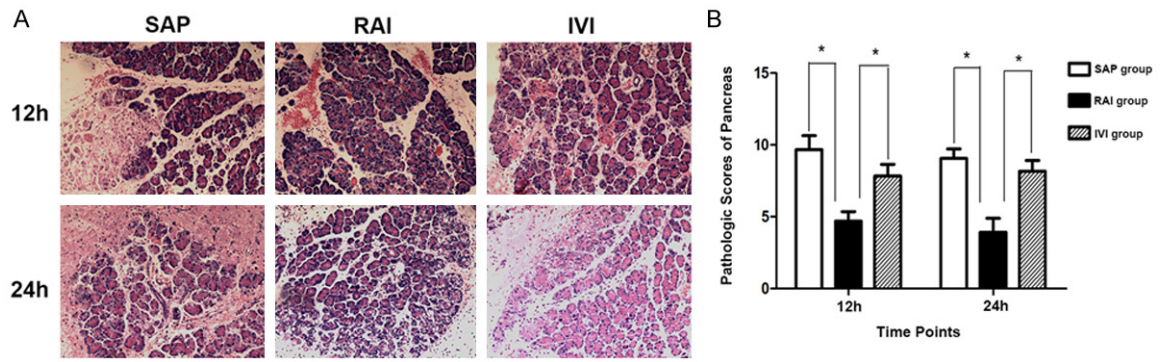

Figure 2. A. Pathological examination of the pancreas in different groups observed using H&E staining (×100). B. The pathological score of the pancreas in the RAI group was significantly lower than the other groups at both time points (*P<0.05).

the RAI group. After the infusion, the proximal site of the left gastric artery was ligated with 4-0 silk thread. An equal dosage of 5-FU was administered through the left femoral vein in the IVI group (with the left gastric artery ligated for strict control). The SAP group was administered the same volume of physiological saline via a regional arterial infusion.

Sampling and determination of the pathological changes

Blood and the pancreas were collected 12 and 24 h after drug delivery. The tissues of pancreas was fixed in 10% buffered formalin and embedded in paraffin, and sections were stained with hematoxylin and eosin (H&E) for light microscopy. The pathological grading of the pancreatic injury takes all factors, such as pancreatic edema, hemorrhage, inflammation, as well as necrosis, into consideration by following the criterion of Schmidt et al. [14]. The pathological sections were examined by an experienced pathologist unaware of the treatment groups, that is, in a blinded fashion.

Serum amylase and lipase activity

Serum amylase activity was determined by means of iodine-amylum colorimetry and the

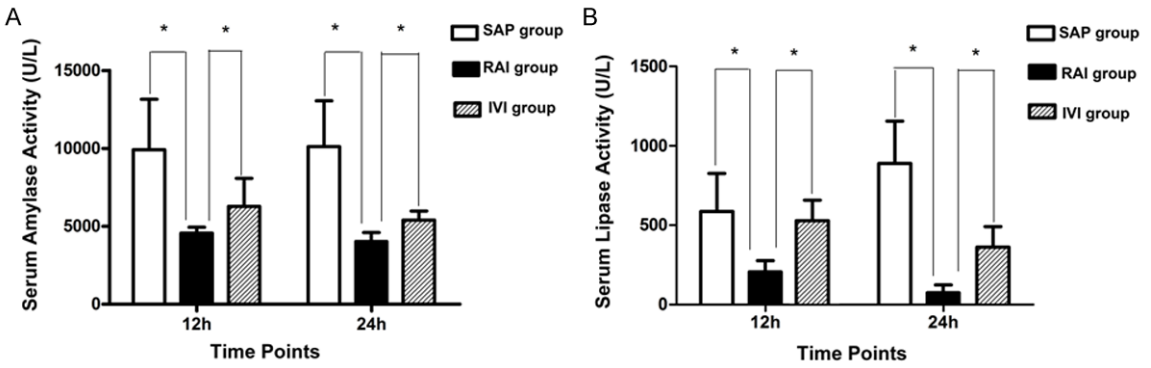

Figure 3. The activities of the serum amylase (A) and lipase (B) in the RAI group were significantly lower than the other groups (*P<0.05).

serum lipase activity was determined by means of enzymatic colorimetry, both expressed in U/L.

Levels of serum IL-1, IL-6, IL-10, and TNF-α

Levels of serum IL-1, IL-6, IL-10, and TNF-α were determined by means of enzyme-linked immunosorbent assay (ELISA) and are expressed in pg/mL.

Statistical analysis

Statistical package for the social sciences software (SPSS, version 18.0) was used to compute the mean ± standard deviation (SD). Statistical analysis was analyzed using one-way analysis of variance (ANOVA) or rank-sum test. The levels of serum IL-1, TNF-α, amylase and lipase among different groups were analyzed using SNK-q test as a post-hoc test. The pathological score of the pancreas and the ratio of IL-6/IL-10 were analyzed using the Nemenyi test. For all analyses, the statistical significance was defined as P<0.05.

Results

Pathological examination of the pancreas

The pathological examination of the pancreas showed that, in the SAP group, the glandular structure was completely deranged, and the pancreatic acinar cells presented massive areas of necrosis infiltrated with a large number of inflammatory cells; these features were compatible with SAP. In the IVI group, inflammatory cell infiltration and hemorrhage were slightly attenuated. However, in the RAI group, necrosis was remarkably ameliorated, and edema and limited inflammatory cells were observed compared with the SAP group and the IVI group (**Figure 2**).

Importantly, the pathological score of the pancreas in the RAI group was significantly less than the other two groups at both time points (P<0.05) (**Figure 2**).

Activity of serum amylase and lipase

The activities of serum amylase and lipase in the RAI group were significantly lower than those in the other two groups at both time points (P<0.05) (**Figure 3**). However, there was no significant difference between the SAP group and the IVI group.

Levels of serum cytokines

Serum samples were collected at two time points for the measurement of IL-1, TNF-α, IL-6, and IL-10, and the results were shown in **Figure 4**. Both the RAI and IVI groups showed a lower level of serum IL-1, TNF-α and the ratio of IL-6/IL-10 after SAP induction, though the level of serum IL-1 and the ratio of IL-6/IL-10 had no significance difference between the IVI group and the SAP group at 12 h. In contrast, RAI with 5-FU significantly reduced the level of serum IL-1, TNF-α, and the ratio of IL-6/IL-10 at 12 h and 24 h (P<0.05). For the concentrations of serum cytokines, the RAI group had a profoundly lower level of serum IL-1 when compared with the other two groups. These data indicated that the onset time of 5-FU in the RAI group was earlier than in the IVI group likely due to the regional arterial infusion had a greater drug concentration than the intravenous injection.

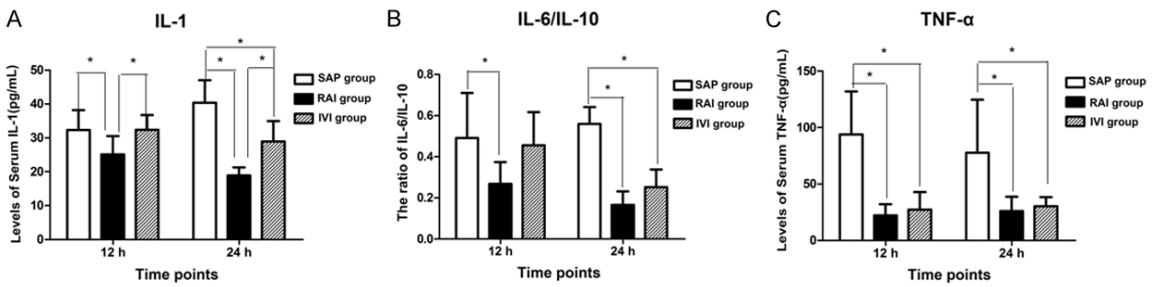

Figure 4. The levels of serum IL-1, TNF-α and the ratio of IL-6/IL-10 in the RAI group were much lower than the SAP group at both time points ($P<0.05$). The RAI group had a significant lower level of serum IL-1 compared with the other two groups ($P<0.05$).

Discussion

The RAI technique is a useful drug delivery system as it delivers the drug to the exact position of the disease, which dramatically increases the tissue concentration of the therapeutic drug [15]. However, the critical point of RAI is to place the catheter as close as possible to the artery that supplies the inflamed area. In pancreatitis, if the main region of the inflammation is located in the pancreatic head, the catheter tip should be placed in the common hepatic artery, the gastroduodenal artery or the superior mesenteric artery. If the main region of inflammation is located in the pancreatic body-tail, the catheter tip should be placed in the splenic artery or the dorsal pancreatic artery. Furthermore, if the inflamed area extends to the entire pancreas, the celiac axis would be the best choice [16].

5-FU is an anti-metabolic and immunosuppressive agent that can induce apoptosis of activated macrophages [17], minimize the abnormal immune cytokine response, and relieve the pathophysiological disorders associated with experimental and clinical acute pancreatitis [6, 9]. However, a clinical trial by Iikka A [8] showed that treatment with 5-FU slightly decreased the amylase and trypsin levels, but did not achieve a significant level. It is generally accepted that a therapeutic trial in humans should have a scientific background based on animal studies. Therefore, before conducting a prospective double-blind clinical trial that investigates the effects of 5-FU in acute pancreatitis, we must answer one essential question: does 5-FU have beneficial effects on experimental pancreatitis?

Histological examination of the pancreas showed that RAI with 5-FU could suppress the sodium taurocholate-induced edema, the infiltration of inflammatory cells, the necrosis and the vacuoles (especially for pancreatic necrosis) in SAP rats. Moreover, RAI with 5-FU resulted in a significantly decrease in serum amylase and lipase activity compared with SAP rats. In contrast, IVI with 5-FU failed to show a significant effect. This discrepancy should be ascribed to the use of RIA technique, which could increase the 5-FU concentration in the pancreatic tissue [18]. 5-FU can directly reach the pancreas via the RAI delivery system, but intravenous administration cannot reach an effective concentration due to the pharmacokinetic characteristics and impaired microcirculation during the course of SAP.

Several studies have indicated that inflammatory mediators, such as IL-1, TNF-α, are essential to the development of AP [19, 20]. Furthermore, the IL-6/IL-10 ratio has been reported to be a more representative biochemical indicator to reveal an increase in a more severe stage of acute pancreatitis [21, 22]. In the present study, both RAI and IVI groups showed lower level of serum IL-1, TNF-α, and the ratio of IL-6/IL-10 after SAP induction. However, the level of serum IL-1 and the ratio of IL-6/IL-10 showed no significant difference between the IVI group and the SAP group at 12 h. In contrast, RAI with 5-FU significantly reduced the level of serum IL-1, TNF-α, and the ratio of IL-6/IL-10 at 12 h and 24 h. These data indicate that 5-FU can reduce the inflammation-associated cytokines, and the onset time of 5-FU in the RAI group was earlier than the IVI group likely because the regional arterial infusion had a greater drug concentration than the intravenous injection.

There were some limitations of this study that need to be clarified. First, the experimental

study of RAI is quite different from the clinical practice, which is conducted using the Seldinger method making use of digital subtraction angiography (DSA). Second, we began RAI soon after the induction of SAP, which is different from real clinical practice in patients. In this condition, the clinical value of this therapy needs further evaluation.

In conclusion, RAI with 5-FU exhibits strong therapeutic effects, including alleviating pancreas injury, decreasing the activity of serum amylase and lipase, and reducing the inflammation-associated cytokines during the course of SAP with great safety. Due to the beneficial effects of RAI with 5-FU on SAP, we support a prospective double-blind, multi-center clinical trial to further evaluate the potential benefits of RAI with 5-FU in patients with acute pancreatitis.

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Disclosure of conflict of interest

None.

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References

[1] Bai Y, Liu Y, Jia L, Jiang H, Ji M, Lv N, Huang K, Zou X, Li Y, Tang C, Guo X, Peng X, Fang D, Wang B, Yang B, Wang L and Li Z. Severe acute pancreatitis in China: etiology and mortality in 1976 patients. Pancreas 2007; 35: 232-237.

[2] Piascik M, Rydzewska G, Milewski J, Olszewski S, Furmanek M, Walecki J and Gabryelewicz A. The results of severe acute pancreatitis treatment with continuous regional arterial infusion of protease inhibitor and antibiotic: a randomized controlled study. Pancreas 2010; 39: 863-867.

[3] Johnson R, Barone R, Das Gupta TK and Nyhus LM. Treatment of experimental acute pancreatitis with 5-fluorouracil (5FU). Rev Surg 1973; 30: 64-66.

[4] Kinami Y, Miyazaki I, Kawamura M, Sugii M and Sakane Y. Clinical effects of anticancer drugs to pancreatic diseases as protein synthesis inhibitors. Gastroenterol Jpn 1976; 11: 123-132.

[5] Mann SK and Mann NS. Effect of chlorophyll-a, fluorouracil, and pituitrin on experimental acute pancreatitis. Arch Pathol Lab Med 1979; 103: 79-81.

[6] Chen XL, Ciren SZ, Zhang H, Duan LG and Wesley AJ. Effect of 5-FU on modulation of disarrangement of immune-associated cytokines in experimental acute pancreatitis. World J Gastroenterol 2009; 15: 2032-2037.

[7] Laptev VV. [5-fluorouracil treatment of destructive pancreatitis]. Khirurgiia (Mosk) 1981; 67-73.

[8] Saario IA. 5-Fluorouracil in the treatment of acute pancreatitis. Am J Surg 1983; 145: 349-352.

[9] Gu F, Liu Y and Pan R. [Local arterial infusion of 5-FU in treatment of acute necrotic pancreatitis]. Zhonghua Wai Ke Za Zhi 1995; 33: 339-341.

[10] Zhou MT, Chen BC, Sun HW, Jin YP, Yang FJ, Zhang X, Andersson R and Zhang QY. Continuous regional arterial infusion with fluorouracil and octreotide attenuates severe acute pancreatitis in a canine model. PLoS One 2012; 7: e37347.

[11] Yong FJ, Mao XY, Deng LH, Zhang MM and Xia Q. Continuous regional arterial infusion for the treatment of severe acute pancreatitis: a meta-analysis. Hepatobiliary Pancreat Dis Int 2015; 14: 10-17.

[12] Hamada T, Yasunaga H, Nakai Y, Isayama H, Horiguchi H, Matsuda S, Fushimi K and Koike K. Continuous regional arterial infusion for acute pancreatitis: a propensity score analysis using a nationwide administrative database. Crit Care 2013; 17: R214.

[13] Aho HJ and Nevalainen TJ. Experimental pancreatitis in the rat. Ultrastructure of sodium taurocholate-induced pancreatic lesions. Scand J Gastroenterol 1980; 15: 417-424.

[14] Schmidt J, Lewandrowsi K, Warshaw AL, Compton CC and Rattner DW. Morphometric characteristics and homogeneity of a new model of acute pancreatitis in the rat. Int J Pancreatol 1992; 12: 41-51.

[15] Satoh H, Harada M, Tashiro S, Shiroya T, Imawaka H and Machii K. The effect of continuous arterial infusion of gabexate mesilate (FOY-007) on experimental acute pancreatitis. J Med Invest 2004; 51: 186-193.

[16] Ganaha F, Yamada T, Yorozu N, Ujita M, Irie T, Fukuda Y, Fukuda K and Tada S. Vascular access system for continuous arterial infusion of a protease inhibitor in acute necrotizing pan-

creatitis. Cardiovasc Intervent Radiol 1999; 22: 436-438.
[17] Wang W, Zhou X, Yang F, Sun H, Xu D, Andersson R, Chen B and Zhou M. The effects of 5-fluorouracil on severe acute pancreatitis-inducing apoptosis of macrophages. Pancreas 2014; 43: 660-663.
[18] Xie Y, Zhou Z, Li M, Duan W, Lv W, Sun H And Zhou M. Pharmacokinetic comparison between regional arterial infusionand intravenous injection with 5-fluorouracilin experimental severe acute pancreatitis. Latin Am J Pharmacy 2013; 32: 1-7.
[19] Hughes CB, Grewal HP, Gaber LW, Kotb M, Eldin AB, Mann L and Gaber AO. Anti-TNFalpha therapy improves survival and ameliorates the pathophysiologic sequelae in acute pancreatitis in the rat. Am J Surg 1996; 171: 274-280.
[20] Liu HS, Pan CE, Liu QG, Yang W and Liu XM. Effect of NF-kappaB and p38 MAPK in activated monocytes/macrophages on pro-inflammatory cytokines of rats with acute pancreatitis. World J Gastroenterol 2003; 9: 2513-2518.
[21] Ino Y, Arita Y, Akashi T, Kimura T, Igarashi H, Oono T, Furukawa M, Kawabe K, Ogoshi K, Ouchi J, Miyahara T, Takayanagi R and Ito T. Continuous regional arterial infusion therapy with gabexate mesilate for severe acute pancreatitis. World J Gastroenterol 2008; 14: 6382-6387.
[22] Ohmoto K and Yamamoto S. Serum interleukin-6 and interleukin-10 in patients with acute pancreatitis: clinical implications. Hepatogastroenterology 2005; 52: 990-994.