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Effects of indomethacin suppositories on serum amylase, inflammatory factors and immune function after endoscopic retrograde cholangiopancreatography

Xiao-Bin Peng✉, Xiao-Yun Wang, Gao-Jue Wu, Zhen Hu, Shuang Tang, Lei Gong

Department of Internal Medicine, Wuxi Second People's Hospital, Wuxi 214000, Jiangsu, China

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ABSTRACT

Objective: To explore the effects of indomethacin suppositories on serum amylase, inflammatory factors and immune function after endoscopic retrograde cholangiopancreatography (ERCP). **Methods:** A total of 85 patients with common bile duct stones or obstructive jaundice were divided into the observation group (n=45) and the control group (n=40) according to the different treatment methods, both two groups patients were treated with ERCP, patients in the observation group was given indomethacin suppositories 50 mg preoperative 30 min. Serum amylase, inflammatory factors and T cell subsets were detected preoperative, postoperative 6 h and postoperative 24 h. Inflammatory factors including interleukin -10 (IL-10), interleukin -6 (IL-6), tumor necrosis factor alpha (TNF-) and interleukin-4 (IL-4). T cell subsets including $CD3^+$, $CD4^+$, $CD8^+$ and calculated $CD4^+/CD8^+$. **Results:** In both two groups, postoperative 6 h, 24 h serum amylase were significantly higher than before surgery; in the observation group, the postoperative 6 h, 24 h serum amylase were significantly lower than in the control group at the same time point and the differences were statistically significant (P<0.05). Both two groups′ postoperative 6 h, 24 h serum pro-inflammatory factor IL-6 and TNF- increased first and then decreased, both were significantly higher than before surgery; both two groups′ postoperative 6 h, 24 h serum anti-inflammatory factor IL-10 and IL-4 gradually increased, both were significantly higher than before surgery, and the differences were statistically significant (P<0.05); In the observation group, anti-inflammatory factor IL-10 and IL-4 significantly increased while pro-inflammatory factor IL-6 and TNF- significantly decreased compared with the control group at the same time point 6 h and 24 h after surgery, the difference between the two groups was statistically significant (P<0.05). Both two groups′ postoperative 6 h, 24 h T cell subsets $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ first decreased and then increased, all were significantly lower than before surgery, and the differences were statistically significant (P<0.05); In the observation group, the postoperative 6 h, 24 h $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ were significantly higher than in the control group at the same time point and the differences were statistically significant (P<0.05). **Conclusions:** Indometacin suppositories could lower the postoperative serum amylase, reduce inflammation reaction and regulate immune function.

1. Introduction

Endoscopic retrograde cholangiopancreatography (ERCP) is an indispensable technique in the diagnosis and treatment of biliary and pancreatic diseases. Although ERCP is a minimally invasive technique, but the bleeding, pancreatitis and other postoperative complications still bring uncertainty to patient's healing, hindering the clinical application of this technology[1–3]. Postoperative pancreatitis (PEP) is a common complication after ERCP, and its prevention is concerned. Indomethacin suppositories is a kind of non-steroidal anti-inflammatory drugs (NSAIDs), which has the effect of reduce or block the inflammatory cascade[4–6]. This

✉Corresponding author: Lei Gong (1963-), Male, Bachelor Degree, Chief Physician.
✉Author introduction: Xiao-Bin Peng (1975 -), Male, Bachelor Degree, Deputy Chief Physician.
Tel: 13812297572
E-mail: pengxiaobin98@163.com
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study explores the influence of indomethacin suppositories on ERCP postoperative serum amylase, inflammatory factors and immune function, we aims to explore whether given indomethacin suppositories after ERCP treatment can prevent the occurrence of high amylase and pancreatitis after ERCP operation.

2. Materials and methods

2.1. Clinical data

Retrospective analysis of patients with ERCP treated in Wuxi Second People's Hospital of from June 2014 to February 2016, all meet the following conditions: ①The primary diseases were common bile duct stones or jaundice obstructive, according to ERCP and treated with ERCP; ②The preoperative blood test, serum amylase and immune and inflammatory factors were normal; ③Exclusion of patients with respiratory failure, heart failure and other serious diseases; ④ Exclusion of patients with family history of pancreatitis; ⑤ Exclusion of patients with surgery and anesthesia; ⑥ Exclusion of patients with peptic ulcer, perforation, bleeding, and with history of allergic asthma; ⑦ Exclusion of patients with drug contraindications; ⑧ Exclusion of patients with combined coagulation abnormalities, respiratory insufficiency, severe liver and kidney diseases, severe cardiovascular and cerebrovascular diseases. A total of 85 cases were included, according to the different treatment methods, they were divided into two groups, the observation group and the control group. In the observation group, total 45 cases, including 25 males and 20 females; Age from 42 to 68 years old, with an average (55.31±13.30) years old; The disease type: 26 cases of common bile duct stones, 19 cases of obstructive jaundice; the operation time from 22 to 40 min with an average (31.19±9.13) min. In the control group, total 40 cases, including 22 males and 18 females; Age from 41 to 67 years old, with an average (54.16±13.12) years old; The disease type: 24 cases of common bile duct stones, 16 cases of obstructive jaundice; the operation time from 24 to 38 min with an average (31.02±8.10) min. The general information of the two groups were comparable.

2.2. Treatment method

All patients were treated with ERCP. Lidocaine 10 g was given preoperative 5 to l0 min, guide wire super selection technique was taken, subsequent stent placement or stone removal operation was taken after successful intubation. They received continuous intravenous infusion of octreotide 0.6 mg postoperative 24 h. On the basis of this, the observation group was given indomethacin suppositories 50 mg preoperative 30 min.

2.3. Observation indexes

Serum amylase, inflammatory factors and T cell subsets were detected preoperative, postoperative 6 h and postoperative 24 h. Inflammatory factors included interleukin-10 (IL-10), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-) and interleukin-4 (IL-4). T cell subsets included $CD3^+$, $CD4^+$, $CD8^+$ and calculated $CD4^+/CD8^+$. A total of 1 mL venous blood was taken at different time points, hepain sodium anticoagulation, the serum was separated and used for amylase detection, using SP-4430 dry biochemical analyzer. A total of 2 mL venous blood was taken at different time points, the serum was separated and IL-6, TNF- , IL-10 and IL-4 were determined by ELISA, the kits were purchased from Beijing Ou Meng Biological Technology Co., ltd. Peripheral venous blood was sampled at different time points, and T cell subsets were determined by FACSCanto flow cytometry (BD company, US).

2.4. Statistical treatment

Statistical software SPSS20.0 was used for data analysis, serum amylase, inflammatory cytokines and immune function indexes were expressed as mean±sd. t test was used for groups comparison, $P<0.05$ was regarded as significant difference.

3. Results

3.1. Serum amylase levels of the two groups at different time points before and after operation

There was no significant difference in preoperative serum amylase between the two groups ($P>0.05$); both two groups′ postoperative 6 h, 24 h serum amylase were significantly higher than before surgery ($P<0.05$); in the observation group, the postoperative 6 h, 24 h serum amylase were significantly decreased compared with in the control group ($P<0.05$) (Table 1).

Table 1
Serum amylase levels of the two groups at different time points before and after operation (U/L).

Groups	Cases	Time	Amylase
Observation group	45	Preoperative	51.14±14.40
		Postoperative 6 h	121.32±45.58*#
		Postoperative 24 h	136.65±56.61*#
Control group	40	Preoperative	50.09±15.51
		Postoperative 6 h	143.68±51.26*
		Postoperative 24 h	153.43±62.93*

Ps：Compared with preoperative, $^*P<0.05$; Compared with the control group at the same time point, $^\#P<0.05$.

3.2. Serum inflammatory factors of two groups at different time points before and after operation

There were no significant differences in preoperative serum inflammatory factors between the two groups ($P>0.05$); both two groups′ postoperative 6 h, 24 h serum pro-inflammatory factor IL-6 and TNF- increased first and then decreased, both were significantly higher than before surgery ($P<0.05$); both two groups′ postoperative 6 h, 24 h serum anti-inflammatory factor IL-10 and IL-4 gradually increased, both were significantly higher than before surgery ($P<0.05$); In the observation group, anti-inflammatory factor IL-10 and IL-4 significantly increased while pro-inflammatory factor IL-6 and TNF- significantly decreased compared with the control group at the same time point 6 h and 24 h after surgery ($P<0.05$) (Table 2).

3.3. T lymphocyte subsets of the two groups at different time points before and after operation

There were no significant differences in preoperative T lymphocyte subsets between the two groups (P>0.05); both two groups′ postoperative 6 h, 24 h T cell subsets $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ first decreased and then increased, all were significantly lower than before surgery, and the differences were statistically significant (P<0.05); There were no significant changes in postoperative 6 h, 24 h $CD8^+$ between the two groups (P>0.05); In the observation group, the postoperative 6 h, 24 h $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ were significantly increased compared with the control group and the differences were statistically significant (P<0.05) (Table 3).

4. Discussion

Studies thought that the uncontrolled release of cytokines and the excessive activation of inflammatory cells are the important reasons for the occurrence of PEP, which is also the key factor of pancreatic necrosis and multiple organ failure[7,8]. When PEP occurs, a variety of inflammatory cells are activated, the number of inflammatory mediators and cytokines release increased significantly, leading to the occurrence of cascade reaction. On the one hand, the release of pro-inflammatory factors such as TNF- , IL-6 can cause the occurrence of systemic inflammatory response syndrome. On the other hand, activated monocyte macrophages could promote the release of endogenous anti-inflammatory mediators such as IL-4, IL-10, which resulted in the compensatory anti-inflammatory response syndrome[9,10]. The abnormal and excessive reaction of pro-inflammatory factors and anti-inflammatory cytokines exacerbate pancreatitis, leading to pancreatic necrosis, pancreas and other organs injured and will induce the generation of inflammatory mediators and cytokines, formed the second cytokine peak and vicious circle, eventually lead to multiple organ failure and even death[11,12]. Although ERCP is a minimally invasive surgery, but the postoperative tissue damage and thermal conduction will induced pancreatic duct congestion and edema, with the pancreatic tissue damage caused by contrast agent, all caused pancreatic excretion disruption PEP[13–14]. Our results found that ERCP can cause the increase of serum amylase levels, promote the release of inflammatory factors, cause the imbalance of pro-inflammatory factors and anti-inflammatory factors which is also an important reason for the occurrence of PEP.

Phospholipase A2 (PLA2) is a kind of important central inflammatory cytokine which is distributed in the plasma membrane and organelle membrane, it′s an initial factor in "waterfall like cascade" inflammatory process. Kochar[15] found that PLA2 was significantly elevated after ERCP, and that the occurrence of PEP may be closely related to the premature activation and excessive release of PLA2. PLA2 can produce oxygen free radicals, induce tissue cells excessive apoptosis to aggravate the tissue damage. Choksi[16] studied the PEP damage mechanism and found that the interaction between neutrophils, endothelial cells, PLA2 and epoxidase played an important role in the injury. Therefore, we hypothesized that inhibition of the PLA2 and epoxidase activity might contribute to the inhibition of the inflammatory response after ERCP. Indomethacin suppositories is a NSAIDs, it′s a potent PLA2 and epoxidase inhibitor, which can significantly weaken the role of neutrophil chemotaxis, inhibit the activation of neutrophils, reduce inflammatory response, and stabilize the lysosomal membrane[17,18]. In our study, the postoperative 6 h, 24 h serum amylase in the observation group were significantly lower than that in the control group at the same time point. In the observation group, anti-inflammatory factor IL-10 and IL-4 significantly increased while pro-inflammatory factor IL-6 and TNF- significantly decreased compared with the control group at the same time point 6 h and 24 h after surgery, the difference between the two groups was statistically significant, which indicated that indomethacin suppositories can reduce the ERCP postoperative serum amylase and reduce

Table 2

Serum inflammatory factors of the two groups at different time points before and after operation (pg/mL).

Groups	Cases	Time	IL-6	TNF-	IL-10	IL-4
Observation group	45	Preoperative	22.34±4.27	45.12±5.12	19.93±3.64	23.65±4.27
		Postoperative 6 h	113.42±14.93$^{*\#}$	170.54±23.31$^{*\#}$	26.67±4.89$^{*\#}$	31.25±5.12$^{*\#}$
		Postoperative 24 h	87.25±10.33$^{*\#}$	132.21±15.52$^{*\#}$	31.19±5.03$^{*\#}$	35.53±5.24$^{*\#}$
Control group	40	Preoperative	24.03±4.30	47.01±5.24	18.87±4.02	24.09±4.42
		Postoperative 6 h	138.67±16.63*	208.21±31.53*	22.15±4.46*	26.69±4.78*
		Postoperative 24 h	101.28±12.83*	168.69±20.64*	25.52±4.91*	30.10±4.66*

Ps：Compared with preoperative, $^{*}P<0.05$; Compared with the control group at the same time point, $^{\#}P<0.05$.

Table 3

T lymphocyte subsets of two groups at different time points before and after operation

Groups	Cases	Time	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$
Observation group	45	Preoperative	63.12±4.65	36.54±4.42	25.63±3.38	1.50±0.26
		Postoperative 6 h	54.42±5.08$^{*\#}$	30.16±4.08$^{*\#}$	24.26±3.12	1.22±0.19$^{*\#}$
		Postoperative 24 h	57.78±4.76$^{*\#}$	34.02±4.16$^{*\#}$	24.08±3.17	1.38±0.20$^{*\#}$
Control group	40	Preoperative	63.09±4.73	34.68±4.36	26.41±3.42	1.47±0.24
		Postoperative 6 h	51.08±5.13*	27.78±4.12*	25.58±3.12	1.03±0.22*
		Postoperative 24 h	54.65±4.92*	31.15±4.24*	25.14±3.23	1.20±0.21*

Ps：Compared with preoperative, $^{*}P<0.05$; Compared with the control group at the same time point, $^{\#}P<0.05$.

inflammatory reaction. Indomethacin suppository cannot damage gastric mucosa, and the bioavailability is high, drug can quickly reach the peak concentration, and also in line with the fasting requirements after ERCP[19].

Our study also found that, both two groups postoperative 6 h, 24 h T cell subsets $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ first decreased and then increased, all were significantly lower than preoperative and the difference was statistically significant. The results showed that ERCP induced a transient immune suppression. Stress factors such as surgery, trauma, anesthesia and other factors can activate the single nuclear macrophage system and reduce the immune function of the body. The postoperative 6 h, 24 h $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ in the observation group were significantly higher than in the control group at the same time point, and the differences were statistically significant. The results showed that indomethacin suppositories can regulate immune function after ERCP. The activation of monocyte macrophage system can induce the expression of the enzyme, which can increase the degree of inflammatory reaction and oxidative stress. On the one hand, indomethacin suppository can directly inhibit the expression of cyclooxygenase. It could also inhibit arachidonic acid conversion to prostaglandin (PG), reduce the synthesis of PG and inhibit the thermal effect of cytokines. On the other hand, indomethacin also can inhibit TNF- and other inflammatory cytokines, alleviate inflammatory reaction degree, reduce protein catabolism and improve the recovery of immune function[20,21].

In summary, our study found that after ERCP, patients with increased postoperative serum amylase, increased inflammatory cytokine release, the imbalance of pro-inflammatory factors and anti-inflammatory factors and the inhibited immune function. Indomethacin could reduce post ERCP serum amylase, reduce inflammation, regulating immune function, may contribute to the prevention of post ERCP pancreatitis and hyperamylasemia occurred and worth further research.

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