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ORIGINAL ARTICLE

Effect of Diclofenac on the Levels of Lipoxin A4 and Resolvin D1 and E1 in the Post-ERCP Pancreatitis

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Abstract
Objectives Acute pancreatitis is one of the most common complications of endoscopic retrograde cholangiopancreatography (ERCP). Numerous studies have shown that administered nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce the incidence of acute pancreatitis after ERCP. Little is known, however, about the mechanism of NSAIDs in preventing pancreatitis (PEP).
Methods In this study, we assigned patients to receive a single dose of intramuscular diclofenac 75 mg immediately after ERCP (diclofenac group) or without (control group). The primary outcome measure was the occurrence of PEP. The serum amylase levels were measured before ERCP and at 3 and 24 h post-procedure in all patients. The Lipoxin A4 (LXA4), Resolvin D1 (Rvd1), and Resolvin E1 (RvE1) levels were measured before ERCP, and 3 and 24 h after the procedure in 30 patients from the diclofenac group and 30 patients from the control group.
Results A total of 120 patients were enrolled and completed the follow-up. The overall incidence of PEP was 13.3 % (16/120). It occurred in four of 60 patients (6.67 %) in the diclofenac group and in 12 of 60 patients (20.00 %) in the control group ($p = 0.032$). The LxA4, RvD1, and RvE1 levels in the diclofenac group at 3 h after ERCP were significantly increased compared with before ERCP ($p < 0.05$). Compared with the control group, the LxA4, RvD1, and RvE1 levels in the diclofenac group at 3 and 24 h after ERCP were significantly increased ($p < 0.05$).
Conclusions Intramuscular diclofenac after ERCP can reduce the incidence of PEP. This may be related to the fact that diclofenac can increase the levels of LxA4, RvD1, and RvE1.

Keywords Diclofenac · Post-ERCP pancreatitis · Resolvin · Lipoxin · Mechanism

Introduction

Acute pancreatitis is the most common major complication of endoscopic retrograde cholangiopancreatography (ERCP), it occurs in 1–10 % of patients overall, but may approach 30 % in high-risk patients [1–3]. Several agents for the pharmacologic prophylaxis of post-ERCP pancreatitis (PEP) have been proposed, including gabexate [4], somatostatin [5–7], octreotide [8, 9], and allopurinol [10]. However, the results are disappointing. There are numerous studies showing that nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce the incidence of PEP. Murray et al. [11] found that rectal diclofenac given immediately after ERCP can reduce the incidence of acute pancreatitis. Elmunzer et al. [12] reported a study showing that among patients at high risk for PEP, rectal indomethacin significantly reduced the incidence of PEP. Meta-analysis [13–15] has shown a statistically significant reduction of PEP with indomethacin or diclofenac given rectally just before ERCP or immediately after ERCP, but the mechanism of NSAIDs to prevent PEP has not been completely elucidated. Phospholipase A2 (PLA2) plays an important role in the pathophysiology of acute pancreatitis [16]. Makela et al. [17] reported that NSAIDs are potent inhibitors of

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PLA2 activity in severe acute pancreatitis patients. Recently, a study found that NSAIDs could increase the activity of endogenous lipid mediators and attenuate the inflammation response.

Lipid mediators, including lipoxins and resolvins, have been shown to control and resolve inflammation in a variety of experimental models of inflammatory disorders. Gewirtz et al.'s [18] study found that oral administration of Lipoxin A4 (LXA4) led to the attenuation of dextran sodium sulfate (DSS)-induced colitis in mice and to the down-regulation of proinflammatory gene expression. Arita et al. [19] found that in TNBS-induced colitis in mice, treatment with Resolvin E1 (RvE1) increased survival, decreased loss of body weight, and improved histological scores. Lipid mediators were triggered by NSAIDs. Therefore, we conducted a trial to determine whether diclofenac given immediately after ERCP can reduce the incidence of PEP and to explore the influence of NSAIDs on lipoxins and resolvins.

Materials and Methods

Subjects

The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University and written informed consent was obtained from each participant.

In this study, we enrolled patients from the First Affiliated Hospital of Anhui Medical University, Anhui Province, China. Entry to the study was restricted to patients with choledocholithiasis and serum amylase levels before ERCP levels were normal. Patients were excluded if they had any contraindications to receiving diclofenac (e.g., patients with perforation or bleeding complications), acute pancreatitis or chronic pancreatitis, with severe heart failure or liver and renal insufficiency patients, pregnancy or lactation, or history of allergy to contrast. Between September 2012 and October 2013, a total of 120 patients fulfilled the entry criteria. These patients, including 52 male and 68 female, were aged 21–89 years old, with an average age of 59.

Intervention

The diagnoses of PEP were according to consensus criteria [20]. Briefly, PEP is most commonly defined as newly emerging or worsening of prior abdominal pain with a serum amylase level at least three times higher than the upper limits of normal within 24 h of ERCP.

Immediately after ERCP, the diclofenac group patients were assigned to receive a single dose of intramuscular diclofenac 75 mg. Blood samples were drawn from all patients at 3 and 24 h post-procedure. Serum amylase levels were determined before ERCP and at 3 and 24 h post-ERCP in all patients, and the levels of the Lipoxin A4, Resolvin D1, and Resolvin E1 were measured in 30 patients from the diclofenac group and 30 patients from the control group. The value of the serum amylase, LxA4, RvD1, and RvE1 were expressed as mean ± SE.

Statistical Analysis

The Chi-square test was used to compare the incidence of PEP in the diclofenac group and the control group. Serum amylase values and Lipoxin A4, Resolvin D1, and Resolvin E1 levels were compared using Student's t test. $p < 0.05$ was considered statistically significant.

Results

The clinical and demographic characteristics of diclofenac group patients and controls were similar and there were no significant deference in the two groups.

A total of 120 patients entered the study, the diclofenac group and the control group both totaled 60 patients. The diclofenac group received 75 mg intramuscular diclofenac immediately after ERCP. The overall incidence of PEP was 13.3 % (16/120). Of these patients, four of 60 (6.67 %) occurred in the diclofenac group and 12 of 60 (20.00 %) occurred in the placebo group. Three hours after the endoscopic procedure, the mean serum amylase level was 328.70 ± 369.55 U/l in the control group and 214.93 ± 309.28 U/l in the diclofenac group. Twenty-four hours after ERCP, the mean serum amylase level was 251.17 ± 175.18 U/l in the control group and 192.50 ± 282.37 U/l in the diclofenac group. The difference in mean serum amylase value between the two groups at 3 and 24 h is not statistically significant.

In the two groups, the levels of the Lipoxin A4, Resolvin D1, and Resolvin E1 before ERCP is not statistically significant. However, the Lipoxin A4 levels in the diclofenac group at 3 and 24 h after ERCP were 401.69 ± 89.08 and 423.99 ± 96.61 ng/ml, respectively, which were significantly increased compared with the control group 357.04 ± 70.70 and 372.12 ± 91.26 ng/ml (Fig. 1). At the same time, the Resolvin D1 levels in the diclofenac group at 3 and 24 h after ERCP were 447.12 ± 91.75 and 414.49 ± 68.78 pg/ml, respectively, which were also significantly increased compared with the control group 387.98 ± 86.39 and 351.09 ± 112.72 pg/ml (Fig. 2). As well as the Lipoxin A4 and the Resolvin D1, the Resolvin E1 levels in the diclofenac group at 3 and 24 h after ERCP were also significantly increased compared with the control group (Fig. 3).

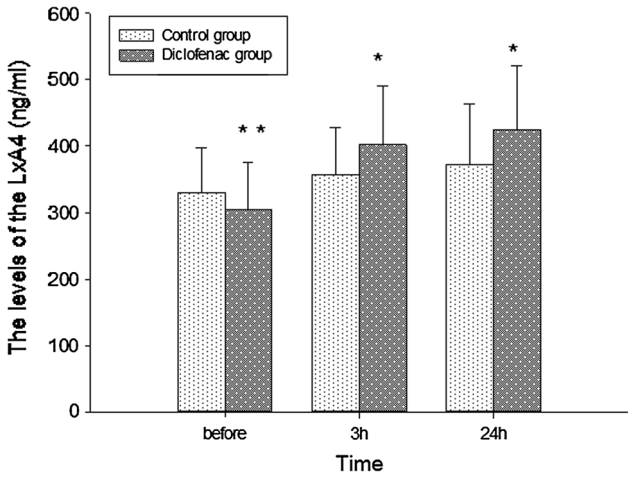

Fig. 1 Levels of LxA4 before ERCP and at 3 and 24 h post-ERCP. In the two groups ($n = 60$ in each group), the levels of Lipoxin A4 before ERCP is not statistically significant. After receiving a single dose of intramuscular diclofenac 75 mg immediately after ERCP, the Lipoxin A4 levels at 3 and 24 h after ERCP were 401.69 ± 89.08 and 423.99 ± 96.61 ng/ml, respectively, which were significantly increased compared with the control group 357.04 ± 70.70 and 372.12 ± 91.26 ng/ml, $*p < 0.05$. In the diclofenac group, the Lipoxin A4 levels at 3 h were significantly increased compared with the before ERCP, $**p < 0.01$

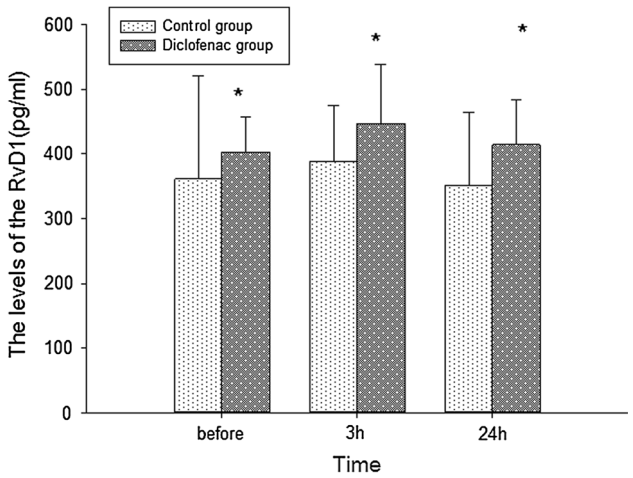

Fig. 2 Levels of RvD1 before ERCP and at 3 and 24 h post-ERCP. In the two groups ($n = 60$ in each group), the levels of Resolvin D1 before ERCP is not statistically significant. After receiving a single dose of intramuscular diclofenac 75 mg immediately after ERCP, the Resolvin D1 levels at 3 h and 24 h after ERCP were 447.12 ± 91.75 and 414.49 ± 68.78 pg/ml, respectively, which were significantly increased compared with the control group 387.98 ± 86.39 and 351.09 ± 112.72 pg/ml, $*p < 0.05$. In the diclofenac group, Resolvin D1 levels at 3 h were significantly increased compared with the before ERCP, $*p < 0.05$

In addition, the levels of Lipoxin A4, Resolvin D1, and Resolvin E1 in the diclofenac group at 3 h after ERCP were significantly increased compared with before ERCP ($p < 0.05$) (Figs. 1, 2, 3).

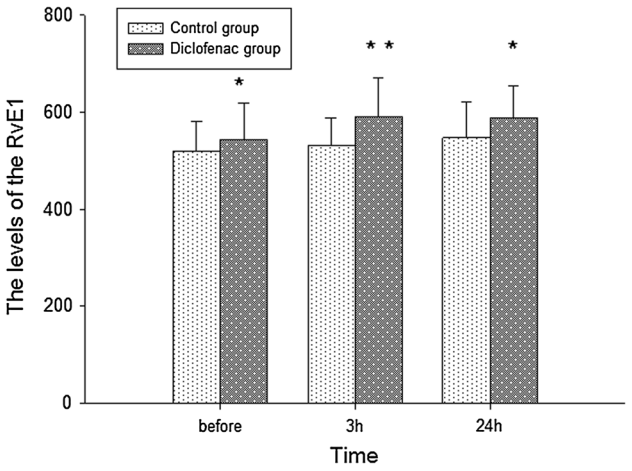

Fig. 3 Levels of the RvE1 before ERCP and at 3 and 24 h post-ERCP. In the two groups ($n = 60$ in each group), the levels of Resolvin E1 before ERCP is not statistically significant. After receiving a single dose of intramuscular diclofenac 75 mg immediately after ERCP, the Resolvin E1 levels at 3 and 24 h after ERCP were 590.69 ± 79.64 and 587.44 ± 65.96 pg/ml, respectively, which were significantly increased compared with the control group 531.40 ± 56.24 and 546.79 ± 74.78 pg/m. $**p < 0.01$, $*p < 0.05$. In the diclofenac group, the Resolvin E1 levels at 3 h were significantly increased compared with the before ERCP, $*p < 0.05$

Discussion

Endoscopic retrograde cholangiopancreatography (ERCP) is a widely applied and indispensable method in the treatment of pancreatobiliary disease. Pancreatitis remains the most common complication of ERCP. A meta-analysis by Ding et al. [21] found that most of the PEP is clinically mild or moderate in severity, but nearly 10 % of cases are severe and potentially fatal.

Multiple studies have shown that pharmacological agent studies for PEP have met with disappointing results in preventing PEP. Andriulli et al.'s [5] study found that no significant differences in incidences of pancreatitis, hyperamylasemia, or abdominal pain were observed among the placebo (4.8, 32.6, and 5.3 %, respectively), somatostatin (6.3, 26.8, and 5.1 %, respectively), and gabexate mesylate groups (5.8, 31.5, and 6.3 %, respectively). Kisli et al. [9] found that a single administration of intravenous octreotide infusion does not prevent ERCP-induced pancreatitis ($p > 0.05$). A randomized, multicenter, double-blind, placebo-controlled study was conducted by Sherman [22], patients was received IL-10 at a dose of either 8 or 20 μg/kg or placebo as a single intravenous injection 15–30 min before ERCP, found that there was no apparent benefit of IL-10 treatment when compared with placebo in reducing the incidence of post-ERCP acute pancreatitis ($p = 0.83$ for IL-10 8 μg/kg vs. placebo and 0.14 for IL-10 20 μg/kg vs. placebo). A study [23] of 806 patients in

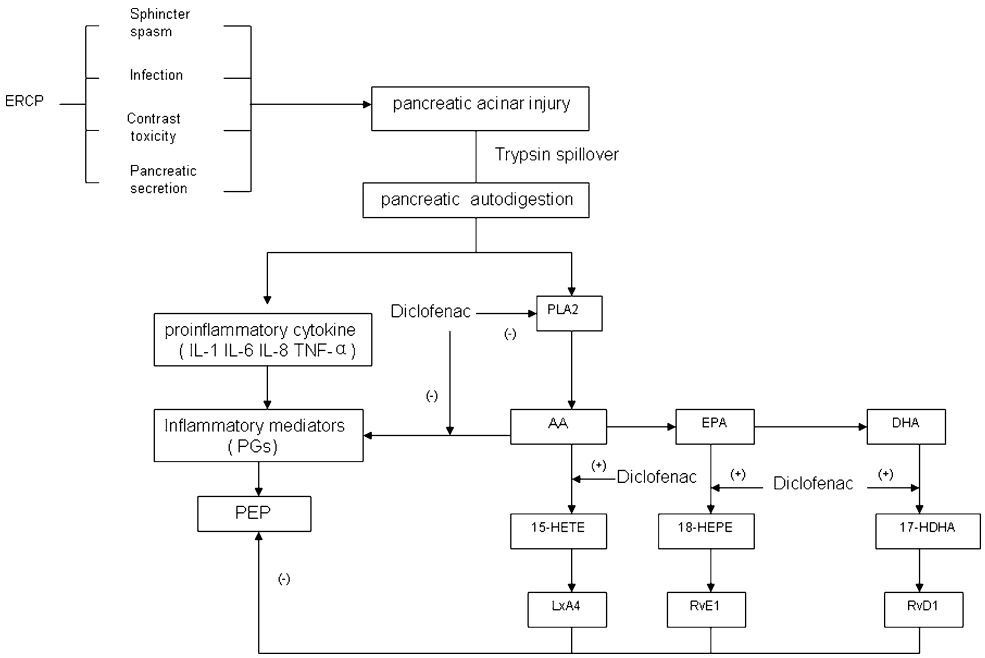

Fig. 4 Scheduled potential site of the diclofenac on the pharmacological prevention of the PEP. *PEP* post-ERCP pancreatitis, *PGs* prostaglandins, *AA* arachidonic acid, *PLA2* phospholipase A2, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid, *LxA4* Lipoxin A4, *RvD1* Resolvin D1, *RvE1* Resolvin E1, *HETE* hydroxyeicosatetraenoic acid, *HDHA* hydroxy docosahexaenoic acid, *HEPE* hydroxyeicosapentaenoic acid

Denmark showed no statistically significant preventive effect of glyceryl nitrate on PEP [18 (4.5 %) in the glyceryl nitrate group and 29 (7.1 %) in the placebo group].

In recent years, NSAIDs prevention of pancreatitis after ERCP has shown the promising results. Murray et al. [11] found that immediately after ERCP, patients were given a suppository containing 100 mg diclofenac can reduce the incidence of acute ancreatitis (7/110 in the diclofenac group vs. 17/110 in the placebo group, $p < 0.05$). A double-blind randomized trial [24] in Iran implicated 490 patients showed that rectal indomethacin given immediately before ERCP can reduce the incidence and moderate to severe pancreatitis was significantly higher in the placebo group ($p = 0.03$). Otsuka et al.'s [25] study found that receiving a saline infusion with 50 mg of rectal diclofenac 30 min before ERCP can prevent PEP [3.9 %(2/51) in the diclofenac group and 18.9 % (10/53)] in the control group, $p = 0.017$). In our study, the incidence of PEP was significantly reduced by the administration of an intramuscular diclofenac immediately following the endoscopic procedure (6.67 vs. 20.00 %, $p = 0.032$). Congruent with Elmunzer et al. [12], previous studies evaluating NSAIDs in the prevention of PEP.

However, the mechanism of NSAIDs prevent of PEP is not clearly understood (Fig. 4). It is thought that PLA2 plays an important role in the initial inflammatory cascade in acute pancreatitis through regulate pro-inflammatory mediators, including prostaglandins, leukotrienes, and platelet-activating agents [16]. Makela et al. [17] reported that NSAIDs are potent inhibitors of PLA2 activity and neutrophil–endothelial interactions in severe acute pancreatitis patients. Recently, many studies have found that lipid mediators inhibit inflammation [26] and were triggered by NSAIDs. The lipid mediators there are Lipoxins and Resolvins, and derived from essential omega-6 and omega-3 polyunsaturated fatty acids (n-3 and n-6 PUFA). Bento et al. [27] found that treatment with aspirin-triggered Resolvin D1 (AT-RvD1) greatly improved disease activity index, body weight loss, colonic damage, and polymorphonuclear infiltration in colitis experimental models, and reduced colonic cytokine levels for TNF-α, IL-1β, MIP-2. However, at present, in the pathophysiology of acute pancreatitis there is lack of study of lipid mediators. Zhou et al.'s [28] study found that serum levels of amylase in the LXA(4)-ME group were significantly lower than those in the acute pancreatitis group respectively at 12 and 24 h post-operation (all $p < 0.01$), and the pathological scores of the LXA(4)-ME group were improved. It has been proven that LXA(4)-ME exerts protective effects in acute pancreatitis rats.

In this study, the Lipoxin A4, Resolvin D1, and Resolvin E1 levels in the diclofenac group at 3 and 24 h after ERCP were significantly increased compared with before ERCP ($p < 0.05$). Compared with the control group, the Lipoxin A4, Resolvin D1, and Resolvin E1 levels in the diclofenac group at 3 and 24 h after ERCP were significantly increased ($p < 0.05$). It is suggested that diclofenac may increase the levels of Lipoxin A4, Resolvin D1, and Resolvin E1. Therefore, diclofenac given immediately after ERCP can reduce the incidence of PEP, and the mechanism may be related to that diclofenac can increase the levels of the Lipoxin A4, Resolvin D1, and Resolvin E1.

Conflict of interest None.

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