BASIC RESEARCH

Effect of emodin on pancreatic fibrosis in rats

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Abstract

AIM: To establish the rats model of chronic fibrosing pancreatitis and to prove the anti-fibrotic effect of emodin in chronic pancreatitis with fibrosis.

METHODS: Fifty rats were randomly divided into five groups, 10 rats in each group. Trinitrobenzene sulfonic acid (TNBS) was infused into the pancreatic duct to induce chronic pancreatitis in rats (except for normal group). Emodin-treated rats were fed with different doses of emodin (20, 40 and 80 mg/kg body weight) for 28 d, while normal group and control group received 0.9% sodium chloride solution. Serum levels of hyaluronic acid (HA) and laminin (LN) were determined by radioimmunoassay. Histopathological alterations were studied by optical microscopy. Expression of collagen was also examined while transforming growth factor-beta-1 (TGF-β_1) was localized by immunochemistry.

RESULTS: In emodin-treated rats, the serum levels of HA and LN were decreased significantly (HA, 62.2 ± 19.3 μg/L *vs* 112.7 ± 26.5 μg/L, $P < 0.05$; LN 44.3 ± 10.4 μg/L *vs* 86.2 ± 16.5 μg/L, $P < 0.05$); the degree of fibrosis was ameliorated observably; the expression of collagen in pancreatic tissue was reduced especially in high-dose emodin-treated group (36% ± 5% *vs* 42% ± 6%, $P < 0.05$); with the increased doses of emodin, the expression of TGF-β_1 was declined, compared with those in control group.

CONCLUSION: Emodin has an anti-fibrotic effect on pancreatic fibrosis in rats. Because of its anti-fibrotic effect, it could be a potential herb for the treatment of chronic pancreatitis.

Key words: Emodin; Fibrosis; Chronic pancreatitis; Animal model

INTRODUCTION

Chronic pancreatitis is characterized morphologically by a progressive pancreatic fibrotic process[1]. Extracellular matrix (ECM) components are distributed in both healthy and diseased pancreas, and there is an increased deposition of disorganized ECM components in chronic pancreatitis[2]. However, the mechanism of the fibrotic process is not fully understood. Progressive fibrosis is a characteristic feature of chronic pancreatitis of various etiologies, thus anti-fibrosis is the key to the therapy of chronic pancreatitis. However, no ideal treatment is available yet. Could traditional Chinese herbs be effective? Emodin (1, 3, 8-trihydroxy-6-methylanthraquinone) isolated from a great deal of herbs is an effective constituent with many effects. Lots of pharmaceutical studies have demonstrated that emodin has many biological effects[3-5], such as anticancer, antimicrobial, and anti-inflammatory effects, *etc*. Moreover, several studies[6,7] have revealed that emodin is efficacious in the management of acute pancreatitis and hepato-cirrhosis, but studies on chronic pancreatitis are rare. We designed this experiment to investigate the effect of emodin on pancreatic fibrosis in rats, and elucidate its potential therapeutic mechnisms.

MATERIALS AND METHODS

Materials

Male Sprague-Dawley rats weighing 200-250 g were provided by the Animal Center of Zhejiang University. Trinitrobeneze sulfonic acid (TNBS) was obtained from Sigma Chemical Company. Radioimmunoassay kits of rat hyaluronic acid (HA) and laminin (LN) were purchased from Shanghai Tiancheng Science and Technology Ltd. Emodin was offered by Hunan Huaguang Chemical Company. TGF-β_1 polyclonal antiboby was provided by Santa Cruz Company. Immunochemistry kits (Envision TM) were offered by DAKO, Denmark.

Grouping and animal model

Fifty male Sprague-Dawley rats were randomly divided into five groups, 10 rats in each group. A chronic pancreatitis model was induced in rats by infusion of TNBS into the pancreatic duct with a modification of the method

Table 1 Grading of pancreatic fibrosis

Grade	Duct dilation	Periductal fibrosis	Intralobular fibrosis	Glandular atrophy
F0	-	-	-	-
F1	+	-	-	-
F2	+	+	-	-
F3	+	+	+	-
F4	+	+	+	+

Table 2 Effect of emodin on serum hyaluronic acid (HA) and laminin (LN) (mean ± SE)

Group	n	HA (μg/L)	LN (μg/L)
1 (Normal)	10	55.2 ± 23.7	40.5 ± 12.2
2 (Control)	8	112.7 ± 26.5[b]	86.2 ± 16.5[b]
3 (emodin, 20 mg/kg)	8	86.6 ± 22.1[a]	66.8 ± 14.4[a]
4 (emodin, 40 mg/kg)	8	78.0 ± 24.5[d]	56.7 ± 12.3[a]
5 (emodin, 80 mg/kg)	9	62.2 ± 19.3[d]	44.3 ± 10.4[d]

[b]$P < 0.01$ *vs* group 1; [a]$P < 0.05$, [d]$P < 0.01$ *vs* group 2.

described by Puig-Divi *et al*[8] (except for normal group). After fasted for 3 d, the rats in group 1 (normal group, nonoperation) and group 2 (control group, nontreating) received oral 0.9% sodium chloride solution. Emodin-treated rats in groups 3-5 were fed with different doses of emodin (20, 40 and 80 mg/kg body mass). Twenty-eight days after treatment, all rats were killed. Pancreatic tissues and blood samples were collected and kept for further study.

Measurement of serum HA and LN
Blood samples were obtained 28 d after treatment, and serum levels of HA and LN were determined by radioimmunoassay.

Histological examination
Paraffin sections of rat pancreatic tissue were stained with hematoxylin and eosin. We evaluated the morphologic changes by light-microscopy. Grading of pancreatic fibrosis was carried out as previously described[9] (Table 1).

Mesurement of collagen expression
Pancreatic fibrosis was observed by the Van Gieson staining, semi-quantitative analysis of collagen fibres was carried out by the KS400 imaging analysis system (KS400 ver. 2.0).

Immunochemistry
Paraffin-embedded tissue sections were subjected to immunostaining using the DAKO EnVisin two-step procedure. Tissue sections were submerged for 10 min in Tris buffered saline (TBS) (10 mmol/L Tris-HCl, 0.85% NaCl, pH 7.4) containing 3% hydrogen peroxide in order to quench the endogenous peroxidase activity. Following washing with distilled water, the sections were incubated in TBS with polyclonal antibodies against TGF-β_1 diluted at 1:200 for 30 min. After 10-min washing with TBS, the sections were incubated with Envision TM for another 30 min. After washing with TBS for 10 min, the sections were incubated with diaminobenzidine (DAB) as a substrate and counterstained. The results were evaluated by microscopy. All samples were classified according to the percentage of TGF-β_1 positive cells (brown colour reaction). We defined 0 as no, + as 0%-25%, +2 as 25%-50%, +3 as 50%-75%, +4 as > 75%.

Statistical analysis
Serum levels of HA and LN and pancreatic collagen content were expressed as mean ± SE. Student's *t* test was

used for statistical analysis. Chi square test was used for the ranked data statistical analysis. If the expected cell count was less than or equal to 5, Fisher's exact test was used. SPSS 11.0 was used for statistical analysis, and $P < 0.05$ was considered statistically significant.

RESULTS

Survival rate of rats
The survival rate was 83.3% in emodin-treated groups (25/30) and 80.0% (8/10) in control group. However, no significant difference was found between these groups.

Serum HA and LN
The mean serum level of HA and LN was the lowest in normal group (group 1, $P < 0.05$), and the levels of HA and LN were significantly lower in emodin-treated groups (groups 3-5) than in control group (group 2, $P < 0.05$). With the increased doses of emodin, the serum levels of HA and LN were decreased in emodin-treated groups, but there was no statistically significant difference between them (Table 2).

Histopathological examination
Pancreatic fibrosis was evaluated according to the criteria described above (Table 1), and the results are shown in Figure 1. The extent of fibrosis was much milder in emodin-treated groups than in control group. Structural changes such as periductal fibrosis, intralobular fibrosis and glandular atrophy were ameliorated. With the increased doses of emodin, the changes were significantly improved.

Pancreatic collagen content
Pancreatic collagen was detected by Van Gieson staining. An extensive accumulation of collagen and glandular atrophy were found in control group, while the accumulation of collagen was decreased and no glandular atrophy was found in emodin-treated groups. We obtained the positive proportions of Van Gieson staining by the imaging analysis system. The positive proportions in each group are shown in Table 3. With the increased doses of emodin, the changes were improved obviously. Only a significant difference was found between group 5 and group 2 ($P < 0.05$).

Immunochemistry
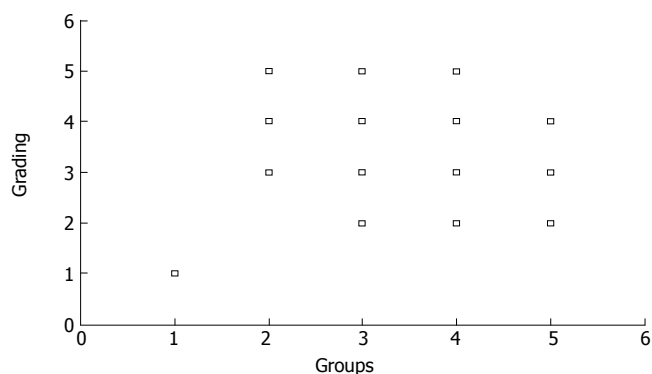Four groups (except for normal group) showed TGF-β_1 expression (brown colour reaction) (Figure 2). The

Figure 1 Effect of emodin on histopathological alterations in rats. Grading: 1 = F0, 2 = F1, 3 = F2, 4 = F3, 5 = F4, 6 = F5.

Table 3 Pancreatic collagen content in different groups (mean ± SE, %)

Group	n	Positive proportion
2 (Control)	8	42 ± 6
3 (emodin, 20 mg/kg)	8	39 ± 7
4 (emodin, 40 mg/kg)	8	38 ± 4
5 (emodin, 80 mg/kg)	9	36 ± 5[a]

[a]$P < 0.05$ vs group 2.

Table 4 Immunochemistry results in different groups (percentage of TGF-β_1 positive cells)

Group	n	0	+	+2	+3	+4
1 (Normal)	10	9	1	0	0	0
2 (Control)	8	0	0	0	4	4
3 (emodin, 20 mg/kg)	8	0	0	4	4	0
4 (emodin, 40 mg/kg)	8	0	4	3	1	0
5 (emodin, 80 mg/kg)	9	4	5	0	0	0

0: No; +: 0%-25%; + 2: 25%-50%; + 3: 50%-75%; + 4: > 75%.

results are shown in Table 4. With the increased doses of emodin, the changes were improved obviously, similar to the above results. For the sake of statistical analysis, we divied all samples into two large groups: low expression of TGF-β_1 (0, +1, +2) and high expression of TGF-β_1 (+3, +4). Fisher's exact test was used for statistical analysis. Compared with group 2 (0 rat in low expression group and 8 rats in high expression group), a significant difference was found between group 4 (7 rats in low expression group and 1 rat in high expression group) and group 5 (9 rats in low expression group and 0 rat in high expression group) ($P < 0.05$). Compared with group 3 (4 rats in low expression group and 4 rats in high expression group), only group 5 had a significant difference ($P < 0.05$). There was no statistically significant difference between any other two groups.

DISCUSSION

The incidence of chronic pancreatitis is increasing, and the most important etiological factor is alcohol. The pathogenesis of chronic pancreatitis has not been completely understood. Nevertheless, it was reported that various factors such as TGF-β_1, ECM, oxidative stress etc, are involved in the progression of pancreatic fibrosis[10,11]. Since pancreatic fibrosis is the characteristic feature of chronic pancreatitis, preventing and inverting pancreatic fibrosis is the key to therapy of chronic pancreatitis and amelioration of its prognosis.

Drugs which could prevent or invert pancreatic fibrosis might be developed and interventioned for the treatment of chronic pancreatitis with fibrosis. Emodin isolated from a great deal of herbs is an effective constituent with many effects[3-5]. It was reported that emodin could inhibit the expression of α-smooth muscle actin (α-SMA) and reduce the transformation and synthesis of TGF-β_1[6]. Some researchers observed that emodin could inhibit transformation of hepatic stellate cells (HSCs) from G₁ phase to S phase and S phase to G₂ phase that could prevent HSC proliferation. It also has been reported that emodin could inhibit lipid peroxidation[12]. However, previous studies of emodin mainly focused on how to inhibit liver fibrosis, while studies on pancreatic fibrosis are rare. The aim of this research was to study the effect

of emodin on pancreatic fibrosis, and the results of our study indicate that emodin has an anti-fibrotic effect on pancreatic fibrosis in rats.

Cells similar to HSCs have been identified and isolated from the pancreas, known as pancreatic stellate cells (PSCs)[13,14]. It was reported that PSCs are activated in both experimental and human pancreatic fibrosis and these activated PSCs are the main cellular source of collagen in chronic pancreatitis[15], suggesting that pathological alteration of pancteatic fibrosis is similar to liver fibrosis, and may share the same mechanism of fibrosis and anti-fibrosis.

The potential mechanisms of emodin include the following aspects. The first is cell protection. As we know, tissue injury is the first step of fibrosis. Apte et al[16] showed that activated PSCs around the pancreatic micrangium express α-SMA, which could aggravate fibrosis of the pancreas, because of vasoconstriction and pancreatic ischemia caused by α-SMA. Emodin could protect pancreatic cells and organs from impairment. In our study, the extent of fibrosis was much milder in emodin-treated groups than in control group. This may be the first mechanism of emodin's anti-fibrotic effect. The second is the effect of emodin on cytokines. PSCs produce ECM after stimulation with TGF-β_1, platelet-derived growth factor (PDGF), etc[17]. It was reported that TGF is the key regulating factor of fibrosis. Modulation of TGF-β signaling may be the therapeutic target for the prevention of chronic pancreatitis[18]. It was reported that emodin could inhibit the expression of TGF-β_1 in liver fibrosis model of rats[6]. Imanishi et al[19] found that emodin could modulate the effect of PDGF, which could slow down the progression of fibrosis. In our study, emodin-treated groups showed low expression of TGF-β_1, especially in high-dose emodin-treated group. Emodin could counteract cytokines and their receptors, which are
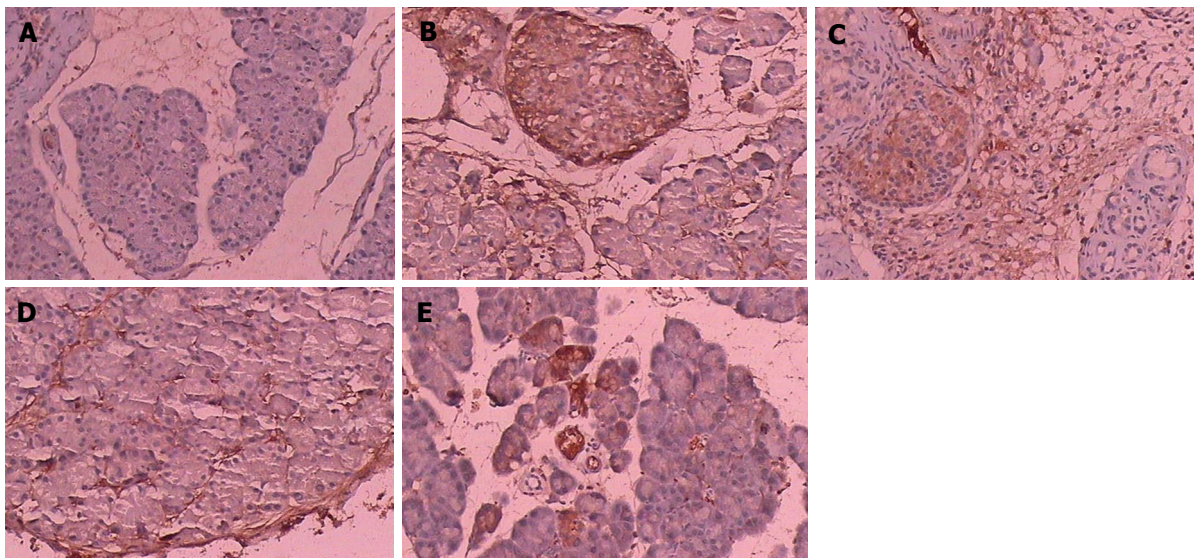
Figure 2 Pancreatic immunochemistry changes in normal group (**A**), control group (**B**) and 20 mg/kg (**C**), 40 mg/kg (**D**) and 80 mg/kg (**E**) emodin-treated groups (× 100). Normal group had no TGF-β_1 expression (brown color reaction), control group had the highest expression of TGF-β_1 while 20, 40 and 80 mg/kg emodin-treated groups had different levels of TGF-β_1 expression.

relevant to the progression of fibrosis, and then brings its anti-fibrotic effect into play. The third is the regulatory effect of emodin on ECM metabolism. Disbalance in ECM metabolism is the common way of fibrosis. Chronic pancreatitis is due to the cumulative intrapancreatic ECM deposition. ECM is consisted of collagens and noncollagenous glycoproteins (fibronectin, laminin). It was reported that serum HA and LN are two important markers responsible for the degrees of fibrosis[20]. Our study revealed that the serum levels of HA and LN were significantly lower in emodin-treated groups than in control group, suggesting that emodin can affect the metabolism of ECM. However, the exact mechanism by which emodin regulates the content of ECM still needs further investigation. The fourth is the effect of emodin on ECM-producing cells. PSCs rapidly proliferate following injury and are the major source of pancreatic ECM component during pancreatic fibrogenesis, and accumulating evidence has placed PSCs at the center of pancreatic fibrosis[15]. Emodin not only inhibits proliferation and activation of PSCs, but also induces their apoptosis. This is another mechanism underlying the anti-fibrotic effect of emodin. The fifth is the antioxidizing effect of emodin. Yoo and co-workers[11] reported that oxidative stress is principally involved in the pathogenesis of chronic pancreatitis with fibrosis and antioxidants could prevent and ameliorate the extent of pancreatic fibrosis, suggesting that antioxidants can be considered in preventing and treating chronic pancreatitis. Eomdin could inhibit lipid peroxidation[12], and antioxidization maybe another mechanism underlying the anti-fibrotic effect of emodin.

Chronic pancreatitis is a progressive pancreatic fibrotic process, and anti-fibrosis is the key therapy for liver fibrosis. However, previous studies on the effect of emodin on chronic pancreatitis in rats are not available. Our study showed that emodin had an effect on pancreatic fibrosis in rats.

The results of this study indicate that emodin can significantly affect the development of pancreatic fibrosis by reducing the serum levels of HA and LN, ameliorating the degrees of fibrosis, diminishing the content of collagen, inhibiting TGF-β_1 expression. Therefore emodin has an excellent prospect in the development of some new praeparatum for the management of chronic pancreatits. However, there are still some questions we need to answer, such as whether emodin can be used in patients with chronic pancreatitis, whether it is safe, since few previous data are available so far. Further detailed experiments and clinical trails are required to prove the potential clinical application. As a traditional herb, emodin also has a long long way to go.

COMMENTS

Background
Chronic pancreatitis is characterized by a progressive pancreatic fibrotic process. However, the mechanism of fibrotic process is not fully understood. Thus anti-fibrosis is the key to the therapy of chronic pancreatitis.

Research frontiers
Emodin has many biological effects, but studies about its application in chronic pancreatitis are rare.

Innovations and breakthroughs
There are few studies on the application of emodin in chronic pancreatitis. We designed this experiment to investigate the effect of emodin on pancreatic fibrosis.

Applications
No ideal therapy is available so far for the treatment of chronic pancreatitis. Emodin may be efficacious in the management of chronic pancreatitis in rats.

Terminology
Emodin (1, 3, 8-trihydroxy-6-methylanthraquinone), isolated from a great deal of herbs, is an effective constituent with many effects, such as anticancer, antimicrobial, and anti-inflammatory effects, *etc*.

Peer review
The topic is of interest because up to now no antifibrotic treatment option is

available in patients with chronic pancreatitis. The positive effect of emodin on development of pancreatic fibrosis described in this paper shows that emodin may be a potential treatment option.

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