Available online at www.sciencedirect.com

ScienceDirect

Journal of the Chinese Medical Association 81 (2018) 437−443

www.jcma-online.com

Original Article

Amygdalin improves microcirculatory disturbance and attenuates pancreatic fibrosis by regulating the expression of endothelin-1 and calcitonin gene-related peptide in rats

Xiangqun Zhang [a], Jiangong Hu [b], Yuzhen Zhuo [c,d], Lihua Cui [c,d], Caixia Li [c,d], Naiqiang Cui [d,e], Shukun Zhang [c,d,*]

[a] *Department of Emergency, Tianjin Medical University General Hospital, Tianjin, China*
[b] *Department of Pathology, The Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine, Tianjin, China*
[c] *Department of Cell and Molecular Biology, Institute of Integrated Traditional Chinese and Western Medicine, Tianjin Nankai Hospital, Tianjin Hospital of Integrated Traditional Chinese and Western Medicine, Tianjin, China*
[d] *Nankai Clinical College, Tianjin Medical University, Tianjin, China*
[e] *Hepatobiliary and Pancreatic Surgery, Nankai Hospital, Tianjin, China*

Received June 21, 2017; accepted September 6, 2017

Abstract

Background: The pathogenesis of chronic pancreatitis (CP) is a complex process of interaction between tissue injury and repair, which involves microcirculatory disturbance. Amygdalin, an effective component extracted from *Semen Persicae* (a kind of Chinese herbal medicine), can decrease blood viscosity and improve microcirculation. In this study, we investigated the therapeutic effects of amygdalin on pancreatic fibrosis in rats with CP.

Methods: The rat CP model was induced by injecting dibutyltin dichloride (DBTC) into the right caudal vein. Amygdalin was administrated via the penile vein at a dose of 10 mg/(kg d) from the next day, after the induction of CP, once a day for the previous 3 days, and then once every 2 days, until the end of the experiment. Body weight was observed every 7 days. Pancreatic blood flow and histopathological changes were assessed at 28 days. The activation of pancreatic stellate cells (PSCs) was estimated by the expression of α-smooth muscle actin (α-SMA). At the same time, the expression of platelet-derived growth factor-BB (PDGF-BB), transforming growth factor β-1 (TGFβ-1), endothelin-1 (ET-1), and calcitonin gene-related peptide (CGRP) of pancreatic tissues were detected.

Results: Treatment of CP rats with amygdalin improved body weight and pancreatic blood flow, as well as alleviated pancreatic fibrosis and acinar destruction, accompanied by the down-regulation of the expressions of α-SMA, PDGF-BB, TGFβ-1, and ET-1, and the up-regulation of the CGRP's expression.

Conclusion: Amygdalin could reduce the production of pro-fibrotic cytokines, inhibit the activation of PSCs, and attenuate pancreatic fibrosis in a rat with CP. The mechanism probably includes improving microcirculatory disturbance by regulating the production of ET-1 and CGRP.

Keywords: Amygdalin; Chronic fibrosis; Cytokine; Microcirculatory disturbance

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials in this article.

* Corresponding author. Dr. Shukun Zhang, Department of Cell and Molecular Biology, Institute of Integrated Traditional Chinese and Western Medicine, Tianjin Nankai Hospital, Tianjin Hospital of Integrated Traditional Chinese and Western Medicine, 6, Changjiang Road, Tianjin 300100, China.
E-mail address: shu1971@163.com (S. Zhang).

https://doi.org/10.1016/j.jcma.2017.09.005

1. Introduction

Chronic pancreatitis (CP) is an intractable disease characterized by progressive fibrosis, which leads to the permanent loss of exocrine and endocrine functions. Pancreatic stellate cells (PSCs) are the major source of collagen and now known to play a major role in the production of pancreatic fibrosis.[1,2] In the normal pancreas, PSCs are in a state of quiescence. When the pancreas gets damaged, PSCs are activated and undergo both morphological and functional changes. The cells enlarge, proliferate, and secrete collagen types I and other extracellular matrix proteins.

Activated PSCs exhibit positive staining for the cytoskeletal protein α-smooth muscle actin (α-SMA) and become responsive to cytokines such as platelet derived growth factor-BB (PDGF-BB) and transforming growth factor β-1 (TGFβ-1). Studies have indicated that PDGF-BB and TGFβ-1 were overexpressed in CP patients.[3−5] They can stimulate PSCs activation, proliferation and migration.[3,6] Actually, PSCs are activated and secrete collagen to repair damaged tissue, but a lot of PSCs become activated and secrete excessive collagen and other extracellular matrix components leading to pancreatic fibrosis. So it is essential for treating CP to terminate the activation of PSCs in a timely and appropriate manner.

Microcirculatory disturbance is a hallmark in pancreatic fibrosis.[7] Both endothelin-1 (ET-1) and calcitonin gene-related peptide (CGRP) are important factors that affect microcirculation. Amygdalin, an effective monomer component of *Semen Persicae* (a kind of Chinese herbal medicine), has analgesic, anti-coagulation, anti-neoplastic, and anti-inflammatory effects.[8] Whether or not amygdalin inhibits the progression of pancreatic fibrosis remains unknown. The aim of this study was to observe the effect of amygdalin on the microcirculation of pancreas and discuss its potential mechanism in rats with CP.

2. Methods

2.1. Animals

Specific pathogen free male Wistar rats weighing 170−180 g were provided by the Animal Center of Institute of Hygiene Environmental Medicine, Military Medical Science Academy of the PLA, Beijing, China. All rats were housed under standard conditions, including a 12 h light/dark cycle and free access to food and drinking water. All experiments were conducted in accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the experimental protocol was approved by the Medicine Ethical Committee of Tianjin Nankai Hospital.

2.2. Experimental procedures

Forty rats were randomly divided into four groups (n = 10): control group, amygdalin group, CP group, and CP + amygdalin group. Dibutyltin dichloride (DBTC, Sigma−Aldrich, China) was dissolved first in 100% ethanol (two parts) and then mixed with glycerol (three parts). CP model was induced by injecting DBTC solution (8 mg/kg body weight) into the right caudal vein, according to a protocol reported by Sparmann et al.[9] For the control group, the rats were injected with vehicle (ethanol:glycerol, 2:3) only. Amygdalin (Sigma−Aldrich, China) was dissolved in saline solution and administered via the penile vein with a dose of 10 mg/(kg d) from the next day after induction of CP, once a day for the previous 3 days, and then once every 2 days, until the end of the experiment. The body weight of each rat was observed every 7 days. At 28 days after the injection of DBTC, all rats were anesthetized and sterilized. Then the abdominal cavity was opened and the pancreas was exposed for the measurement of the blood flow using laser Doppler flowmeter (PeriFlux 5001 Master monitor, Perimed AB, Sweden). Moreover, the pancreatic tissues were harvested for hematoxylin and eosin (H&E) staining to evaluate the histopathology injury of pancreas. The expression of α-SMA, PDGF-BB, TGFβ-1, ET-1, and CGRP in pancreatic tissues was detected by using western-blot and real-time polymerase chain reaction (PCR), respectively. Experimental protocol was carried out according to the time schedule (Table 1).

2.3. Evaluation of pancreatic histopathology change

Half of the pancreatic tissues were fixed in 4% paraformaldehyde, embedded in paraffin, cut into 3 μm-thick slices and stained with H&E. Histopathologic assessment was performed by two pathologists who blinded to the source of the histology sections they evaluated. Two sections of each sample were taken to acquire image from different five fields of view at 100× magnification. The score was determinated using scoring acinar destruction and fibrosis as follows: 0 = 0%, 1 = 0−25%, 2 = 26%−50% and 3 = 51%−100% in the whole field of view. The alteration of the tissues was calculated as the sum of lesion size scores per one rat.

2.4. Determination of pancreatic blood flow

Rats were anesthetized and placed on a constant temperature (37 °C) heated metal plate. The abdominal cavity was opened along the midline, the pancreas was found along with the duodenum, and then the probe of Laser Doppler flowmetry was placed on the surface of the pancreas at the side of the biliary pancreatic duct and near the duodenum. After it remained stable for 5 min, the microcirculation blood flow was recorded as perfusion unit (PU).

2.5. Detection of the protein expressions of a-SMA, ET-1, and CGRP

The total protein was prepared from the pancreatic tissue and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto a polyvinylidene difluoride membrane. The membrane was incubated with primary antibodies (a-SMA, diluted 1:1000, Abcam, China; ET-1 and CGRP, diluted 1:500, Santa Cruz, USA) at 4 °C overnight, washed with Tris-buffered saline with Tween 20 for 3 times, and incubated with horseradish peroxidase-

Table 1
Time schedule.

Group	0 d	1 d	2 d	3 d	5 d (7 d, 9 d,…27 d)	28 d
Control	vehicle	saline	saline	saline	saline	sacrifice
Amygdalin	vehicle	amygdalin	amygdalin	amygdalin	amygdalin	sacrifice
CP	DBTC	saline	saline	saline	saline	sacrifice
CP + amygdalin	DBTC	amygdalin	amygdalin	amygdalin	amygdalin	sacrifice

conjugated goat anti-rabbit IgG (1:1000, Boster, China) for 2 h at room temperature. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:1000, Cell Signaling, USA) was used as internal control.

2.6. Detection of the mRNA expressions of a-SMA, PDGF-BB, TGFβ-1, ET-1, and CGRP

Total RNA was extracted from the pancreatic homogenization with SV Total RNA Isolation System (Promega, USA), and 1.0 μg of total RNA was reverse-transcribed into cDNA with Reverse Transcription System (Promega, USA). Then 1.0 μl of cDNA product was used for PCR in a 25 μl reaction volume by using the following primers (Table 2) and real-time Master Mix (Tiangen Biotech, China). PCR amplification was performed on iCycler iQ™ Multicolor Real-Time PCR Detection System (Bio-Rad, USA).

2.7. Statistical analysis

The results were expressed as mean ± standard deviation (SD). All data were analyzed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). One-Way ANOVA test followed by the LSD test for multiple comparisons were used for statistical analysis. $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effect of amygdalin on the body weight

During the 7 days after DBTC injection, rats in CP group did not gain weight. Moreover, final body weight in the CP group over the 28-day observation period was found to be significantly lower when compared to the control animals, administration of amygdalin increased the body weight of CP rats (Table 3).

3.2. Effect of amygdalin on the development of DBTC-induced rat pancreatitis

At gross inspection, in contrast to the normal appearance of the pancreas in both the control and amygdalin groups (Fig. 1A and B), the pancreas of the CP rats presented with paleness, marked atrophy, and dilatation of the biliopancreatic duct (Fig. 1C). Treatment of the CP rat with amygdalin could alleviate pancreatic injury (Fig. 1D). Sections from the rats of both the control and amygdalin groups exhibited a normal

Table 2
Sequences of primer in real-time PCR.

Gene	Sequences of the primer	Lengths of product (bp)
a-SMA (NM_031004)	sense: 5′ TGTGCTGGACTCTGGAGATG 3′ antisense: 5′ GATCACCTGCCCATCAGG 3′	292
PDGF-BB (NM_031524)	sense: 5′ CCCAAGAACCTGGGACAAGC 3′ antisense: 5′ GAGAGTTCCTCCAGTCCGTG 3′	192
TGF-β1 (NM_021578)	sense: 5′ CTCAACACCTGCACAGCTCC 3′ antisense: 5′ AGTTGGCATGGTAGCCCTTG 3′	159
ET-1 (NM_012548)	sense: 5′ GAGCAGAGACACAGTGCCAT 3′ antisense: 5′ GCCTGAGTCAGACACGAACA 3′	114
CGRP (NM_017338)	sense: 5′ CCAGATCTAAGCGGTGTGGG 3′ antisense: 5′ TGCCAAAATAGGGGTGGTGG 3′	168
GAPDH (NM_017008)	sense: 5′ GGCTCATGACCACAGTCCAT 3′ antisense: 5′ ACATTGGGGGTAGGAACACG 3′	202

Table 3
The body weight of rats in four groups (n = 10, mean ± SD).

Groups	Body weight (g)				
	0 day	7 day	14 day	21 day	28 day
Control	176.6 ± 7.6	193.0 ± 12.2	220.3 ± 18.7	252.3 ± 23.4	273.3 ± 28.7
Amygdalin	177.1 ± 7.8	190.2 ± 12.6	221.4 ± 18.1	256.2 ± 20.8	275.2 ± 29.1
CP	176.8 ± 6.8	175.4 ± 14.5	188.3 ± 17.6[a]	198.5 ± 21.8[a]	219.4 ± 33.2[a]
CP + amygdalin	177.6 ± 8.0	179.5 ± 13.6	203.5 ± 19.2	230.8 ± 23.1[b]	268.5 ± 32.6[b]

[a] $p < 0.05$, vs the control group; [b] $p < 0.05$, vs the CP group.

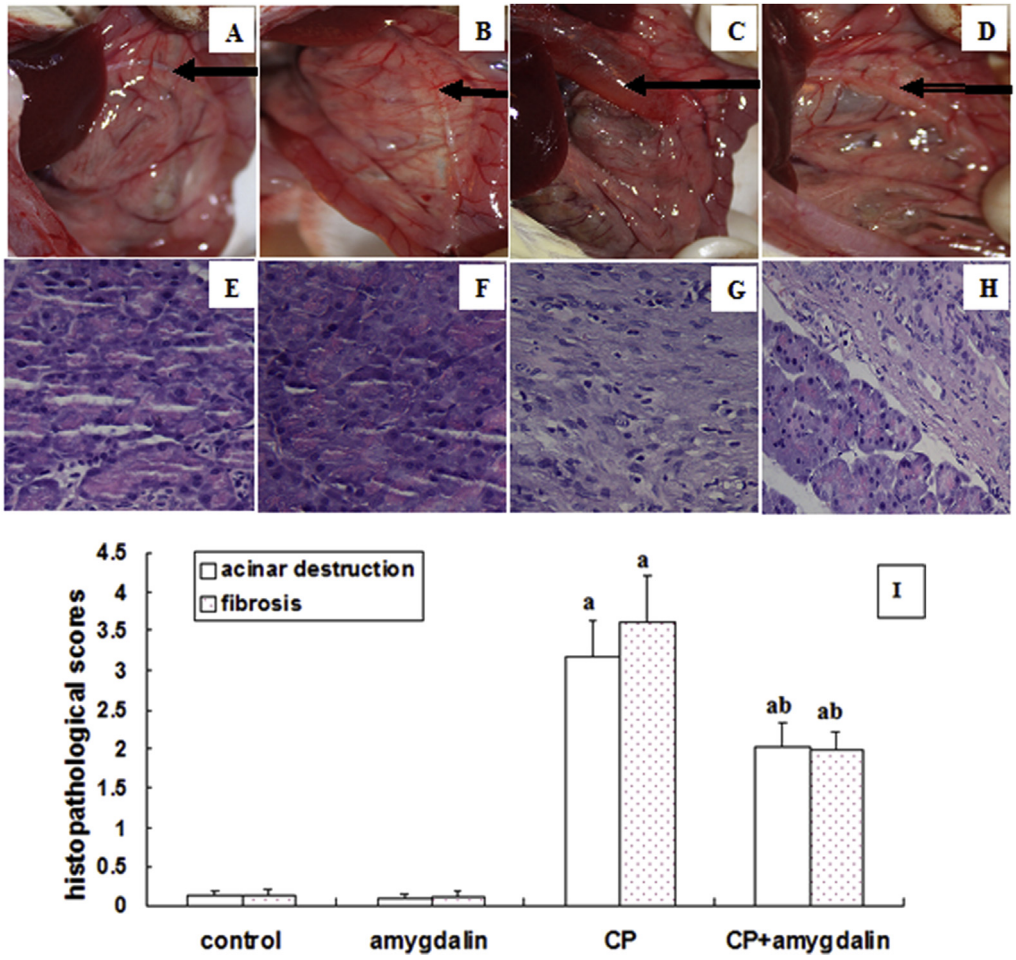

Fig. 1. Gross and microscopic findings in the pancreas of the four groups. **A**–**D**: Gross finding. **A**: Control group, normal appearance of pancreatic tissue. **B**: amygdalin group, normal. **C**: CP group, four weeks after administration of DBTC, the pancreatic tissue was pale, had marked atrophy, and a dilated biliopancreatic duct (arrow). **D**: CP + amygdalin group, both the pancreatic atrophy and the dilatation of pancreatic duct were ameliorated. **E**–**H**: H&E stain (×200). **E**: Control group, normal structure of pancreatic tissue. **F**: amygdalin group, normal. **G**: CP group, destroyed acinar cells and extensive fibrosis were observed. **H**: CP + amygdalin group, acinar cells atrophy and some lobules structure was destroyed, and only intralobular fibrosis was observed. **I**: The scores of acinar degeneration and fibrosis in the CP group were increased compared to those of the control group, and treatment of the CP rats with amygdalin decreased the histopathologic scores significantly. $^{a}p < 0.05$, vs control group; $^{b}p < 0.05$, vs CP group.

structure (Fig. 1E and F). But in the sections of the pancreas from the CP rats, there were found destroyed acinar cells and extensive diffuse fibrosis (Fig. 1G). Amygdalin administration decreased acinar cell atrophy and loss, and substantially reduced fibrosis (Fig. 1H). Both the scores of acinar degeneration and fibrosis in the CP group were increased compared to those in the control group, while the treatment of the CP rats with amygdalin decreased the histopathologic scores significantly ($p < 0.05$, Fig. 1I).

3.3. Effect of amygdalin on pancreatic blood flow

Pancreatic blood flow was decreased in rats with CP compared to that in the control group. Treatment of the rat CP model with amygdalin increased the pancreatic blood flow ($p = 0.014$, Fig. 2).

3.4. Effect of amygdalin on PSCs activation

Activated PSCs were demonstrated by the expression of a-SMA using both western-blot and real-time PCR assay. The mRNA and protein expression of a-SMA were all significantly increased in the CP group compared to those of the control group, and treatment of the CP rats with amygdalin could reduce the expression of PSCs significantly ($p < 0.05$, Fig. 3).

3.5. Effects of amygdalin on the expressions of PDGF-BB and TGFβ-1

The mRNA expressions of PDGF-BB and TGFβ-1, were all markedly increased in the CP group than that in the control group. While treatment of the rat CP model with amygdalin could significantly decrease the expressions (Fig. 4).

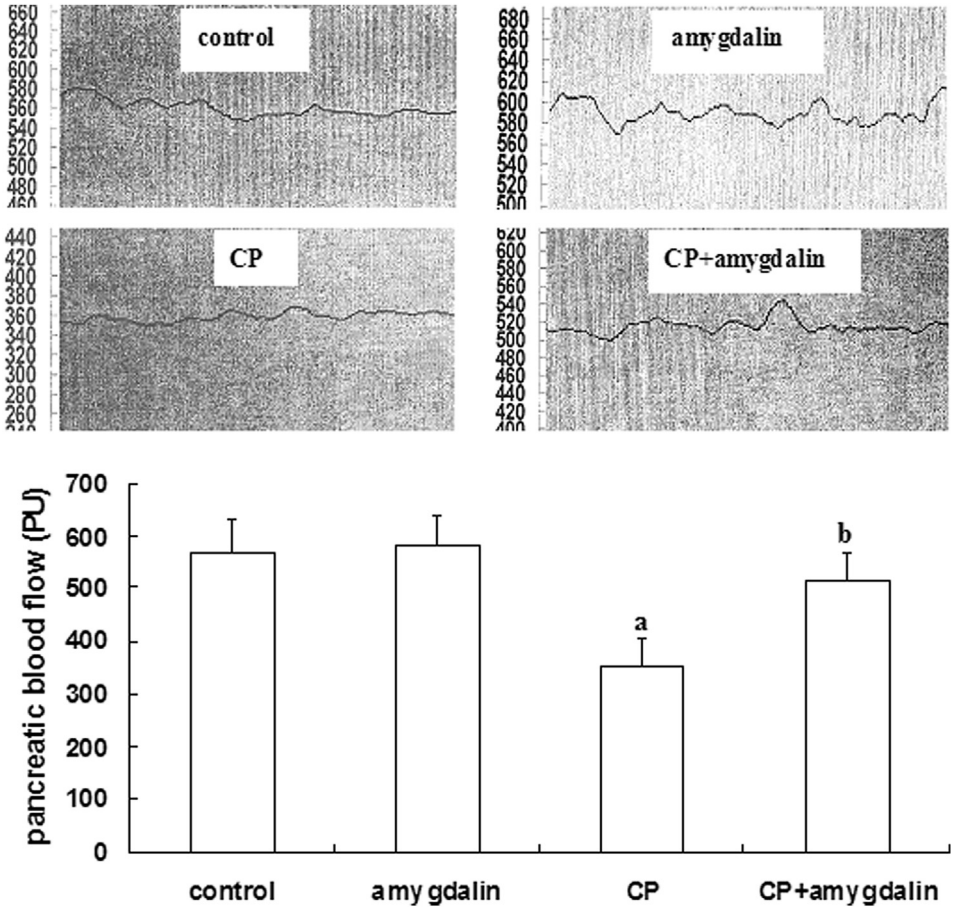

Fig. 2. Pancreatic blood flow in the four groups. The pancreatic blood flow was analyzed by using Laser Doppler flowmetry and was recorded as perfusion unit (PU). The pancreatic blood flow was decreased in rats with CP as compared to the control group. Treatment with amygdalin increased the pancreatic blood flow significantly. [a]$p = 0.000$, vs control group; [b]$p = 0.014$, vs CP group.

3.6. Effects of amygdalin on the expressions of ET-1 and CGRP

Both the protein and the mRNA expression of ET-1 were increased, and the expressions of CGRP were decreased in the CP group. On the other hand, treatment of the rat CP model with amygdalin could significantly down-regulate the expression of ET-1 and up-regulate the expression of CGRP (Fig. 5).

4. Discussion

CP is an irreversible, progressive disease and there is still no specific drug used for treatment. Citric acid, enzyme, and surgery can only relieve relative symptoms temporarily, but cannot stop the progression of pancreatic fibrosis. Amygdalin is a cyanogenic glucoside that widely exists in the seeds of rosaceae plants, including peach, almond, plum, and apricot. It has been used for promoting blood circulation to dissipate blood stasis for many years in China. A recent study confirmed that amygdalin could effectively decrease plasma viscosity, prolong thromboplastin time, and decrease fibrinogen content in rats.[10] Another study by Wu et al. demonstrated that amygdalin could suppress kidney fibroblast proliferation, reduce TGF-β1 secretion, and postpone the process of renal interstitial fibrosis in animal models with unilateral ureteral obstruction.[11] As the usage of amygdalin has been increasingly recognized, we therefore observed the effects of amygdalin on pancreatic microcirculation and fibrosis in rats with CP.

DBTC can specifically induce the necrosis and aggregation of biliary epithelial cell, which cause blockage of bile duct, and then leads to pancreatic acute inflammation in the early stage and fibrosis in late stage. At 28 days, after a single intravenous administration, we observed that the pancreas presented with paleness and atrophy, and the common biliopancreatic duct was dilated to a diameter of 6–15 mm. Histologic changes of the pancreas including fibrosis, tubular complex formation, destruction of acinar cells, and inflammatory cell infiltration were observed in model rats. Along with the fibrotic progression, we observed that the weight of rats with CP decreased at 21 days and 28 days after the application of DBTC, as previous report.[12] According to previous reports, amygdalin at doses in the range of 3–60 mg/kg were shown to protective effects against renal fibrosis and hypoxic brain injury in rats.[11,13,14] In the current study, we demonstrate that amygdalin at a dose of 10 mg/kg ameliorates both the pancreatic appearance and the

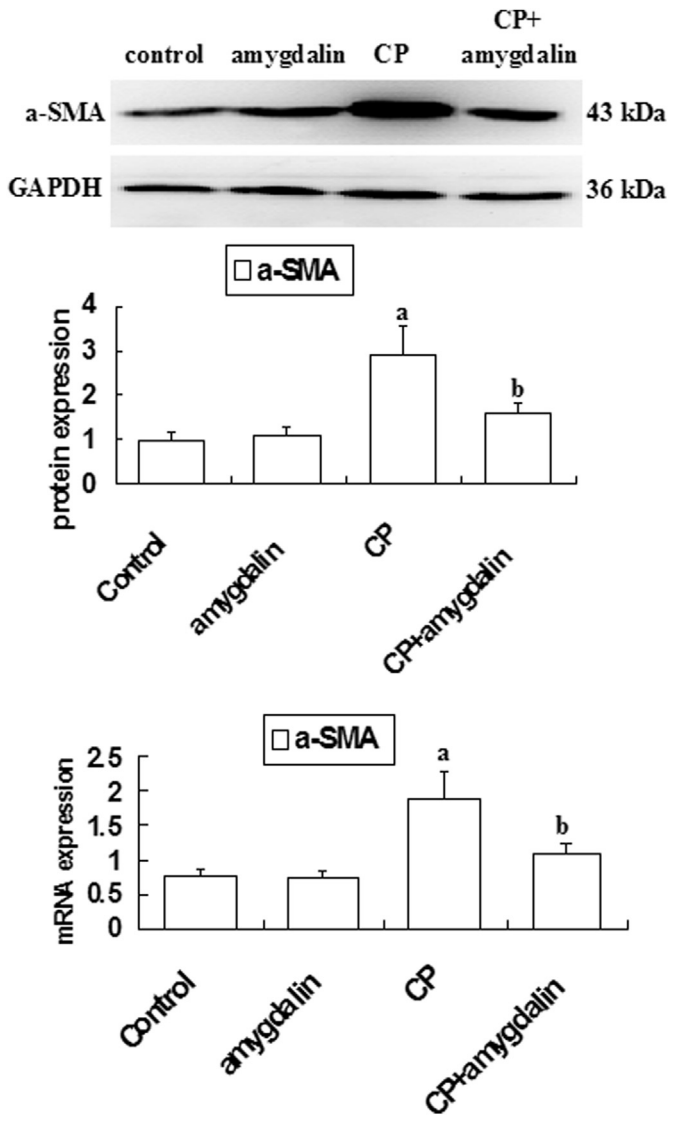

Fig. 3. The protein and mRNA expressions of a-SMA in the pancreas of the four groups. Both the protein and the mRNA expressions of a-SMA were markedly increased in the CP group compared with the control group, treatment of the CP rats with amygdalin significantly decreased the expressions. [a]$p = 0.000$, vs control group; [b]$p = 0.013$, vs CP group.

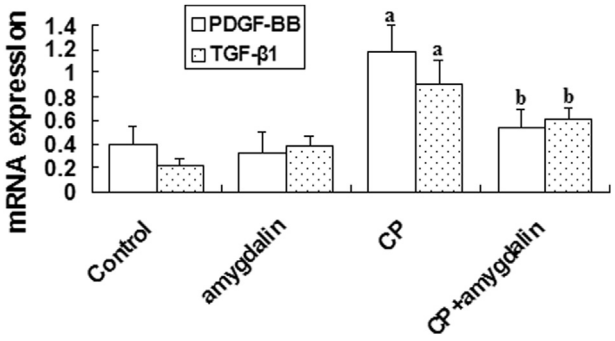

Fig. 4. The mRNA expressions of PDGF-BB and TGFβ-1 in the pancreas of the four groups. mRNA expressions were analyzed by using real time PCR. Both the PDGF-BB and the TGFβ-1 mRNA expressions were increased in the CP group compared with the control group, treatment of the CP rat with amygdalin could significantly decrease the expressions. [a]$p < 0.05$, vs control group; [b]$p < 0.05$, vs CP group.

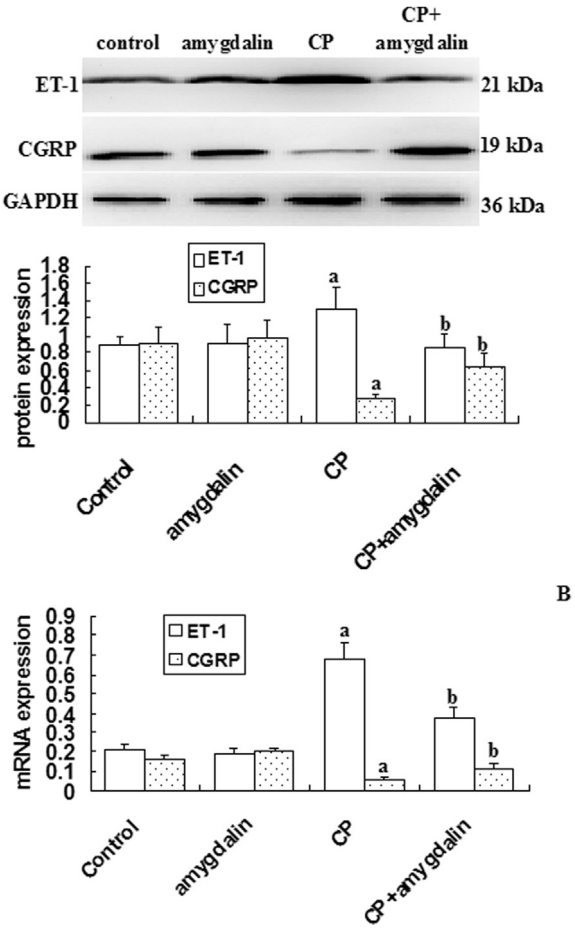

Fig. 5. The protein and mRNA expressions of ET-1 and CGRP in the pancreas of the four groups. The protein and mRNA expressions of ET-1 were markedly increased in the CP group compared with the control group, but the protein and mRNA expressions of CGRP were decreased. Treatment of the CP rat with amygdalin could significantly decrease the expression of ET-1, and increase the expression of CGRP. [a]$p < 0.05$, vs control group; [b]$p < 0.05$, vs CP group.

histopathological changes, and improves the body weight. The results implicates that amygdalin can be a potential remedy for pancreatic fibrosis.

PSCs play a pivotal role in pancreatic fibrogenesis. In response to pancreatic injury or inflammation, quiescent PSCs are activated, and then obtain the ability to proliferate rapidly and secrete lots of extracellular matrix components, such as type I and type III collagen.[15] The expression of a-SMA is a specific marker of the activated PSCs, so the mRNA and protein expression of a-SMA were demonstrated in this study, which was originally derived from platelets, is one of the most powerful stimulating factors that promotes cell mitosis. It has been confirmed that PDGF-BB can accelerate the activation and proliferation of PSCs, and in turn, activated PSCs can secret some kinds of cytokines (such as PDGF-BB and TGF-β1). PDGF-BB and TGF-β1 also promote collagen synthesis and secretion of extracellular matrix synthesis.[16,17] At the same time, PDGF-BB has a chemotactic effect on PSCs by attracting activated PSCs to the damaged site of the pancreas, and aggravates fibrosis.[18] The interactions between inflammatory cytokines and

activated PSCs, aggravate the progress of pancreatic fibrosis. In this study, we found that amygdalin could decrease the mRNA and protein expressions of a-SMA, PDGF-BB and TGF-β1 in pancreatic tissue of CP rats. It is suggested that amygdalin reduced the production of PDGF-BB and TGF-β1, and inhibited the activation of PSCs.

The blood flow of the pancreatic tissue is supplied by the internal carotid artery which is a terminal artery. The anatomical feature determines that pancreas is very sensitive to ischemia. Reduction of blood flow in the pancreatic microcirculation may be the primary reason of pancreatic inflammatory, leading to the capillary thrombosis, ischemia, activation of macrophages and leukocytes, and the production of inflammatory mediators, which can activate PSCs.[19,20] Further research found that the activated PSCs surrounding the pancreatic microvasculature has an effect of contracting the microvessel, which reduces the pancreatic microvascular perfusion; microvascular perfusion deficiency in turn aggravates pancreatic fibrosis and parenchymal atrophy of pancreas.[21] ET-1, as a medium of inflammation, is the strongest long-term vasoconstrictor peptide.[22] While CGRP is a potent vasodilator neuropeptide which can protect myocardium against damage evoked by ischemia reperfusion bind.[23] Our results showed that the expression of ET-1 was reduced and the expression of CGRP was raised with the treatment of the CP rat with amygdalin. It is suggested that the role of amygdalin in improving pancreatic microcirculation may be achieved by down-regulation of ET-1 and up-regulation of CGRP.

In conclusion, our results suggest that amygdalin improves microcirculatory disturbance, attenuates PSCs activation and reduces inflammatory factors production in pancreatic fibrosis, with a probable mechanism by regulating the expression of ET-1 and CGRP *in vivo*.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant numbers 81102686). We thank Professor Sun Yan (Tianjin Medical University) for assistance with the language modification.

References

1. Talukdar R, Tandon RK. Pancreatic stellate cells: new target in the treatment of chronic pancreatitis. *J Gastroenterol Hepatol* 2008;**23**:34–41.
2. Neuschwander-Tetri BA, Bridle KR, Wells LD, Marcu M, Ramm GA. Repetitive acute pancreatic injury in the mouse induces procollagen alpha1(I) expression colocalized to pancreatic stellate cells. *Lab Investig* 2000;**80**:143–50.
3. Nishida A, Andoh A, Imaeda H, Inatomi O, Shiomi H, Fujiyama Y. Expression of interleukin 1-like cytokine interleukin 33 and its receptor complex (ST2L and IL1RAcP) in human pancreatic myofibroblasts. *Gut* 2010;**59**:531–41.
4. Adrych K, Smoczynski M, Stojek M, Sledzinski T, Korczynska J, Goyke E, et al. Coordinated increase in serum platelet-derived growth factor-BB and transforming growth factor-β1 in patients with chronic pancreatitis. *Pancreatology* 2011;**11**:434–40.
5. Stojek M, Adrych K, Rojek L, Smoczynski M, Sledzinski T, Szrok S, et al. Decreased serum platelet derived growth factor BB levels in acute and increased in chronic pancreatitis. *World J Gastroenterol* 2014;**20**:13127–32.
6. Kordes C, Brookmann S, Häussinger D, Klonowski-Stumpe H. Differential and synergistic effects of platelet-derived growth factor-BB and transforming growth factor-beta1 on activated pancreatic stellate cells. *Pancreas* 2005;**31**:156–67.
7. Grauvogel J, Daemmrich TD, Ryschich E, Gebhard MM, Werner J. Chronic alcohol intake increases the severity of pancreatitis induced by acute alcohol administration, hyperlipidemia and pancreatic duct obstruction in rats. *Pancreatology* 2010;**10**:603–12.
8. Wang RF, Fan LG, Gao WY, Zhang JY. Advances in research of chemical constituents and pharmacological activities of Persicae Semen. *Drugs Clin* 2010;**25**:426–9.
9. Sparmann G, Merkord J, Jäschke A, Nizze H, Jonas L, Löhr M, et al. Pancreatic fibrosis in experimental pancreatitis induced by dibutyltin dichloride. *Gastroenterology* 1997;**112**:1664–72.
10. Liu L, Duan JA, Tang Y, Guo J, Yang N, Ma H, et al. Taoren-Honghua herb pair and its main components promoting blood circulation through influencing on hemorheology, plasma coagulation and platelet aggregation. *J Ethnopharmacol* 2012;**139**:381–7.
11. Guo J, Wu W, Sheng M, Yang S, Tan J. Amygdalin inhibits renal fibrosis in chronic kidney disease. *Mol Med Rep* 2013;**7**:1453–7.
12. Glawe C, Emmrich J, Sparmann G, Vollmar B. In vivo characterization of developing chronic pancreatitis in rats. *Lab Investig* 2005;**85**:193–204.
13. Park JH, Seo BI, Cho SY, Park KR, Choi SH, Han CK, et al. Single oral dose toxicity study of prebrewed armeniacae semen in rats. *Toxicol Res* 2013;**29**:91–8.
14. Zhao LG, Wu XZ, Wu XX. Beneficial effect of HHI-I on cerebral microcirculation, blood-brain barrier in rats and anti-hypoxic activity in mice. *Chin J Integr Med* 2009;**15**:128–32.
15. Masamune A, Shimosegawa T. Pancreatic stellate cells–multi-functional cells in the pancreas. *Pancreatology* 2013;**13**:102–5.
16. Tang Y, Liao Y, Kawaguchi-Sakita N, Raut V, Fakhrejahani E, Qian N, et al. Sinisan, a traditional Chinese medicine, attenuates experimental chronic pancreatitis induced by trinitrobenzene sulfonic acid in rats. *J Hepatobiliary Pancreat Sci* 2011;**18**:551–8.
17. Masamune A, Suzuki N, Kikuta K, Ariga H, Hayashi S, Takikawa T, et al. Connexins regulate cell functions in pancreatic stellate cells. *Pancreas* 2013;**42**:308–16.
18. Luttenberger T, Schmid-Kotsas A, Menke A, Siech M, Beger H, Adler G, et al. Platelet-derived growth factors stimulate proliferation and extracellular matrix synthesis of pancreatic stellate cells: implications in pathogenesis of pancreas fibrosis. *Lab Investig* 2000;**80**:47–55.
19. Cuthbertson CM, Christophi C. Disturbances of the microcirculation in acute pancreatitis. *Br J Surg* 2006;**93**:518–30.
20. Chen HM, Sunamura M, Shibuya K, Yamauchi JI, Sakai Y, Fukuyama S, et al. Early microcirculatory derangement in mild and severe pancreatitis models in mice. *Surg Today* 2001;**31**:634–42.
21. Apte MV, Phillips PA, Fahmy RG, Darby SJ, Rodgers SC, McCaughan GW, et al. Does alcohol directly stimulate pancreatic fibrogenesis? Studies with rat pancreatic stellate cells. *Gastroenterology* 2000;**118**:780–94.
22. Sliwińska-Mossoń M, Sciskalska M, Karczewska-Górska P, Milnerowicz H. The effect of endothelin-1 on pancreatic diseases in patients who smoke. *Adv Clin Exp Med* 2013;**22**:745–52.
23. Homma S, Kimura T, Sakai S, Yanagi K, Miyauchi Y, Aonuma K, et al. Calcitonin gene-related peptide protects the myocardium from ischemia induced by endothelin-1: intravital microscopic observation and (31)P-MR spectroscopic studies. *Life Sci* 2014;**118**:248–54.