

Expression of sonic hedgehog signaling pathways in a rat model of chronic pancreatitis

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ABSTRACT

الأهداف: إنشاء نموذج للجرذان تعاني من التهاب البنكرياس لإثبات فعالية طرق (SHH) في التهاب البنكرياس المزمن.

الطريقة: أجريت هذه الدراسة خلال الفترة ما بين يناير 2008 ويوليو 2008م - قسم الجراحة العامة - مستشفى ووهان العام - مدينة ووهان - الصين. تم تقسيم عدد 30 جرذ ويستار عشوائياً إلى 3 مجموعات: (A) مجموعة التحكم، (B) مجموعة التجربة، (C) مجموعة النموذج، بعدد 10 جرذان لكل مجموعة. تم تسريب حمض سولفونيك ترينتروبنزين إلى القناة البنكرياسية لتحريض التهاب البنكرياس المزمن في نموذج مجموعات الجرذان. تم فتح التجويف البطني في جرذان مجموعة التجربة وأجري لها التسريب بمعدل 0.9% من محلول كلوريد الصوديوم. تم تحديد مستويات مصل البيلوروبين وأميلييس بواسطة الطريقة المناعية الإشعاعية. تم دراسة التعاقب النسيجي المرضي بواسطة المجهر البصري. تم اكتشاف ظهور باتشيد (PTCH-1) وسموئند (SMO) و SHH بواسطة الكيمياء النسيجية المناعية.

النتائج: مقارنة مع مجموعة التحكم (A) تبين أن مصل البيلوروبين وأميلييس في مجموعة النموذج قد ازداد بشكل ملحوظ بعد 7 يوم من المعالجة وتبين وجود التكاثر التليفى لأنسجة البنكرياس خلال 35 يوم. ازداد ظهور PTCH-1 و SMO و SHH في النسيج البنكرياسي بشكل ملحوظ في مجموعة النموذج.

خاتمة: يمكن أن يزيد حمض السلفونيك ترينتروبنزين من تحريض التهاب البنكرياس لدى الجرذان. تم تفعيل طرق SHH لدى الجرذان المصابة بالتهاب البنكرياس.

Objectives: To establish a rat model of chronic pancreatitis, and to prove the activation of sonic hedgehog (SHH) signaling pathways in chronic pancreatitis.

Methods: This study was conducted between January and July 2008 in the Department of General Surgery, Wuhan General Hospital, Guangzhou Military Command, Wuhan, China. Thirty Wistar rats were randomly divided into 3 groups: control group (A),

experimental control group (B), and model group (C) (10 rats in each group). Trinitrobenzene sulfonic acid was infused into the pancreatic duct to induce chronic pancreatitis in the model group rats. In the experimental control group, we opened the abdominal cavity and infused with 0.9% sodium chloride solution. Serum levels of bilirubin and amylase were determined by radioimmunoassay. Histopathological alterations were studied using the optical microscopy. Expression of patched-1 (PTCH-1), smoothed (SMO), and SHH were detected by immunohistochemistry.

Results: Compared with the control group (A), the serum bilirubin and amylase in the model group increased significantly after 7 days of treatment, and fibrotic proliferation of pancreatic tissues were found after 35 days; the expression of PTCH-1, SMO, and SHH in the pancreatic tissue increased significantly in the model group.

Conclusion: Trinitrobenzene sulfonic acid can induce chronic pancreatitis in rat. The SHH signaling pathway is activated in rats with chronic pancreatitis.

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Sonic hedgehog (SHH) and its receptors patched (PTC), and smoothed (SMO) are important parts in the hedgehog signaling family. Sonic hedgehog signal plays an important role in pancreas organogenesis and differentiation. Berman et al¹ reported the role of inappropriate activation of the SHH signaling pathway in gastrointestinal tumors, including pancreatic cancer.

Chronic pancreatitis (CP) is a chronic clinical disorder, which is characterized by inflammation and fibrosis, and finally the destruction of pancreas tissue.² As we know, patients with chronic pancreatitis have a high risk of developing pancreatic cancer, but the molecular basis of this process is not clear.³ The expression of SHH and its receptors in chronic pancreatitis tissues of a rat model has not been analyzed before. Since chronic pancreatitis is frequently associated with pancreatic cancer, and since SHH signaling has an important role in pancreatic cancer, we established a chronic pancreatitis model and evaluated the activation of SHH signal in chronic pancreatitis tissues.

Methods. The present study was conducted between January and July 2008 in the Department of General Surgery, Wuhan General Hospital, Guangzhou Military Command, Wuhan, China. All animal experimental protocols were approved by the Animal Care and Use Committee of Wuhan General Hospital of Guangzhou Military Command, Wuhan, China and conformed to the Guide for the Care and Use of Laboratory Animals (National Research Council, Chinese Version, 1996).⁴ Male Wistar rats weighing 200-250g were provided by the Animal Center of Wuhan University. Trinitrobenzene sulfonic acid (TNBS) was obtained from the Sigma Chemical Company (Munich, Germany). Radioimmunoassay kits of rat bilirubin and amylase were purchased from Wuhan Tiangen Science and Technology Ltd (Wuhan, China) Sonic hedgehog, expression of patched-1 (PTCH-1), and SMO polyclonal antibody were provided by the Santa Cruz Company (Santa Cruz, CA, USA).

Chronic pancreatitis model. Thirty Wistar rats were randomly divided into 3 groups: control group (A), experimental control group (B), and model group (C) (10 rats in each group). A chronic pancreatitis model was induced in rats by infusion of TNBS into the pancreatic duct. We opened the abdominal cavity of the rats in the experimental control group and infused with 0.9% sodium chloride solution. No treatment was carried out in the control group.

Measurement of serum bilirubin and amylase. Blood samples were obtained at different time points after treatment, and serum levels of bilirubin and amylase were determined by radioimmunoassay.

Measurement of collagen expression. Five weeks after the treatment, all rats were killed and the pancreatic tissue samples were prepared for further analysis. Pancreatic fibrosis was observed by masson staining of paraffin sections of rat pancreatic tissue.

Immunohistochemistry staining. Paraffin-embedded rat pancreatic tissue was sliced with a thickness of 5 μ m.

One of the slices was stained with hematoxylin-eosin, and the others prepared for immunohistochemistry staining. After all the slices went through an autoclave (120°C, 15 lb) for 15 minutes to expose the antigens fixed by formalin, we treated them with H₂O₂ for 30 minutes to eliminate intrinsic peroxidase. For immunohistochemical staining, the sections were incubated with the rabbit polyclonal anti-PTCH-1 antibody (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA) diluted in 1:25, goat polyclonal anti-SMO antibody (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA) diluted in 1:25, and goat polyclonal anti-SHH antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) diluted in 1:50, at room temperature for 3 hours.

Student's t test and SPSS version 11.0 were used for statistical analysis and $p < 0.05$ was considered statistically significant. Statistical analysis was performed by t-test and χ^2 test.

Results. Rats in the model group had chronic pancreatitis 7 days after the treatment. Cellulose adhesion could be observed around the pancreas of the model rats. There was no such cellulose adhesion in the control and experimental control groups.

Serum bilirubin and amylase increased gradually after treatment in the model group. But in the control and experimental control groups, no changes were observed. This difference is statistically significant after 7 days of treatment ($p = 0.017$). The difference of serum bilirubin and amylase between the control and experimental control groups were not significant (Table 1). Masson staining showed a high expression of collagen in the chronic pancreatitis tissue from the model group. Whereas in the other 2 groups, the expression of collagen is much lower than the model group (Figure 1). The PTCH-1 immunoreactivity was present in the specimens from the model group. No signal was present in the experimental control and control groups. The SMO immunoreactivity was present mainly in cytoplasmic specimens from the model group, but it was negative in the other 2 groups. In the chronic pancreatitis model group, the pancreatic tissue displayed stronger immunoreactivity of SHH, compared with the weak expression of SHH in the experimental control group and the control group (Figure 2).

Discussion. The SHH signal pathway plays an important role in embryo development and organ patterning, such as the pancreas. The SHH ligand has 2 transmembrane receptors: PTCH-1 and SMO. In the absence of SHH, PTCH-1 inhibits SMO activity. When SHH is binded to PTCH-1, SMO will be

Table 1 - Serum bilirubin and amylase in the 3 groups (mean±SD).

Day	Amylase (U/ L)			Bilirubin (μmol/ L)		
	Control group	Experimental control group	Model group	Control group	Experimental control group	Model group
1	1014 ± 115	1123 ± 104	1124 ± 143	2.45 ± 0.32	3.44 ± 0.54	3.17 ± 0.21
3	1040 ± 107	1102 ± 121	1245 ± 105	3.24 ± 0.45	3.24 ± 0.72	4.32 ± 0.16
7	1107 ± 124	1034 ± 142	1475 ± 203	3.57 ± 0.34	3.31 ± 0.73	8.36 ± 2.38
14	1054 ± 110	1125 ± 122	1782 ± 221	3.05 ± 1.72	3.51 ± 0.20	10.54 ± 2.52
21	1053 ± 125	1122 ± 105	1931 ± 451	3.27 ± 1.04	3.45 ± 0.82	13.57 ± 3.37
28	1130 ± 151	1028 ± 217	1743 ± 382	3.35 ± 1.24	3.28 ± 0.34	10.55 ± 2.64
35	1013 ± 142	1007 ± 141	1522 ± 169	3.27 ± 1.43	3.76 ± 0.15	9.28 ± 2.15

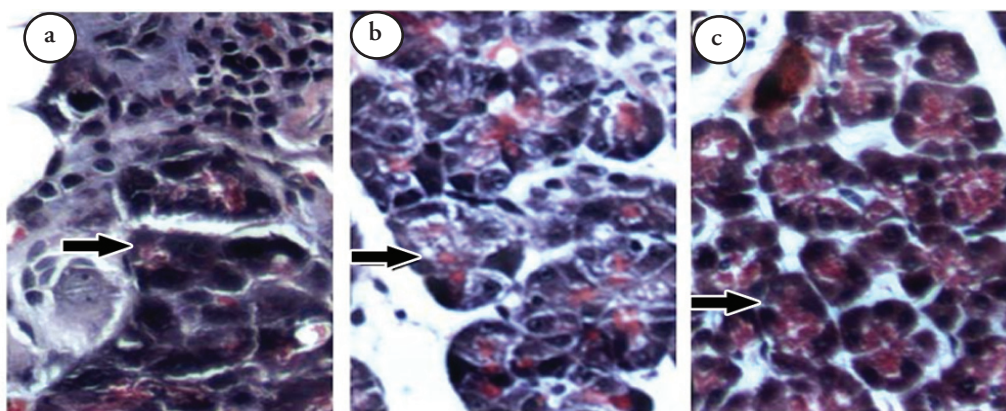


Figure 1 - Collagen expression in the 3 groups. a) masson staining of collagen in the control group (400x); b) masson staining of collagen in the experimental control group (400x) and, c) masson staining of collagen in the model group (400x).

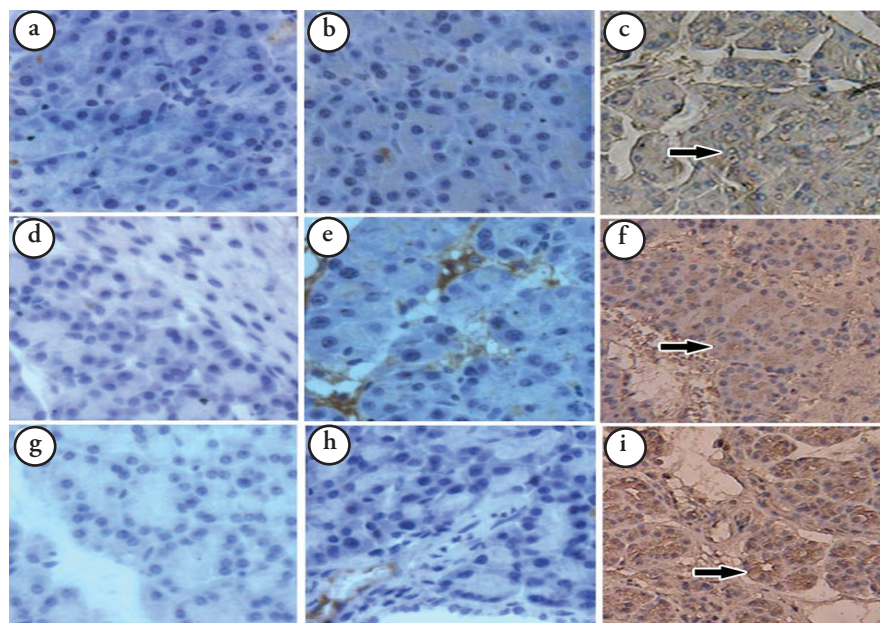


Figure 2 - Expression of expression of patched-1 (PTCH-1), smoothened (SMO), and sonic hedgehog (SHH) in the 3 groups. a) PTCH-1 expression in control group (200x); b) PTCH-1 expression in experimental control group (200x); c) PTCH-1 expression in model group (200x) d) SMO expression in control group (200x); e) SMO expression in experimental control group (200x); f) SMO expression in model group (200x), g) SHH expression in control group (200x); h) SHH expression in experimental control group (200x) and, i) SHH expression in model group (200 x).

activated. This will lead to the activation of the glioma-associated oncogene homolog 1-GLI-1 transcription factor, which induces the activation of some target genes that regulate proliferation and differentiation.⁵ Persistent inflammation could induce the malignant transformation of pancreatic ductal cells and cancer. Some of these changes are observed in samples from chronic pancreatitis.⁶ In chronic pancreatitis, the acinar injury leads to inflammation with continuous infiltration of inflammatory cells and fibrosis.⁷ Hereditary pancreatitis patients have higher risk of developing pancreatic cancer than other patients.⁸ The dysregulation of the SHH signaling has been reported in pancreatic cancer.⁹ For this reason, we established a rat model of chronic pancreatitis in the present study, and investigated the expression of SHH signaling pathways in chronic pancreatitis. After we successfully built the rats model of chronic pancreatitis, we found that the model group had chronic pancreatitis 7 days after the treatment, and cellulose adhesion could be observed around the pancreas of the model rats. There was no such cellulose adhesion in the control and experimental control groups. Accordingly, serum bilirubin and amylase increased gradually after the treatment in the model group, but in the control and experimental control groups, no changes were observed. Masson staining also showed the high expression of collagen in the chronic pancreatitis tissue from the model group. Whereas in the other 2 groups, the expression of collagen is much lower than the model group. These data show that our method of creating a rat model of chronic pancreatitis was available.

In our study, we found that in the chronic pancreatitis model group, the pancreatic tissue displayed stronger immunoreactivity of PTCH-1, SMO, and SHH, compared with the weak expression of these 3 moleculars in the experimental control and control groups. Our results show that SHH signaling is not only active during human pancreatic development; there is also an activation in pancreatic tissues during the pathogenesis of CP.

The correlation of pancreatic cancer in patients with chronic pancreatitis has provided evidence for a link between pancreatic inflammation and cancer. Recently, a report indicated that india hedgehog, another member of the hedgehog family, may be the dominant ligand expressed in both pancreatic cancers and chronic pancreatitis.¹⁰

Our data suggest that in CP, expression of SHH, and its receptors may have the capacity to modulate the growth of pancreatic cells, the co-localization of PTCH-1, SMO, and SHH in CP further suggests that activation of the SHH signaling pathway has a potential role in the pathogenesis of CP. Sonic hedgehog signal pathways may also play an important role in the development from CP to pancreatic cancer. There is a limitation in our study: we did not investigate SHH signal in chronic pancreatitis tissue of patients. We will study this issue in the future.

References

1. Berman DM, Karhadkar SS, Maitra A, Montes De Oca R, Gerstenblith MR, Briggs K, et al. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. *Nature* 2003; 425: 846-851.
2. Church NI, Pereira SP, Churchill D, Cairns S, Hatfield AR, Webster GJ. Chronic pancreatitis: diagnosis and management of complications. *Gut* 200; 56: 1189-1190.
3. Etemad B, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 2001; 120: 682-707.
4. Guide for the Care and Use of Laboratory Animals (Chinese Version). (ISBN 030909285X). Washington (DC): Commission on Life Sciences, National Research Council; 1996.
5. Mfopou JK, De Groote V, Xu X, Heimberg H, Bouwens L. Sonic hedgehog and other soluble factors from differentiating embryoid bodies inhibit pancreas development. *Stem Cells* 2007; 25: 1156-11565.
6. Witt H, Apte MV, Keim V, Wilson JS. Chronic pancreatitis: challenges and advances in pathogenesis, genetics, diagnosis, and therapy. *Gastroenterology* 2007; 132: 1557-1573.
7. Chari ST. Chronic pancreatitis: classification, relationship to acute pancreatitis, and early diagnosis. *J Gastroenterol* 2007; 17: 58-59.
8. Thoufееq M. Comments on hereditary pancreatitis. *Gut* 2009; 58: 1171
9. Thayer SP, di Magliano MP, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature* 2003; 425: 851-856.
10. Kaye H, Kleeff J, Keleg S, Guo J, Ketterer K, Berberat PO, et al. Indian hedgehog signaling pathway: expression and regulation in pancreatic cancer. *Int J Cancer* 2004; 110: 668-676.