

Protective effects and mechanisms of Baicalin and octreotide on renal injury of rats with severe acute pancreatitis

Xi-Ping Zhang, Hua Tian, Yue-Hong Lai, Li Chen, Ling Zhang, Qi-Hui Cheng, Wei Yan, Yun Li, Qing-Yu Li, Qing He, Fei Wang

Xi-Ping Zhang, Department of General Surgery, Hangzhou First People's Hospital, Hangzhou 310006, Zhejiang Province, China

Hua Tian, Li Chen, Department of General Surgery, 2nd Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou 310009, Zhejiang Province, China

Yue-Hong Lai, Zhejiang University of Traditional Chinese Medicine, Hangzhou 310053, Zhejiang Province, China

Ling Zhang, Department of Seven Year's Clinical Medicine, Shanxi Medical University, Taiyuan 310001, Shanxi Province, China

Qi-Hui Cheng, Department of Gynecology and Obstetrics, Hangzhou First People's Hospital, Hangzhou 310006, Zhejiang Province, China

Wei Yan, Yun Li, Qing-Yu Li, Qing He, Fei Wang, Manufacturing Laboratory, Hangzhou First People's Hospital, Hangzhou 310006, Zhejiang Province, China

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Correspondence to: Xi-Ping Zhang, MD, Department of General Surgery, Hangzhou First People's Hospital, 261 Huansha Road, Hangzhou 310006, Zhejiang Province, China. xzp99688@vip.163.com

Telephone: +86-571-87065701 Fax: +86-571-87914773

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Abstract

AIM: To investigate the protective effects and mechanisms of Baicalin and octreotide on renal injury of rats with severe acute pancreatitis (SAP).

METHODS: One hundred and eighty SD rats were randomly assigned to the model group, Baicalin-treated group, octreotide-treated group and sham operation group. The mortality, plasma endotoxin level, contents of blood urea nitrogen (BUN), creatinine (CREA), phospholipase A₂ (PLA₂), nitrogen monoxide (NO), tumor necrosis factor (TNF)- α , IL-6 and endothelin-1 (ET-1) in serum, expression levels of renal Bax and Bcl-2 protein, apoptotic indexes and pathological changes of kidney were observed at 3, 6 and 12 h after operation.

RESULTS: The renal pathological changes were milder in

treated group than in model group. The survival at 12 h and renal apoptotic indexes at 6 h were significantly ($P < 0.05$) higher in treated group than in model group [66.67% vs 100%; 0.00 (0.02)% and 0.00 (0.04)% vs 0.00 (0.00)%, respectively]. The serum CREA content was markedly lower in octreotide-treated group than in model group at 3 h and 6 h ($P < 0.01$, 29.200 ± 5.710 $\mu\text{mol/L}$ vs 38.400 ± 11.344 $\mu\text{mol/L}$; $P < 0.05$, 33.533 ± 10.106 $\mu\text{mol/L}$ vs 45.154 ± 17.435 $\mu\text{mol/L}$, respectively). The expression level of renal Bax protein was not significantly different between model group and treated groups at all time points. The expression level of renal Bcl-2 protein was lower in Baicalin-treated group than in model group at 6 h [$P < 0.001$, 0.00 (0.00) grade score vs 3.00 (3.00) grade score]. The Bcl-2 expression level was lower in octreotide-treated group than in model group at 6 h and 12 h [$P < 0.05$, 0.00 (0.00) grade score vs 3.00 (3.00) grade score; 0.00 (0.00) grade score vs 0.00 (1.25) grade score, respectively]. The serum NO contents were lower in treated groups than in model group at 3 h and 12 h [$P < 0.05$, 57.50 (22.50) and 52.50 (15.00) $\mu\text{mol/L}$ vs 65.00 (7.50) $\mu\text{mol/L}$; $P < 0.01$, 57.50 (27.50) and 45.00 (12.50) $\mu\text{mol/L}$ vs 74.10 (26.15) $\mu\text{mol/L}$, respectively]. The plasma endotoxin content and serum BUN content (at 6 h and 12 h) were lower in treated groups than in model group. The contents of IL-6, ET-1, TNF- α (at 6 h) and PLA₂ (at 6 h and 12 h) were lower in treated groups than in model group [$P < 0.001$, 3.031 (0.870) and 2.646 (1.373) pg/mL vs 5.437 (1.025) pg/mL; 2.882 (1.392) and 3.076 (1.205) pg/mL vs 6.817 (0.810) pg/mL; 2.832 (0.597) and 2.462 (1.353) pg/mL vs 5.356 (0.747) pg/mL; 16.226 (3.174) and 14.855 (5.747) pg/mL vs 25.625 (7.973) pg/mL; 18.625 (5.780) and 15.185 (1.761) pg/mL vs 24.725 (3.759) pg/mL; 65.10 (27.51) and 47.60 (16.50) pg/mL vs 92.15 (23.12) pg/mL; 67.91 ± 20.61 and 66.86 ± 22.10 U/mL, 63.13 ± 26.31 and 53.63 ± 12.28 U/mL vs 101.46 ± 14.67 and 105.33 ± 18.10 U/mL, respectively].

CONCLUSION: Both Baicalin and octreotide can protect the kidney of rats with severe acute pancreatitis. The therapeutic mechanisms of Baicalin and octreotide might be related to their inhibition of inflammatory mediators and induction of apoptosis. Baicalin might be a promising therapeutic tool for severe acute pancreatitis.

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Key words: Severe acute pancreatitis; Baicalin; Octreotide; Renal injury; Rats; Tissue microarrays

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INTRODUCTION

Severe acute pancreatitis (SAP) is a fatal systemic disease featuring acute onset, serious conditions, high incidence of complications and 20%-30% of mortality mainly due to multiple organ failure at its early stage^[1-4]. Octreotide has been shown to exert its therapeutic effects on SAP mainly *via* inhibiting pancreatin secretion, release of inflammatory mediators and platelet aggregation, and reducing endotoxin generation^[5-8]. It is found to improve prognosis and lower mortality by enhancing the kidney protection during SAP. Baical skullcap root is an essential of "Qingyitang", a representative prescription of Traditional Chinese Medicine for SAP. Baicalin is its main effective ingredient (monomer). The *in vitro* experiment of Baicalin has confirmed it has anti-bacterial, antiviral and anti-inflammatory activities. It also can inhibit platelet aggregation and eliminate oxygen-free radicals. It was found in animal experiments that Baicalin could reduce the generation of endotoxin. In addition, Baicalein, which is the initial metabolite of Baicalin, has potent effect in inhibiting pancreatin. All these pharmacologic actions can inhibit SAP during its multiple stages^[9]. It is difficult to popularize octreotide especially in remote areas with poor economy since it features high price, short half-life and inconvenient administration, while Baicalin features low price, extensive routes of administration and preparation, multiple pharmacologic actions and precise therapeutic effects.

The idea of Baicalin treatment of pancreatitis was brought forward in 1999 and validated in 2000. At the beginning, the one-time Baicalin injection *via* vena dorsalis penis or vena femoralis injection was applied, which resulted in poor therapeutic effects. Later, it was found one-time injection was inappropriate due to the short half-life of Baicalin. The expected therapeutic effects could hardly be met with one-time injection. In 2001, the intravenous drip and large dosage were applied, which resulted in sound therapeutic effects. The idea was originated from the study of the principal on pancreatitis treated by Baicalein injection. Baicalin is hydrolyzed into Baicalein. The stability, solubility and therapeutic effects of Baicalin injection are all superior to those of Baicalein injection. In this experiment, the feasibility of Baicalin treatment for SAP has been studied by comparing the protective effects and mechanisms of Baicalin and octreotide on kidneys of rats with SAP.

MATERIALS AND METHODS

Experimental animals

Clean grade healthy male Sprague-Dawley (SD) rat, weighing 250-300 g, were purchased from the Experimental

Animal Center of Medical School of Zhejiang University, China.

Experimental reagents

Sodium taurocholate and sodium pentobarbital were purchased from USA Sigma Company. Octreotide was purchased from Swiss Pharmaceutical Company Novartis, and 5% Baicalin injection (China National Invention Patent Number ZL200310122673.6) was prepared by the first author at 305 mmol/L osmotic pressure. Plasma endotoxin tachypleus amebocyte lysate kit was purchased from Shanghai Yihua Medical Science and Technology Corporation (Institute of Medical Analysis, Shanghai, China); the calculation unit for content is EU/mL. Serum nitrogen monoxide (NO) was purchased from Nanjing Jiancheng Bioengineering Research Institute; the calculation units for content is $\mu\text{mol/L}$. TNF- α ELISA kit was purchased from Jingmei Bioengineering Corporation; the calculation unit for content is pg/mL (ng/L). IL-6 ELISA kit was purchased from Shanghai Shenxiong Biotech Company (China); the calculation unit for content is pg/mL (ng/L). Serum secretory phospholipase A₂ enzyme Assay ELA kit (PLA₂) was purchased from R&D system Ins; the calculation unit for content is U/mL. The serum endothelin-1 ELA kit (ET-1) was purchased from Cayman Chemical Company (Catalog Number: 583151), the calculation unit for content is ng/L (pg/mL). The Bax and Bcl-2 antibodies were purchased from Santa Cruz Company, USA. The main reagents for DNA *in situ* nick end-labeling (TUNEL) staining (Takara *In Situ* Apoptosis Detection Kit) was purchased from TaKaRa Biotechnology (Dalian) Co., Ltd. PK (protease K) was purchased from Sigma Company (USA). DAB (biphenyldiamine) was purchased from China Hua-meimei Company, China.

Preparation of animal models

The improved Aho's method^[10] was adopted to prepare 135 SAP rat models *via* retrograde injection of 35 g/L sodium taurocholate to the pancreatic duct through epidural catheter and duodenal papilla. The 135 SAP rat models were randomly assigned to the model group, Baicalin-treated group and octreotide-treated group, 45 rats in each group, while other 45 rats were assigned to the sham operation group (SO group). In sham operation group, only exploratory laparotomy (i.e., entering abdominal cavity, checking the pancreas and duodenum and then abdomen closure) was performed. Thereafter, the above-mentioned groups were randomly subdivided into 3-h, 6-h and 12-h groups, 15 rats in each group. The rats were observed at 3, 6 and 12 h after operation for: (1) Mortalities of rats in all groups followed by execution of rats and observation of gross pathological changes of kidney; (2) Kidney tissue samples were collected, fixed in accordance with relevant requirements and observed for the pathological score changes of kidney under HE staining; (3) Tissue microarray was applied to prepare the tissue microarray sections (2 mm in diameter) and immunostained using SP (streptavidin-peroxidase) method. Expressions of Bax and Bcl-2 protein in the kidney tissue were observed under light microscope, and grading was carried out based on the percentage of positive cells as follows: (-) = positive

cell count < 10%, (+) = positive cell count 10%-20%, (++) = positive cell count 20%-50%, and (+++) = positive cell count > 50%; (4) TUNEL staining technique was applied to detect apoptotic cells in the kidney and then apoptotic index was calculated as follows: Apoptotic index = apoptotic cell count/total cell count \times 100%; (5) The changes in blood urea nitrogen (BUN), creatinine (CREA), phospholipase A₂ (PLA₂), nitrogen monoxide (NO), tumor necrosis factor (TNF)- α , IL-6 and endothelin-1 (ET-1) contents in blood samples obtained from the heart were determined; and (6) The correlations among these indexes were analyzed.

Procedures

Fast but water restraint was imposed on all rat groups 12 h prior to the operation. The rats were anesthetized by intra-peritoneal injection of 20 g/L sodium pentobarbital (0.25 mL/100 g), laid and fixed on table, routinely shaved, disinfected and draped. After establishing the right external jugular vein transfusion passage by using the microinfusion pump for continuous transfusion (1 mL/h per 100 g), 35 g/L sodium taurocholate was administered to prepare SAP model. To establish model control group, through median epigastrium incision, the bile-pancreatic duct and hepatic hilus common hepatic duct were confirmed, the pancreas was disclosed, the duodenal papilla inside the duodenum duct wall was identified, and then a No. 5 needle was used to drill a hole in the avascular area of mesentery. After inserting a segmental epidural catheter into the duodenal cavity *via* the hole, the bile-pancreatic duct was inserted toward the direction of papilla in a retrograde way, a microvascular clamp was used to nip the duct end temporarily and meanwhile another microvascular clamp was used to temporarily occlude the common hepatic duct at the confluence of hepatic duct. After connecting the anesthetic tube end with the transfusion converter, 35 g/L sodium taurocholate (0.1 mL/100 g) was transfused by retrograde transfusion using the micro-injection pump (made by Zhejiang University) at a speed of 0.2 mL/min. After 4 to 5 min post-injection, the microvascular clamp and epidural catheter were removed. After checking for bile leakage, the hole in the lateral wall of duodenum was sutured. The anesthetic in the abdominal cavity was absorbed up by disinfected cotton ball and then the abdomen was closed. Sham operation group received laparotomy *via* upper midline incision, turning over of the pancreas and duodenum and finally closure of the abdomen.

Dosage

In Baicalin-treated group, the animal experiments of 5% Baicalin injection have been completed including the acute toxicity test and SAP rat treatment by small, middle and large dose. The large dose (10 mg/h per 100 g) can achieve the best therapeutic effect and the dosage referred to the result of the previous preliminary experiment^[10]. Ten minutes after successful modeling, Baicalin-treated group was first injected 5% Baicalin injection (10 mg/100 g) *via* the external jugular vein, followed by continuous intravenous administration (10 mg/h per 100 g) by microinfusion pump. Octreotide-treated group was

first injected octreotide (0.2 μ g/100 g) *via* the external jugular vein, followed by continuous intravenous transfusion (10 mg/h per 100 g) by microinfusion pump at a transfusion speed of 0.2 μ g/h per 100 g. All above-mentioned dosages have been proved as effective dosages in the previous preliminary experiment^[10]. Both the sham operation group and model control group were injected normal saline of equivalent volume at the corresponding time points after operation. The diameter of the drilling needle is 2.0 mm.

Statistical analysis

The values were presented as mean \pm SD for normal distribution variables or median and quartile range for highly skewed variables. The significance of differences among the four groups was analyzed using Kruskal-Wallis test for highly skewed data and analysis of variance (ANOVA) for normal distribution data. Multiple comparisons were subjected to Bonferroni correction test. Chi-square test was used to evaluate equality of frequencies for discrete variables. Correlations were tested using Spearman rank correlation coefficients. A *P* value less than or equal to 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS version 11.5 for windows.

RESULTS

Survival rate

The mortalities of model group were 0% (0/15), 13.33% (2/15) and 33.33% (5/15) at 3, 6 and 12 h, respectively, while those of Baicalin-treated group and octreotide-treated group were 0% at different time points, indicating a marked difference at 12 h (*P* < 0.05). The whole sham operation group survived at different time points.

Serum BUN content

Serum BUN content was markedly higher in model group and treated groups than in sham operation group at all time points (*P* < 0.001). However, the content was not significantly different between Baicalin- and octreotide-treated groups at all time points. The content was lower in Baicalin-treated group than in model group at 3 and 12 h (*P* < 0.05). The content was not different between octreotide-treated group and model group at 3 h. The content was lower in Baicalin-treated group than in model group at 6 h (*P* = 0.001), lower in octreotide-treated group than in model group (*P* < 0.05), and lower in octreotide-treated group than in model group at 12 h (*P* < 0.01) (Table 1).

Serum CREA content

The CREA content was significantly higher in model group and treated groups than in sham operation group at all time points (*P* < 0.001). However, no significant difference was found between Baicalin-treated group and model group at all time points. The content was lower in octreotide-treated group than in Baicalin-treated group at 3 h and 12 h (*P* < 0.01), and also lower in octreotide-treated group than in model group at 3 h (*P* < 0.01) and 6 h (*P* < 0.05). But no marked difference was observed between Baicalin-

Table 1 Comparison of different indexes level in blood [*M (Q_n)*]

		Sham operation group	Model group	Baicalin treated group	Ocreotide treated group
Endotoxin (EU/mL)	3 h	0.016 (0.005)	0.053 (0.029)	0.027 (0.005)	0.033 (0.006)
	6 h	0.016 (0.010)	0.059 (0.037)	0.039 (0.019)	0.031 (0.010)
	12 h	0.014 (0.015)	0.060 (0.022)	0.034 (0.015)	0.042 (0.014)
BUN (mmol/L)	3 h	5.310 (0.940)	12.050 (4.030)	10.530 (3.625)	9.850 (3.020)
	6 h	5.500 (2.200)	17.390 (3.850)	12.220 (4.530)	13.930 (5.500)
	12 h	4.860 (1.590)	22.270 (11.375)	13.720 (4.380)	13.520 (9.810)
NO (μmol/L)	3 h	7.500 (5.000)	65.000 (7.50)	57.500 (22.50)	52.500 (15.00)
	6 h	7.500 (5.000)	62.500 (38.75)	47.500 (37.50)	57.500 (15.00)
	12 h		74.100 (26.15)	57.500 (27.50)	45.000 (12.50)
TNF-α (pg/mL)	3 h	3.900 (3.200)	41.440 (37.72)	44.930 (45.84)	39.300 (30.60)
	6 h	4.000 (1.700)	92.150 (23.12)	65.100 (27.51)	47.600 (16.50)
	12 h	5.3000 (3.000)	65.020 (26.81)	47.650 (25.52)	54.500 (41.40)
IL-6 (pg/mL)	3 h	1.846 (0.346)	5.437 (1.025)	3.031 (0.870)	2.646 (1.373)
	6 h	1.743 (0.838)	6.817 (0.810)	2.882 (1.392)	3.076 (1.205)
	12 h	2.036 (0.818)	5.356 (0.747)	2.832 (0.597)	2.462 (1.353)
ET-1 (pg/mL)	3 h	15.293 (4.231)	24.745 (1.011)	19.635 (6.065)	16.827 (3.775)
	6 h	16.275 (3.180)	25.625 (7.973)	16.226 (3.174)	14.855 (5.747)
	12 h	14.173 (2.556)	24.725 (3.759)	18.625 (5.780)	15.185 (1.761)

Table 2 Comparison of serum CREA content (mean ± SD, μmol/L)

Groups	3 h	6 h	12 h
Sham operation group	17.867 ± 2.890	21.467 ± 3.044	19.733 ± 3.150
Model group	38.400 ± 11.344	45.154 ± 17.435	41.500 ± 12.122
Baicalin-treated group	37.615 ± 9.483	39.867 ± 13.648	50.733 ± 29.310
Ocreotide-treated group	29.200 ± 5.710	33.533 ± 10.106	33.933 ± 9.145

and ocreotide-treated groups. Moreover, the content was not different between ocreotide-treated group and model group at 12 h (Table 2).

Gross changes and light microscopic changes of kidney

Sham operation group: Macroscopically, the morphous of kidney was normal without swelling, with no bleeding points on surface of renal cortex. Microscopically, there were normal structure of renal glomerulus, tubule and interstitium in most rats without visible pathological change; however, swelling and blurry boundary of renal tubular epithelial cells, and stenosis of lumens were found in very few rats.

Model group: Macroscopically, there was no gross change in the kidney at 3 h; but were kidney swelling, tension of renal envelope, scattered bleeding points on surface of renal envelope, and slightly hemorrhagic urine in pelvis in severe cases at 6 h and 12 h. Microscopically, there were capillary congestion of renal glomerulus, swelling, scattered necrosis and blurry boundary of renal tubule epithelial cell, stenosis or atresia of lumens, visible protein cast (Figure 1A), interstitial edema and inflammatory cell infiltration at 3 h; and capillary congestion of renal glomerulus, swelling and scattered necrosis of epithelial cell of renal tubule (Figure 1B), interstitial edema (Figure 1C) and inflammatory cell infiltration at 6 and 12 h. The floss and red cell with eosinophilic stainings were found in renal glomerulus and homogenous or red cell cast with eosinophilic staining in renal tubule (Figure 1D).

There was lamellar necrosis of epithelial cell of renal tubule in few rats.

Baicalin- and ocreotide-treated groups: Macroscopically, the gross renal pathological changes were milder in Baicalin- and ocreotide-treated group than in model group at 6 h and 12 h. Microscopically, there were less capillary congestion of renal glomerulus, swelling of renal tubular epithelial cell, floss and red cell with eosinophilic staining in renal capsule and inflammatory cell infiltration in treated group than in model group. Mild red cell cast was found occasionally in renal tubule of treated group. There were also renal interstitial edema and scattered necrosis of renal tubular epithelial cell in few cases. There was no visible difference between Baicalin- and ocreotide-treated groups. Better therapeutic effects were achieved in ocreotide-treated group.

Changes of pathological score of kidney in all groups

Pathological grading of kidney: The pathological grading of kidney was used (Table 3) and two pathologists performed the evaluation of degree of pathological changes in pancreatic tissue in double-blind fashion.

Comparison of pathological score of kidney: The score was significantly higher in model group, Baicalin- and ocreotide-treated groups than in sham operation group at different time points ($P < 0.001$). However, the score was lower in Baicalin- and ocreotide-treated groups than in model group at 6 h ($P < 0.05$). The score was lower in ocreotide-treated group than in model group at 12 h ($P < 0.05$). There was not significant different between Baicalin- and ocreotide-treated groups at different time points (Table 4).

Expression of Bax protein in renal tissue

Bax-positive staining was located in the kytoplasm of renal tubular epithelial cell (Table 5 and Figure 2A-E). The expression level was not different among all groups at 12 h. The level was higher in model group and Baicalin-treated group than in sham operation group at 3 h and

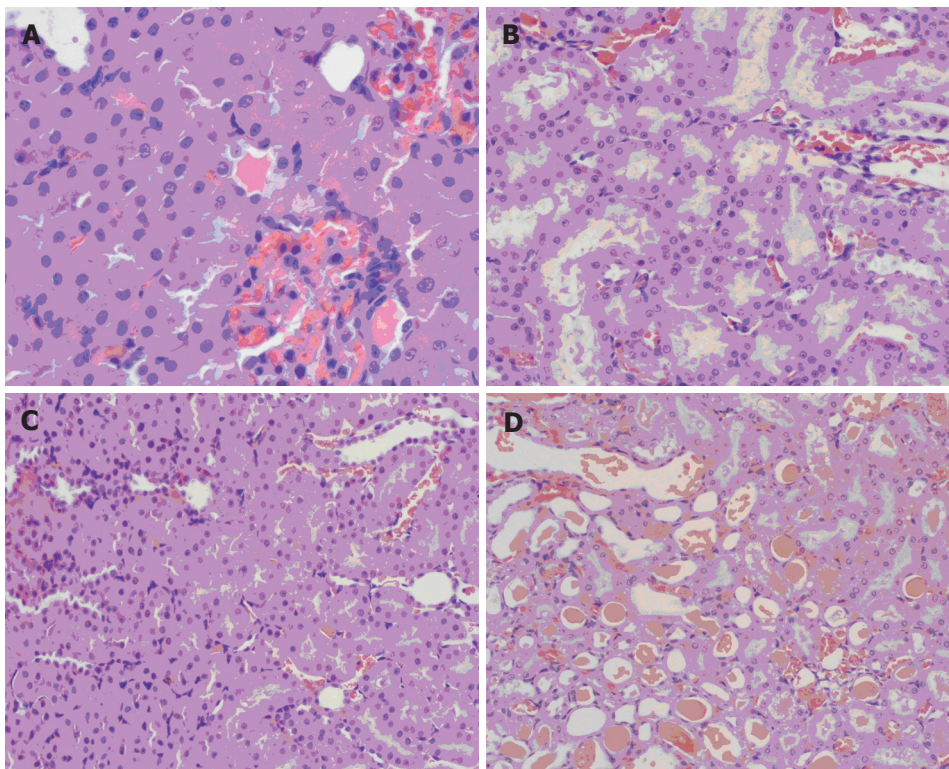


Figure 1 Light microscopic changes of kidney (HE, ×400). **A:** 3-h model group showing protein cast in renal tubule; **B** and **C:** 6-h model group showing scattered degenerative necrosis in renal tubular epithelial cells, and renal interstitial edema, respectively; **D:** 12-h model group showing visible red cell cast.

Table 3 Pathological score standard of kidney

Grade	Observation indexes
I	No cellular proliferation or fibrosis in renal glomerulus; no capillary congestion or microthrombus; swelling and blurry boundary of renal tubular epithelial cell; stenosis or atresia of lumens; protein cast and renal interstitial edema
II	Glomerular capillary congestion, scattered necrosis in renal tubular epithelial cell, interstitial edema and inflammatory cell infiltration
III	II + lamellar necrosis of renal tubular epithelial cell

Table 4 Comparison of pathological score of kidney in all groups [$M(Q_R)$]

Groups	3 h	6 h	12 h
Sham operation group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Model group	2.00 (1.00)	2.00 (1.00)	2.00 (1.25)
Baicalin treated group	1.00 (1.00)	1.00 (1.00)	2.00 (1.00)
Octreotide-treated group	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)

6 h ($P < 0.05$), and also higher in octreotide-treated group than in sham operation group at 6 h ($P < 0.05$). The expression level was not different between model group and Baicalin-treated group at all time points. Similarly, the expression level was not different between Baicalin- and octreotide-treated groups. The level was lower in octreotide-treated group than in model group at 3 h ($P < 0.05$) (Table 6).

Expression of Bcl-2 protein in renal tissue

Bcl-2-positive staining was located in the cytoplasm of renal tubular epithelial cells. (Table 7 and Figure 3A-C). The

Table 5 Expression of Bax protein in kidney

Groups	<i>n</i>	Pathologic grade			
		-	+	++	+++
Sham operation group	(3 h) 15	15			
	(6 h) 15	15			
	(12 h) 15	15			
Model group	(3 h) 15	7	4	4	
	(6 h) 13	10	1	2	
	(12 h) 10	7	1	1	1
Baicalin treated group	(3 h) 15	11	3	1	
	(6 h) 15	10	4	1	
	(12 h) 15	12	3		
Octreotide treated group	(3 h) 15	12	3		
	(6 h) 15	11	2	2	
	(12 h) 15	10	4	1	

level was higher in model group than in sham operation group at all time points ($P < 0.05$). The level was higher in Baicalin-treated group than in sham operation group at 12 h ($P < 0.05$), lower in octreotide-treated group than in Baicalin-treated group ($P < 0.05$), lower in Baicalin-treated group than in model group at 6 h ($P < 0.001$), and also lower in octreotide-treated group than in model group at 6 and 12 h ($P < 0.05$) (Table 8).

Comparison of renal apoptotic index

The apoptotic cells were renal tubular epithelial cells. The index was not different between model group and sham operation group at different time points. Moreover, the index was not different among all groups at 3 and 12 h. The index was higher in Baicalin- and octreotide-treated groups than in sham operation group and model group at 6 h ($P < 0.05$). The index was not different between Baicalin- and octreotide-treated groups at all time points (Table 9 and Figure 4A-D).

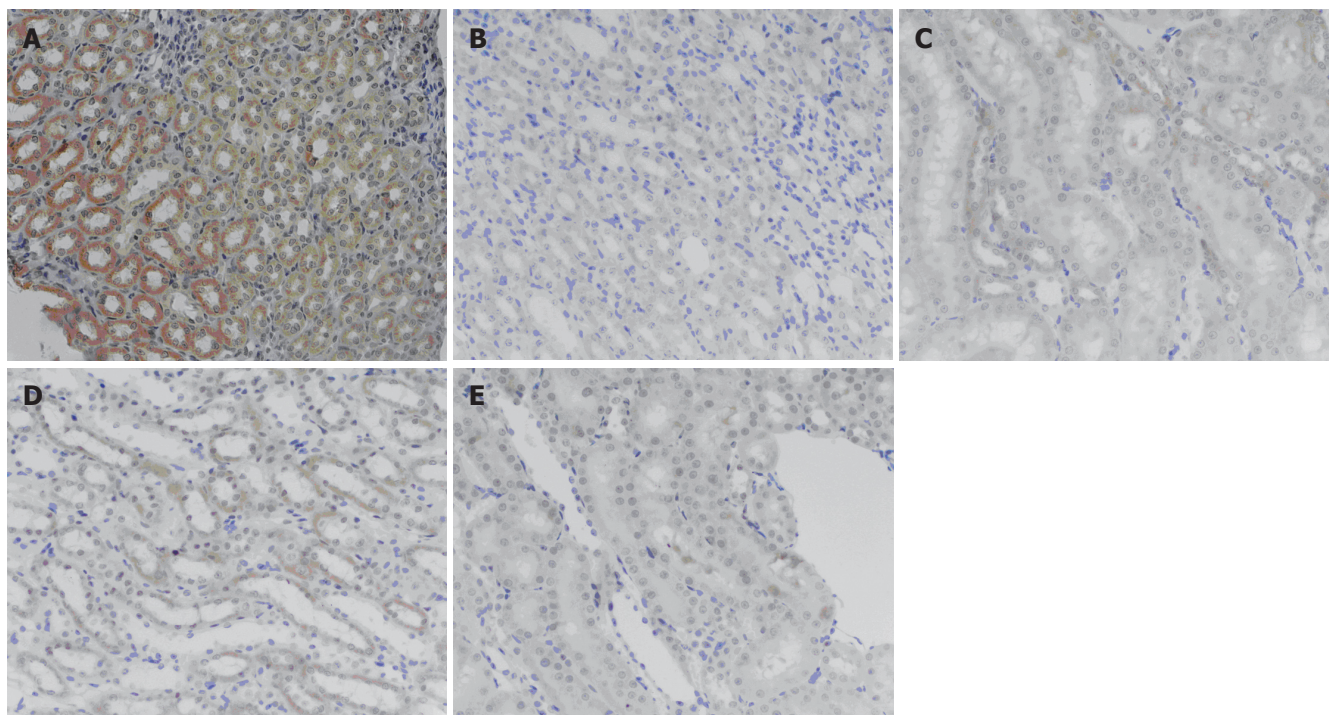


Figure 2 Bax expression in different groups ($\times 200$). **A:** 12-h model group showing high (+++) Bax expression; **B:** 3-h Baicalin-treated with negative (-) Bax expression; **C:** 12-h model group showing moderate (++) Bax expression; **D and E:** 6-h octreotide-treated group showing mild (+) Bax expression.

Table 6 Comparison of Bax protein in kidney [$M(Q_2)$] grade score

Groups	3 h	6 h	12 h
Sham operation group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Model group	1.00 (2.00)	0.00 (0.50)	0.00 (1.25)
Baicalin-treated group	0.00 (0.50)	0.00 (1.00)	0.00 (0.00)
Octreotide-treated group	0.00 (0.00)	0.00 (1.00)	0.00 (1.00)

Table 7 Expression of Bcl-2 protein in kidney

Groups	<i>n</i>	Pathologic grade			
		-	+	++	+++
Sham operation group	(3 h)	15	15		
	(6 h)	15	15		
	(12 h)	15	15		
Model group	(3 h)	15	10	2	2
	(6 h)	13	4	1	8
	(12 h)	10	7	2	
Baicalin-treated group	(3 h)	15	14	1	
	(6 h)	15	15		
	(12 h)	15	11	4	
Octreotide-treated group	(3 h)	15	13	2	
	(6 h)	15	15		
	(12 h)	15	15		

Comparison of plasma endotoxin content

The content was higher in model group and treated group than in sham operation group at all time points ($P < 0.001$). The content was not different between Baicalin- and octreotide-treated groups at 6 and 12 h. The content was lower in Baicalin- and octreotide-treated groups than in model group at 3 h ($P < 0.001$), lower in Baicalin-treated

group than in octreotide-treated group at 3 h ($P < 0.01$), lower in Baicalin-treated group than in model group at 6 h ($P < 0.05$), lower in octreotide-treated group than in model group at 6 h ($P = 0.001$), lower in Baicalin-treated group than in model group at 12 h ($P < 0.001$), and also lower in octreotide-treated group than in model group at 12 h ($P < 0.01$) (Table 1).

Comparison of serum PLA₂ content

Serum PLA₂ content in model group and treated groups significantly exceeded sham operation group at different time points ($P < 0.001$). At 3 h, PLA₂ content in Baicalin-treated group was significantly less than model group and octreotide-treated group ($P < 0.01$), but no marked difference was observed between octreotide-treated group and model group. At 6 h and 12 h, PLA₂ content in Baicalin- and octreotide-treated groups was significantly less than model group ($P < 0.001$). There was no marked difference between Baicalin-treated group and octreotide-treated group at 6 h, while octreotide-treated group had significantly less PLA₂ content than Baicalin-treated group at 12 h ($P < 0.001$) (Table 10).

Comparison of serum NO content

Serum NO content in model group, Baicalin-treated group and octreotide-treated group significantly exceeded sham operation group at different time points ($P < 0.001$). At 3 h and 12 h, Baicalin-treated and octreotide-treated groups had significantly less serum NO content than model group ($P < 0.05$). There was no marked difference in serum NO content between Baicalin-treated group and octreotide-treated group at different time points (Table 1).

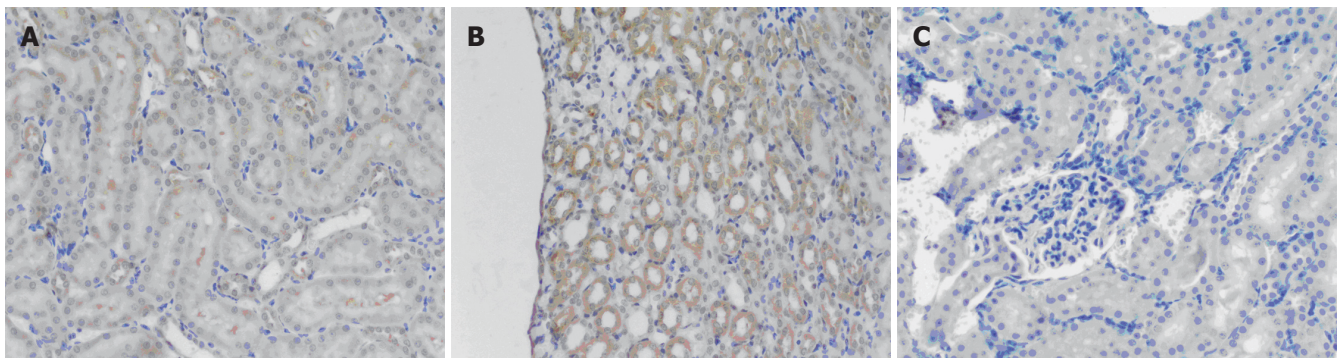


Figure 3 Bcl-2 expression in different groups (× 200). **A** and **B**: 6-h model group showing high (+++) Bcl-2 expression; **C**: 6-h octreotide-treated group with negative (-) Bcl-2 expression.

Table 8 Comparison of Bcl-2 protein in kidney [$M(Q_R)$] grade score

Groups	3 h	6 h	12 h
Sham operation group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Model group	0.00 (2.00)	3.00 (3.00)	0.00 (1.25)
Baicalin-treated group	0.00 (0.00)	0.00 (0.00)	0.00 (1.00)
Octreotide-treated group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

Table 9 Apoptotic index of kidney [$M(Q_R)$] (%)

Groups	3 h	6 h	12 h
Sham operation group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Model group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Baicalin-treated group	0.00 (0.01)	0.00 (0.02)	0.00 (0.00)
Octreotide-treated group	0.00 (0.00)	0.00 (0.04)	0.00 (0.00)

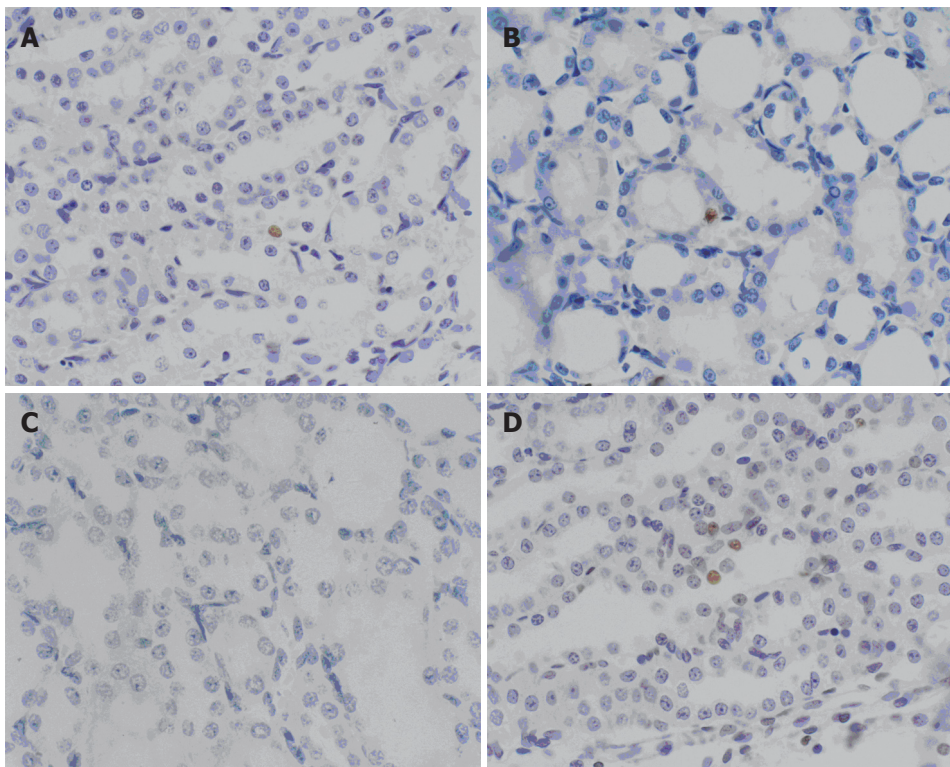


Figure 4 Apoptosis in different groups (TUNEL, × 400). **A**: 6-h octreotide-treated group showing apoptosis of renal tubular epithelial cells; **B**: 6-h Baicalin-treated group showing apoptosis of renal tubular epithelial cells; **C**: 6-h model group with no (-) apoptosis; **D**: 6-h octreotide-treated group showing apoptosis of renal tubular epithelial cells.

Comparison of serum TNF- α content

Serum TNF- α content in model group and treated groups significantly exceeded sham operation group at different time points ($P < 0.001$). There was no significant difference among model group, Baicalin-treated group and octreotide-treated group at 3 h and 12 h. At 6 h, serum TNF- α contents in Baicalin-treated group and octreotide-treated group were significantly less than model control group ($P < 0.001$); and octreotide-treated group had signif-

icantly less serum TNF- α content compared to Baicalin-treated group ($P < 0.01$) (Table 1).

Comparison of serum IL-6 content

Serum IL-6 contents at 3 h and 6 h were significantly higher in model control group and treated groups than in sham operation group ($P < 0.001$). Baicalin-treated group and octreotide-treated group had no significant difference in serum IL-6 content at all time points. Baicalin-

Table 10 Comparison of serum PLA₂ content (mean \pm SD, U/mL)

Groups	3 h	6 h	12 h
Sham operation group	14.62 \pm 3.02	17.49 \pm 3.82	19.02 \pm 5.07
Model group	76.10 \pm 16.70	101.46 \pm 14.67	105.33 \pm 18.10
Baicalin-treated group	56.25 \pm 22.43	67.91 \pm 20.61	66.86 \pm 22.10
Octreotide-treated group	74.37 \pm 19.94	63.13 \pm 26.31	53.63 \pm 12.28

and octreotide-treated groups had significantly lower serum IL-6 content compared to model control group at all time points ($P < 0.001$). The model control group had significantly higher serum IL-6 content than sham operation group at 12 h ($P < 0.001$), and so was Baicalin-treated group ($P < 0.01$), but no significant difference was found between octreotide-treated group and sham operation group (Table 1).

Comparison of serum ET-1 contents

Serum ET-1 content in model group was significantly higher than in sham operation group at all time points ($P < 0.001$). At all time points, Baicalin- and octreotide-treated groups had significantly lower serum ET-1 content than model group ($P < 0.001$). Octreotide-treated group had significantly lower ET-1 content compared to Baicalin-treated group at 3 h and 12 h ($P < 0.01$). At 3 h, Baicalin-treated group had significantly higher ET-1 content than sham operation group ($P < 0.01$), but no significant difference was found between octreotide-treated group and sham operation group. At 6 h, there was no marked difference between Baicalin-treated group or octreotide-treated group and sham operation group, or between Baicalin-treated group and octreotide-treated group. At 12 h, octreotide-treated group and sham operation group had no marked difference, and Baicalin-treated group had significantly higher ET-1 content than sham operation group ($P < 0.001$) (Table 1).

Comparison of correlations among various indexes

Correlation between apoptotic indexes and Bax and Bcl-2 expression in the kidney: The apoptotic index of Baicalin-treated group at 3 h and that of octreotide-treated group at 3 h and 6 h was positively correlated with Bax expression ($P < 0.001$). However, there was no correlation between apoptotic index and Bcl-2 expression.

Correlation between pathological score change and BUN and CREA of kidney: The pathological score of sham operation group at 12 h was positively correlated with BUN ($P < 0.01$) and CREA levels ($P < 0.05$). The pathological score of model group was positively correlated with CREA at 3 h ($P = 0.01$) and 6 h ($P < 0.001$). The score of Baicalin-treated group at 3 h and 6 h was positively correlated with CREA ($P < 0.05$). The pathological score of Baicalin-treated group was positively correlated with BUN at 6 h ($P < 0.05$) and 12 h ($P < 0.01$). The pathological score of octreotide-treated group at 3 h was positively correlated with BUN and CREA ($P < 0.05$), and that at 6 h and 12 h was positively correlated with CREA ($P < 0.01$) and BUN ($P < 0.01$), respectively.

Correlation analysis among inflammatory mediators: PLA₂ content at 12 h was positively correlated with TNF- α in model group ($P < 0.05$), and TNF- α was positively correlated with PLA₂ content ($P < 0.01$) at 3 h.

DISCUSSION

This study demonstrated that there were milder renal pathological changes and lower serum BUN content in treated groups as compared with model group. The survival rate was higher in treated groups compared to model group. All these indicate the potent therapeutic effect of Baicalin and octreotide on rats with severe acute pancreatitis. Baicalin showed superiority over octreotide in decreasing plasma endotoxin and PLA₂ content in SAP rats at 3 h, while octreotide was found to be superior to Baicalin in alleviating renal pathological changes and decreasing CREA, Bcl-2, TNF- α , PLA₂ (at 12 h) and ET-1 contents.

Regarding the mechanisms *via* which these two drugs improve renal pathological changes, we mainly hypothesized inhibition of inflammatory mediators and induction of apoptosis.

The endotoxin^[11] in plasma, and PLA₂^[12,13], NO^[14-18], TNF- α ^[19-21], IL-6^[22] and ET-1^[23,24] in serum are all important inflammatory mediators during SAP complicated with multiple organ injury. They are important indexes of severity and prognosis of acute pancreatitis and have two important features in common: (1) Dual effects: These inflammatory mediators, especially NO and ET-1, will protect body in low concentration and injure body in high concentration; and (2) There are interactions among different inflammatory mediators. This experiment confirmed a positive correlation between PLA₂ and TNF- α . According to many studies as well as our experiment, the concentrations of these inflammatory mediators increase during SAP^[25-31]. Our experiment demonstrated that almost all indexes of inflammatory mediators were lower in treated groups than in model group, while the indexes were not different between Baicalin- and octreotide-treated groups, thereby indicating that both drugs, with similar effects, could lower the concentration of inflammatory mediators, inhibit them and protect kidney.

Both necrosis and apoptosis are ways of death of injured cells^[32]. In contrast to necrosis, apoptosis does not cause intense inflammatory reaction^[33], while necrosis will cause systemic inflammatory response syndrome^[34]. At present, a consensus has been reached on apoptosis of pancreas during SAP^[35,36]. When necrosis and apoptosis coexist in pancreas and necrosis prevails, induction of pancreatic apoptosis will result in a protective effect. We believe this conclusion is also applicable to renal apoptosis, which has been demonstrated by this experiment.

This experiment clearly showed that the renal pathological changes were milder in treated group than in model group. The renal apoptotic indexes at 6 h were markedly higher in treated group than in model group. All these indicate the renal pathological changes have been alleviated after apoptosis of renal tubular epithelial cells. In addition, the renal apoptotic indexes were not different between model group and sham operation group, possibly

because apoptosis had not occurred in model group or its incidence was too low to be detected. The occurrence of apoptosis of renal cells in treated groups, however, demonstrated that both Baicalin and octreotide could induce apoptosis. But some researchers believe the pathological changes would be aggravated by renal apoptosis during SAP^[37,38], which is different from our view and therefore worth discussing.

Bax and Bcl-2 are two important apoptosis-regulating factors. The homo- or heterodimerization between anti-apoptotic Bcl-2 and proapoptotic Bax plays an important role in the apoptosis regulating function of the Bcl-2-related proteins. Interestingly, in an excess of Bax, Bax/Bax homodimers predominate, which promote apoptosis, whereas an excess of Bcl-2 leads to the formation of Bcl-2/Bax heterodimers, which inhibit apoptosis. Thus, the ratio of Bcl-2 to Bax appears to be a critical determinant of a cell's threshold for undergoing apoptosis. No expression of Bcl-2 gene has been found normal pancreatic tissue^[39]. In addition, there has been no report on expression of Bcl-2 gene in normal renal tissue. It was found in this experiment that the expression levels of both Bax and Bcl-2 protein had increased during SAP, possibly because the apoptosis-inducing and -inhibiting factors had been enhanced simultaneously. As a result of the conflict of the two factors, the apoptotic indexes were not different between model group and sham operation group. However, the apoptotic indexes at 6 h were higher in treated group than in sham operation group and model group, indicating that the apoptosis of renal tubular epithelial cells occurred because the apoptosis-inducing factor prevailed in treated group. It was also found that the apoptotic indexes at 3 h were positively correlated with Bax in Baicalin-treated group. The apoptotic indexes at 3 h and 6 h were positively correlated with Bax in octreotide-treated group. But there was no correlation between apoptotic indexes and Bcl-2. Thus these data indicate that Bax might have participated in the apoptosis of renal tubular epithelial cells. Compared to model group, the expression level of Bcl-2 protein was lower in Baicalin-treated group at 6 h, and in octreotide-treated group at 6 h and 12 h, thereby indicating that both Baicalin and octreotide can lower the expression level of Bcl-2 protein, enhance the apoptosis-promoting effect of Bax dimmer and thus protect kidney and alleviate its pathological changes.

The traditional histopathological section technique that has been surpassed by tissue microarray (TMA) features single sample and low efficiency^[40]. The TMA we adopted has advantages such as high throughput and reliable results and great potential in oncopathological study^[41,42]. Current studies are also mainly focused in this field^[43-50]. To our knowledge, this is the first report on the application of TMA to the pathological examination of pancreatitis around the world.

In conclusion, both Baicalin and octreotide can alleviate the renal pathological changes and improve the survival of SAP rats by inhibiting inflammatory mediators, decreasing the expression level of Bcl-2 protein and enhancing the apoptosis-promoting effect of Bax dimmer to induce the apoptosis of renal tubular epithelial cells. Tissue microarray is time- and energy-saving, highly efficient and

well representative in the pathological examination of pancreatitis. We believe Baicalin, a low-priced new drug with precise therapeutic effects, is hopeful to play certain role in SAP treatment in future.

COMMENTS

Background

Up to now, severe acute pancreatitis (SAP) is still an acute clinical disease featuring multiple complications, high mortality and difficult treatment. Recent studies found octreotide, a somatostatin analogue, could effectively treat SAP. However, octreotide is expensive, which has hindered its clinical application. Therefore, an important direction of the current study is to find other cheap and effective drugs. Baicalin injection (China National Invention Patent Number ZL200310122673.6) prepared by the first author would be one of the best choice to treat SAP. In this experiment, the feasibility of Baicalin treatment of SAP has been studied by comparing the protecting effects and mechanisms of Baicalin and octreotide on kidneys of rats with SAP.

Research frontiers

Both Baicalin and octreotide can alleviate inflammatory reactions by inhibiting the generation of inflammatory mediators and inducing renal cell apoptosis, and thereby exert therapeutic effects on SAP. The mechanism of Baicalin- and octreotide-induced renal cell apoptosis may be related to regulation of Bax and Bcl-2 protein expressions. The therapeutic effects and mechanism of Baicalin on SAP rats are similar to those of octreotide.

Innovations and breakthroughs

As a cheap medicine with extensive pharmacological actions, few side effects and convenient administration, Baicalin can hopefully become a new drug for treating SAP. The application of tissue microarrays in pathological examination of SAP has several advantages, including time- and energy-saving, high efficiency and good representativeness, and therefore is worth popularizing.

Applications

Both Baicalin and octreotide can alleviate the renal pathological changes and improve the survival of SAP rats by inhibiting inflammatory mediators, regulating the expression level of Bax and Bcl-2 protein and inducing the apoptosis of renal tubular epithelial cell. Tissue microarray is time- and energy-saving, highly efficient and well representative in the pathological examination of pancreatitis. We believe Baicalin, a low-priced new drug with precise therapeutic effects, is hopeful to play certain role in future SAP treatment.

Terminology

Baicalin is an important monomer of Baical skullcap root. Severe acute pancreatitis (SAP) is a fatal systemic disease featuring acute onset, serious conditions, high incidence of complications and 20%-30% of mortality.

Peer review

The authors analyzed the protecting effects and mechanism of Baical skullcap root in the treatment of SAP in rats. The authors showed that Baicalin seems to be equally effective as octreotide in terms of reduction of renal pathological alterations. This study is well performed and the results merit further investigation.

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