Turk J Gastroenterol 2009; 20 (2): 108-115

Protective effects of baicalin and octreotide on intestinal mucosa of rats with severe acute pancreatitis

Şiddetli akut pankreatitli ratların intestinal mukozalarında baicalin ve oktreotid'in koruyucu etkisi

Xiping ZHANG[1], Guanghua FENG[1], Weihong WENG[2], Jianfeng LIANG[3], Naigong LIN[1], Yang CAI[1], RuJun XU[4], Ninin ZHOU[1], Meijuan YUAN[1], Wenqin YUAN[1], Xiaofeng XIA[1]

Departments of [1]General Surgery and [4]Pathology, Hangzhou First People's Hospital, [2]Traditional Chinese Medical Science, Zhejiang University, Children's Hospital, [3]Zhejiang University, School of Medicine, Statistics Center, Hangzhou, Zhejiang Province, China

Background/aims: *To compare the protective effects of baicalin and octreotide on intestinal mucosa of rats with severe acute pancreatitis and to explore the application value of baicalin as a new drug.* ***Methods:*** *Severe acute pancreatitis rats were randomly divided into a model control group, baicalin-treated group and octreotide-treated group. An equal number of normal rats were included in a sham-operated group. At 3, 6 and 12 hours (h) after operation, mortality rate, pathological changes in the intestinal mucosa of the terminal ileum, expression levels of nuclear factor (NF)-κB, Bax and Bcl-2 proteins, and apoptosis indices in the rats in each group were evaluated. Endotoxin and tumor necrosis factor (TNF)-α contents in blood were also determined.* ***Results:*** *At 12h after operation, the survival rates in both the baicalin-treated group and octreotide-treated group were higher than in the model control group, and the difference was significant ($p<0.05$). At all time points after the operation, endotoxin and TNF-α values as well as the expression levels of NF-κB protein and pathological severity scores in the intestinal mucosa in the two treated groups were, to varying degrees, significantly lower than those in the model control group ($p<0.05$, $p<0.01$ and $p<0.001$, respectively). Moreover, the expression level of Bax protein at 3h postoperatively as well as the expression level of Bax protein and apoptosis indices at 6h postoperatively in the two treated groups were significantly higher than those in the model control group ($p<0.01$).* ***Conclusions:*** *Baicalin and octreotide exert significant protective effects on severe acute pancreatitis-induced intestinal mucosa injury via a mechanism that is associated with inhibiting inflammatory mediators and inducing apoptosis. In comparison with the pharmacological action of octreotide, we believe that baicalin, as a new drug, has similar protective effects on the intestinal mucosa of severe acute pancreatitis rats, and therefore deserves further study and development.*

Key words: Severe acute pancreatitis, baicalin, octreotide, intestinal mucosa injury, inflammatory mediators, apoptosis

Amaç: *Şiddetli akut pankreatitli ratların intestinal mukozalarında baicalin ve oktreotid'in koruyucu etkisini karşılaştırmak ve Baicalin'in yeni bir ilaç olarak kullanım değerini araştırmak.* ***Yöntem:*** *Şiddetli akut pankreatitli ratlar random olarak model kontrol grubu, baicalin verilen grup ve oktreotid verilen grup olmak üzere ayrıldı. Aynı sayıda normal rat sham-operasyonlu grupta yer aldı. Operasyondan sonra 3, 6 ve 12. saatte, her bir grupta yer alan ratlardaki mortalite oranları ve terminal ileum intestinal mukozasındaki patolojik değişiklikler, NF-κB, Bax ve Bcl-2 proteinlerinin ekspresyon seviyeleri ve apoptozis indeksleri incelendi. Bunun yanında, kandaki endotoksin ve TNF-α düzeyleri saptandı.* ***Bulgular:*** *Operasyondan sonra 12. saatte, sağ kalım oranları baicalin verilen grupta ve oktreotid verilen grupta kontrol grubuna göre daha yüksekti ve fark önemliydi ($p<0.05$). Operasyondan sonra tüm zaman noktalarında, endotoksin ve TNF-α düzeyleri yanında NF-κB protein ekspresyon seviyeleri ve intestinal mukozadaki patolojik şiddet skorları tedavi verilen iki grupta da, değişen derecelerde, model kontrol grubuna göre daha düşüktü ($p<0.05$, $p<0.01$ ve $p<0.001$, sırasıyla). Ayrıca, operasyondan sonra 3. saatteki Bax protein ekspresyon seviyesi yanında, operasyondan sonra 6. saatteki Bax protein ekspresyon seviyesi ve apoptozis indeksleri tedavi verilen iki grupta model kontrol gruba göre önemli oranda daha yüksekti ($p<0.01$).* ***Sonuç:*** *Baicalin ve oktreotid şiddetli akut pankreatitin indüklediği intestinal mukozal hasar üzerinde, inflamatuvar mediatörlerin inhibisyonu ve apotozisin indüklenmesi ile ilişkili bir mekanizma aracılığıyla önemli koruyucu etki sağlar. Oktreotid'in farmakalojik etkisi ile karşılaştırarak, baicalin'in yeni bir ilaç olarak, şiddetli akut pankreatitli ratların intestinal mukozalarında oktreotid'e benzer koruyucu etkiye sahip olduğuna ve böylece ileri çalışmaları ve gelişmeleri hak ettiğine inanıyoruz.*

Anahtar kelimeler: Şiddetli akut pankreatit, baicalin, oktreotid, intestinal mukozal hasar, inflamatuvar mediatörler, apoptozis

This study was supported by the Technological Foundation Project of Traditional Chinese Medical Science of Zhejiang Province, No. 2003C130 and No. 2004C142; Foundation Project for Medical Science and Technology of Zhejiang Province, No. 2003B134; Grave Foundation Project for Technology and Development of Hangzhou, No. 2003123B19; Intensive Foundation Project for Technology of Hangzhou, No. 2004Z006; Foundation Project for Medical Science and Technology of Hangzhou, No. 2003A004; and Foundation Project for Technology of Hangzhou, No. 2005224.

Address for correspondence: Xiping ZHANG
Department of General Surgery, Hangzhou First People's Hospital
261 Huansha Road, Hangzhou 310006, Zhejiang, China
Fax: +86 571 87914773
E-mail: zxp99688@vip.163.com

Manuscript received: 27.05.2008 **Accepted:** 03.12.2008

INTRODUCTION

Severe acute pancreatitis (SAP) is a common acute abdomen in clinical practice, and has become a hot spot of research in recent years because of its acute onset, rapid progression and high mortality. The pathogenesis of SAP has also been further recognized in recent years (1, 2). The intestinal mucosal barrier is injured during SAP mainly by bacteria and endotoxin translocation through mesenteric lymph node, thoracic duct and systemic circulation, leading to secondary infection or even systemic inflammatory response syndrome (SIRS), inducing multiple organ dysfunction syndrome (MODS) and resulting in death. Octreotide is one of the common SAP medications at present (3, 4). However, its high cost and inconvenient administration has made it difficult to popularize its clinical application, resulting in the necessity of finding other cheap and effective alternatives. "Qingyitang" is a representative traditional Chinese preparation for treating AP, and its sound therapeutic effects on AP has been demonstrated in a large number of clinical practices (5, 6).

Baicalin, as the most important monomer of Baical skullcap root, which is an essential ingredient of "Qingyitang", has most of the pharmacologic actions of Baical skullcap root (7-10). With multiple administration routes, including intravenous and oral medication, baicalin can overcome the shortcoming of "Qingyitang" (5, 11), namely inconvenient administration. Its low cost and extensive pharmacologic actions also ensure its prospects for promising applications.

This experiment investigated the protective effects of baicalin on intestinal mucosa injury due to SAP. Baicalin was compared with octreotide to explore the protective effects and mechanism of baicalin and octreotide on the intestinal mucosa of SAP rats. This experiment is also the first to report on the application of tissue microarray to pathohistological examination of intestinal mucosa in order to increase study efficiency.

MATERIALS and METHODS

Animals and Reagents

Clean grade healthy male Sprague-Dawley (SD) rats, 250-300 g in body weight, were obtained from the Experimental Animal Center of the Medical School of Zhejiang University, China. Sodium taurocholate and sodium pentobarbital were obtained from USA Sigma Company. Octreotide was obtained from Swiss pharmaceutical company Novartis; 5% baicalin injection (China national invention patent number ZL200310122673.6) was prepared by the first author with 305 mmol/L osmotic pressure. Plasma endotoxin tachypleus amebocyte lysate kit was obtained from Shanghai Yihua Medical Science and Technology Corporation (Institute of Medical Analysis in Shanghai, China), with calculation unit for content in EU/ml. Serum tumor necrosis factor (TNF)-α ELISA kit was obtained from Jingmei Bioengineering Corporation, China, with calculation unit for content in pg/ml (ng/L). Nuclear factor (NF)-κB, Bax and Bcl-2 antibody were obtained from Santa Cruz Company (USA); DNA nick in situ end-labeling (TUNEL) kit was obtained from Takara Company (Japan). The above determinations were all performed according to the instructions of the kits.

Animal Grouping

The improved Aho's method was adopted to prepare SAP rat models via retrograde injection of 3.5% sodium taurocholate to the pancreatic duct through an epidural catheter and duodenal papilla. After preparation, the 135 SAP rat models were randomly divided into a model control group, baicalin-treated group and octreotide-treated group with 45 rats in each group; another 45 were selected as the sham-operated group, which underwent only abdominal- opening surgery. The above-mentioned groups were then randomly divided into 3 hour (h), 6h and 12h groups with 15 rats in each group (7-10).

Preparation Methods of Animal Models

The rats were anesthetized by intraperitoneal injection of 2% sodium pentobarbital (0.25 ml/100 g). We first established the right external jugular vein transfusion passage using the microinfusion pump for continuous transfusion (1 ml/h/100 g) and then used 3.5% sodium taurocholate to prepare the SAP model. After connecting the anesthetic tube end with the transfusion converter, we transfused 3.5% sodium taurocholate (0.1 ml/100 g) by retrograde transfusion via the microinjection pump (made by Zhejiang University) at the speed of 0.2 ml/minute. Rats were waited for 4 to 5 minutes after injection and the microvascular clamp and epidural catheter were removed. After checking for bile leakage, the hole in the duodenum lateral wall was sutured. A disinfected cotton ball was used to absorb the anesthetic in the abdominal cavity and the abdomen was closed. In the sham-operated group, after abdominal opening,

the pancreas and duodenum were turned over and the abdomen closed (7-10).

Dosage and Methods

Baicalin-treated group: the results from the guinea pig sensitivity test, rabbit hemolysis test and vascular stimulation test of 5% baicalin injection were all negative. According to the mouse acute toxicity test, the LD_{50} of baicalin injection was 2.7354 ± 0.2588 g/kg. The animal experiments of 5% baicalin injection were completed including the acute toxicity test and SAP rat treatment using low, medium and high dose. The high dose (10 mg/h/100 g) achieved the best therapeutic effect and the dosage referred to the result of the previous preliminary experiment. Ten minutes after successful modeling, the baicalin-treated group was first injected with 5% baicalin injection 10 mg/100 g via external jugular vein passage followed by continuous intravenous administration (10 mg/h/100 g) by microinfusion pump.

The octreotide-treated group was first injected with octreotide 0.2 ug/100 g via external jugular vein passage followed by continuous intravenous transfusion (10 mg/h/100 g) by microinfusion pump at a transfusion speed of 0.2 ug/h/100 g. All the above dosages were proven as effective dosages in the previous preliminary experiment. The sham-operated group and model control group were injected with saline of equivalent volume at the corresponding time points postoperatively (7-10).

Observation Indices

Rat mortality was examined at 3h, 6h and 12h after operation, survival was calculated, and gross changes in the terminal ileum were observed.

Pathological changes of intestinal mucosa: After mercy batch execution of rats, the tissue samples of the terminal ileum were collected to observe the pathological changes in the intestinal mucosa. The pathological severity score of intestinal mucosa was calculated in accordance with the self-made standard (12) (Table 1).

Plasma endotoxin and serum TNF-α values were determined via blood sampling from the heart.

NF-κB, Bax and Bcl-2 protein expression and apoptotic index of the intestinal mucosa of the terminal ileum were determined.

Preparation of Tissue Microarrays of the Terminal Ileum

Tissue microarrays technique was applied to prepare microarray sections of the terminal ileum, with a drilling needle diameter of 2.0 mm.

NF-κB, Bax and Bcl-2 Protein Immunohistochemical Staining

We adopted SP (streptavidin peroxidase) method for immunohistochemical staining in microarray sections of the terminal ileum; expression of the three proteins of the intestinal mucosa were observed under light microscope and the comprehensive assessment according to the positive cell percentage was carried out: positive cell count <10% indicates (–); positive cell count 10-20% (+); positive cell count 20-50% (++); and positive cell count >50% (+++).

TUNEL Staining

DNA nick in situ end-labeling (TUNEL) technology for staining in microarray sections of the terminal ileum was adopted. The apoptotic cells of the intestinal mucosa were observed and the apoptotic index calculated. The TUNEL staining technique was applied to observe the changes in apoptotic cells of the intestinal mucosa and the apoptotic index was calculated as: apoptotic cell count / total cell count x100%.

Statistical Methods

Values are presented as mean and standard deviation for normally distributed variables or median and quartile range for highly skewed variables. The significance of differences between the four groups was tested using the Kruskal Wallis test for highly skewed data and analysis of variance (ANOVA) for normal distribution data. Multiple

Table 1. Standard of pathological severity score for intestinal mucosa (12)

Grade	Observation indices
0	Integrated mucosa without necrosis (epithelium mucosae and glandular epithelium), edema in proper layer, submucosa and placenta percreta
1	Incomplete mucosa, focal necrosis, or inflammatory cell infiltration in proper layer, submucosa and placenta percreta (neutrophilic granulocyte, eosinophile granulocyte and large mononuclear cell)
2	Incomplete mucosa, focal necrosis, and edema in proper layer, submucosa and placenta percreta
3	Incomplete mucosa, focal necrosis, inflammatory cell infiltration in proper layer, submucosa and placenta percreta (neutrophilic granulocyte, eosinophile granulocyte and large mononuclear cell)

comparisons were subjected to Bonferroni correction test. The chi-square test was used to evaluate equality of frequencies for discrete variables. Correlations were tested using the Spearman rank correlation coefficients. A P value ≤0.05 was considered statistically significant, and all statistical analyses were conducted using SPSS version 11.5 for Windows.

RESULTS

Comparison of Survival Rate

The mortality rates of the model control group were 0% (0/15), 13.33% (2/15) and 33.33% (5/15) at 3h, 6h and 12h, respectively, while mortality rates in the baicalin- and octreotide-treated groups were 0% at the different time points. The whole sham-operated group also survived at the different time points. The survival rate of the model control group was 66.67% (10/15) at 12h, while the survival rates of both the baicalin- and octreotide-treated groups were 100% at 12h, indicating a significant difference ($p<0.05$) (7-10).

Comparison of Plasma Endotoxin Content

Plasma endotoxin values in the model control group and treated groups were significantly higher than in the sham-operated group at all time points ($p<0.001$), with no significant difference between the baicalin-treated group and octreotide-treated group at 6 and 12h ($p>0.05$). Values in both the baicalin-treated group and octreotide-treated group were significantly lower than in the model control group at 3h ($p<0.001$), and the baicalin-treated group values were significantly lower than those of the octreotide-treated group ($p<0.01$). Values in the baicalin-treated group and octreotide-treated group were significantly lower than in the model control group at 6h ($p<0.05$, $p=0.001$, respectively). Values in the baicalin-treated group and octreotide-treated group were also significantly lower than in the model control group at 12h ($p<0.001$, $p<0.01$, respectively) (Table 2).

Comparison of Serum TNF-α Content

Values in the model control group and treated groups significantly exceeded those of the sham-operated group at the different time points ($p<0.001$). There was no significant difference between the model control group, baicalin- treated group and octreotide-treated group at 3h and 12h ($p>0.05$). At 6h, values in both the baicalin-treated group and octreotide-treated group were significantly lower than in the model control group ($p<0.001$). The values in the octreotide-treated group were significantly lower than in the baicalin-treated group ($p<0.01$) (Table 2).

Gross Changes and Changes Under Light Microscope of Intestinal Mucosa

Sham-operated group

Gross changes

No visible intestinal dilation, wall hyperemia or edema was found. Smooth intestinal mucosa surface without bleeding, ulcer, etc. was seen in all groups.

Changes under light microscope

Integrated epidermis and microvillus structure of intestinal mucosa, no exfoliation or necrosis, edema in proper layer, submucosa and placenta percreta of some rats, and necrosis focus as well as inflammatory cell infiltration of proper layer, submucosa, and placenta percreta in rare cases.

Model control group

Gross changes

No visible intestinal change at 3h; at 6 and 12h, visible intestinal dilation accompanied by gas and liquid retention, wall hyperemia and edema, visible bleeding points on intestinal mucosa surface, and in a few severe cases, mucosal ulcers.

Changes under light microscope

Focal necrosis of intestinal mucosa and inflammatory cell infiltration of all mucosa layers in most

Table 2. Comparison of different indices in blood (M (Q_R))

Indices	Time	Sham-operated group	Model control group	Baicalin-treated group	Octreotide-treated group
Endotoxin	3h	0.016 (0.05)	0.053 (0.029) ***	0.027 (0.05) ***+++	0.033 (0.06)***++
(EU/ml)	6h	0.016 (0.01)	0.059 (0.037)***	0.039 (0.019)***+	0.031 (0.010)***++
	12h	0.014 (0.015)	0.060 (0.022) ***	0.034 (0.015) ***	0.042 (0.014) ***++
TNF-α					
(pg/ml)	3h	3.90 (3.20)	41.44 (37.72)***	44.93 (45.84)***	39.30 (30.60) ***
	6h	4.00 (1.70)	92.15 (23.12)***	65.10 (27.51)***+++	47.60 (16.50)***+++
	12h	5.30 (3.00)	65.02 (26.81)***	47.65 (25.52)***	54.50 (41.40)***

Note: Compared to sham-operated group, *p<0.05, **p<0.01 and ***p<0.001; compared to model control group, +p<0.05, ++p<0.01 and +++p<0.001.

rats, visible hyperemia and edema of intestinal mucosa proper layer, central chyle vessel dilation, exuviation of epidermis microvillus end of intestinal mucosa, and disordered structure arrangement at 6 and 12h.

Baicalin- and octreotide-treated groups

Gross changes

Slight intestinal dilation, little gas and liquid retention, milder wall hyperemia and edema in treated groups than in the model control group, few bleeding points or mucosal ulcer in intestinal mucosa. The most visible therapeutic effects were obtained in the octreotide-treated group.

Changes under light microscope

Mild hyperemia and edema in intestinal mucosa proper layer most visible at 12h, mild central chyle vessel dilation, neat structure arrangement in intestinal mucosa epithelial glands, little exfoliation of mucosa epidermis microvillus. More integrated as a whole in treated groups than in the model control group; less inflammatory cell infiltration of wall muscular layer and blood capillary periphery in treated groups than in model control group. The most visible effects were obtained in the octreotide- treated group.

Comparison of Pathological Severity Score

A quantitative score standard for severity was made based on pathological changes of intestinal mucosa in the various groups. A blind method was used for evaluation in accordance with the following standard (Table 2). The pathological severity score was significantly higher in the model control group and baicalin- treated group than in the sham-operated group at the different time points ($p<0.001$), significantly lower in the octreotide-treated group than in the model control group at 6h ($p<0.05$), and significantly lower in the baicalin- and octreotide-treated groups than in the model control group at 12h ($p<0.05$); there were no marked differences between groups at the other time points ($p>0.05$) (Table 3).

Comparison of NF-κB Expression Levels

Positive staining was located in the cytoplasm of intestinal mucosa epithelial cells. The level was significantly higher in the model control group than in the sham-operated group at the different time points ($p<0.05$), significantly lower in the baicalin-treated group and octreotide-treated group than in the model control group at 3h ($p<0.05$), significantly lower in the octreotide-treated group than in model control group at 6h ($p<0.05$), significantly lower in the baicalin-treated group than in model control group at 12h ($p<0.01$), and significantly lower in the octreotide-treated group than in model control group at 12h ($p<0.05$) (Table 3).

Comparison of Bax Expression Levels

The Bax positive staining was located in the cytoplasm of intestinal mucosa epithelial cells. The level was significantly higher in the octreotide-treated group than in the sham-operated group at the different time points ($p<0.05$), significantly higher in the baicalin-treated group than in the sham-operated group at 3h and 6h ($p<0.01$), and signifi-

Table 3. Comparison of different pathological indices (M (Q_R))

Indices	Time	Sham-operated group	Model control group	Baicalin-treated group	Octreotide-treated group
Pathological score	3h	0.00 (1.00)	3.00 (2.00)***	3.00 (2.00)***	3.00 (3.00)
	6h	0.00 (1.00)	3.00 (1.00)***	2.00 (2.00)***	0.00 (3.00)+
	12h	0.00 (1.00)	3.00 (0.00)***	2.00 (2.00)***+	0.00 (3.00)+
NF-κB expression	3h	0.00 (0.00)	0.00 (1.00)	0.00 (1.00)	0.00 (0.00)
	6h	0.00 (0.00)	0.00 (2.00)	0.00 (0.00)	0.00 (0.00)
	12h	0.00 (0.00)	0.50 (2.00)	0.00 (0.00)	0.00 (0.00)
Bax expression	3h	0.00 (0.00)	0.00 (0.00)	3.00 (3.00)**+	0.00 (1.00)*
	6h	0.00 (0.00)	0.00 (1.00)	0.00 (2.00)**	0.00 (1.00)*
	12h	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (1.00)*
Bcl-2 expression	3h	0.00 (0.00)	3.00 (0.00)**	3.00 (1.00)**	0.00 (0.00)
	6h	0.00 (0.00)	0.00 (0.00)**	0.00 (3.00)**++	0.00 (0.00)
	12h	0.00 (0.00)	3.00 (0.00)**	3.00 (1.00)**	0.00 (0.00)
Apoptosis index	3h	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	6h	0.00 (0.00)	0.00 (0.00)	0.00 (12.00)** +	0.00 (0.00)
	12h	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

Note: Compared to sham-operated group, *$p<0.05$, **$p<0.01$ and ***$p<0.001$; compared to model control group, +$p<0.05$, ++$p<0.01$ and +++$p<0.001$.

cantly higher in the baicalin-treated group than in the model control group and octreotide-treated group at 3h ($p<0.01$) (Table 3).

Comparison of Bcl-2 Expression Levels

The Bcl-2 positive staining was located in the cytoplasm of intestinal mucosa epithelial cells. The level was significantly higher in the model control group and baicalin-treated group than in the sham-operated group and octreotide-treated group at the different time points ($p<0.01$), and significantly higher in the baicalin-treated group than in the model control group at 6h ($p<0.01$); there were no marked differences between groups at the other time points ($p>0.05$) (Table 3).

Comparison of Apoptotic Indices

The apoptotic cells were intestinal mucosa epithelial cells, intestinal proper layer lymphocytes and intestinal gland epithelial cells. The apoptotic index was significantly higher in the baicalin-treated group than in the sham-operated group ($p<0.01$) as well as model control group ($p<0.05$) at 6h; there were no marked differences between groups at the other time points ($p>0.05$) (Table 3).

DISCUSSION

The excessive release of inflammatory mediators is the main cause of intestinal mucosa injury during SAP, while severe intestinal mucosa injury is the main cause of SAP death (13-15). The results of this experiment and our previous experiments showed the expression levels of manifold inflammatory mediators such as endotoxin, PLA_2, NO, TNF-α and NF-κB. We also showed that ascites/body weight comparison coefficients were significantly higher in SAP-induced rats than in the sham-operated group, and the inflammatory mediator level was positively correlated with the level of injury to the intestinal mucosa, indicating a cascade reaction among inflammatory mediators that has also been proven by the correlation analysis of this experiment. Endotoxin can invade the body to cause intestinal-originated endotoxemia, increase permeability of the intestinal mucosa, promote the invasion of intestinal bacteria and endotoxin (16), resulting in a vicious cycle. TNF-α, an important cytokine in SAP (17, 18), can participate in the generation of interleukin (IL)-1β and IL-6, and stimulate the generation of NO and oxygen free radicals, leading to cascade and amplification effects (19-21). Plasma endotoxin and serum TNF-α (22, 23) are important indices for evaluating AP severity and prognosis. The two drugs inhibited the inflammatory reactions and changes in SAP rats, alleviated injury to the intestinal mucosa, and improved the survival of SAP rats.

NF-κB activation is the key initial step in the inflammatory reaction, regulating the expression of inflammatory mediators (24, 25). Activated NF-κB can stimulate cytokine generation, while cytokines will then activate NF-κB, resulting in a cascade reaction. The experimental results showed the terminal ileum NF-κB expression levels were significantly lower in the baicalin-treated group and octreotide-treated group than in the model control group at all time points, demonstrating that both baicalin and octreotide can inhibit the intestinal mucosa NF-κB expression in SAP rats, inhibit inflammatory reaction and thus protect the intestinal mucosa.

Some studies found that pancreas injury can be alleviated by induction of pancreas acinus epithelial cells (26, 27). However, there has been a dispute regarding the influence of apoptosis on intestinal mucosa injury. Some scholars believe apoptosis can promote intestinal mucosa injury, while others think apoptosis is helpful in preventing SAP aggravation (28-30). This experiment also found that the apoptotic indices are negatively correlated with intestinal mucosa injury level and inflammatory mediator content, indicating that apoptosis can also protect intestinal mucosa. Bax and Bcl-2 are two important components of the apoptosis regulating system. Bax protein is homologous with Bcl-2 in the same cell. When Bax forms dimer, it will induce apoptosis; as Bcl-2 expression increases, more and more Bax dimers will split to combine Bcl-2 to form Bax-Bcl-2 heterodimers that are more stable than Bax dimers, and thus inhibit the apoptosis promoting effect of Bax dimers (31-33). Our experiment results showed that Bax and Bcl-2 expression levels and apoptotic indices of intestinal tissue were all significantly higher in the baicalin-treated group than in the model control group ($p<0.05$, $p<0.01$). We believe Bax and Bcl-2 are contradictory and unified. They both showed increased expression in the baicalin-treated group, indicating the enhancement of both apoptosis-inducing and -inhibiting factors. However, the result is that the inducing factor prevails, which suggests the existence of other apoptosis-inducing factors responsible for the rise in the apoptotic index. This experiment adopted tissue microarray (34-36), which is economical

and time saving, and features high-throughput, multiple samples, error reduction, convenience for empirical control design, capability of combination with other biological technologies, extensive applications, and other advantages, and it thus has significantly reduced study cost, improved the efficiency of pathohistological study, and achieved a satisfactory result. We think the technique of tissue microarray merits its popularization.

Recent studies have demonstrated manifold pharmacologic actions of octreotide and baicalin (7-10, 37, 38). By comparing the therapeutic effects of octreotide and baicalin, this experiment has convincingly proven the therapeutic effects of baicalin in SAP rats. Advantages of the baicalin injection are its low cost, long half-life, convenient administration, and extensive pharmacologic actions, and it therefore shows prospect for a broad application.

REFERENCES

1. Skipworth JR, Pereira SP. Acute pancreatitis. Curr Opin Crit Care 2008; 14: 172-8.
2. Al Mofleh IA. Severe acute pancreatitis: pathogenetic aspects and prognostic factors. World J Gastroenterol 2008; 14: 675-84.
3. Czakó L, Hegyi P, Takács T, et al. Effects of octreotide on acute necrotizing pancreatitis in rabbits. World J Gastroenterol 2004; 10: 2082-6.
4. Suzuki M, Shimizu T, Kudo T, et al. Octreotide prevents L-asparaginase-induced pancreatic injury in rats. Exp Hematol 2008; 36: 172-80.
5. Qiu Y, Li YY, Li SG, et al. Effect of Qingyitang on activity of intracellular Ca2+-Mg2+-ATPase in rats with acute pancreatitis. World J Gastroenterol 2004; 10: 100-4.
6. Li YY, Li XL, Yang CX, et al. Effects of Tetrandrine and QYT on ICAM-1 and SOD gene expression in pancreas and liver of rats with acute pancreatitis. World J Gastroenterol 2003; 9: 155-9.
7. Zhang XP, Zhang L, Yang P, et al. Protective effects of baicalin and octreotide on multiple organ injury in severe acute pancreatitis. Dig Dis Sci 2008; 53: 581-91.
8. Zhang XP, Zhang L, He JX, et al. Experimental study of therapeutic efficacy of Baicalin in rats with severe acute pancreatitis. World J Gastroenterol 2007; 13: 717-24.
9. Zhang XP, Tian H, Lai YH, et al. Protective effects and mechanisms of Baicalin and octreotide on renal injury of rats with severe acute pancreatitis. World J Gastroenterol 2007; 13: 5079-89.
10. Xiping Z, Hua T, Hanqing C, et al. The protecting effects and mechanisms of Baicalin and Octreotide on heart injury in rats with SAP. Mediators Inflamm 2007; 2007: 19469.
11. Li YY, Sibaev A, Zhou MZ, et al. The Chinese herbal preparation Qing Yi Tang (QYT) improves intestinal myoelectrical activity and increases intestinal transit during acute pancreatitis in rodents. Phytother Res 2007; 21: 324-31.
12. Zhang X, Chen L, Luo L, et al. Study of the protective effects of dexamethasone on ileum mucosa injury in rats with severe acute pancreatitis. Pancreas 2008; 37: e74-82.
13. Flint R, Windsor J. The role of the intestine in the pathophysiology and management of severe acute pancreatitis. HPB (Oxford) 2003; 5(2): 69-85.
14. Akhija R, Kingsnorth AN. Cytokine storm in acute pancreatitis. J Hepatobil Pancreat Surg 2002; 9: 401-10.
15. Zhang XP, Zhang J, Song QL, et al. Mechanism of acute pancreatitis complicated with injury of intestinal mucosa barrier. J Zhejiang Univ Sci B 2007; 8: 888-95.
16. Zhang XP, Wang L, Zhang J. Study progress on mechanism of severe acute pancreatitis complicated with hepatic injury. J Zhejiang Univ Sci B 2007; 8: 228-36.
17. Pereda J, Sabater L, Cassinello N, et al. Effect of simultaneous inhibition of TNF-alpha production and xanthine oxidase in experimental acute pancreatitis: the role of mitogen activated protein kinases. Ann Surg 2004; 240: 108-16.
18. Strobel O, Wachter D, Werner J, et al. Effect of a pneumoperitoneum on systemic cytokine levels, bacterial translocation, and organ complications in a rat model of severe acute pancreatitis with infected necrosis. Surg Endosc 2006; 20: 1897-903.
19. Mole DJ, Taylor MA, McFerran NV, et al. The isolated perfused liver response to a 'second hit' of portal endotoxin during severe acute pancreatitis. Pancreatology 2005; 5: 475-85.
20. Granger J, Remick D. Acute pancreatitis: models, markers, and mediators. Shock 2005; 24: 45-51.
21. Cuzzocrea S, Mazzon E, Dugo L, et al. Absence of endogenous interleukin-6 enhances the inflammatory response during acute pancreatitis induced by cerulein in mice. Cytokine 2002; 18: 274-85.
22. Rau B, Schilling MK, Beger HG. Laboratory markers of severe acute pancreatitis. Dig Dis 2004; 22: 247-57.
23. Zhang XP, Wang L, Zhou YF. The pathogenic mechanism of severe acute pancreatitis complicated with renal injury: a review of current knowledge. Dig Dis Sci 2008; 53: 297-306.
24. Suk K, Yeou Kim S, Kim H. Regulation of IL-18 production by IFN gamma and PGE2 in mouse microglial cells: involvement of NF-ÎB pathway in the regulatory processes. Immunol Lett 2001; 77: 79-85.
25. Vaquero E, Gukovsky I, Zaninovic V, et al. Localized pancreatic NF-kappaB activation and inflammatory response in taurocholate-induced pancreatitis. Am J Physiol Gastrointest Liver Physiol 2001; 280: G1197-208.
26. Kataoka K, Yasuda H, Sakaqami J. Role of apoptosis in severe acute pancreatitis. Nippon Rinsho 2004; 62: 2021-6.
27. Bhatia M. Apoptosis of pancreatic acinar cells in acute pancreatitis: is it good or bad? J Cell Mol Med 2004; 8: 402-9.
28. Zhang XP, Lin Q, Zhou YF. Progress of study on the relationship between mediators of inflammation and apoptosis in acute pancreatitis. Dig Dis Sci 2007; 52: 1199-205.
29. Sha HC, Ma QY, Rajiv KJ, et al. Protective effect of resveratrol on the intestinal mucosal cells in rats with severe acute pancreatitis and the mechanism. Nan Fang Yi Ke Da Xue Xue Bao 2008; 28: 1542-5.
30. Nakajima T, Ueda T, Takeyama Y, et al. Protective effects of vascular endothelial growth factor on intestinal epithelial apoptosis and bacterial translocation in experimental severe acute pancreatitis. Pancreas 2007; 34: 410-6.
31. Yuan Y, Gong Z, Lou K, et al. Effects and mechanisms of somatostatin analogs on apoptosis of pancreatic acinar cells in acute pancreatitis in mice. J Gastroenterol Hepatol 2001; 16: 683-8.

32. Gomez G, Lee HM, He Q, et al. Acute pancreatitis signals activation of apoptosis-associated and survival genes in mice. Exp Biol Med 2001; 226: 692-5.
33. Gu JC, Wang Y, Zhang ZT, et al. Effects of human interleukin 10 gene transfer on the expression of Bcl-2, Bax and apoptosis of hepatocyte in rats with acute hemorrhagic necrotizing pancreatitis. Chin Med J (Engl) 2005; 118: 1658-60.
34. Merseburger AS, Anastasiadis AG, Hennenlotter J, et al. Tissue microarrays: applications in urological cancer research. World J Urol 2006; 24: 579-84.
35. Waterworth A, Horgan K, Speirs V, et al. Tissue microarrays--big potential from small samples (review). Int J Oncol 2004; 25: 167-71.
36. Zellweger T, Ninck C, Mirlacher M, et al. Tissue microarray analysis reveals prognostic significance of syndecan·1 expression in prostate cancer. Prostate 2003; 55: 20-9.
37. Zhang XP, Tian H, Cheng QH. The current situation in pharmacological study on Baicalin. Chinese Pharmacological Bulletin 2003; 19: 17-20.
38. Zhang XP, Li ZF, Liu XG, et al. Effects of emodin and baicalein on rats with severe acute pancreatitis. World J Gastroenterol 2005; 11: 2095-100.