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Full Length Research Paper

Baicalin inhibited nuclear factor κB (NF-κB) activation and attenuated sodium taurocholate of induced experimental pancreatitis in rats

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Nuclear factor κB (NF-κB) plays a critical role in development of acute pancreatitis. Baicalin, a root extract of *Scutellaria baicalensis* Georgi, had protective effects in a rat model of severe acute pancreatitis (SAP). This study investigated the influence of baicalin on pancreatitis and the mechanism of anti-inflammatory effects associated with NF-κB activation. Sixty rats were assigned to four groups (n = 15). SAP rat models were prepared via retrograde injection of 3.5% sodium taurocholate in the pancreatic duct. Sham-treated rats only received open abdomenal surgery. Ten minutes post-surgery, rats were administered 5% baicalin solution or saline. Measures of pancreatic inflammation and pathology were assessed 6 h later. Compared to controls, sodium taurocholate-treated rats had greater pancreatic injury and neutrophil infiltration, stronger activation of lipid peroxidation and oxidative stress, enhanced levels of intercellular adhesion molecule-1 (ICAM-1) and P-selectin, tumor necrosis factor (TNF)-α and IL-6, and decreased IκBα. Baicalin markedly reduced the degree of inflammation and tissue injury, lipid peroxidation, poly (ADP-ribose) and nitrotyrosine production, upregulation/formation of ICAM-1 and P-selectin, neutrophil infiltration, TNF-α and IL-6 production, and increased IκBα in SAP rats. Baicalin attenuated rat pancreatitis induced by sodium taurocholate, and the anti-inflammatory effect was attributed in part to NF-κB inhibition.

Key words: Baicalin, nuclear factor-κB, acute pancreatitis, inflammation, neutrophil infiltration, cytokines.

INTRODUCTION

Acute pancreatitis is a common disease that varies greatly in severity. The mortality rate for severe pancreatitis is about 29.8%, whereas that for moderate pancreatitis is about 2.3%. The main causes of death include circulatory shock, cardiac insufficiency, and renal, respiratory and hepatic failure. Therefore, it has been demonstrated that many patients with pancreatitis develop dysfunction of two organs on average, signaling the occurrence of multiple organ failure (MOF) (Ogawa, 1998). Acute pancreatitis is caused by various factors, but once proteolytic enzymes become activated and pancreatic tissues digested, patients show a similar pattern of aggravation and MOF follows. Thus, the prognosis of severe acute pancreatitis (SAP) is highly dependent on appropriate measures to prevent MOF (Gloor et al., 2001; Lowham et al., 1999; Powell and Parks, 2003). The mechanism of disease progression from acinar cell injury to an overwhelming life-threatening illness is still not fully understood (Powell and Parks, 2003). Recently, signaling molecules and pathways which are responsible for the initiation and progression of this disease have been intensely investigated. One important signaling molecule, nuclear factor κB (NF-κB), has been shown to play a critical role in the development of acute pancreatitis (Algul et al., 2002; Chen et al., 2002; Ethridge et al., 2002; Meng et al., 2005; Rakonczay et al.,2008; Vaquero et al., 2001; Virlos et al., 2003; Xue et al., 2006).

NF-κB is a ubiquitous inducible transcription factor responsible for mediating the expression of a large

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number of genes involved in inflammation, tissue injury and repair (Luo et al., 2010; Rakonczay et al., 2003). Normally, NF-κB is inactive and resides in the cytoplasm, where it is sequestered by the inhibitory protein IκBα. After activation, NF-κB complexes translocate into the nucleus and activate transcription of target genes. It has been demonstrated that localized pancreatic NF-κB activation is an early and central phenomenon in the host response to acinar cell injury in acute pancreatitis (Chen et al., 2002; Meng et al., 2005; Rakonczay et al., 2008; Vaquero et al., 2001; Xue et al., 2006). NF-κB can bind to many types of cytokines and adhesion molecules at the κB site of their gene promoters to enhance transcription of a variety of inflammatory mediators involved in SAP. Inhibition of NF-κB activation causes a marked decrease in the expression of these inflammatory cytokines and significant protective effects on organs (Altavilla et al., 2003; Meng et al., 2005; Rakonczay et al., 2008; Virlos et al., 2003). As a pleiotropic transcription factor, NF-κB can be activated by low levels of reactive oxygen species (ROS) and inhibited by anti-oxidants (Altavilla et al., 2003; Cuzzocrea et al., 2004; Meng et al., 2005; Virlos et al., 2003). Therefore, anti-oxidants may, in part, mediate their beneficial effects by inhibiting NF-κB activation, and subsequently preventing induction of the cytokine cascade and upregulation of adhesion molecules (Altavilla et al., 2003; Cuzzocrea et al., 2004; Meng et al., 2005; Shen et al., 2003; Virlos et al., 2003).

Baicalin (5,6,7-trihydroxyflavone-7-O-D-glucuronic acid. $C_{21}H_{18}O_{11}$) is a flavonoid compound purified from the medicinal plant *Scutellaria baicalensis* Georgi. The anti-oxidative, anti-inflammatory and lipid peroxidation preventive effects of baicalin and its metabolite baicalein have been reported (Huang et al., 2006; Shen et al., 2003). Many studies have shown the protective effects of baicalin on multiple organs besides the pancreas, and it was shown to attenuate pancreatic pathological lesions and reduce SAP-related mortality (Huang et al., 2006; Meng et al., 2005; Shen et al., 2003; Tian et al., 2009; Xiping et al., 2009; Zhang et al., 2007, 2008, 2009a). Antioxidants have been proven to modulate the interaction between inflammatory target cells and peripheral leukocytes through which they exert anti-inflammatory effects (Oruc et al., 2004; Park et al., 2003; Shen et al., 2003). It has also been shown that the oxidative state of the cell influences the induction of NF-κB. Reactive oxygen intermediates likely induce IκBα phosphorylation by influencing the activity of tyrosine kinases. Baicalin or baicalein was demonstrated in a previous study to have anti-inflammatory activity by production of scavenging reactive oxygen intermediates in neutrophils or monocytes (Shen et al., 2003). Therefore, the potential protective anti-oxidant effects of baicalin may be partly attributed to the inhibition of NF-κB activation (Meng et al., 2005; Xiping et al., 2009). Although baicalin was previously observed to inhibit NF-κB activation in the pancreas, lung, liver and kidney of rats with acute pancreatitis (Meng et al., 2005; Xiping et al., 2009), the protective mechanism of NF-κB inhibition on acute pancreatitis needs to be further clarified.

The present study was designed to test the hypothesis that the inhibition of NF-κB activation by baicalin ameliorates the indices of pancreatic injury in a rat model of SAP induced by sodium taurocholate administration. More specifically, this study investigated the effects of baicalin on measures of pancreatic pathology and inflammation, such as serum amylase and lipase levels, lipid peroxidation, poly (ADP-ribose) (PAR) and nitrotyrosine production, neutrophil infiltration, intercellular adhesion molecule-1 (ICAM-1) and P-selectin expression, proinflammatory cytokines tumor necrosis factor (TNF)-α and IL-6 production.

MATERIALS AND METHODS

Experimental animals

Male Sprague-Dawley rats (weight 250 to 300 g) were obtained from the animal center at the Fourth Military Medical University (FMMU). Animals were kept in a controlled environment and provided with standard rodent chow and water. All animal procedures were approved by the Institutional Ethical Committee of the FMMU.

Induction of pancreatitis and administration of drugs

Sixty rats were randomly assigned to four groups, 15 rats in each group. The improved Aho method was adopted to prepare 30 SAP rat models via retrograde injection of 35 g/L sodium taurocholate (0.1 ml/100 g, Sigma Company, USA) to the pancreatic duct through an epidural catheter (Zhang et al., 2009a). Rats in the sham operation group were only given exploratory laparotomy (that is, opening the abdominal cavity, checking the pancreas and duodenum and then closing the abdomen). The first group (sham + vehicle) was treated with vehicle solution (saline) via femoral vein passage 10 min after exploratory laparotomy. The second group (sham + baicalin) group was first injected with 5% baicalin injection (Xi'an Scidoor Organic-Tech Co., Ltd, China; purity >98%, HPLC) at 10 mg/100 g via the femoral vein passage followed by continuous intravenous administration (10 mg/h/100 g) using a microinfusion pump 10 min after exploratory laparotomy. The third group (sodium taurocholate + vehicle) was treated with an equivalent volume of saline as the first group 10 min after successful modeling. The fourth group (sodium taurocholate + baicalin) was treated with an equivalent volume of baicalin injection as the second group 10 min after successful modeling. Rats were sacrificed by exsanguination at 6 h after induction of pancreatitis. Blood samples were obtained by direct intracardiac puncture. Pancreases were removed, immediately frozen in liquid nitrogen and stored at -80°C until assayed. Portions of these organs were also fixed in formaldehyde for histological and immunohistochemical examination.

Biochemical assays

Serum amylase and lipase levels were measured at 6 h after operations by a clinical laboratory. Results are expressed in international units per liter.

Morphological examination

Paraffin-embedded pancreatic tissue samples were sectioned (5 µm) and stained with hematoxylin/eosin. Pancreatic sections were examined by an experienced morphologist blinded to the sample identity. Pancreatic histological grading of edema, necrosis and infiltration of inflammatory cells was scored by assigning a subjective value described previously (Zhang et al., 2008): 0, absent; 1, mild; 2, moderate; and 3, severe. Grading of vacuolization was based on the approximate fraction of cells involved: 0, absent; 1, 0 to 5%; 2, 5 to 20%; and 3 to >20%.

Determination of pancreatic edema

The extent of pancreatic edema was assayed by measuring the difference between wet and dry pancreatic tissue weight. For these latter measurements, freshly obtained blotted pancreatic samples were weighed on aluminium foil, dried for 12 h at 95°C, and reweighed. The difference between wet and dry tissue weight was calculated and expressed as a percent of tissue wet weight.

Immunohistochemical localization of PAR, nitrotyrosine, P-selectin, TNF-α and ICAM-1

At 6 h after sodium taurocholate administration, the pancreatic tissues were fixed in 10% (w/v) phosphate-buffered saline (PBS, 0.01 M, pH 7.4) -buffered formaldehyde, and 8 µm sections were prepared from paraffin embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 0.3% (v/v) hydrogen peroxide in 60% (v/v) methanol for 30 min. The sections were permeabilized with 0.1% (w/v) Triton X-100 in PBS for 20 min. Non-specific adsorption was minimized by incubating the sections in 2% (v/v) normal goat serum in PBS for 20 min. Endogenous biotin and avidin binding sites were blocked by sequential incubation for 15 min with avidin and biotin, respectively. Sections were incubated for 2 h at room temperature with rabbit anti-PAR polyclonal antibody, rabbit anti-TNF-α antibody, rabbit anti-P-selectin antibody, or rabbit anti-ICAM-1 antibody (CD54) (all antibodies were from Beijing Biosynthesis Biotechnolgy Co., Ltd, China., diluted 1:500 in PBS, v/v). Subsequently, the sections were washed three times with PBS with 0.1% Tween-20 (TBS) and incubated with goat anti-rabbit horseradish peroxidase (HRP)-labeled secondary antibody (1:500 in PBS, v/v). For nitrotyrosine detection, sections were incubated with mouse anti-nitrotyrosine polyclonal antibody (1:500 in PBS, v/v, Millipore Co., Bedford, MA, USA), washed three times in TBS and incubated with goat anti-mouse HRP-labeled secondary antibody (1:500 in PBS, v/v, Tiangen biotech Co., Ltd, China). To verify the binding specificity for each antibody, some sections were also incubated with primary antibody only (no secondary) or with secondary antibody only (no primary).

Measurement of myeloperoxidase (MPO) activity and malondialdehyde (MDA)

MPO activity, an index of polymorphonuclear leukocyte accumulation, was determined according to the manufacturer's protocols. Pancreatic tissues, collected at 6 h after induction of pancreatitis, were homogenized in a solution containing 0.5% hexadecyltrimethylammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged at 20,000×*g* for 30 min at 4°C. An aliquot of the supernatant was then allowed to react with a solution of tetra-methyl-benzidine (1.6) and 0.1 mM H_2O_2. The rate of change in absorbance was measured using a spectrophotometer at 460 nm. MPO activity is defined as the quantity of enzyme degrading 1 µmol of peroxide/min at 37°C and was expressed in units per gram weight of wet tissue.

Pancreatic MDA was determined according to the manufacturer's protocols. Briefly, at 6 h after sodium taurocholate injection, the pancreatic tissues were homogenized in a 1.15% (w/v) KCl solution. All homogenization procedures were performed on ice. A 100 µl aliquot of homogenate was added to a reaction mixture containing 200 µl of 8.1% (w/v) sodium laurylsulfate, 1.5 ml of 20% (v/v) acetic acid, 1.5 ml of 0.8% (w/v) thiobarbituric acid and 700 µl of distilled water. Samples then were boiled for 1 h at 95°C and centrifuged at 3000×*g* for 10 min. The absorbance of the supernatant was measured by spectrophotometry at 650 nm. MDA concentrations were expressed in nanogram MDA per ml (ng/ml) of wet tissue.

Measurement of pancreatic TNF-α and IL-6

Pancreatic tissues were homogenized in PBS containing 2 mmol/L of phenyl-methyl sulfonyl fluoride. The pancreatic levels of TNF-α and IL-6 were determined by radioimmunoassay according to the manufacturer's protocols (Nanjing Jiancheng Bioengineering Institute, China).

Western blot analysis of IκBα in cytoplasm

Pancreatic tissue was collected 6 h after sodium taurocholate administration. Briefly, both nuclear and cytoplasmic proteins from pancreatic lobules of each experiment were extracted using the Nuclear-Cytosol Extraction Kit (Applygen Technologies, Beijing, China) following the manufacturer's instructions. Protein concentrations were determined by the Bradford method. Cytosolic protein was loaded on 12% polyacrylamide gels and after electrophoresis was transferred onto a nylon membrane. The membranes were blocked with 5% nonfat milk solution overnight and then incubated for 1.5 h with primary antibodies to IκBα (Santa Cruz, Heidelberg, Germany) at a dilution of 1:1000 in TBST with 5% nonfat milk. After washing with TBS containing 0.2% Tween-20, membranes were incubated with HRP-conjugated secondary antibodies (Beijing Biosynthesis Biotechnology) for 30 min and bands visualized using the Enhanced Chemiluminescence system (ECL, Amersham Pharmacia Biotech, Buckinghamshire, UK). Each sample was analyzed in duplicate or triplicate and at least three repetitions were performed for each experiment described earlier.

Statistical analysis

All values in the figures are presented as the mean ± standard error (S.E.M) of *n* observations. Statistical analysis was performed by using the Mann–Whitney non-parametric t-test. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

Effects of baicalin on the degree of acute pancreatitis

Sodium taurocholate-induced pancreatitis in vehicle-treated rats was associated with significant rises in the serum levels of lipase and amylase (Figure 1), which were significantly reduced in baicalin-treated rats (Figure 1). In sham-treated rats, the histological features of the pancreas showed the typical architecture of a normal organ (Figure 2A). At 6 h after the injection with sodium taurocholate, histological examination of pancreatic

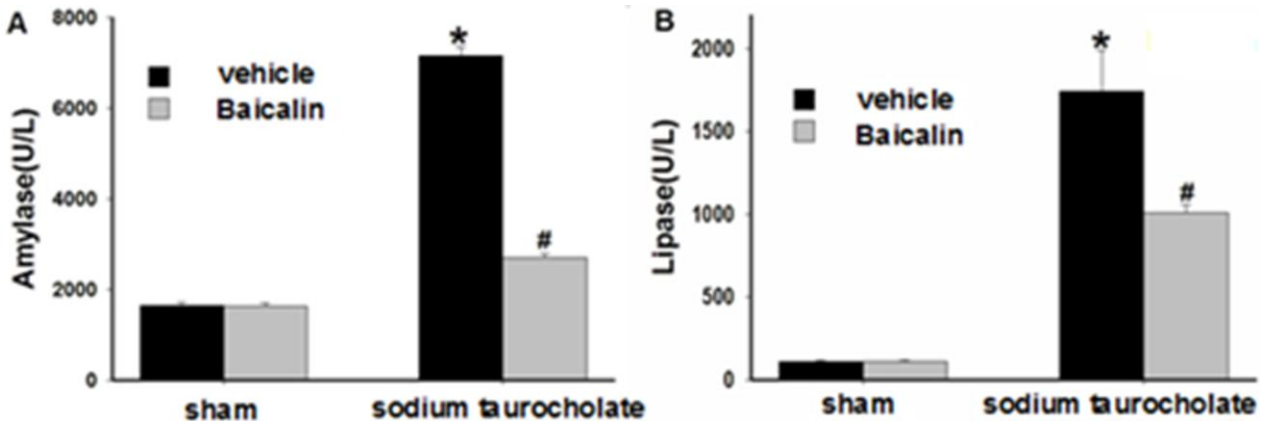

Figure 1. Amylase (A) and lipase (B) serum levels (U/L). The treatment with baicalin significantly reduced the increase of amylase and lipase induced by sodium taurocholate. Data are mean ± SEM of 15 rats for each group. *, $p < 0.01$ versus sham, #, $p < 0.01$ versus sodium taurocholate + vehicle.

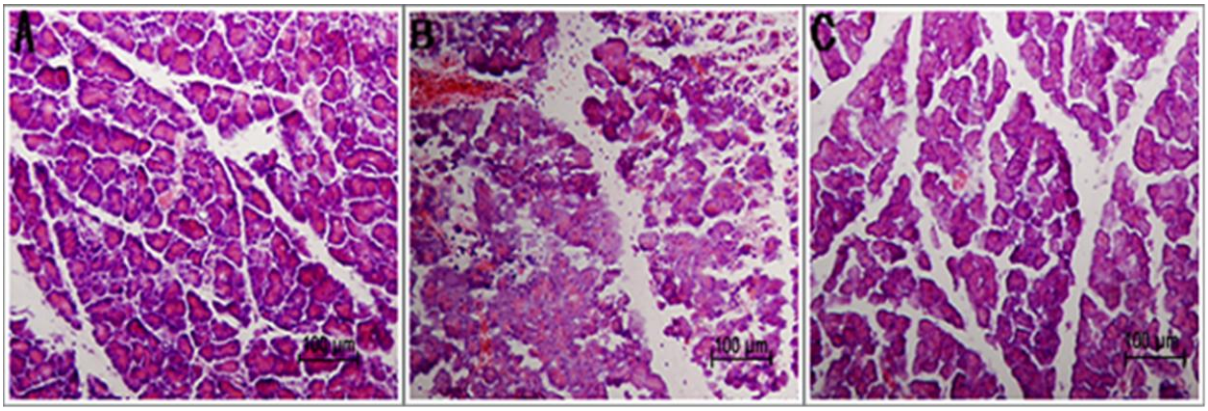

Figure 2. Morphologic changes of pancreas at 6 h after sodium taurocholate administration. No histological alterations were observed in the pancreas tissues from sham-treated rats (A). Pancreas section from sodium taurocholate-induced rats showed interstitial necrosis and infiltration of the tissue with inflammatory cells (B). The treatment with baicalin significantly reduced the extent and severity of the histological signs of pancreas injury (C). Magnification ×100. The figure is a representative of at least 3 experiments performed on different experimental days.

sections from vehicle-treated rats revealed tissue damage characterized by inflammatory cell infiltrates and acinar cell necrosis (Figure 2B). Furthermore, sodium taurocholate elicited an inflammatory response characterized by edema formation in the pancreatic tissues (Figure 3A). Baicalin treatment significantly reduced the extent and severity of the histological signs of pancreatic injury (Figures 2C and 3B), as well as the accumulation of water in the tissue (Figure 3A).

Effect of baicalin on lipid peroxidation, PAR and peroxynitrite production

The pancreatic injury in sodium taurocholate-treated rats was characterized by an increase in the tissue levels of MDA indicative of lipid peroxidation (Figure 4A). MDA levels were significantly reduced in the pancreas (Figure 4A) in baicalin-treated rats with acute pancreatitis. Positive staining for nitrotyrosine, a marker of oxidative stress and injury, was found in the acinar glandular surfaces and in acinar duct of the injured pancreas (Figure 5B1) of sodium taurocholate-treated rats. Meanwhile, a reduction in the level of nitrotyrosine staining was observed in the pancreas (Figure 5C1) from sodium taurocholate-treated animals that received baicalin. The rat pancreatic injury was also characterized by an increase in PAR polymerase activation caused by oxidative stress in the tissue. In fact, positive staining for PAR, a marker of PAR polymerase activation, was found in the injured pancreas of sodium taurocholate-treated rats (Figure 5B2). PAR staining was markedly reduced in

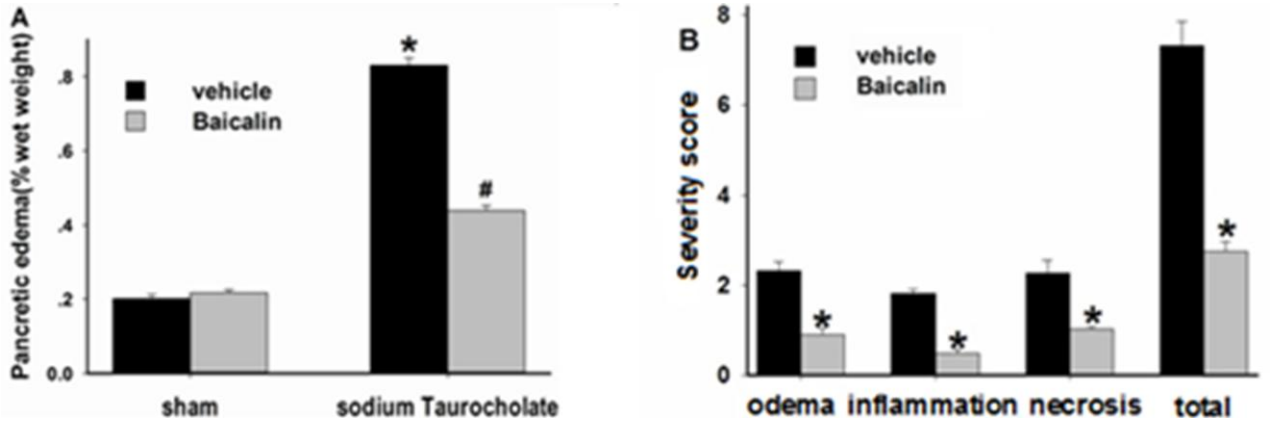

Figure 3. Effects of baicalin treatment on pancreas injury at 6 h after sodium taurocholate administration. (A) The treatment with baicalin significantly reduced edema formation in the pancreas. Data are mean ± SEM of 8 rats for each group. *, $p < 0.01$ versus sham. #, $p < 0.01$ versus sodium taurocholate + vehicle; (B) The treatment with baicalin significantly reduced the histological score of the pancreas injury (B). Data are mean ± SEM of 8 rats for each group. *$p < 0.01$ versus sodium taurocholate + vehicle.

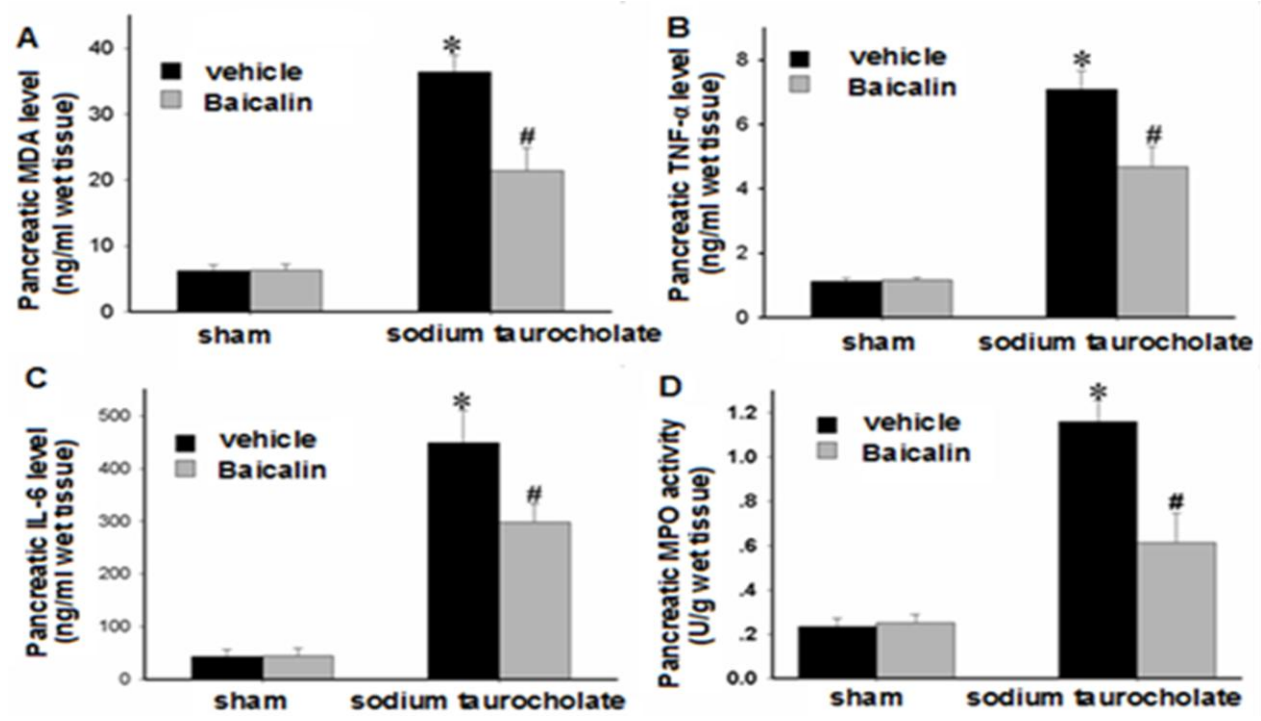

Figure 4. Effects of baicalin treatment on the production of MDA, TNF-α, IL-6 and neutrophil infiltration in pancreas tissues at 6 h after sodium taurocholate administration. Vehicle-treated rats showed a significant increase of the production of MDA (A), TNF-α (B) and IL-6 (C) at 6 h after sodium taurocholate administration. The treatment with baicalin significantly reduced pancreas levels of MDA (A), TNF-α (B) and IL-6 (C). MPO activity also showed a significant increase at 6 h after sodium taurocholate administration and the level was reduced by treatment of baicalin significantly (D). Data are mean ± SEM of 15 rats for each group. *, $p < 0.01$ versus sham. #, $p < 0.01$ versus sodium taurocholate + vehicle.

Baicalin-treated rats with induced acute pancreatitis (Figure 5C2). However, there was no staining for either nitrotyrosine or PAR in pancreatic tissue obtained from sham-treated rats (Figures 5A1 and A2).

Effects of baicalin on production of proinflammatory cytokines

In order to observe whether baicalin could regulate the

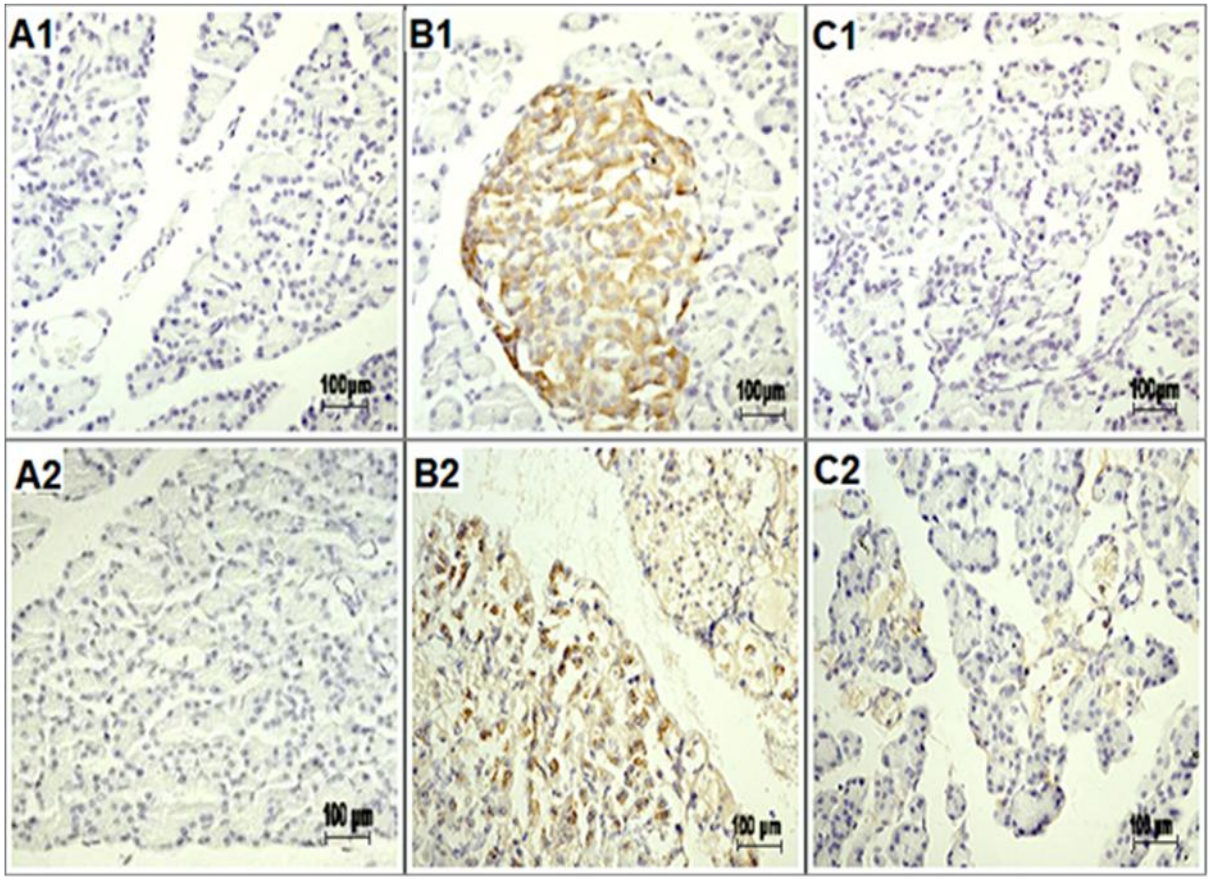

Figure 5. Immunohistochemical localization of PAR and nitrotyrosine in the pancreas at 6 h after sodium taurocholate administration. No positive staining for PAR (A1) or nitrotyrosine (A2) was observed in pancreas tissues from sham-treated rats. Section obtained from sodium taurocholate-treated rat showed intense positive staining for PAR (B1) or nitrotyrosine (B2) in the pancreas. The degree of pancreas staining for PAR (C1) or nitrotyrosine (C2) was markedly reduced from Baicalin-treated rats with acute pancreatitis. Magnification ×100. The figure is a representative of at least 3 experiments performed on different experimental days.

secretion of these proinflammatory cytokines through the modulation of NF-κB activation, the pancreatic TNF-α and IL-6 levels were assayed. No positive staining for TNF-α was found in pancreatic tissue sections from sham-treated rats (Figure 6A1). At 6 h after the sodium taurocholate injection, positive staining for TNF-α was observed in the pancreases collected from vehicle-treated rats (Figure 6B1), and the extent of immunostaining (Figure 6C1) was markedly reduced in those from baicalin-treated rats subjected to acute pancreatitis. The TNF-α levels in pancreatic samples were also evaluated by radioimmunoassays. A substantial increase in TNF-α was found in pancreatic samples collected from vehicle-treated rats, while the level was significantly reduced in those from baicalin-treated rats at 6 h after sodium taurocholate administration (Figure 4B), which was consistent with the change of TNF-α immunostaining in pancreatic tissues. In contrast to the substantial increase of pancreatic IL-6 in vehicle-treated rats with acute pancreatitis, the level was significantly reduced in pancreatic samples from baicalin-treated rats at 6 h after sodium taurocholate administration (Figure 4C). Therefore, the secretion of both proinflammatory cytokines was inhibited by baicalin during the development of SAP.

Effects of baicalin on ICAM-1, P-selectin expression and neutrophil infiltration

Acute pancreatitis is characterized by the accumulation of neutrophils in the pancreas, which augments the tissue damage. Therefore, we evaluated the extent of expression of ICAM-1 and P-selectin, which play pivotal roles in the rolling and firm attachment of neutrophils to the endothelium. No positive staining for ICAM-1 and P-selectin was found in pancreatic tissue sections from sham-treated rats (Figures 6A2 and A3). At 6 h after the sodium taurocholate injection, positive stainings for ICAM-1 and P-selectin were observed (Figures 6B2 and

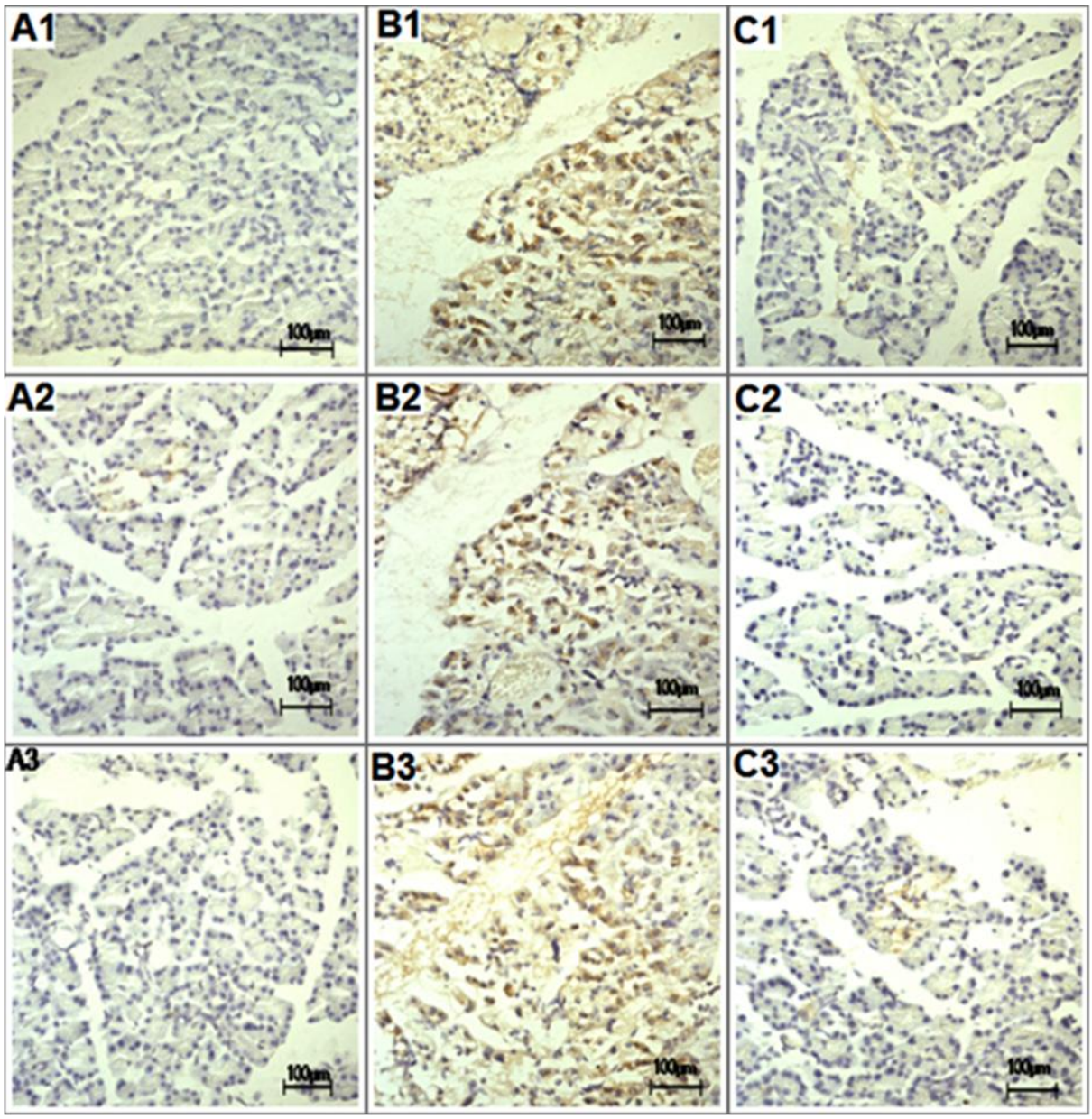

Figure 6. Immunohistochemical localization of TNF-α, ICAM-1 and P-selectin in the pancreas at 6 h after sodium taurocholate administration. No positive staining for TNF-α, ICAM-1 and P-selectin was observed in pancreas tissues from sham-treated rats (A1, A2 and A3). Section obtained from sodium taurocholate-treated rats showed intense positive staining for TNF-α, ICAM-1 and P-selectin in the pancreas (B1, B2 and B3).The staining degree for TNF-α, ICAM-1 and P-selectin (C1,C2 and C3) was markedly reduced in pancreas tissue section obtained from Baicalin-treated rats with acute pancreatitis. Magnification ×100. The figure is a representative of at least 3 experiments performed on different experimental days.

B3) in the pancreases collected from vehicle-treated rats. Meanwhile, immunostainings for ICAM-1 and P-selectin (Figures 6C2 and C3) were markedly reduced in pancreatic tissues from baicalin-treated rats with acute pancreatitis.

It has been demonstrated that the expression of adhesion molecules correlates with the influx of leukocytes into the pancreas tissue. Therefore, we investigated the effect of baicalin on neutrophil infiltration by measuring the activity of MPO, which was significantly elevated at 6 h after sodium taurocholate administration in vehicle-treated rats (Figure 4D). By contrast, neutrophil infiltration was significantly reduced in pancreatic tissues from sodium taurocholate-treated rats which had received

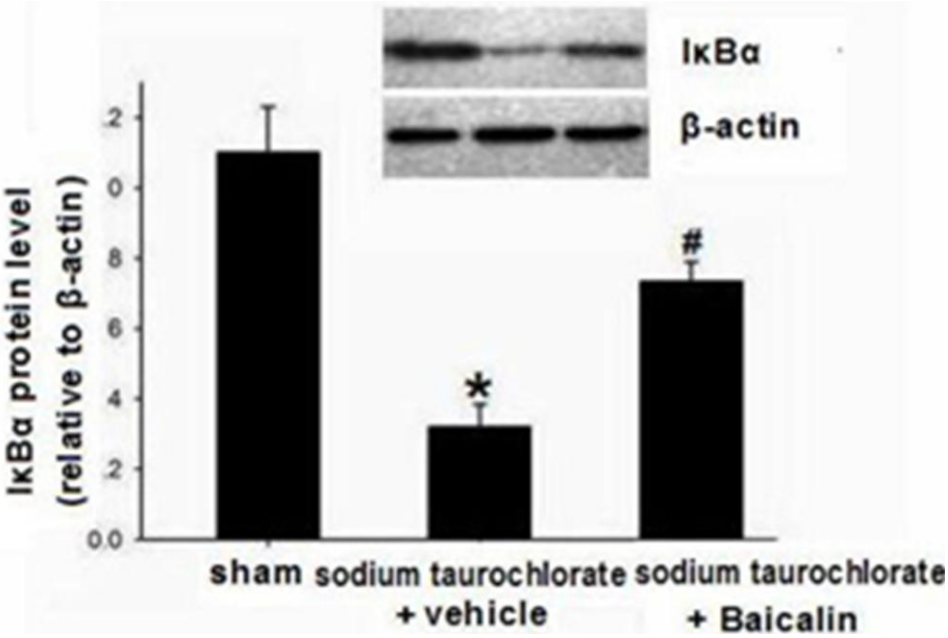

Figure 7. Western blot analysis shows the effect of baicalin treatment on degradation of IκBα in cell/tissue pellet from pancreatic lobules collected at 6 h after sodium taurocholate administration. Basal level of IκBα band was present in the cytosolic fraction of cell collected from sham-treated rats: IκBα band has significantly decreased from the cytosolic fraction of cells collected from sodium taurocholate-treated rats. The treatment with baicalin significantly reduced the decrease of IκBα band in the cytosolic fraction induced by sodium taurocholate. The data illustrated are representative of at least 3 experiments performed on different experimental days. *, $p < 0.01$ versus sham. $^{\#}$, $p < 0.01$ versus sodium taurocholate + vehicle.

baicalin (Figure 4D).

Degradation of IκBα in cytoplasm of pancreatic cells

Finally, we tested whether baicalin treatment could prevent the degradation of IκBα in pancreatic lobules after treatment with sodium taurocholate. Cytoplasmic extracts were analyzed by western blotting using an anti-IκBα antibody. A basal level of IκBα was detectable in the cytosolic fraction of the cell/tissue pellets from pancreatic samples of sham-treated rat, whereas, 6 h after sodium taurocholate, IκBα decreased significantly in the cell/tissue pellet. This IκBα degradation was significantly inhibited in the cell/tissue pellet collected from baicalin-pretreated rats subjected to sodium taurocholate induced SAP (Figure 7).

DISCUSSION

Baicalin has been reported to possess anti-oxidant and anti-inflammatory effects. It can protect cells and tissues by eliminating oxidants, blocking oxidant production, modulating lipoprotein metabolism and inhibiting lipid peroxidation. The anti-oxidative effects of this drug were shown to modulate the interaction between inflammatory target cells and peripheral leukocytes through which it displays anti-inflammatory effects (Shen et al., 2003). In the present study, we also demonstrated that baicalin markedly attenuated the severity of pancreatitis at least partly by its inhibition of NF-κB activation in a rat model of SAP induced by sodium taurocholate.

Lipid peroxidation, ROS and peroxynitrite contribute to the tissue injury observed during inflammation (Ethridge et al., 2002; Park et al., 2003). Our results are in close agreement with the idea that quenching oxidative stress could be a valuable approach for the management of acute pancreatitis. Here, we demonstrated that the levels of MDA and nitrotyrosine staining, indicative of lipid peroxidation and oxidative stress in the pancreatic tissue, were reduced in baicalin-treated SAP rats. PAR staining, representing the activation of PAR polymerase which is possibly involved in oxidant-induced injury, was also markedly reduced in baicalin-treated SAP rats. In fact, baicalin caused a reduction in the biochemical markers of pancreatitis, such as increased serum amylase and lipase activity, improved the histolopathological profile and decreased tissue edema.

Indeed, the protective effects from pancreatitis of baicalin may be attributed to its anti-oxidant effects. Studies have shown NF-κB activation to be localized in

areas of oxidative stress, and it is now generally accepted that many lipid peroxidation inhibitors and anti-oxidants are efficient inhibitors of NF-κB activation (Altavilla et al., 2003; Ethridge et al., 2002; Virlos et al., 2003). Furthermore, baicalin has been shown to exert its anti-inflammatory activity by scavenging reactive oxygen intermediates in certain inflammatory cells (Shen et al., 2003). Therefore, NF-κB inhibition may represent an important component of the protective effects of baicalin against acute pancreatitis.

NF-κB activation is a key event in triggering the local cascade of inflammatory mediators associated with acute pancreatitis. NF-κB inhibition has been demonstrated in acute pancreatitis to impede systemic inflammation at the nuclear level and restrain the progression of the characteristic systemic inflammatory response (Ethridge et al., 2002; Meng et al., 2005; Rakonczay et al., 2008; Virlos et al., 2003). As a key pluripotent protein with its gene transcription regulatory function, NF-κB can participate in modulation of many inflammatory cytokines. It is now generally accepted that activation of NF-κB can result in increased expression of cytokines such as TNF-α, monocyte chemoattractant protein-1, iNOS, IL-6, IL-1, IL-10, ICAM-1 and P-selectin, leading to immune and inflammatory responses in organisms (Chen et al., 2002; Meng et al., 2005; Rakonczay et al., 2008; Vaquero et al., 2001; Xiping et al., 2009; Xue et al., 2006; Zhang et al., 2009b). As a consequence, activated neutrophils and pro-inflammatory cytokines are released into the systemic circulation, allowing interactions with the vascular endothelium of distant organs and contributing to the systemic inflammatory response.

Pro-inflammatory cytokines, such as TNF-α and IL-6, play a major role in the inflammation associated with acute pancreatitis (Chen et al., 2002; Oruc et al., 2004; Rakonczay et al., 2008; Tian et al., 2009; Vaquero et al., 2001). Activation of NF-κB in the pancreas has been demonstrated in pancreatitis to improve the corresponding gene transcription and to synthesize TNF-α and IL-6, and inhibition of NF-κB activation results in a decrease in the expression of cytokines TNF-α and IL-6 (Chen et al., 2002; Ethridge et al., 2002; Meng et al., 2005; Rakonczay et al., 2008; Vaquero et al., 2001; Virlos et al., 2003; Xue et al., 2006). In the present study, we found that sodium taurocholate-induced rat acute pancreatitis led to a substantial increase of TNF-α and IL-6 levels in the pancreatic tissues, and that both levels were markedly reduced by baicalin treatment, which is consistent with previous findings in an experimental model of endotoxin shock and SAP (Cheng et al., 2007; Xue et al., 2006). The results demonstrated that baicalin could modulate the expression of these pro-inflammatory cytokines in experimental pancreatitis, perhaps in association with its inhibition of NF-κB.

NF-κB activation has been associated with upregulated P-selectin, ICAM-1 and adhesion molecules in acinar and endothelial cells in the early stage after taurocholate administration. These effects lead to a large increase of adherent and transmigrated polymorphonuclear neutrophils. Once activated, neutrophils help increase ROS production, which in turn leads to further pancreatic NF-kB activation (Rakonczay et al., 2008). It has been demonstrated that baicalin can inhibit NF-κB activation in multiple organs of rats in the early stage of SAP, reduce inflammatory factors production and downregulate the expression of P-selectin (Xiping et al., 2009; Zhang et al., 2009a). In this study, we also found that experimental acute pancreatitis led to markedly increased expression of ICAM-1 and P-selectin, accompanied by a significant increase in MPO activity directly correlated with leukocyte infiltration. As baicalin could reduce the upregulation of ICAM-1 and P-selectin, MPO activity was consequently reduced. These results suggested that baicalin may interfere with the interaction of neutrophils and endothelial cells both at the early rolling phase mediated by P-selectin and at the late firm adhesion phase mediated by ICAM-1. The finding that baicalin significantly reduced neutrophil infiltration and tissue injury in this experimental setting is in agreement with previous studies (Shen et al., 2003; Xiping et al., 2009; Zhang et al., 2009a). Therefore, it is plausible that the reduction in leukocyte infiltration caused by baicalin could at least partly be attributed to its inhibitory effect on NF-κB activation.

In the early phase of sodium taurocholate-induced rat SAP in our experiments, the marked loss of IκBα indicative of NF-κB activation was prevented by baicalin treatment, suggesting that baicalin may inhibit NF-κB activation via stabilization of IκBα. This result is consistent with previous findings that baicalin prevents LPS-induced IκBα degradation and thus inhibits NF-κB activation *in vivo* in a model of endotoxic shock in the rat (Chen et al., 2007). As NF-κB activation is a key event in triggering the local cascade of inflammatory mediators associated with acute pancreatitis, NF-κB inhibition may represent an important component of its protective effects on acute pancreatitis.

In summary, our results demonstrated that sodium taurocholate-induced pancreatitis led to the activation of NF-κB in the pancreas, and that baicalin inhibited the activation of NF-κB *in vivo*. As a consequence, baicalin attenuated pancreatic inflammation and tissue injury, lipid peroxidation, PAR and nitrotyrosine production, upregulation/formation of ICAM-1 and P-selectin, neutrophil infiltration, and production of proinflammatory cytokines TNF-α and IL-6 in sodium taurocholate-induced acute pancreatitis. Therefore, this study showed that the baicalin inhibition of NF-κB activation was partly responsible for its protective effects in the rat model of experimental acute pancreatitis.

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