Involvement of Redox-Sensitive Extracellular-Regulated Kinases in Angiotensin II-Induced Interleukin-6 Expression in Pancreatic Acinar Cells

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ABSTRACT

Angiotensin II has been shown to play a role in the pathogenesis of acute pancreatitis (AP). The present investigation aimed at elucidating redox-sensitive mechanistic pathway involved in proinflammatory actions of angiotensin II during an episode of AP; in particular, the regulation of expression of cytokine interleukin (IL)-6. Exogenous angiotensin II induced IL-6 expression, activation of extracellular-regulated kinase (ERK) 1/2, and superoxide generation in pancreatic acinar cell line AR42J, which were reversed by the angiotensin II type 1 (AT₁) receptor antagonist, losartan (2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-methanol monopotassium salt, C₂₂H₂₃ClN₆O). Pharmacological blockade of ERK1/2 improved angiotensin II-induced IL-6 expression. Moreover, angiotensin II-induced ERK1/2 activation was suppressed by antioxidant, indicating that redox-regulated ERK1/2 mediates the cytokine expression. cAMP-responsive element-binding protein (CREB) might be involved in ERK1/2-induced IL-6 expression because phosphorylation of CREB was observed after angiotensin II treatment, which was reversed by losartan and the ERK1/2 inhibitor. These results were in close agreement with the in vivo findings using an obstructive model of AP. Obstruction of the common biliopancreatic duct time-dependently enhanced angiotensinogen levels, which correlated well with superoxide generation, ERK1/2 and CREB phosphorylation, and subsequent IL-6 expression. It is more important that changes in these parameters were antagonized by prophylactic administration of losartan. These in vitro and in vivo results indicate that angiotensin II induces redox-regulated ERK1/2 and CREB activation, thus leading to IL-6 expression in an AT₁ receptor-mediated manner in pancreatic acinar cells during the pathogenesis of AP.

Interleukin (IL)-6 is a multifunctional and proinflammatory cytokine that regulates the progression of self-limiting acute pancreatitis (AP) to the systemic inflammation. Patients with AP exhibit elevated serum IL-6 levels within 48 h after the onset of symptoms, and these levels correlate well with disease severity (Davies and Hagen, 1997). Neutralization of IL-6 using the monoclonal antibody has been demonstrated to attenuate experimental AP and its associated pulmonary injury (Chao et al., 2006). In this regard, pharmacological antagonism of IL-6 action is suggested to serve as one of targets for the management of pancreatitis.

Emerging body of evidence has shown that the local pancreatic renin-angiotensin system (RAS) plays a crucial role in the pathophysiology of AP. Pancreatic expression of the major components of RAS was up-regulated in experimental AP (Tsang et al., 2004a; Chan and Leung, 2007a). The nonspecific angiotensin II receptor blocker, saralasin, inhibited the effects of losartan are not only restricted to local pancreatic levels but also extended to AP-induced systemic inflammation (Chan and Leung, 2006). We have reported recently that angiotensin II, via the AT₁ receptor, mediates the pancreatic injury initiated by hyperstimulation with caerulein (Tsang et al., 2004b). The protective effects of losartan are not only restricted to local pancreatic levels but also extended to AP-induced systemic inflammation (Chan and Leung, 2006).

We have reported recently that angiotensin II, via the AT₁

ABBREVIATIONS: IL, interleukin; AP, acute pancreatitis; RAS, renin-angiotensin system; AT₁, angiotensin II type 1; losartan, 2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl) benzyl] imidazole-5-methanol monopotassium salt, C₂₂H₂₃ClN₆O; NF, nuclear factor; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; ERK, extracellular-regulated kinase; PD123319, 2-[4-(dimethylamino)-3-methylphenyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid; PBS, phosphate-buffered saline; PD98059, 2’-amino-3’-methoxy flavone; DHE, dihydro-β-erythroidine; CBPD, common biliopancreatic duct; CREB, cAMP-responsive element binding protein; DAPI, 4,6-diamidino-2-phenylindole; PCR, polymerase chain reaction.
receptor, exerts oxidative stress to activate nuclear factor (NF) xB during experimental pancreatitis (Chan and Leung, 2007a). However, it is well documented that reactive oxygen species (ROS) not only switch on NFkB but also trigger the activation of redox-sensitive mitogen-activated protein kinase (MAPK) in a variety of cell types. MAPKs, in particular extracellular-regulated kinase (ERK) 1/2, are responsive to such stimuli as cytokines, growth factors, ROS, and cellular stress, which ultimately regulate multiple cellular processes, including cytoskeleton arrangement, transcription factor activation, apoptosis, proliferation, and differentiation (Leung and Chan, 2009). In isolated pancreatic acini, H2O2 and menadione, a strong superoxide generator, trigger ERK1/2 activation that is comparable with that induced by CCK (Dabrowski et al., 2000). Angiotensin II, via ROS generation, triggers activation of ERK1/2 in renal proximal tubule cells (Tanifuji et al., 2005), vascular smooth muscle cells (Kranzhofer et al., 1999; Viedt et al., 2000), and neutrophils (El Bekay et al., 2003). Which particular cell type contributes to the redox-sensitive pathways during AP pathogenesis, however, remains ambiguous. In this study, we employed in vivo models to investigate the role of ROS generation in ERK1/2 activation. Redox-sensitive pathways during AP pathogenesis, how-

In Vitro Studies. The AR42J cell line (American Type Culture Collection, Manassas, VA), which is a rat pancreatic cell line derived from excocrine pancreas of Wistar rat, was employed in the present study. They were cultured in Kaighn’s modification of Ham’s F-12 medium (Invitrogen, Carlsbad, CA) with the supplement of 20% fetal bovine serum (Invitrogen) and antibiotics (100U/ml penicillin and 100 µg/ml streptomycin) (Invitrogen). The cells were treated with exogenous angiotensin II (Tocris Bioscience, Ellisville, MO) at different concentration and time. Losartan (Merck Research Labs, West Point, PA), PD123319 (Sigma-Aldrich, St. Louis, MO), and N-acetyl cysteine (Sigma-Aldrich) were dissolved in phosphate-buffered saline (PBS) and incubated with the cells 10 min before treatment. PD98059 (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (Sigma-Aldrich), and they were pretreated for 30 min before peptide stimulation. After treatment, the cells were rinsed with PBS and lysed with lysis buffer to collect protein. For dihydro-β-erythroidine (DHE) (Sigma-Aldrich) staining, the cells were immediately fixed with fixative and then subjected to staining.

In Vivo Studies. Male Wistar rats (250–300 g) were obtained from the Laboratory Services Centre of the Chinese University of Hong Kong. The experimental procedures were approved by the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong (Ref. 04/036/ERG). They had free access to water and standardized rat chow but were fasted for 24 to 28 h before being subjected to the experiments. Obstructive pancreatitis, mimicking gallstone obstruction-induced AP, was induced by ligation of common bileopancreatic duct (CBDP), as described previously (Chan and Leung, 2007a,b). In brief, the animal (250–300g) underwent laparotomy, and the CBDP was double-ligated. For the losartan treatment group, rats were given 30 mg/kg losartan, an AT1 receptor antagonist, intragastrically 1 h before surgery. The animals were killed 6 h after surgery. The pancreas was isolated and snap-frozen for further analysis. Tissues for immunohistochemistry processing were isolated, fixed in 4% paraformaldehyde (ICN, Irvine, CA) in PBS, and embedded in paraffin for sectioning. For cryosection (DHE staining), the tissues were quickly embedded in Tissue-Tek O.C.T. Compound (Sakura Finetek Europe, Zoeterwoude, The Netherlands) at −20°C for further analysis.

Western Blot Analysis. Pancreatic protein was extracted for SDS-polyacrylamide gel electrophoresis and subsequently blotted onto a polyvinylidene difluoride membrane as described previously (Chan and Leung, 2007a). Antibodies against IL-6 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA; 1:1000), phospho-ERK (Cell Signaling Technology Inc., Danvers, MA; 1:1000), or phospho-cAMP-responsive element-binding protein (CREB) (Cell Signaling Technology Inc.; 1:2000) or CREB (Cell Signaling Technology Inc.; 1:1000) for internal control. Immunolabeled membranes were subjected to probing with the appropriate horseradish peroxidase-linked secondary antibodies and subsequently visualized by enhanced chemiluminescence with an enhanced chemiluminescence kit. The intensity of the band was quantified using the software FluorChem (Alpha Innotech, San Leandro, CA).

Immunohistochemistry and DHE Staining. Paraffin-embedded tissues were sectioned, dewaxed, rehydrated, and blocked as described in previous study (Chan and Leung, 2007a). The slides were incubated with goat anti-IL-6 (Santa Cruz Biotechnology, Inc.; 1:100) together with mouse anti-a-amylase (Santa Cruz Biotechnolog, Inc.; 1:100) antibodies overnight at 4°C and subsequent fluorescein-tagged secondary antibodies [Cy3-anti mouse (Jackson ImmunoResearch Laboratories Inc., West Grove, PA; 1:100); FITC-anti goat secondary antibodies (Jackson ImmunoResearch Laboratories Inc.; 1:100)]. For DHE staining, the O.C.T.-embedded tissue was sectioned (10 µm) at −20°C using the Benchtop Cryostat (Leica, Wetzlar, Germany; CM 1100). After fixation, the slides were allowed to react with 5 µM DHE in the presence of DAPI (Jackson ImmunoResearch Laboratories Inc.; 1:1000) in dimethyl sulfoxide at 37°C for 30 min. Both immunofluorescent- and DHE-stained slides were mounted with Vectashield fluorescence mounting medium (Vector Laboratories, Burlingame, CA). Images were then captured with a Leica digital camera mounted on a fluorescence microscope attached to a personal computer running Leica software.

RNA Extraction, Reverse Transcription, and Real-Time PCR. Pancreatic RNA was extracted by TRIzol reagent (Invitrogen) as described previously (Wong et al., 2007). Reverse transcription was performed using Superscript First-Strand Synthesis System (Invitrogen) as described by the kit. Semiquantification of mRNA was achieved by real-time PCR using the SYBR Green detection method. In brief, cDNA was mixed with primers (β-actin forward, AGC TAT GAG CTG CCT GACG; reverse, GGA TGC CAC AGG ATT CCA; angiotensinogen forward, GCA AATCAGTGCCCTCACC; reverse, AACAAACCCCTCACCCGAGG; IL-6 forward, CCA CCC ACA ACA GAC CAG T; reverse, ACA GTG CAT CAT CGG TGT TTC) and allowed to react with SYBR Green PCR Master Mix Reagent (Applied Biosystems, Foster City, CA) for 40 cycles. The polymerase reaction was monitored by real-time PCR System (Prism 7700; Applied Biosystems). Primers were designed by the Web site Primer 3.

Results

Effects of Angiotensin II on IL-6 and ERK1/2 Activation. Exogenous administration of angiotensin II dose-depen
dently (0.01–10 µM) augmented IL-6 protein expression (Fig. 1A). Angiotensin II (1 µM) led to elevated protein expression of IL-6 in AR42J cells at 6 h (by ~1.8-fold) but not 3 h (by 1.2-fold; Fig. 1B). Angiotensin II could trigger ERK1/2 activation concomitantly, peaking at 1 µM concentration (Fig. 1C). Incubation of pancreatic acinar cells with angiotensin II leads to ERK activation at 5 min (~2-fold of control) (Fig. 1D). Continuous treatment also yielded elevated ERK

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activation, but it is not statistically significant compared with the control (Fig. 1D).

Pretreatment of losartan, a specific AT\(_1\) receptor blocker (0.5 and 5 \(\mu\)M), did not exert any significant effects on ERK1/2 activation after stimulation of angiotensin II for 5 min, representing 20 and 8.7% decrease, respectively. However, the protective effect was observed when 50 \(\mu\)M losartan was given, exerting a 57% reduction (Fig. 2A). The reversal effect on ERK activation was confirmed by pretreatment of the ERK inhibitor PD98059. Pretreatment of PD98059 at 5 \(\mu\)M, but not 0.05 and 0.5 \(\mu\)M, revealed a significant reversal effects on ERK activation (by nearly 70% reduction) (Fig. 2B). Likewise, pretreatment of 50 \(\mu\)M losartan significantly abolished the angiotensin II-induced IL-6 protein expression by 45% (Fig. 2C). It is interesting that angiotensin II-induced IL-6 expression was antagonized by PD98059 at the 5 \(\mu\)M concentration by 52% (Fig. 2D), indicating that angiotensin II-induced superoxide generation-mediated ERK activation.

**Effects of Angiotensin II on ROS Generation and Its Role in the ERK1/2 Pathway.** To examine the role of ROS-mediated ERK1/2 activation in AR42J cell line, DHE staining was performed after stimulation of angiotensin II for 5 min (minimal time for ERK1/2 activation). Exogenous treatment of angiotensin II triggered superoxide free radical generation after 5 min, as evidenced by positive intense red fluorescent staining (Fig. 3A). Pretreatment of pancreatic acinar cells with 50 \(\mu\)M losartan, but not the AT\(_2\) receptor blocker PD123319 at the same concentration, abolished angiotensin II-induced ROS generation (Fig. 3A). It is more important that activation of ERK1/2 was significantly diminished by pretreatment of the antioxidant NAC (Fig. 3B), indicative of angiotensin II-induced superoxide generation-mediated ERK activation.

**Involvement of CREB in Angiotensin II Signaling.** Exogenous treatment of angiotensin II for 1 h induced phosphorylation of CREB (Fig. 4A). Pretreatment of AR42J cells with 50 \(\mu\)M losartan and 5 \(\mu\)M PD98059 (effective dose of two drugs in inhibiting ERK activation) could significantly abolish the effect on angiotensin II-induced CREB activation by 53 and 45%, respectively (Fig. 4B), indicating that ERK might be an upstream mediator in CREB activation.
Expression of Angiotensin II Precursor in Obstruction-Induced Pancreatitis and the Effects of Losartan on Angiotensin II Signaling in Vivo. To confirm the role of angiotensin II in pancreatic acinar cells during pathogenesis of AP, an in vivo model of pancreatitis were employed. Obstruction of CBPD time-dependently increased the gene expression of angiotensinogen, the precursor of angiotensin II, in the pancreas (Fig. 5A). Obstruction of CBPD led to superoxide free radical generation, as indicated by DHE staining in pancreatic section. ROS generation was antagonized by pretreatment of losartan (Fig. 5B), indicating superoxide generation is mediated via the AT1 receptor. Obstruction of CBPD resulted in significant increase in phosphorylation of ERK1/2 by 5.6-fold. Prophylactic administration of losartan reversed the obstruction-induced ERK1/2 activation by 74%, compared with the untreated AP group (Fig. 5C). Likewise, AP induction resulted in phosphorylation of CREB in pancreatic tissue, which was blunted by losartan treatment (2.2-fold increase in AP versus control and 45% reduction by losartan treatment) (Fig. 5D). Obstruction of CBPD resulted in 11- and 2.1-fold enhancement in pancreatic mRNA and protein expression of IL-6, respectively (Fig. 6, A and B). The augmentation was significantly suppressed nearly to half by losartan treatment (Fig. 6, A and B). Consistent with Western blot analysis, immunostaining results revealed that pancreatic section from obstructive AP exhibited higher IL-6 immunoreactivity, compared with sham-operated control (red stain in Fig. 6C). Colocalization of IL-6 and amylase expression (green stain) confirmed that the expression of IL-6 originates from pancreatic acinar cells during obstructive AP (orange stain in Fig. 6C).

Discussion

This is the first report to have demonstrated the involvement of redox-sensitive ERK1/2 and CREB activation in angiotensin II-induced IL-6 expression in pancreatic acinar cells during the pathogenesis of AP. It is more important that this work, together with our previous findings (Chan and Leung, 2007a), provides an additional mechanistic pathway involving the angiotensin II-NADPH oxidase-NFκB axis in the regulation of proinflammatory action, thus implicating the potent protective effects of RAS blockade against pancre-
atic inflammation (Ip et al., 2003a; Tsang et al., 2003, 2004b; Chan and Leung, 2006).

Prevalent therapy for pancreatitis targeting IL-6 includes neutralizing antibodies against IL-6 itself and the IL-6 receptor (Gentiletti and Fava, 2003). However, the peptidic nature of antibodies hampers its use as a pharmacological agent in treating AP. The present study provides evidence showing the effectiveness of oral active AT1 receptor blocker, losartan, in suppressing the expression of IL-6. In this regard, losartan has been reported to inhibit an array of proinflammatory cytokines and proteins, including IL-1, intercellular adhesion molecule-1, and cyclooxygenase-2, thus further improving pancreatic inflammation (Chan and Leung, 2007a). In the present study, it was shown that ROS serve a pivotal role in transducing signal to ERK and subsequently lead to IL-6 transcription in pancreatic acinar cells during AP. ROS has been shown to regulate a number of intracellular signaling during pancreatitis (Leung and Chan, 2009). Angiotensin II has been demonstrated concomitantly to trigger oxidative stress in several pancreatic cells, such as β cells (Chipitsyna et al., 2007), pancreatic islets (Chu and Leung, 2007), and pancreatic stellate cells (Masamune et al., 2008). These findings are consistent with our data indicating that angiotensin II, via the AT1 receptor, could result in generation of superoxide in pancreatic acinar cells in vitro and in vivo during AP. In the present study, administration of the antioxidant NAC could reverse ERK1/2 activation, suggesting that ERK1/2 is subjected to be redox-regulated to express IL-6 in pancreatic acinar cells.

One of the potential sources of ROS triggered by angiotensin II stimulation might be NADPH oxidase. It has been shown that AR42J cells express the key components of NADPH oxidase, which are subjected to be regulated upon stimulus (Yu et al., 2005a,b, 2007). Preliminary data demonstrated that the NADPH oxidase inhibitor, diphenyleneiodonium chloride, could significantly reverse the angiotensin II-induced superoxide generation in AR42J cells (Supplemental Fig. 1). Moreover, angiotensin II-induced IL-6 expression was significantly blunted after diphenyleneiodonium chloride pretreatment (Supplemental Fig. 2), implying NADPH oxidase might serve as a central executor of angiotensin II-induced ROS generation. These findings are in line with the results from a previous study showing that pancreatitis-induced up-regulation of NADPH oxidase subunits p67

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**Fig. 3.** Effects of angiotensin II in superoxide generation (A). Top, representative diagram showing the DHE staining of AR42J cell after treatment of angiotensin II (1 μM) for 5 min with/without the AT1 receptor blocker, losartan, or the AT2 receptor blocker, PD123319. Slides from control (a, e, i), angiotensin II alone (b, f, j), angiotensin II pretreated with losartan (c, g, k), and angiotensin II pretreated with PD123319 (d, h, l) were allowed to react with DHE to detect the superoxide generation (red) and were stained with DAPI (blue) to localize the nucleus. Merged images (i–l) reveal signals from nucleus of AR42J cells (purple). Bottom, statistical analysis of DHE staining. Effects of N-acetylcysteine on angiotensin II-induced ERK1/2 activation (B). Bottom, representative Western blot results after treatment of angiotensin II (1 μM) in the absence or presence of NAC. Bar chart, statistical analysis. *, $p < 0.05$ versus controls; ***, $p < 0.001$ versus control. +, $p < 0.05$ versus angiotensin II alone; ++, $p < 0.01$ versus angiotensin II alone ($n = 5$).
and p22 was associated with AT₁ receptor stimulation (Chan and Leung, 2007a).

Nevertheless, the underlying mechanism of redox-regulated ERK1/2 during pancreatitis has yet to be elucidated. Oxidative stress could activate membrane-bound receptors such as epidermal growth factor receptor and platelet-derived growth factor receptor in a ligand-independent manner, subsequently leading to activation of Ras and ERK1/2 (Knebel et al., 1996; Zhuang and Schnellmann, 2004). Protein tyrosine kinase Src could also be stimulated by ROS (Lee and Esselman, 2002), which in turn directly activate ERK in a Ras-dependent manner (Servitja et al., 2003). In addition, redox-activated Src kinase could trigger phospholipase Cγ activation, resulting in the release of inositol triphosphate and diacylglycerol (Banan et al., 2001). Intracellular calcium is then mobilized and released, leading to ERK activation in a calmodulin-dependent manner (Schmitt et al., 2004). Elevated intracellular calcium also triggers protein kinase C activation, turning on the Raf pathway, and thus results in ERK activation (Zou et al., 1996). On the other hand, ERK could be redox-regulated by suppressing the phosphatase activity; oxidative stress inhibited protein phosphatase, thus resulting in activation of ERK (Lee and Esselman, 2002). In this regard, ERK1/2 also might be activated in a similar manner; however, the precise mechanism by which ROS switch on ERK1/2 in AP awaits further elucidation.

The association between ERK1/2 and IL-6 expression depends on cooperation with other nuclear proteins because ERK1/2 does not serve directly as a transcription factor. ERK1/2 controls many downstream mediators for the transcription of gene expression. Among them, CREB is one of the candidates that is closely related to inflammatory response. It has been shown that the promoter of IL-6 contains a number of cAMP-responsive elements (Ichiki, 2006). Target disruption of the cAMP-responsive element site of the IL-6 promoter impairs stimulus-induced proinflammatory gene expression, indicating that CREB is critical in mediating inflammatory response (Sano et al., 2001; Ichiki, 2006). Besides, angiotensin II has been shown to promote the phosphorylation of CREB at Ser113 in an ERK1/2-dependent manner (Cammarota et al., 2001). Taken together, our in vitro and in vivo data are in line with those previous reports, describing that CREB may be involved in ERK1/2-dependent angiotensin II-induced IL-6 expression in pancreatic acinar cells.

The beneficial effects of losartan undoubtedly are due to the blockade of the AT₁ receptor and, thus, subsequent abolishment of downstream proinflammatory pathways. However, it is possible that the protective effects might also be attributed to stimulation of the AT₂ receptor, the well-characterized AT₁-counteracting receptor that expresses in the exocrine pancreas and AR42J cell line. From the results in Fig. 3A, blockade of the AT₂ receptor exerts no exaggerated effects in ROS generation compared with angiotensin II treatment only. Moreover, angiotensin II-induced ERK activation is not affected after blockade of AT₂ receptor by PD123319 (Supplemental Fig. 3), suggesting that the AT₂ receptor, despite its abundance in expression, is not involved in attenuation of redox-sensitive ERK-signaling. A previous study from our group actually showed that PD123319, administrated either prophylactically or therapeutically, exerts an insignificant effect on caerulein-induced AP (Tsang et al., 2004b).

It is worthwhile to note that the effective dose of angiotensin II used in the in vitro experiment is 1 μM, which is far higher than normal circulating levels. Previous studies have shown that basal intrapancreatic generation of angiotensin II in canine pancreas was substantially higher than that
detected in peripheral blood (Chappell et al., 1991). Moreover, local pancreatic RAS is subjected to up-regulation during AP; the expression of the precursor of angiotensin II, angiotensinogen, is significantly augmented by 3-fold during obstructive pancreatitis. On top of this, our previous report indicated that experimental pancreatitis results in a 6-fold increase in local pancreatic angiotensin-converting enzyme activity (Ip et al., 2003a). Increase in both angiotensinogen and angiotensin-converting enzyme activity will result in enhanced production of local angiotensin II levels. On the other hand, infiltration of inflammatory cells also could contribute to the local generation of angiotensin II during AP. It has been reported that neutrophil expresses cathepsin G on their membrane, which could actively convert angiotensinogen to angiotensin II (Tonnesen et al., 1982). Neutrophil could also promote angiotensin II generation via activation of prorenin (Dzau et al., 1987). On top of that, inflammatory cells themselves have their own intrinsic RAS, which could also enhance the pool of angiotensin II in the pancreas (Wintroub et al., 1981, 1984). Nevertheless, there are still no substantial data available to justify that a high amount of angiotensin II may exist within pancreatic tissues, particularly during the episode of AP.

It should be mentioned that pancreatic acinar cells are not the only cell population that contributes to the proinflammatory actions. Ample studies have shown the involvement of other pancreatic cells during the inflammatory process. It has been demonstrated that pancreatic ductal cells express profibrogenic proteins during obstructive pancreatitis (Fukumura et al., 2007). Immunoreactivity of IL-6 has been detected in acinar, pancreatic islet, and duct cells during human pancreatitis (Jablonowska et al., 2008). Experimental induction of AP leads to elevated expression of toll-like receptor 4 in pancreatic ductal epithelium, vascular endothelium, and islet, further complicating the inflammatory signals (Li et al., 2005). Taken these findings together, an intricate cross talk between pancreatic cells in regulating inflammatory response might exist. Further investigations are required to elucidate the role of nonacinar cell in cytokine expression during AP.

In conclusion, angiotensin II could generate superoxide radical in an AT₁ receptor-mediated manner in pancreatic acinar cells. Oxidative stress could lead to ERK1/2 activation and CREB phosphorylation and, thus, subsequent expression of IL-6, which would control the severity of AP. Blockade of AT₁ receptors represents a potential therapeutic approach to...
treating pancreatitis by targeting oxidative stress management and preventing systemic inflammation.

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References


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