Pentoxifylline Prevents Loss of PP2A Phosphatase Activity and Recruitment of Histone Acetyltransferases to Proinflammatory Genes in Acute Pancreatitis

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ABSTRACT
Mitogen-activated protein kinases (MAPKs) are considered major signal transducers early during the development of acute pancreatitis. Pentoxifylline is a phosphodiesterase inhibitor with marked anti-inflammatory properties through blockade of extracellular signal regulated kinase (ERK) phosphorylation and tumor necrosis factor α production. Our aim was to elucidate the mechanism of action of pentoxifylline as an anti-inflammatory agent in acute pancreatitis. Necrotizing pancreatitis induced by taurocholate in rats and taurocholate-treated AR42J acinar cells were studied. Phosphorylation of ERK and ERK kinase (MEK1/2), as well as PP2A, PP2B, and PP2C serine/threonine phosphatase activities, up-regulation of proinflammatory genes (by reverse transcription-polymerase chain reaction and chromatin immunoprecipitation), and recruitment of transcription factors and histone acetyltransferases/deacetylases to promoters of proinflammatory genes (egr-1, atf-3, inos, icam, il-6, and trf-α) were determined in the pancreas during pancreatitis. Pentoxifylline did not reduce MEK1/2 phosphorylation but prevented the marked loss of serine/threonine phosphatase PP2A activity induced by taurocholate in vivo without affecting PP2B and PP2C activities. The rapid loss in PP2A activity induced by taurocholate in acinar cells was due to a decrease in cAMP levels that was prevented by pentoxifylline. Pentoxifylline also reduced the induction of early (egr-1, atf-3) responsive genes and abrogated the up-regulation of late (inos, icam, il-6, trf-α) responsive genes and recruitment of transcription factors (nuclear factor κB and C/EBPβ) and histone acetyltransferases to their gene promoters during pancreatitis. In conclusion, the beneficial effects of pentoxifylline—and presumably of other phosphodiesterase inhibitors—in this disease seem to be mediated by abrogating the loss of cAMP levels and PP2A activity as well as chromatin-modifying complexes very early during acute pancreatitis.

Acute pancreatitis begins with a local inflammation of the pancreatic tissue that, in the severe forms, leads to a systemic inflammatory response, and eventually 20 to 30% of cases result in death due to multiple organ failure (Bhatia et al., 2001). The early events and signaling mechanisms that occur in the pancreas, and in particular, in acinar cells, are considered of special pathophysiological relevance because they may be translated into long-term inflammatory responses that would determine the development of pancreatitis (Ji et al., 2003). The early inflammatory cascade in acute pancreatitis should be ascribed not only to the inflammatory infiltrate but also to acinar cells, which may behave as inflammatory cells (de Dios et al., 2005). Indeed, they respond to, produce, and release cytokines, chemokines, and adhesion molecules (Grady et al., 1997; Gukovskaya et al., 1997; Zaninovic et al., 2000; Ramudo et al., 2005; de Dios et al., 2006).

Numerous inflammatory mediators such as activated pancreatic enzymes, cytokines, chemokines, free radicals, Ca²⁺, platelet-activating factor, adenosine, and neurogenic factors have been involved in the pathogenesis of acute pancreatitis...
(Denham et al., 1997; Grady et al., 1997; Bhatia et al., 1998; Satoh et al., 2000; Pereda et al., 2006). At present, elucidation of the signaling pathways involved in this inflammatory network is underway. Protein kinases, and especially mitogen-activated protein kinases (MAPKs), are considered major signal transducers early during the development of pancreatitis (Höfken et al., 2000). Activation of MAPK requires phosphorylation of threonine and tyrosine residues by upstream MAPK kinases, and strength and duration of MAPK activation may determine their biological effects (Marshall, 1995). Inhibition of p38 decreased pancreatic and pulmonary injury in severe acute pancreatitis in rats (Yang et al., 1999).

Pentoxifylline [3,7-dimethyl-1-(5-oxyhexyl)purine-2,6-dione] exhibits marked anti-inflammatory properties mediated mainly by inhibition of tumor necrosis factor α (TNF-α) production and by prevention of ERK phosphorylation and NF-κB activation (Schandene, 1992; Haddad et al., 2002a,b; Pereda et al., 2004). Accordingly, in knockout mice deficient in TNF-α receptors, the rate of mortality due to necrotizing acute pancreatitis decreased because the systemic response was restrained (Denham et al., 1997). Simultaneous blockade of the three major MAPKs—that is, extracellular signal regulated kinase (ERK), c-jun N-terminal kinase (JNK), and p38 kinase—by pentoxifylline and oxy- purinol greatly reduced the local and systemic inflammatory response in necrotizing acute pancreatitis and decreased the mortality rate (Pereda et al., 2004). Pentoxifylline also shows beneficial effects in edematous pancreatitis (Gómez-Cambronero et al., 2000; Pereda et al., 2004; de Campos et al., 2008). However, the precise mechanism of action of pentoxifylline responsible for these positive effects is not yet elucidated.

Pentoxifylline is a well known phosphodiesterase inhibitor (Meskini et al., 1994) and this effect may be involved in its anti-inflammatory action (Haddad et al., 2002b). The beneficial effects of phosphodiesterase inhibitors are associated with reduced secretion of proinflammatory cytokines, adherence of leukocytes to endothelium, and inhibition of leukocyte activation (Klemm et al., 1995; Sekut et al., 1995). Rolipram, a phosphodiesterase IV-specific inhibitor, inhibits lymphocyte-activated IL-6 production (Haddad et al., 2002a) and ameliorates cerulein-induced acute pancreatitis (Sato et al., 2006). cAMP might affect MAPK activation by acting on phosphatase activities, such as serine threonine phosphatase PP2A (Feschenko et al., 2002). Indeed, inactivation of MAPK by dephosphorylation is critical and may be triggered by serine threonine (Ser/Thr) phosphatases.

The aim of the present study was to investigate the mechanism of action of pentoxifylline as anti-inflammatory agent and potential therapy in acute pancreatitis, in particular, to explain its effects on MAPK activation and TNF-α production.

**Materials and Methods**

**Animals.** Young male Wistar rats were used in the experiments. Animals were cared for and handled in accordance with the Declaration of Helsinki and the European regulations (Council Directive 86/609/EEC), and the studies were approved by the Research Committee of the University of Valencia. Animals were anesthetized with intraperitoneal administration of ketamine (80 mg/kg b.w.t.) and acepromazine (2.5 mg/kg b.w.t.) before sacrifice.

**Experimental Model of Acute Pancreatitis and Treatment with Pentoxifylline.** Acute necrotizing pancreatitis was induced in rats by retrograde infusion into the biliopancreatic duct of sodium taurocholate (3.5%) (Sigma-Aldrich, St. Louis, MO) (Pereda et al., 2004). Lipase activity was measured and histological studies were performed to confirm the appropriate induction of necrotizing pancreatitis. Lipase activity in plasma was 47 ± 19 IU/liter (n = 6) in controls and it increased markedly at 6 h after pancreatitis induction (1280 ± 230 IU/liter; n = 6) as well as at 6 h in rats with pancreatitis treated with pentoxifylline (945 ± 310 IU/liter; n = 6). Rats were anesthetized as previously mentioned before induction of pancreatitis and before sacrifice at 0, 30 min, 1, 3, and 6 h after the infusion of taurocholate. Where indicated, rats were treated with pentoxifylline (12 mg/kg b.w.t.) just after taurocholate treatment. Pentoxifylline (12 mg/kg b.w.t.) was infused into the femoral vein (0.066 ml/min) for 30 min, as in Pereda et al. (2004).

**Culture of AR42J Acinar Cells.** The AR42J cell line, derived from an exocrine rat pancreatic tumor (CRL 1492; American Type Culture Collection, Manassas, VA), was grown in Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA) containing 25 mM glucose, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 25 μg/ml fungizone, supplemented with 10% fetal bovine serum.

**Measurement of Serine/Threonine Phosphatase Activities.** Pancreata were removed from control rats and rats with acute pancreatitis at 1 h after induction. Pancreatic tissue was homogenized in 50 mM Tris-HCl, pH 7.4, containing 150 mM NaCl, 5 mM dithiothreitol, 2 mM EDTA, 0.1% Triton X-100, and the following protease inhibitor cocktail (Sigma-Aldrich): 1.5 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, 1.2 μM aprotinin, 60 μM bestatin, 21 μM E-64, 30 μM leupeptin, 15 μM pepstatin A. Samples were centrifuged for 1 h at 100,000 g at 4°C. Supernatants were collected and subjected to exclusion chromatography on Sephadex G-25 columns to eliminate free phosphates. Eluates were used to measure the activities of PP2A, PP2B (calcineurin), and PP2C serine/threonine phosphatases using a serine/threonine phosphopeptide and the Serine/Threonine Phosphatase Assay System provided by Promega and following the manufacturer instructions.

**Measurement of cAMP Levels.** cAMP levels were measured in AR42J cells using the cyclic AMP EIK kit (Cayman Chemical Co., Ann Arbor, MD) following the instructions of the manufacturer.

**Western Blotting.** Pancreatic specimens were frozen at −80°C until they were homogenized in extraction buffer (100 mg/ml) on ice. The extraction buffer contained 10 mM Tris-HCl, pH 7.5, 0.25 M sucrose, 5 mM EDTA, 50 mM NaCl, 30 mM sodium pyrophosphate, 50 mM sodium fluoride, 100 μM sodium orthovanadate, and the previously mentioned protease inhibitor cocktail (Sigma-Aldrich). Phospho ERK 1/2 (p42/p44) and phospho-MEK1/2 were determined by Western blotting and chemiluminescence detection by use of the Phototope-HRP Detection kit (Cell Signaling Technology, Danvers, MA). The following antibodies were used: phospho-p42/p44 MAPK (ERK1/2)(Tyr 202/Tyr 204) and phospho-MEK1/2 (Ser217/221) antibodies (Cell Signaling Technology).

**Chromatin Immunoprecipitation (ChIP) Assay and RNApol ChIP.** Rats were sacrificed at the indicated times after taurocholate treatment and their pancreas were excised and immersed in 1% formaldehyde during 10 min at room temperature to cross-link the chromatin. ChIP and RNApol ChIP procedures were performed as described previously (Sandoval et al., 2004).

The following antibodies were used against AP-1 (sc-1694), ATF2 (sc-187), ATF3 (sc-188), CBP (sc-369), C/EBPβ (sc-150), SP1 (sc-59), EGR1 (sc-110), α-HD1 (sc-6298), NF-κB (sc-109), PCAP (sc-8999), and Sin3A (sc-994) from Santa Cruz Biotechnology (Santa Cruz, CA) for ChIP assay, and against RNA polymerase II (sc-899 from Santa Cruz Biotechnology) for RNApol ChIP.

**PCR Analysis of Immunoprecipitated Chromatin.** Input, IP, and NoAb fractions were analyzed by PCR with the appropriate primer pairs to amplify products corresponding to either the promoter or the coding regions of the target genes. Primer sequences
were as follows: *egr-1* (promoter): forward 5′-GTGAGAGGCTGCTGACTTC-3′ and reverse 5′-AGGCTCTGGAGTTCGACGC-3′; *tnf-α* (promoter): forward 5′-GGTCACTGGTCCTGACTTAC-3′ and reverse 5′-GCCACTTTCCCAGAATCCT-3′; *il-6* (promoter): forward 5′-CTGTTGCCTGGATAGGATT-3′ and reverse 5′-AGCGGCTCCATGACTCTCA-3′; *atf-3* (promoter): forward 5′-ACTGGACCACCGCTGTCAGG-3′ and reverse 5′-GGGTCACTGGTGTTTGAGGATT-3′; *inos* (coding region): forward 5′-GGTGCAGCTAA-3′ and reverse 5′-CCTGCTT- CAGCCTGGAGTTCCCAGC-3′; *egr-1* (coding region): forward 5′-CTCACCTGACCTGCAGTCC-3′ and reverse 5′-TGGGCCTAGCTGACACTG-3′; *il-6* (coding region): forward 5′-AGAGGCACCTCAGTGGCTGC-3′ and reverse 5′-AGGCTCCTGGAGTTCCCAGC-3′; *tnf* (coding region): forward 5′-CTCACCTGACCTGCAGTCC-3′ and reverse 5′-TGGGCCTAGCTGACACTG-3′; *inos* (coding region): forward 5′-ACTGGACCACCGCTGTCAGG-3′ and reverse 5′-GGGTCACTGGTGTTTGAGGATT-3′.

RNA Extraction and Determination of Steady-State Levels of mRNA. A small piece of the pancreas was excised and immediately immersed in 1 ml of RNA-later solution (Ambion, Austin, TX) to stabilize the RNA. Total RNA was isolated from pancreas and from AR42J cell cultures by the guanidinium thiocyanate method (Chomczynski and Sacchi, 1987). The isolated RNA was size-fractioned by electrophoresis (2×). The isolated RNA was then extracted as real-time RT-PCR control. The following specific primers that only a single product was amplified. 18S rRNA was also analyzed by agarose gel electrophoresis (2×). The isolated RNA was size-fractioned by electrophoresis (2×). The isolated RNA was then extracted as real-time RT-PCR control. The following specific primers that only a single product was amplified. 18S rRNA was also analyzed by agarose gel electrophoresis (2×). The isolated RNA was size-fractioned by electrophoresis (2×). The isolated RNA was then extracted as real-time RT-PCR control. The following specific primers that only a single product was amplified. 18S rRNA was also analyzed by agarose gel electrophoresis (2×). The isolated RNA was size-fractioned by electrophoresis (2×). The isolated RNA was then extracted as real-time RT-PCR control. The following specific primers that only a single product was amplified. 18S rRNA was also analyzed by agarose gel electrophoresis (2×).

**Statistical Analysis.** Results are expressed as mean ± S.D. with the number of experiments given in parentheses. Statistical analysis was performed in two steps. First, a one-way analysis of variance was carried out to identify the overall comparison of groups. Then, differences between individual groups were investigated by the Scheffé test. Differences were considered to be significant at p < 0.05.

**Results**

**Phosphorylation of ERK and MEK1/2 in Acute Pancreatitis. Effect of Pentoxifylline.** Phosphorylation of ERK and MEK1/2 was measured by Western blotting as an index of their activation in pancreas in the course of acute pancreatitis. Figure 1A and B, shows a rapid and intense phosphorylation of both ERK and MEK1/2 after induction of taurocholate-induced pancreatitis. Indeed, at 30 min after induction the phosphorylation of ERK and MEK1/2 was maximal and declined progressively thereafter, especially in the case of ERK.

In a previous work, we demonstrated that pentoxifylline diminished ERK phosphorylation in pancreas in taurocholate-induced pancreatitis (Pereda et al., 2004). To test whether this effect of pentoxifylline was mediated by inhibition of MEK1/2, the MAPK kinase mainly responsible for ERK phosphorylation, the effect of pentoxifylline on MEK1/2 phosphorylation was measured. Our results show that pentoxifylline does not prevent MEK1/2 phosphorylation at 1 h after induction of pancreatitis (Fig. 1C). The absence of pentoxifylline effect on MEK1/2 phosphorylation was also confirmed at 6 h after induction (Fig. 1C). Alternatively, and to explain the prevention of ERK phosphorylation by pentoxifylline,

**Fig. 1.** Phosphorylation of ERK (A) and MEK1/2 (B) in the course of acute pancreatitis. Effect of pentoxifylline (PTX) (C). Phospho-ERK1/2 and phospho-MEK1/2 were determined by Western blotting by use of tubulin as reference. Shown are representative Western blottings of four different experiments.
toxifylline, we decided to focus on serine/threonine phosphatase activities as a potential target for this anti-inflammatory agent.

**Serine/Threonine Phosphatase Activities in Acute Pancreatitis and in AR42J Acinar Cells. Effects of Pentoxifylline.** The activities of PP2A, calcineurin, and PP2C serine/threonine (Ser/Thr) phosphatases were measured in pancreas at 1 h after pancreatitis induction. Figure 2 shows that the activities of these three phosphatases were reduced by 50, 57, and 29%, respectively, at 1 h after induction. Pentoxifylline abrogated the loss in PP2A activity, but it did not prevent the decreases in PP2B (calcineurin) and PP2C. The activities of dual MAPK phosphatases (MKP1, MKP2, and MKP3) were also measured, and pentoxifylline showed no effect on these phosphatases (results not shown).

To elucidate the mechanism responsible for the loss of PP2A activity in taurocholate-induced pancreatitis and its prevention by pentoxifylline, this phosphatase activity was measured in AR42J acinar cells incubated with taurocholate. Figure 3A shows that in vitro taurocholate induced a rapid and transient decrease in PP2A activity in AR42J cells. This fall in PP2A activity was prevented by pentoxifylline or dibutyryl cAMP (see Fig. 3B). Therefore, the transient loss of PP2A activity seems to be mediated by cAMP. Accordingly, taurocholate caused a rapid decrease in cAMP levels that was abrogated by pentoxifylline (Fig. 3C). Furthermore, pentoxifylline also prevented the rapid ERK phosphorylation that occurred in AR42J cells incubated with taurocholate under the same conditions (Fig. 3D).

**Expression of Early Responsive Genes (egr-1 and atf-3) in Acute Pancreatitis. Effect of Pentoxifylline.** EGR-1 and ATF-3 are transcription factors induced rapidly in signaling pathways triggered by stress and cytokines (Ji et al., 2003). Our results show that egr-1 was rapidly and markedly up-regulated in pancreas—more than 20-fold—after pancreatitis induction (Figs. 4 and 5). Nevertheless, after pentoxifylline administration the induction of this gene at 1 h, although still significant, was only half the level of those in the untreated group (Fig. 5). Likewise, the rapid and marked up-regulation of atf-3 was also reduced by pentoxifylline (Fig. 5).

**Expression of Late Responsive Genes (inos, icam-1, il-6, and tnf-α) in Pancreas in Pancreatitis. Effect of Pentoxifylline.** Inducible nitric oxide synthase (inos), intercellular adhesion molecule 1 (icam-1), and interleukin 6 (il-6) were up-regulated in the course of taurocholate-induced pancreatitis, as demonstrated by binding of RNA polymerase II to their coding regions (Fig. 4), an index of actual transcriptional activity (Sandoval et al., 2004). The expression of inos, icam-1, and il-6 was measured by RT-PCR in pancreas in the course of acute pancreatitis at 0, 30 min, 1, 3, and 6 h. inos was up-regulated more than 3-fold at 1 h and later more than 6-fold at 3 and 6 h, whereas icam-1 was up-regulated more than 3-fold at 1 h after induction and maintained around this level thereafter (results not shown). Il-6 was already up-regulated 15-fold at 1 h after induction and later it was further induced (results not shown). Pentoxifylline reduced to a great extent the increase in inos expression and abrogated the up-regulation of both icam-1 and il-6 at 6 h after induction (Fig. 5). In accordance with previous studies (Gómez-Cambronero et al., 2000; Pereda et al., 2004), pentoxifylline also completely prevented the increase in tnf-α expression in the course of acute pancreatitis in the present study (results not shown).

**Recruitment of Transcription Factors, Histone Acetyltransferases, and Histone Deacetylases to the Promoters of Proinflammatory Genes. Effects of Pentoxifylline.** Epigenetic mechanisms that modulate chromatin structure, in particular, recruitment of transcription factors and histone acetylation, determine the up-regulation of proinflammatory genes. Figure 6A shows that egr-1 induction is mediated, at least in part, by binding of SP-1 factor to its promoter. At 6 h, binding of EGR-1 itself to its own
promoter might lead to negative feedback because egr-1 expression started to decrease at 6 h after induction, concomitantly with EGR-1 recruitment. Pentoxifylline clearly diminished the binding of SP-1 to the promoter of egr-1 at 1 h after induction.

Up-regulation of inos, icam and il-6 is mediated, at least in part, by NF-κB and C/EBP recruitment (Fig. 6, B–D). Pentoxifylline markedly reduced the recruitment of both NF-κB and C/EBP to the promoter of the three genes.

The level of histone acetylation is regulated by the opposite action of histone acetyltransferases and histone deacetylases, and their recruitment to gene promoters. In our experimental model of acute pancreatitis, the induction of proinflammatory genes was associated with recruitment of histone acetyltransferases to the gene promoters. Thus, up-regulation of icam-1 and tnf-α was associated with binding of CBP and PCAF, two major histone acetyltransferases, whereas egr-1 and inos induction was associated only with CBP recruitment (Fig. 7). Pentoxifylline increased binding of histone deacetylases to the promoters of icam-1 and inos, and in the case of egr-1, icam-1, and tnf-α it prevented the binding of histone acetyltransferases (Fig. 7). Consequently pentoxifylline reduced the level of acetylation in the histones located at the promoters of the proinflammatory genes leading to their repression.

Discussion

Inactivation of MAPK by dephosphorylation is critical and may occur through serine/threonine (Ser/Thr) phosphatases, dual-specificity (Thr/Tyr) phosphatases (MKPs), and protein tyrosine phosphatases. The role of protein phosphatases in acute pancreatitis seems to be complex. Calcineurin—or Ser/Thr phosphatase PP2B—mediates pancreatic zymogen activation in acinar cells (Husain et al., 2007). Inhibition of protein tyrosine phosphatases causes dissociation of cell contacts in pancreatic acini as a prerequisite for the development of pancreatic edema (Schnekenburger et al., 2005).
Furthermore, expression of the transmembrane protein tyrosine phosphatase CD45 decreases in the course of acute pancreatitis in parallel with the up-regulation of TNF-α (de Dios et al., 2006). Hence, CD45 seems to negatively control the production of cytokines. On the other hand, up-regulation of Thr/Tyr phosphatases (MKPs) and protein tyrosine phosphatases SHP-1 and SHP-2 is an early event during acute pancreatitis (Hoefken et al., 2000).

Our results point to Ser/Thr phosphatase PP2A as a key modulator of the inflammatory cascade through the ERK pathway and histone acetylation. It is known that PP2A may act as a negative regulator of MAPK signaling through the ERK pathway (Letourneux et al., 2006). On the other hand, histone acetylation is associated with chromatin-modifying complexes and gene activation (Shahbazian and Grunstein, 2007).

Under basal conditions there is a well-regulated balance between protein kinases and Ser/Thr phosphatases (Hunter, 1995). Hence, any loss of phosphatase activity either triggers activation of MAPK pathways or keeps them activated, leading to the uncontrolled inflammatory network. In acute pancreatitis we have found a rapid and marked decrease simultaneously in three of the major Ser/Thr phosphatase activities, that is, PP2A, calcineurin, and PP2C. Among
Furthermore, this process is relevant in the pathophysiology of acute pancreatitis because administration of a selective A1 agonist induces several features of the disease, such as leukocyte infiltration and interstitial edema in the pancreas and hyperamylasemia (Satoh et al., 2000). Based on our findings, activation of A1 receptors would lead to the decrease in PP2A contributing decisively to the inflammatory cascade and leukocyte infiltration in acute pancreatitis.

Nevertheless, it should be taken into account that the effects of extracellular adenosine on immune cells depend on the interplay of A1 and A2 receptors and on the concentration of adenosine (Sitkovsky et al., 2004). High-affinity A1 receptors inhibit adenyl cyclase, but A2 receptors activate it. Furthermore, extracellular adenosine exhibits anti-inflammatory effects acting through high-affinity A2A receptors (Lukashev et al., 2004; Sitkovsky and Ohta, 2005). Consequently, A1 receptors might be activated first at very low adenosine levels followed later on by the stimulation of immunosuppressive A2A receptors to trigger “OFF” signals in the immune response.

cAMP seems to play a key role in the inflammatory response in acute pancreatitis. Accordingly, phosphodiesterase inhibitors—such as rolipram or pentoxifylline—ameliorate taurocholate and cerulein-induced acute pancreatitis (Gómez-Cambronero et al., 2000; Pereda et al., 2004; Sato et al., 2006). The beneficial actions of phosphodiesterase inhibitors seem to be mediated by inhibition of leukocyte activation and infiltration decreasing secretion of TNF-α and IL-6 and adherence of leukocytes to the endothelium (Klemm et al., 1995; Sekut et al., 1995; Haddad et al., 2002a; Pereda et al., 2004). On one hand, cAMP presumably enhances the activity of cAMP-dependent protein kinase A and reduces the production of initiator proinflammatory cytokines TNF-α and IL-1β. On the other hand, and in accordance with our findings, maintenance of cAMP levels would avoid the loss of PP2A activity reducing the activation of ERK as well as the induction of proinflammatory cytokines. Regarding the early responsive gene egr-1, the reduction of its up-regulation mediated by pentoxifylline is also relevant in pancreatitis since the induction of MCP-1, IL-6, and ICAM-1 were diminished in EGR-1-deficient mice (Ji et al., 2003).

Chromatin remodelling during induction of proinflammatory genes depends on phosphorylation of transcription factors and subsequently their recruitment to gene promoters and histone acetylation. The latter mechanism opens the chromatin favoring anchorage of the transcriptional complex containing RNA polymerase II and gene up-regulation. Consequently, down-regulation of ERK phosphorylation eventually may abrogate recruitment of transcription factors and histone acetyltransferases, in favor of histone deacetylases that lead to deacetylation-mediated chromatin compaction. In acute pancreatitis, induction of proinflammatory genes such as inos, icam-1, and il-6 involves mainly recruitment of NF-κB and C/EBPβ, both subjected to activation by phosphorylation. Therefore, prevention of ERK phosphorylation with pentoxifylline abrogates recruitment of NF-κB, C/EBPβ, and histone acetyltransferases to the promoters together with release of histone deacetylases from them. All these mechanisms lead to a significant blockade of the inflammatory cascade.

These findings are in accordance with the well known role of NF-κB as an early key event sufficient to initiate the

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**Fig. 7.** Binding of histone deacetylases (mSin3A and HD1) and histone acetyltransferases (CBP and PCAF) to the promoters of egr-1 (A), inos (B), icam-1 (C), and tnf-α (D) in pancreas at 3 h after induction of necrotizing pancreatitis. Effect of pentoxifylline (PTX). No Ab, no antibody. These images are representative of four different experiments.
inflammatory response in acute pancreatitis (Gukovsky et al., 1998; Chen et al., 2002). In addition, our results support the hypothesis that the earliest events in the evolution of acute pancreatitis occur within acinar cells (Steer and Saluja, 1993) and that acinar cells behave as inflammatory cells (de Dios et al., 2005).

It is also noteworthy that the beneficial effects of pentoxifylline are mediated not only by inhibition of TNF-α up-regulation but also by blocking expression of two other relevant mediators of the inflammatory response such as icam-1 and il-6. Indeed, up-regulation of icam-1 mediates neutrophil adhesion to pancreatic acinar cells (Zaninovic et al., 2000) and IL-6 is considered a reliable marker of severity in acute pancreatitis. Consequently, pentoxifylline exhibits broad anti-inflammatory effects by keeping normal PP2A activity and it is not acting specifically on TNF-α production. In this regard it should be highlighted that pentoxifylline reduces the up-regulation of early responsive genes—such as egr-1—before blocking induction of late-responsive genes such as TNF-α.

Nevertheless, it should be emphasized that the anti-inflammatory action of pentoxifylline as a phosphodiesterase inhibitor in acute pancreatitis seems to be mediated, at least in part, by prevention of the rapid and presumably transient inflammatory action of pentoxifylline as a phosphodiesterase inhibitor. Up-regulation of proinflammatory cytokine (interleukin-6 and tumor necrosis factor-alpha) biosynthesis in alveolar epithelial cells. J Pharmacol Exp Ther 300:559–566.


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