Alcohol Activates Activator Protein-1 and Mitogen-Activated Protein Kinases in Rat Pancreatic Stellate Cells

ATSUSHI MASAMUNE, KAZUHIRO KIKUTA, MASASHIRO SATOH, AKIHIKO SATOH, and TOORU SHIMOSEGAWA
Division of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai, Japan

Received December 26, 2001; accepted March 11, 2002 This article is available online at http://jpet.aspetjournals.org

ABSTRACT
Alcohol is a major cause of both acute and chronic pancreatitis. Activated pancreatic stellate cells (PSCs) have recently been implicated in the pathogenesis of pancreatic inflammation and fibrosis. Herein, we examined the effect of ethanol and acetaldehyde on the activation of transcription factors and mitogen-activated protein (MAP) kinases in PSCs. PSCs were isolated from rat pancreas tissue and used in their culture-activated, myofibroblast-like phenotype. PSCs were treated with ethanol and acetaldehyde at clinically relevant concentrations (50 mM and 200 μM, respectively). Ethanol and acetaldehyde activated activator protein-1 but not nuclear factor-κB. In addition, they activated three classes of MAP kinases: extracellular signal-regulated kinase 1/2, c-Jun N-terminal kinase/stress-activated protein kinase, and p38 MAP kinase. Ethanol- and acetaldehyde-induced activation of activator protein-1 and MAP kinases was blocked by the antioxidant N-acetyl-cysteine, suggesting a role of oxidative stress in the signal transduction. Ethanol and acetaldehyde induced α1(I) procollagen gene expression but did not induce intercellular adhesion molecule-1 and monocyte chemoattractant protein-1. The acetaldehyde-induced increase of α1(I) procollagen gene expression was inhibited by the p38 MAP kinase inhibitor 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)imidazole (SB203580) but not by the MAP kinase inhibitor 2′-amino-3′-methoxyflavone (PD98059). Specific activation of these signal transduction pathways may play a role in the pathogenesis of alcohol-induced pancreatic injury.

Recently, star-shaped cells in the pancreas, namely pancreatic stellate cells (PSCs), have been identified and characterized (Apte et al., 1998a; Bachem et al., 1998). They are morphologically similar to the hepatic stellate cells that play a central role in the fibrogenesis of the liver (Friedman, 1993). In normal pancreas, stellate cells are quiescent and can be identified by the presence of vitamin A-containing lipid droplets in the cytoplasm. In response to pancreatic injury or inflammation, they are transformed (“activated”) from their quiescent phenotype into highly proliferative myofibroblast-like cells, which express the cytoskeletal protein α-smooth muscle actin (α-SMA), and produce type I collagen and other extracellular matrix components. PSCs are activated in response to cytokines such as transforming growth factor-β and platelet-derived growth factor, which are secreted during inflammation and tissue repair (Apte et al., 1998a; Bachem et al., 1998). Many of the morphological and metabolic changes associated with the activation of PSCs in animal models of fibrosis also occur when these cells are grown in culture on plastic. There is accumulating evidence that PSCs, like hepatic stellate cells, are responsible for the development of pancreatic fibrosis and inflammation (Wells and Crawford, 1998; Haber et al., 1999).

A relationship between alcohol and pancreatitis has been suggested for more than 50 years (Comfort et al., 1946; Apte et al., 1998b), and alcohol is now accepted as a major cause of both acute and chronic pancreatitis in industrialized nations worldwide (Apte et al., 1998b). It was reported that abundant vitamin A-storing cells were surrounded by collagen fibers in areas of fibrosis in the pancreas from human subjects with chronic pancreatitis, suggesting a role of PSCs in the alcohol-induced pancreatic fibrosis (Ikejiri, 1990). One mechanism relevant to alcohol-induced pancreatic fibrosis might be PSC activation by inflammatory mediators released during pancreatic injury. Recently, Apte et al. (2000) reported that both ethanol and acetaldehyde increased the synthesis of type I collagen in rat PSCs, suggesting a direct effect of alcohol on PSCs. To elucidate the underlying molecular mechanisms, we examined the effect of ethanol and acetaldehyde on the activation of transcription factors and mitogen-activated protein (MAP) kinases. Herein, we report that ethanol and ac-
ethanol at clinically relevant concentrations activated activator protein-1 (AP-1), but not nuclear factor-κB (NF-κB), in PSCs. Ethanol and acetaldehyde induced the activation of three classes of MAP kinases. In addition, acetaldehyde-induced expression of α1(I) procollagen gene was inhibited by the p38 MAP kinase inhibitor SB203580. Specific activation of these signal transduction pathways may play a direct role in the pathogenesis of alcohol-induced pancreatic injury.

**Experimental Procedures**

**Materials.** Poly(dI:dC)-poly(dI:dC), [α-32P]dCTP, and [γ-32P]ATP were purchased from Amersham Biosciences UK, Ltd. (Buckinghamshire, England). Recombinant human interleukin (IL)-1β was from Roche Applied Science (Mannheim, Germany). Rabbit antibodies against phosphospecific MAP kinases, pan-MAP kinases, and IκBα were purchased from Cell Technologies, Inc. (Beverly, MA). PD98059 and SB203580 were from Calbiochem (La Jolla, CA). All other reagents were from Sigma-Aldrich (St. Louis, MO) unless specifically described.

**Cell Culture.** Rat PSCs were prepared from the pancreas tissues of male Wistar rats (Japan SLC, Inc., Hamamatsu, Japan), weighing 200 to 250 g, as previously described using the Nycodenz solution (Nycoderm Pharma, Oslo, Norway) (Apte et al., 1998a). All animal procedures were performed in accordance with the National Institutes of Health Animal Care and Use Guidelines. Isolated stellate cells were cultured in Ham’s F-12 medium containing 10% heat-inactivated fetal bovine serum, penicillin sodium, and streptomycin sulfate. On the day of experimentation, cells were refed with serum-free medium, incubated for 6 h, and treated with IL-1β (10 ng/ml), ethanol (50 mM), or acetaldehyde (200 μM). In experiments involving N-acetyl-cysteine (NAC), PD98059, or SB203580, these reagents were added at 30 min before the addition of ethanol or acetaldehyde. All experiments were performed using cells between passages two and five.

**Nuclear Extract Preparation and Electrophoretic Mobility Shift Assay.** Nuclear extracts were prepared and electrophoretic mobility shift assay performed as previously described (Masamune et al., 1996). Double-stranded oligonucleotide probes for NF-κB (5′-AGTGGAGGGGACTTTCCGAGG-3′) or AP-1 (5′-CCTTGCAGGAGTCCTTCGATTCTCCAG-3′) were end-labeled with [γ-32P]ATP. Nuclear extracts (approximately 5 μg) were incubated with the labeled oligonucleotide probe for 20 min at 22°C and electrophoresed through a 4% polyacrylamide gel. Gels were dried and autoradiographed at −80°C overnight. A 100-fold excess of unlabeled oligonucleotide was incubated with nuclear extracts for 10 min before the addition of the radiolabeled probe in the competition experiments.

**Luciferase Assay.** Luciferase expression vectors containing two consensus AP-1 binding sites (TGACTCA) or two consensus NF-κB binding sites (GGGACTTTCC) were kindly provided by Dr. Naofumi Mukaida (Kanazawa University, Kanazawa, Japan). For the luciferase assay, approximately 1 × 10^6 PSCs were transfected with 2 μg of each luciferase expression vector, along with 40 ng of pRL-TK vector (Promega, Madison, WI) as an internal control, using the LipofectAMINE reagent (Invitrogen, Rockville, MD). After 24 h, the transfected cells were treated with ethanol or acetaldehyde for an additional 24 h. At the end of the incubation, cell lysates were prepared using a Pica Gene kit (Tayo Ink Co., Tokyo, Japan), and the light intensities were measured using a model Lumat LB9507 luminescence reader (EG&G Berthold, Bad Wildbad, Germany).

**Western Blotting.** The levels of activated, phosphorylated MAP kinases in the samples were determined by Western blotting using anti-phosphospecific MAP kinase antibodies [extracellular signal-regulated kinase (ERK)1/2, c-Jun N-terminal kinase/stress-activated protein kinase (JNK/ASAP), or p38 MAP kinase], as previously described (Masamune et al., 2002c). Briefly, cells were treated with ethanol or acetaldehyde and lysed in SDS. The samples were then sonicated, heated, and centrifuged to remove insoluble cell debris. Whole cell extracts (approximately 100 μg) were fractionated on a 10% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA). The membrane was incubated with anti-phosphospecific MAP kinase antibodies overnight. After incubation with the secondary antibody (goat anti-rabbit, hors eradish peroxidase conjugated), proteins were visualized by using the ECL kit (Amersham Biosciences UK, Ltd.). Levels of pan-MAP kinase and IκBα were examined in a similar manner.

**Northern Blotting.** Total RNA was isolated using an RNasea total RNA preparation kit (Qiagen, Valencia, CA). Ten micrograms of total RNA were separated on a 1% agarose-2.2 M formaldehyde gel and transferred to a nylon membrane filter (Amersham Biosciences UK, Ltd.). Blots were hybridized for 16 h at 42°C to the 32P-labeled DNA probes of c-jun, c-fos, or α1(I) procollagen generated by a polymerase chain reaction. Specific primer sets were as follows (listed 5′-3′, sense and antisense, respectively): c-jun, CCCTAAGATTCT- GAAGAGAG and TCTCGAGCATCAGGGCAG; c-fos, ACAG- GACCTTTCCGGCAGATC and AGGTACTGGGATTCTGGC; α1(I) procollagen, CCTGTCGTAGCCGGGAGAAAC and TCACACAGT- TCACAGGTT. Polymerase chain reactions were performed by 30 cycles at 94°C (for 1 min), at 55°C (for 1 min), and at 72°C (for 1 min). The identity of the products was confirmed by direct sequencing. After the hybridization, the filter was washed three times with 2× standard saline citrate (3 M NaCl, 0.3 M sodium citrate) and 0.1% SDS at 42°C for 10 min. The washed filter was subjected to autoradiography at −80°C for 3 days for c-jun and c-fos or for 2 h for α1(I) procollagen.

**Enzyme-Linked Immunosorbent Assay.** After the incubation for 24 h, culture supernatants were harvested and stored at −80°C until the measurement. Monocyte chemoattractant protein-1 (MCP-1) levels in the culture supernatants were measured by enzyme-linked immunosorbent assay (Endogen, Woburn, MA), according to the manufacturer’s instructions. Cell-surface expression of intercellular adhesion molecule-1 (ICAM-1) was determined by enzyme-linked immunosorbent assay, as previously reported (Masamune et al., 1999).

**Statistical Analysis.** Differences between experimental groups were evaluated by the two-tailed unpaired Student’s t test. A p value less than 0.05 was considered statistically significant.

**Results**

Ethanol and Acetaldehyde Increased AP-1, but not NF-κB, Binding Activity. It has been shown that PSCs are activated (express α-SMA) after approximately 48 h of culture on plastic (Apte et al., 1998a; Bachem et al., 1998). As mentioned earlier, all experiments used passage 3 stellate cells, which were considered activated. Indeed, the increased expression of α-SMA was confirmed by immunostaining (data not shown). We first examined the effects of ethanol and acetaldehyde on the activation of the transcription factors NF-κB and AP-1. These transcription factors are important regulators of gene expression in response to many stimuli, including proinflammatory cytokines and growth factors (Grilli et al., 1993; Karin et al., 1997). Culture-activated PSCs were incubated with ethanol or acetaldehyde at clinically relevant concentrations (50 mM and 200 μM, respectively) or IL-1β for 1 h. Nuclear extracts were prepared, and the specific binding activities of NF-κB and AP-1 were examined by electrophoretic mobility shift assay. Both ethanol and acetaldehyde, as well as IL-1β, increased the AP-1 binding activity (Fig. 1). The specificity of AP-1-specific DNA binding activity was demonstrated by the addition of a 100-fold molar excess of unlabeled AP-1 oligonucleotide, but not by unrelated NF-κB oligonucleotide, in competition assays (Fig. 1;
data not shown). In these experiments, the treatment did not affect the morphology or cell viability as assessed by a trypan blue exclusion test (data not shown). In contrast, NF-κB binding activity was induced by IL-1β but not by ethanol and acetaldehyde (Fig. 1). Octamer transcription factor-1 binding activity was not altered by the treatment (data not shown), suggesting that the effect of ethanol and acetaldehyde on AP-1 was specific.

Ethanol and Acetaldehyde Increased AP-1-Dependent, but not NF-κB-Dependent, Transcriptional Activity. To confirm that AP-1 activation observed by electrophoretic mobility shift assay was functional, the effects of ethanol and acetaldehyde on AP-1-dependent transcriptional activity was examined. PSCs were transiently transfected with AP-1- or NF-κB-luciferase gene reporter constructs and assayed for the luciferase activity. As shown in Fig. 2A, ethanol and acetaldehyde increased AP-1-dependent, but not NF-κB-dependent, luciferase activity. Phosphorylation and degradation of the inhibitory protein IkB-α and subsequent dissociation of this protein from NF-κB are thought to be necessary for the activation (Grilli et al., 1993). We also examined the effect on the degradation of IkB-α by Western blotting. IL-1β, but not ethanol and acetaldehyde, induced transient degradation of IkB-α, further supporting that ethanol and acetaldehyde did not activate NF-κB (Fig. 2B).

Ethanol and Acetaldehyde Activated MAP Kinases. Induction of the expression of AP-1 components c-Fos and c-Jun by a variety of stimuli such as growth factors and cytokines is mediated by the activation of three distinct MAP kinases: ERK1/2, JNK/SAPK, and p38 MAP kinase (Karin et al., 1997). The ability of ethanol and acetaldehyde to activate AP-1-dependent transcriptional activity predicted that they would activate transcription of their products (Karin et al., 1997). The levels of pan-MAP kinases were unaffected by the treatment, indicating that the lanes had been equally loaded.

Activation of AP-1 and MAP Kinases Was Inhibited by Antioxidant NAC. There is accumulating evidence of
oxidant stress in the pancreas after both short-term and long-term ethanol exposure in experimental animals (Al-
tomare et al., 1996; Norton et al., 1998). On the other hand,
activation of MAP kinases and AP-1 may be dependent on the
intracellular production of oxygen free radicals (Karin et al.,
1997; Robinson and Cobb, 1997). We examined whether the
activation of AP-1 and MAP kinases was mediated by reac-
tive oxygen species. We pretreated PSCs with an antioxidant,
NAC, and examined the effects on the activation of AP-1 and
MAP kinases. NAC has been shown to enter cells readily and
scavenge oxidants directly or indirectly by its conversion to
L-cysteine and by increasing intracellular glutathione
(Aruoma et al., 1989). NAC inhibited the inducible AP-1
binding activity and transcriptional activity (Fig. 5, A and B).
In addition, NAC inhibited the activation of MAP kinases
(Fig. 5C), suggesting a role of oxidative stress in the activa-
tion of these signal transduction pathways.

Acetaldehyde Induced Type I Collagen Gene Expression
through p38 MAP Kinase. It has been shown that activated
PSCs express α-SMA and produce type I collagen.
Indeed, α-SMA expression has been accepted as a marker of
PSC activation (Apte et al., 1998a), and in situ hybridization
techniques showed that α-SMA-positive cells were the prin-
cipal source of collagen in the fibrotic pancreas (Haber et al.,
1999). In addition, activated PSCs acquire the proinflamma-
tory phenotype; they may modulate the recruitment and
activation of inflammatory cells through the expression of
IL-8, MCP-1 (Andoh et al., 2000; Masamune et al., 2002a),
and ICAM-1 (Masamune et al., 2002b). In agreement with
the article of Apte et al. (2000), both ethanol and acetalde-
hyde increased the steady-state mRNA levels of α(I) procoll-
agen (Fig. 6A). In contrast, ethanol and acetaldehyde did not
increase ICAM-1 and MCP-1 expression (Fig. 6B). To clarify
the role of MAP kinases for the acetaldehyde-induced expres-
sion of α(I) procollagen gene, we used specific inhibitors to
block these pathways. The selective p38 MAP kinase inhibi-

Fig. 3. Ethanol and acetaldehyde induced activation of MAP kinases.
PSCs were treated with ethanol (50 mM) or acetaldehyde (200 μM) for the
indicated time. Total cell lysates were prepared, and the levels of acti-
vated, phosphorylated MAP kinases were determined by Western blot-
ting using anti-phosphospecific MAP kinase antibodies. Levels of pan-
MAP kinases were also determined to indicate that the lanes had been
equally loaded. Both ethanol and acetaldehyde activated three classes of
MAP kinases in a time-dependent manner with peak around 5 to 15
min.

Fig. 4. Alcohol increased c-jun and c-fos mRNA expression. PSCs were
-treated with ethanol (Et; 50 mM) or acetaldehyde (Ac; 200 μM) in serum-
free medium for 1 h. Total RNAs were extracted, and the levels of c-jun
and c-fos mRNAs were determined by Northern blotting. Con, control
(medium only).

Fig. 5. NAC inhibited the activation of MAP kinases and AP-1. A, PSCs
were transfected with the luciferase vector (2× AP-1) along with pRL-TK
vector as an internal control. After 24 h, the transfected cells were treated
with ethanol (Et; 50 mM) or acetaldehyde (Ac; 200 μM) in the absence or
presence of NAC (10 mM). After another 24-h-incubation, intracellular
luciferase activities were determined. The data represent mean values ±
S.D., calculated from three independent experiments as fold induction
compared with the activity observed in control (Con). B, PSCs were
treated with NAC (10 mM) for 30 min before the addition of ethanol or
acetaldehyde. After incubation for 10 min with ethanol (50 mM) or
acetaldehyde (200 μM), total cell lysates were prepared, and the levels of
activated, phosphorylated MAP kinases were determined by Western
blotting. **, p < 0.01 versus untreated control; *, p < 0.05 versus corresponding ethanol or acetaldehyde-treated controls; RLU, rel-
ative light units.
intravenous (Peters and Ward, 1988) administration of ethanol but not dissimilar to those reported during experimental ethanol administration to rats (Eriksson and Sippel, 1977). It is also possible that tissue concentrations of acetaldehyde are significantly higher than circulating levels of the compound, as has been shown in the liver of alcohol-fed rats (Eriksson and Sippel, 1977).

Because antioxidant NAC inhibited the alcohol-induced activation of MAP kinases and AP-1 in this study, reactive oxygen species may mediate the activation of these signal transduction pathways. This is in agreement with the idea that activation of MAP kinases and AP-1 is dependent on the intracellular production of oxygen free radicals (Karlin et al., 1997; Robinson and Cobb, 1997). Indeed, hydrogen peroxide activated MAP kinases and AP-1 in PSCs (K. Kikuta, unpublished observation). There is accumulating evidence of oxidant stress in the pancreas after both short-term and long-term ethanol exposure in experimental animals (Altomare et al., 1996; Norton et al., 1998). Such an oxidative stress has been observed in the absence of pancreatic necrosis or inflammation, suggesting that it is a primary, not secondary, event to ethanol-induced tissue injury. The development of oxidative stress within the pancreas during ethanol consumption may be related to increased free radical generation during ethanol metabolism and to compromised antioxidant defense mechanisms (Altomare et al., 1996). It is likely that PSCs are exposed to oxidative stress during ethanol consumption because PSCs, in addition to pancreatic acinar cells (Haber et al., 1998), have the capacity to metabolize ethanol to acetaldehyde via alcohol dehydrogenase-mediated oxidation of ethanol (Apte et al., 2000). Apte et al. (2000) showed that intracellular oxidant stress is an early occurrence in PSCs exposed to ethanol and acetaldehyde. They also showed that the alcohol-induced activation of PSCs and increased collagen synthesis were prevented by the antioxidant vitamin E, suggesting that these effects occurred by the oxidative stress that developed during ethanol consumption.

Activated PSCs acquire the proinflammatory phenotype; they may modulate the recruitment and activation of inflammatory cells. ICAM-1 and MCP-1 are important cell adhesion molecules of leukocytes and PSC interactions. We and others have reported that activated PSCs express MCP-1 (Andoh et al., 2000; Masamune et al., 2002a) and ICAM-1 (Masamune et al., 2002b) in response to IL-1B activators. Li and Karin (2000) have emphasized that transcription factor NF-kB in vitro. Indeed, MCP-1 expression by activated PSCs is shown to be increased in fibrous tissue sections from patients with chronic pancreatitis (Saurer et al., 2000). In this study, alcohol failed to induce NF-juB activation and the consequent expression of ICAM-1 and MCP-1. The failure of alcohol to induce ICAM-1 and MCP-1 expression is not surprising because the activation of NF-kB has been shown to play a central role in ICAM-1 and MCP-1 expression in PSCs (Andoh et al., 2000; Masamune et al., 2002b). This is in contrast to human endothelial cells in which the AP-1 proteins c-Fos and c-Jun directly induce expression of ICAM-1 and MCP-1 independently of the NF-juB pathway (Wang et al., 1999). Recent articles (Li and Karin, 1999; Bowie and O’Neill, 2000) have emphasized that a redox-dependent activation of NF-kB is cell- and stimulus-specific as opposed to the idea that oxidative stress is a common mediator of diverse NF-kB activators. Li and Karin (1999) reported that when a redox-regulated effect on NF-juB

Discussion

There is accumulating evidence that activated PSCs play a central role in the pathogenesis of pancreatic fibrosis and inflammation (Wells and Crawford, 1998; Haber et al., 1999). However, alcohol-induced signal transduction pathways in PSCs remain unknown. In this study, we have shown for the first time that ethanol and acetaldehyde at clinically relevant concentrations (50 mM and 200 mM, respectively) activated AP-1 but not NF-juB. This selective pattern of activation is distinct from that elicited by IL-1B and tumor necrosis factor-ju, which also activate NF-juB (Masamune et al., 2002b). In addition, ethanol and acetaldehyde activated three classes of MAP kinases (ERK1/2, JNK/SAPK, and p38 MAP kinase). Acetaldehyde-induced expression of α1(I) procollagen gene was inhibited by SB203580, a p38 MAP kinase inhibitor, suggesting a role of p38 MAP kinase in the collagen gene expression. The concentrations of ethanol and acetaldehyde used in this study were higher than the mean blood levels reported in alcoholics after oral (Korsten et al., 1987) or intravenous (Peters and Ward, 1988) administration of ethanol but not dissimilar to those reported during experimental ethanol administration to rats (Eriksson and Sippel, 1977). It is also possible that tissue concentrations of acetaldehyde are significantly higher than circulating levels of the compound, as has been shown in the liver of alcohol-fed rats (Eriksson and Sippel, 1977).

Because antioxidant NAC inhibited the alcohol-induced activation of MAP kinases and AP-1 in this study, reactive oxygen species may mediate the activation of these signal transduction pathways. This is in agreement with the idea that activation of MAP kinases and AP-1 is dependent on the intracellular production of oxygen free radicals (Karlin et al., 1997; Robinson and Cobb, 1997). Indeed, hydrogen peroxide activated MAP kinases and AP-1 in PSCs (K. Kikuta, unpublished observation). There is accumulating evidence of oxidant stress in the pancreas after both short-term and long-term ethanol exposure in experimental animals (Altomare et al., 1996; Norton et al., 1998). Such an oxidative stress has been observed in the absence of pancreatic necrosis or inflammation, suggesting that it is a primary, not secondary, event to ethanol-induced tissue injury. The development of oxidative stress within the pancreas during ethanol consumption may be related to increased free radical generation during ethanol metabolism and to compromised antioxidant defense mechanisms (Altomare et al., 1996). It is likely that PSCs are exposed to oxidative stress during ethanol consumption because PSCs, in addition to pancreatic acinar cells (Haber et al., 1998), have the capacity to metabolize ethanol to acetaldehyde via alcohol dehydrogenase-mediated oxidation of ethanol (Apte et al., 2000). Apte et al. (2000) showed that intracellular oxidant stress is an early occurrence in PSCs exposed to ethanol and acetaldehyde. They also showed that the alcohol-induced activation of PSCs and increased collagen synthesis were prevented by the antioxidant vitamin E, suggesting that these effects occurred by the oxidative stress that developed during ethanol consumption.

Activated PSCs acquire the proinflammatory phenotype; they may modulate the recruitment and activation of inflammatory cells. ICAM-1 and MCP-1 are important cell adhesion molecules of leukocytes and PSC interactions. We and others have reported that activated PSCs express MCP-1 (Andoh et al., 2000; Masamune et al., 2002a) and ICAM-1 (Masamune et al., 2002b) in response to IL-1B activators. Li and Karin (2000) have emphasized that transcription factor NF-kB in vitro. Indeed, MCP-1 expression by activated PSCs is shown to be increased in fibrous tissue sections from patients with chronic pancreatitis (Saurer et al., 2000). In this study, alcohol failed to induce NF-juB activation and the consequent expression of ICAM-1 and MCP-1. The failure of alcohol to induce ICAM-1 and MCP-1 expression is not surprising because the activation of NF-kB has been shown to play a central role in ICAM-1 and MCP-1 expression in PSCs (Andoh et al., 2000; Masamune et al., 2002b). This is in contrast to human endothelial cells in which the AP-1 proteins c-Fos and c-Jun directly induce expression of ICAM-1 and MCP-1 independently of the NF-juB pathway (Wang et al., 1999). Recent articles (Li and Karin, 1999; Bowie and O’Neill, 2000) have emphasized that a redox-dependent activation of NF-kB is cell- and stimulus-specific as opposed to the idea that oxidative stress is a common mediator of diverse NF-kB activators. Li and Karin (1999) reported that when a redox-regulated effect on NF-juB
is observed, it appears to occur downstream from the IκB kinase, at the level of ubiquitination and/or degradation of IκB. Lindros et al. (1999) reported that acetaldehyde protected against necrosis and inflammation in the liver of ethanol-fed rats through the decreased activation of NF-κB in the Kupffer cells. On the other hand, it has been shown that alcohol inhibits the expression of proinflammatory cytokines through the decreased activation of NF-κB in monocytes (Mandrekar et al., 1999), at least in part accounting for the immune dysfunction frequently observed in patients who abuse alcohol (Lieber, 1992).

Effects of alcohol on MAP kinases depend on the method (i.e., in vivo or in vitro) and duration of the exposure (i.e., acute or chronic) and on the cell type. For example, acute exposure to ethanol had no effect on either basal or serum-stimulated activity of MAP kinases in a normal mouse embryonic liver cell line (BNCL2), whereas chronic exposure to ethanol potentiated the serum-stimulated activity (Reddy and Shukla, 1996). In vascular smooth muscle cells, acute ethanol treatment reduced serum-stimulated ERK1/2 activity in a dose-dependent manner (Hendrickson et al., 1998). Treatment of rat hepatocytes in vitro for 16 h prolonged the activation of ERK 1/2 and p38 MAP kinase induced by various agonists (Chen et al., 1998). Such a treatment also increased basal JNK/SAPK activity but did not potentiate or prolong agonist-induced JNK/SAPK activation. In contrast, chronic ethanol consumption in vivo inhibited the activation of ERK1/2, p38 MAP kinase, and JNK/SAPK either by partial hepatectomy or by various agonist (Chen et al., 1998). Although little is known about the MAP kinase cascades in PSCs, MAP kinase pathways in hepatic stellate cells have been previously examined. Reeves et al. (2000) reported that constitutive activity of p38 MAP kinase was higher in transformed versus quiescent cells and that its inhibitor reduced activation of hepatic stellate cells in culture as assessed by α-SMA expression, suggesting a role of p38 MAP kinase in the activation of hepatic stellate cells. Whether similar mechanisms are responsible for the activation of PSCs remains to be determined.

Pancreatic fibrosis is an important morphological feature of alcohol-induced pancreatic injury, and activated PSCs are the principal source of collagen, mainly type I, during the pancreatic fibrosis. In agreement with the previous study (Apte et al., 2000), alcohol increased the steady-state mRNA levels of α1(I) procollagen in this study. This finding is particularly important in light of the generation of fibrosis in the pancreas during ethanol abuse. The up-regulation of α1(I) procollagen mRNA was inhibited by SB203580 but not by PD98059, suggesting a role of p38 MAP kinase pathway in acetaldehyde-induced α1(I) procollagen gene expression. The precise mechanism of type I collagen expression is unclear in PSCs. In hepatic stellate cells, there are several articles dealing with this topic. For example, serum stimulated α1(I) collagen gene expression via ERK and JNK/SAPK pathways through different regions of the 5′-upstream promoter sequence of the gene (Chen and Davis, 1999). Although the ERK-stimulatory signal was mapped to the most proximal nuclear factor-1 and specificity protein-1 (Sp-1) binding domains, a distal guanine cytosine (GC) box located at −1484 to −1476 base pairs played a central role in receiving extracellular signals through the JNK pathway (Chen and Davis, 1999). JNK/SAPK and AP-1 activation were also required for the ultraviolet- and acetaldehyde-induced increase in α1(I) collagen gene expression (Chen and Davis, 1999, 2000). The ultraviolet- and acetaldehyde-responsive elements were located in the distal GC box, and the GC box was bound by a DNA-binding protein termed basic transcription-binding protein. On the other hand, treatment of rat hepatic stellate cells with a 5-lipoxygenase-specific inhibitor reduced α1(I) procollagen mRNA transcript abundance, which suggested that leukotriene production might be involved in maintaining the activated cell’s high level of collagen production (Chen et al., 1996). Suppression of the gene transcription was localized to a nuclear factor-1 binding domain in the proximal promoter and an AP-2 binding domain adjacent to it. An increase in AP-2 binding adjacent to the nuclear factor-1 site was likely to be the transmodulator responsible for the suppression of the nuclear factor-1-dependent gene expression (Chen et al., 1996).

In summary, we have shown that ethanol and acetaldehyde induced the activation of MAP kinases and AP-1, but not NF-κB, in activated PSCs. This specific activation of signaling pathways may depend on the generation of reactive oxygen species and reflect gene activation pathways distinct from that used by proinflammatory cytokines. The signaling pathways in PSCs remain largely unknown and elucidation of the pathways would provide better understanding and rational approaches for the control of pancreatic inflammation and fibrosis targeting PSCs.

Acknowledgments

We thank Dr. Naofumi Mukaida for the luciferase vectors.

References


**Address correspondence to:** Dr. Atsushi Masamune, Division of Gastroenterology, Tohoku University Graduate School of Medicine, 1-1 Seiryo-cho, Aoba-ku, Sendai 980-8574 Japan. E-mail: amasamune@int3.med.tohoku.ac.jp