Chronic Pancreatitis: Evolving Paradigms

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Introduction

Chronic pancreatitis (CP) is a dynamic inflammatory process which is characterized by progressive fibrosis, pain and/or loss of exocrine and endocrine functions [1, 2]. Even though CP was first described in 1788 by Cawly [3], an understanding of its pathogenesis and biological behavior has eluded the researchers until recently. However, the recent identification and characterization of pancreatic stellate cells (PSCs) and unraveling of newer molecular mediators in pancreatic tissues has given us a better insight into the pathogenetic mechanisms leading to CP. The link between diverse etiologies and the common end point of parenchymal destruction and fibrosis is beginning to be unveiled. This has also led to the identification of newer therapeutic targets that will transcend the treatment of CP beyond pain control and enzyme supplementation.

This review looks into the recent developments in the pathogenesis of CP and the potential new therapeutic agents.

Pancreatic Stellate Cell Biology

In 1982 Watari et al. [4] reported the presence of vitamin A-containing cells in the vitamin A-fed rat pancreas. These were later described and characterized as stellate cells in rat and human pancreas [5–7]. PSCs are morphologically similar to hepatic stellate cells (HSCs) and are associated with fibrosis. Furthermore, molecular pathways involving mitogen-activated protein kinases, phosphatidylinositol 3-kinase, Ras superfamily G proteins, serine threonine protein kinase Raf-1 and peroxisome proliferator-activated receptor-γ (PPAR-γ) have been elucidated. Newer pathobiologic concepts concerning pain generation have also been put forward. Understanding the pathogenesis has led to the identification of novel molecular targets and the development of newer potential therapeutic agents.

Abstract

Chronic pancreatitis (CP) is characterized by progressive fibrosis, pain and/or loss of exocrine and endocrine functions. With the identification and characterization of pancreatic stellate cells (PSCs), the pathogenesis of CP and pancreatic fibrosis is now better understood. Molecular mediators shown to regulate the pathogenesis include transforming growth factor-β, platelet-derived growth factor, and proinflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor-α. Besides these, the roles of cyclooxygenase (COX)-2 and apoptosis-related proteins have also been implicated in the pathogenesis. Furthermore, molecular pathways involving mitogen-activated protein kinases, phosphatidylinositol 3-kinase, Ras superfamily G proteins, serine threonine protein kinase Raf-1 and peroxisome proliferator-activated receptor-γ (PPAR-γ) have been elucidated. Newer pathobiologic concepts concerning pain generation have also been put forward. Understanding the pathogenesis has led to the identification of novel molecular targets and the development of newer potential therapeutic agents. Those found to retard the progression of experimental CP and fibrosis in animal models include antioxidants, a Japanese herbal medicine called Saiko-keisi-to (TJ 10), the PPAR-γ ligand troglitazone, the protease inhibitor Camostat mesilate, and Lovastatin.

Key Words

Chronic pancreatitis · Pancreatic stellate cell · Transforming growth factor-β · Platelet-derived growth factor · Proinflammatory cytokines · Interleukin · Tumor necrosis factor-α

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Published online: July 13, 2006

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platelet-derived growth factor (PDGF) on the PSCs by TGF-β in acinar cells points to a possible paracrine stimulation of growth factor (TGF-β). Besides this, the positive staining for transforming growth factor (TGF-β) has also been demonstrated in the perifibrotic areas of the pancreas. mRNA for PDGF-β receptor and mRNA for PDGF-β in the ductal cells, fibroblasts and leukocytes of the pancreas in patients with CP [18]. Finally, the high concentration of 4-hydroxynonenal (a product of lipid peroxidation) in the areas of fibrosis suggests the role of oxidant stress in CP. In vitro studies using cultured PSCs have further qualified their role in the pathogenesis of CP. Apte et al. [8] have shown that oxidation of ethanol to acetaldehyde in the PSCs can directly activate these cells in the quiescent state without any pre-activation. This generates a state of oxidant stress within the PSCs which subsequently activates the downstream pathways of fibrogenesis. This finding implicates that in the human pancreas PSCs may be stimulated early during chronic alcohol intake even in the absence of necro-inflammation. PDGF has been shown to be a potent mitogen and chemoattractant for PSCs [12, 19], which explains the accumulation of PSCs in CP tissues (vide supra) found in vivo studies. Studies have also shown that TGF-β stimulates the synthesis and secretion of type-1 collagen, fibronectin, laminin and MMPs 2, 3 and 13 [10, 11, 13]. It has therefore been hypothesized that the destruction of normal collagen (matrix) by MMP facilitates pancreatic remodeling by deposition of fibrillar collagen, thereby promoting fibrosis. The proinflammatory cytokines TNF-α, IL-1 and IL-6 have also been shown to induce PSC activation as evidenced by proliferation of these cells, SMA-α expression and collagen synthesis. The cytokines IL-1 and TNF-α have been shown to stimulate MMP 1 secretion from human PSCs in a dose- and time-dependent manner [20]. PSC activation has also been induced experimentally by exposure to oxidant complexes like iron sulfate/ascorbic acid [21]. Besides being stimulated by cytokines, oxidant or growth factors via paracrine pathways, PSCs have also been shown to possess self-activating autocrine pathways mediated by TGF-β [21]. This probably partly explains the progression of CP even after cessation of the inciting acute event.

The role of PSCs in the pancreas in the quiescent stage is an aspect that needs to be elucidated. Since these cells secrete both MMPs and tissue inhibitors of MMPs, they may play a role in maintaining normal pancreatic anatomy. Another aspect that remains to be addressed is the fate of the active PSCs after producing pancreatic fibrosis; whether it reverts back to the quiescent phase. PSC activation by oxidant stress and cytokines has been convincingly shown by in vitro experiments. However, the genetic influence, thereby inter-individual variability, in PSC activation also needs to be studied.

Role of Apoptosis in the Pathogenesis of CP

Besides autocrine pathways, another factor that may result in persistence of inflammation in CP is the IL-15-induced inhibition of lymphocyte apoptosis, which subsequently activates PSCs via the cytokine network [22]. Acinar cell and probably islet cell destruction in CP has been shown to be the result of early onset apoptosis [23–27] mediated by the Fas/Fas-L pathways [27, 28] in both
animal models and human pancreas. Both in vivo and in vitro experiments have shown that there is early expression of two genes, namely pancreatitis-associated protein (PAP) [29] and P8 [30] by acinar cells in CP. These act as anti-apoptotic factors in acinar cell injury. Another earlier study by Su et al. [31] showed that PAP mRNA is expressed in CP tissue even before appearance of histological changes and that this may reflect an early stage of ischemic change. The expression of PAP mRNA is induced by the cytokine IL-6. Moreover, P8 expression is also associated with cell growth promotion, thereby implicating a regenerative role [31]. Therefore the balance between the pro-apoptotic and anti-apoptotic factors appears to be important in determining acinar cell death or survival and the severity of CP.

**Role of Cyclooxygenase-2 in the Pathogenesis of CP**

Immunohistochemical analysis has revealed the over-expression of the inducible enzyme cyclooxygenase-2 (COX-2) in CP tissues, mostly in the islets (96%) followed by the hyperplastic ductal cells (86%) and the atrophic acinar cells (80%) [32]. It has been shown that the normal pancreas continually and dominantly expresses COX-2 [33, 34] that can be upregulated by IL-1β [35, 36] subsequently leading to production of the inflammatory mediator prostaglandin E2 (PGE2). This upregulation results in inhibition of glucose-induced insulin secretion from β-cells [37]. COX-2 is also induced by PDGF and TGF-β [38]. This enzyme persists abundantly in the ductal systems even in advanced CP. Therefore, it can be hypothesized that COX-2 overexpression in CP can modulate the degree of pancreatic inflammation and contribute to the occurrence of both exocrine as well as endocrine insufficiency [38].

**Molecular Signaling Pathways in the Pathogenesis of CP**

A number of molecular pathways that modulate PSC pathobiology have recently been elucidated (fig. 1). Ethanol, acetaldehyde and oxidant stress activate PSCs via three mitogen-activated protein kinase pathways [39], namely extracellular signal kinase (ERK 1/2), p38 kinase and c-jun amino terminal kinase [40–42]. In addition, ethanol and acetaldehyde have also been shown to activate phosphatidylinositol 3-kinase and protein kinase C [43]. PDGF-induced proliferation and migration of PSCs are mediated by the ERK-1 and 2 [40, 43] and phosphatidylinositol 3-kinase [44] pathways, respectively. ERK activation on the other hand occurs via a signal transduction pathway that involves G-protein Ras and serine threonine protein kinase Raf-1 [45, 46]. The Ras superfamily G proteins undergo post-translational modification involving isoprenylation, a process that requires intermediates of the cholesterol biosynthesis [47, 48] which is regulated by HMG CoA reductase [49]. The paracrine pro-fibrogenic effect of TGF-β on PSCs is mediated via SMAD while the autocrine effect is mediated through the ERK pathway [50]. Recent studies have also implicated the role of peroxisome proliferator-activated receptor-γ (PPAR-γ) in the activation of PSCs [51, 52]. PPAR-γ is a member of the nuclear receptor family of transcription factors that controls growth, differentiation and inflammation.

**Pain in Chronic Pancreatitis**

Pain in CP has been as enigmatic as the disease itself. Its expression may range from a conspicuous absence to varying degrees of severity that may be episodic or persistent. The mechanism of pain in CP is multifactorial and may be mediated by a combination of factors. Reported factors include recurrent tissue inflammation and necrosis, pancreatic ductal hypertension [53] (which may be a result of parenchymal fibrosis or intraductal calcification), increased interstitial fluid pressure (compartment syndrome) [54–56], pancreatic ischemia [57, 58] and fibrotic encasement of sensory nerves.

However, recent studies have diverted the interest in pain generation of CP from mechanical to neurobiologic concepts. Proliferation of unmyelinated nerve fiber and perineural mononuclear infiltration are found in CP [59]. There is also an upregulation of pain mediators like substance P and calcitonin gene-related peptide in CP tissue [59] and nerve growth factor-dependent nociceptors [60]. Friess et al. [61] demonstrated that nerve growth factor is overexpressed in acinar cells and its high affinity receptor TrkA has been found in the perineurium of enlarged nerve fibers and blood vessels which may serve as a link between inflammation and hyperalgesia. Brain-derived neurotrophic factor, a neurotrophin ligand for TrkB receptors [62], has been shown to modulate inflammatory pain both peripherally and centrally in animal experiments [63–65]. Studies on human CP tissue have shown that the brain-derived neurotrophic factor level increases by 5.6-fold and correlates well with pain intensity and frequency [66]. Another protein called growth-associa-
ed protein-43, a neuroplasticity marker, was found to be highly expressed in human CP tissues and correlated well with pain scores [67]. Animal experiments have further revealed expression of proteinase-activated receptor-2 in sensory neurons [68, 69] and its direct activation by trypsin [70], thereby stimulating nociceptive neurons. Persistent noxious stimulation of the pancreas sensitizes the entire nociceptive system, in such a way that pain is produced even with a physiologic or minimal rise in pressure in the pancreatic ducts or parenchyma, a phenomenon called ‘mechanical allodynia’ [70]. Another phenomenon that may be operational is ‘inflammatory hyperalgesia’, in which minor flare-ups of inflammation in CP may stimulate the pancreatic nociceptors to react in an amplified response [70].

**Newer Treatment Modalities**

Traditionally, treatment of CP has been aimed at control of pain and replacement of the lost exocrine and endocrine functions. Conservative modalities include anal-
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Drug</th>
<th>Pancreatitis</th>
<th>Results</th>
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</table>
| Gomez et al. [75],   | Vitamin E             | Cerulein-induced pancreatitis in male Wistar rats | Reduced TGF-β levels  
Reduced pancreatic weight  
Reduced pancreatic hydroxyproline and plasma hyaluronic acid  
Reduction in myofibroblast number  
Improvement in fibrosis score |
| 2004                 |                       |                                       |                                                                          |
| Woo et al. [77],     | DA 9601               | Cerulein-induced CP in mice            | Reduction in pancreatic fibrosis  
Reduction in NFκB binding activity  
Reduction in myeloperoxidase and iNOS  
Reduced expression of α-SMA and type-1collagen (in vitro) |
| 2005                 |                       |                                       |                                                                          |
| Su et al. [31],      | Saiko-keisi-to (TJ-10)| Spontaneous CP in WBN/Kob and Wistar rats | Increased pancreatic weight at 16 weeks (p < 0.05)  
Reduced serum amylase at all ages  
Normal histology at 12 weeks and slight interstitial edema and inflammatory cell infiltrate at 16 weeks (p < 0.05)  
CP changes evident at 20 weeks in treated rats compared to 12 weeks in untreated ones  
Slight expression of PAP mRNA in inflamed tissue at 16 weeks in treated rats compared to full expression at 8 weeks in untreated ones |
| 1999                 |                       |                                       |                                                                          |
| Su et al. [89],      | Saiko-keisi-to (TJ-10)| Spontaneous CP in WBN/Kob rats        | Increase in pancreatic wet weight at 16 (p < 0.001) and 24 weeks (p < 0.01)  
No histologic changes in CP at 12 weeks and a significant reduction in CP histologic scores at 16 weeks (p < 0.001)  
Significant reduction in acinar degeneration at 20 weeks (p < 0.05)  
Detection of TGF-β mRNA from 12 weeks in treated rats compared to 4 weeks in untreated ones |
| 2001                 |                       |                                       |                                                                          |
| Van Westerloo et al. | Troglitazone          | Cerulein-induced CP in female C57BL/6 mice | Significant attenuation of markers of CP (p < 0.05)  
Significant reduction in myeloperoxidase concentration (p < 0.05)  
Partial prevention of reduction in intrapancreatic acinar cell/HPF in pancreatic tissue (p < 0.05)  
Complete prevention of rise in active TGF-β levels (p < 0.05) in pancreatic tissue |
| [95], 2005           |                       |                                       |                                                                          |
| Su et al. [103],     | Camostat              | Spontaneous CP in WBN/Kob rats        | Increased pancreatic wet weight at 8 weeks (p < 0.001)  
Significant reduction of pathologic scores (p < 0.001 to 0.05) at 16 weeks  
Significant reduction in mRNA expression of PAP, P8, IL-6 and TGF-β at 12 weeks (p < 0.05) |
| 2001                 |                       |                                       |                                                                          |
| Emori et al. [104],  | Camostat              | DDC-induced CP in male Wistar rats     | Reduction in pancreatic fibrotic area in treated rats (0.38 ± 0.18%) compared to untreated rats (4.79 ± 3.17%, p < 0.05)  
Reduction in ratio of α-SMA-positive cells to desmin-positive cells in treated rats (0.4 ± 0.2) compared to untreated rats (1.0 ± 0.3, p < 0.05)  
Reduction in prolyl hydroxylase in pancreas of treated rats (1.89 ± 0.73 ng/ml) compared to untreated rats (12.3 ± 5.3 ng/ml, p < 0.05) |
| 2005                 |                       |                                       |                                                                          |
| Jaster et al. [107], | Lovastatin            | In vitro study in cultured PSC isolated from pancreas of male LEW.1W inbred rats | Retraction in cytoplasmic extension and cell rounding after 24-hour exposure to 3 μM  
Dose-dependent increase in apoptotic cells (TUNEL assay) at 5 μM (p < 0.05)  
Significant reduction in DNA synthesis in a concentration range between 0.3 and 1.0 μM, with or without PDGF activation  
Dose-dependent prevention of the increase in α-SMA expression from PSC  
Increase in basal ERK 1 and 2 phosphorylation (PDGF independent)  
Reduction in the peak of phospho ERK-1 and 2 in response to PDGF stimulation |
| 2003                 |                       |                                       |                                                                          |

DDC = Diethyldithiocarbamate; TUNEL = TdT-mediated X-dUTP nick end labeling.  
Glossary to the experimental agents: DA 9601 = anti-inflammatory phytochemical; Siko-keishi-to = Japanese herbal drug; troglitazone = PPAR-γ ligand; camostat mesilate = serine protease inhibitor; lovastatin = HMG-CoA reductase inhibitor.
shown to reduce TGF-β and to improve fibrosis score. It has also shown a reduction in the number of myofibroblasts in animal models. Following agents have shown promise.

Newer developments in the understanding of the pathogenesis of CP have now shifted the treatment goal towards developing modalities that would prevent or even reverse the progression of fibrosis with early initiation. Table 1 summarizes the results of experiments using the potential therapeutic agents in experimental CP in animal models. Following agents have shown promise.

Antioxidants

Treatment of experimental CP in rats with vitamin E has shown a reduction in the number of myofibroblasts and an improvement in fibrosis score. It has also been shown to reduce TGF-β, pancreatic hydroxyproline and plasma hyaluronic acid levels besides reducing oxidative stress [75]. Vitamin A has also been shown to inhibit PSC activation in a study by McCarron et al. [76]. A novel antioxidant and anti-inflammatory phytochemical DA-9601 has been shown to decrease the levels of myeloperoxidase and inducible nitric oxide synthetase levels along with a reduction in nuclear factor κB-binding activities in cerulein-induced CP in a mouse model [77]. It also decreased expression of α-SMA and type-I collagen in cultured PSCs and reduced pancreatic fibrosis significantly. Cytoprotective proteins such as heat shock protein-70 and the metallothionein level also increased after treatment with DA-9601 [77]. These findings can probably explain the analgesic effect and reduction in the frequency of acute flares after the use of antioxidants (L-methionine, β-carotene, vitamin C, vitamin E, organic selenium) as found in earlier trials [78, 79].

Herbal Medicines

Saiko-keishi-to (TJ-10) is a Japanese herbal derivative with anti-inflammatory, analgesic, anti-emetic and immunomodulatory properties [80]. It is a mixture of extracts from nine medicinal herbs with different pharmacological actions [81–87] (table 2). Su et al. [31] have shown that in spontaneous CP in rats this compound suppresses PAP, which is likely to be through regulation of cytokines. They also demonstrated a histopathological delay in the development of CP. Similar findings were confirmed histopathologically and biologically by Motoo et al. [88]. TJ-10 has also been shown to suppress pancreatic fibrosis through suppression of oxidant stress-mediated expression of TGF-β [89]. Baicalein, one of the components of TJ-10, has a direct inhibitory effect on PSC activation [90]. These findings thus justify the use of this drug in human CP in Japanese medicine [91].

PPAR-γ Ligands

The most well-studied PPAR-γ ligand for CP is the thiazolidinedione derivative troglitazone. Its utility in CP was suggested by the findings that PPAR-γ ligands inhibited the inflammatory response [92] and reduced TGF-β production in hepatic stellate cells, aortic smooth muscles and the kidney [93, 94]. In a recent experiment on rat pancreatitis by van Westerloo et al. [95], troglitazone was indeed shown to reduce the levels of active TGF-β1 and the number of PSCs, thereby inhibiting inflammation, pancreatic damage and fibrosis. Shimuzu et al. [96] observed similar findings in an earlier study. The same authors also observed that troglitazone inhibited cell proliferation by blocking cell-cycle progression beyond the G1 phase by a PPAR-γ-independent mechanism [97].

Camostat Mesilate

This is an orally administered low molecular weight serine protease inhibitor that was found to increase pancreatic secretion and pancreatic weight in rats [98, 99]. This was thought to be mediated by cholecystokinin alone or with secretin through feedback control in both

Table 2. Composition and pharmacological action of different components of Saiko-keishi-to (TJ-10)

<table>
<thead>
<tr>
<th>SI.</th>
<th>Components</th>
<th>Pharmacological action</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Bupleurium root</td>
<td>Adjustment of T-lymphocyte function</td>
</tr>
<tr>
<td>2</td>
<td>Scutellera root</td>
<td>Interleukin 10 (IL-10) stimulation Anti-tumor action</td>
</tr>
<tr>
<td>3</td>
<td>Glycyrrhiza root</td>
<td>Interleukin 10 (IL-10) stimulation Anti-inflammatory action</td>
</tr>
<tr>
<td>4</td>
<td>Cinnamon bark</td>
<td>Inhibitory effect on bacterial endotoxin</td>
</tr>
<tr>
<td>5</td>
<td>Ginseng root</td>
<td>Nitric oxide scavenging action Protection from ischemic disease</td>
</tr>
</tbody>
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animals [100, 101] and humans [102]. It has further been shown that camostat mesilate suppresses gene expression of PAP, p8, IL-6 and TGF-β, thereby inhibiting pancreatic inflammation, PSC stimulation and the resulting fibrosis [103]. In another recent experiment, Emori et al. [104] have shown that camostat mesilate prevents oxidant injury-induced pancreatic fibrosis in a rat model.

Lovastatin

This is an HMG-CoA reductase inhibitor that has recently been found to have antiproliferative and pro-apoptotic activities, besides its long established cholesterol-lowering action [105, 106]. Lovastatin has been shown to suppress proliferation of fibroblast-like cells indirectly in in vitro experiments using rat PSCs. It blocks mevalonic acid, which is a precursor of lipid moieties required for isoprenylation of the Ras superfamily G proteins [107]. At the molecular level, it inhibits the PDGF-activated Raf-Ras ERK pathway [48]. It also interferes with the membrane translocation RhoA in response to mitogen (PDGF) stimulation that results in regulation of the actin cytoskeleton, adhesion and motility [108].

Others

A new carboxamide derivative IS-741 has been shown to bestow a beneficial effect on rat CP in vivo by inhibiting the induction of the cytokine-induced neutrophil chemoattractant and IL-6, PAP and p8. It also protected acinar cells from injury in vitro [109]. Other approaches that have been tried include oxypurinol, pentoxyfylline [110], TGF-β-neutralizing antibodies [111], and TNF-α antibody and receptor blocker [112].

Conclusion

Recent understanding of the molecular mechanisms leading to fibrosis in CP has led to identification of novel potential therapeutic targets to check the progression of the disease. At the same time, many new therapeutic agents have been shown to retard the progression of CP, justifying the existing practice of using antioxidants and the herbal medicine Saiko-keis-to (TJ-10). This has definitively paved the way for the evolution of a logical and scientific approach to the management of CP.

However, the beneficial results of these agents have only been demonstrated in experimental pancreatitis in animal models. Randomized controlled trials need to be conducted to reproduce these beneficial effects in human CP and study the dynamics of response in CP due to different etiologies.

References


