Published in final edited form as:
Pancreas. 2010 January ; 39(1): e24–e30. doi:10.1097/MPA.0b013e3181bc45e9.

Protection Against Chronic Pancreatitis and Pancreatic Fibrosis in Mice Over-Expressing Pancreatic Secretory Trypsin Inhibitor

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Abstract

Objectives—Mutations in the gene encoding for pancreatic secretory trypsin inhibitor (PSTI) can contribute to chronic pancreatitis. In the current study, we tested whether over-expression of pancreatic secretory trypsin inhibitor-I in mice protects against chronic pancreatitis and pancreatic fibrosis.

Methods—Rat PSTI-I expression was targeted to pancreatic acinar cells in transgenic mice. Chronic pancreatitis was achieved by intraperitoneal injection of caerulein for 10 weeks. Pancreatitis severity was assessed by histological grading of inflammatory infiltrate, atrophy, and fibrosis; quantitation of myeloperoxidase (MPO) activity; quantitative morphometric analysis of collagen content; and measurements of type I collagen, fibronectin, and TGFβ mRNA expression.

Results—Caerulein administration to nontransgenic mice produced histological evidence of inflammatory infiltrate, glandular atrophy, and parenchymal fibrosis, and increased collagen production, MPO activity, and collagen I and fibronectin mRNA levels. In caerulein-treated PSTI transgenic mice, there were significant reductions in inflammatory infiltrate, MPO activity, fibrosis, and collagen I and fibronectin mRNA levels. Transgenic mice treated with caerulein had significantly less collagen than nontransgenic mice.

Conclusions—The severity of chronic pancreatitis and pancreatic fibrosis is significantly reduced in mice expressing rat pancreatic secretory trypsin inhibitor-I. We propose that pancreatic trypsin inhibitors play a protective role in the pancreatic response to repeated injurious events.

Keywords

pancreas; caerulein; trypsin; collagen; serine protease inhibitor; Kazal type (SPINK)

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Introduction

Chronic pancreatitis is an irreversible process characterized by chronic inflammation and progressive fibrosis of the pancreas. Destruction of the gland leads to eventual loss of exocrine and endocrine function [1]. Several risk factors for the disease have been identified, including alcoholism, anatomic variation (e.g., pancreas divisum), hypertriglyceridemia, autoimmune disorders and hereditary factors [1, 2]. Chronic pancreatitis is characterized by activation of pancreatic stellate cells [3].

It is generally accepted that chronic pancreatitis derives from repeated episodes of acute pancreatitis [4]. Based on information obtained from hereditary pancreatitis studies, it has been demonstrated that premature activation of trypsin in the pancreas leads to acinar cell injury accompanied by necrosis [5, 6]. It is believed that trypsin activation triggers a localized inflammatory response mediated by pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, IL-8), the platelet activator PAF, and substance P. This response is then balanced by a systemic anti-inflammatory response acting through IL-10 and other anti-inflammatory cytokines [7]. During this process pancreatic stellate cells are activated by pro-inflammatory cytokines. Upon activation, they proliferate, migrate and produce α-smooth muscle actin (α-SMA) and extracellular matrix proteins such as collagen type I, fibronectin, and ICAM-1 [3, 8]. Thus repeated acute pancreatitis leads to chronic changes characterized by fibrosis [9]. In addition, pancreatic calcification, pancreatic ductal dilation and exocrine and endocrine insufficiency occur during late stages of chronic pancreatitis [10, 11].

Barriers exist to protect the pancreas from inappropriate activation of trypsinogen, as intrapancreatic activation of small quantities of this enzyme may occur under physiological conditions. Pancreatic secretory trypsin inhibitor (PSTI), found in the mammalian pancreas and pancreatic juice, is one such barrier [12, 13]. PSTI is a Kazal-type trypsin inhibitor that binds irreversibly to trypsin forming an inactive, stable complex. Studies have suggested that up to 20% of the potentially available trypsin activity in the pancreas may be inhibited by PSTI [14].

It has been hypothesized that impairment in the function of PSTI by genetic mutation may result in loss of protection from trypsin-induced autodigestion. Witt et al. found that 23% of children with chronic pancreatitis had mutations in the gene encoding the serine protease inhibitor Kazal type I *(SPINK1)*, a pancreatic secretory trypsin inhibitor [15]. Drenth et al. demonstrated that mutations in *SPINK1*, were found in over 12% of patients with alcoholic and idiopathic chronic pancreatitis [16]. More recently, Chandak et al. reported that the N34S mutation in the *SPINK1* gene was identified in 73% of patients with hereditary pancreatitis and 31% of patients with non-hereditary chronic pancreatitis [17]. Together, these studies suggest that PSTI plays an important role in protecting against chronic pancreatitis.

We have recently demonstrated that pancreas-specific expression of rat PSTI-I in a transgenic mouse model confers an increase in trypsin inhibitor capacity [18]. Furthermore, we found that the severity of caerulein-induced acute pancreatitis was significantly ameliorated in mice expressing PSTI-I, and pancreatic trypsin activity was significantly reduced. Based on these findings and the recent data demonstrating an association between *SPINK1* mutations and chronic pancreatitis, we designed the current study to determine if endogenous trypsin inhibitors play a protective role against chronic pancreatitis and pancreatic fibrosis.

In this study, we used a PSTI-I transgenic mouse model in which the rat PSTI-I gene had been targeted and expressed in the pancreas through the mouse elastase promoter [18]. We tested the hypothesis that pancreatic over-expression of rat PSTI-I in mice prevents secretagogue-induced chronic pancreatitis and pancreatic fibrosis. We demonstrate that mice over-expressing PSTI-I are protected from caerulein-induced chronic pancreatitis and pancreatic fibrosis. These

data suggest that endogenous pancreatic trypsin inhibitors may play a protective role in the pancreatic parenchymal response to repeated injury.

Methods

Animal protocol and experimental design

Mice were housed in climate-controlled rooms with a 12:12 hour light-dark cycle, and given water and chow ad libitum. The PSTI-expressing transgenic mouse with pancreas-specific expression of rat pancreatic secretory trypsin inhibitor-I was described elsewhere [18]. Male C57Bl/6-PSTI-I transgenic and nontransgenic mice were randomly assigned to receive either vehicle or the cholecystokinin analog caerulein (Bachem California Inc., Torrance, California, USA). All animal experiments were performed with approval of the Duke University Institutional Animal Care and Use Committee.

Caerulein-induced chronic pancreatitis

Caerulein was dissolved in 0.1 M $NaHCO_3$ followed by dilution in isotonic saline, and was administered by intraperitoneal injection every hour × 7 at a supramaximal stimulating dose of 50 μg/kg per injection twice weekly for ten weeks [19]. Control mice received injections of isotonic saline. After ten weeks of repeated injections, animals were euthanized, and the pancreata were quickly removed, rinsed in saline, blotted, and divided for histological grading, MPO quantification, RNA extraction, and determination of collagen content by Sirius red staining.

Histological grading

Pancreatic specimens were fixed overnight at room temperature in 10% neutral buffered formalin. The fixed tissue was embedded in paraffin, sectioned (5 μm sections) stained with hematoxylin and eosin, and coded for examination by a pathologist blinded to the experimental design. The pathologist graded the severity of pancreatitis using the scoring criteria shown in Table I, as modified from Van Laethem et al. [20]. The results were expressed as a score of 0 to 3 for the histological parameters of inflammatory infiltrate and atrophy. The fibrosis score was subdivided into scores for intralobular fibrosis, perilobular fibrosis, and interlobular fibrosis, each ranging from 0 to 3. Total histological score was the combined scores of inflammatory infiltrate, atrophy, and fibrosis.

Myeloperoxidase (MPO) assay

Portions of the pancreas were immediately frozen then prepared as previously described [21]. Assays were performed in microtiter plates using a Safire plate reader from Tecan-Austria GmbH with measurement wavelength of 450 nm and reference wavelength of 650 nm. Human MPO (Sigma, St Louis, MO) was used as standard. Protein concentrations of the pancreatic extract were determined using the micro bicinchoninic acid protein assay (Pierce). Results were expressed in mUnits/mg protein.

Real time PCR

RNA from pancreas were prepared as described [22]. Real time PCR was performed using Quantitect Reverse transcriptase (Qiagen) and Quantitest SYBR green protocols (Qiagen). Primer sets for the mouse transforming growth factor-β *(Tgfβ1)*, procollagen type 1, alpha *(Col1a1)*, *fibronectin (Fn1)*, smooth muscle alpha-actin *(Acta2)* and β-actin *(Actb)* genes were purchased from Qiagen. PCR reactions were performed in an Mx3000P cycler (Stratagene) and analyzed using the MxPro QPCR software (Stratagene).

The fold increase in mRNA expression was determined using the formula $2^{-\Delta\Delta Ct}$ where $\Delta\Delta C_t$ is the difference between the sample ΔC_t and the reference ΔC_t. The range given for the expression level of each gene is determined by evaluating the expression $2^{-\Delta\Delta Ct}$ with $\Delta\Delta C_t$ + SD and $\Delta\Delta C_t$ − SD, when SD is the standard variation of the $\Delta\Delta C_t$ value.

Histomorphometric analysis of pancreatic collagen content

Pancreatic specimens were fixed overnight at room temperature in 10% neutral buffered formalin. Sections (10 μm) were stained with 0.1% sirius red F3B in saturated picric acid (both from Sigma Chemical Co., St. Louis, Missouri, USA) as previously described [23]. Collagen surface density was quantified using a computerized image analysis system (Metavue, Universal Imaging Corp., Downingtown, Pennsylvania, USA). Variation in individual measurements was tested and determined to be less than 10%. The average of the score taken from 10 random fields was used to generate a single score for each animal's pancreas. Data were collected in a blinded fashion.

Statistical analysis

Results are expressed as mean ± standard error of mean (SEM). Statistical methods consisted of one-way analysis of variance with the Tukey post test (GraphPad Prism version 3.03, GraphPad Software Inc., San Diego, California, USA). Statistical significance was set at $p < 0.05$.

Results

Caerulein-induced chronic pancreatitis

Twice weekly administration of supramaximal stimulating doses of caerulein to nontransgenic mice for 10 weeks produced evidence of chronic pancreatitis as demonstrated histologically by extensive inflammatory infiltrate, glandular atrophy, and pancreatic fibrosis (Figure 1, compare panels A and B). The inflammatory infiltrate was characterized predominantly by lymphocytes, and the pancreatic fibrosis involved intralobular, perilobular, and interlobular components. All histological parameters of severity were significantly elevated in nontransgenic mice receiving caerulein compared to controls (Table II). The total chronic pancreatitis histological score in nontransgenic mice increased from 0.2 ± 0.1 to 8.6 ± 0.8 ($p < 0.001$; $n = 4$; Figure 2) after caerulein treatment.

Compared to nontransgenic mice, PSTI-I transgenic mice that received caerulein developed markedly less inflammatory infiltrate, glandular atrophy, and pancreatic fibrosis, as illustrated histologically (Figure 1, compare panels B and D). As shown in Table II, the inflammatory infiltrate and pancreatic fibrosis scores were reduced by 64% ($p < 0.05$) and 56% ($p < 0.01$), respectively, in PSTI-I transgenic mice. The atrophy score was reduced by 29%, however, this parameter failed to reach statistical significance. The total histological severity score of chronic pancreatitis was reduced by 50% in PSTI-I transgenic mice compared to nontransgenic mice ($p < 0.01$; Figure 2). Pancreatic MPO activity which is a measure of neutrophil infiltration was increased 3.9 fold in nontransgenic mice ($p < 0.05$). In contrast, there was no significant increase in pancreatic MPO activity in PSTI-1 transgenic mice treated with caerulein (Figure 3).

Pancreatic collagen content

To examine the relationship between caerulein-mediated pancreatic injury and fibrosis and to determine the significance of PSTI-I over-expression in this process, pancreatic collagen deposition was evaluated histologically and quantified morphometrically (Figures 4 and 5). Caerulein administration caused a marked increase in collagen expression in nontransgenic

mice. Collagen production was significantly less in PSTI-I transgenic mice than in non-transgenic mice; collagen content increased from 1.9 ± 0.1 % surface area to 20.8 ± 2.9 % following caerulein administration in nontransgenic mice ($p < 0.001$; $n = 4$). PSTI-I over-expression led to a 76% reduction in collagen content after long-term caerulein treatment to 5 ± 2.1 % area ($p < 0.001$). This level of collagen content in caerulein-treated transgenic mice was not significantly different from vehicle-treated nontransgenic mice.

Expression of fibrosis-related genes

Measurements by RT real-time PCR of mRNA levels for *Acta2, Col1a1, Fn1 and Tgfβ1* genes indicated that the level of gene expression of both *procollagen I* and *fibronectin 1* were increased 13- and 18-fold, respectively, in caerulein-treated non-transgenic mice (Table III). The level of expression for *TGF-β1* in the caerulein-treated nontransgenic pancreas was also elevated (6-fold), however, this change did not reach statistical significance. Conversely, these genes were not upregulated in PSTI-I mice treated with caerulein.

Discussion

The current study utilized a recently developed mouse model for overexpression of trypsin inhibitor within pancreatic acinar cells. This enabled us to determine if inhibition of endogenous pancreatic trypsin activity could affect the development of chronic pancreatitis and pancreatic fibrosis following repeated injury with caerulein hyperstimulation. In nontransgenic mice, repeated caerulein injections for 10 weeks produced histological evidence of chronic pancreatitis manifest by chronic inflammatory cell infiltration, elevated MPO activity, glandular atrophy, and parenchymal fibrosis with a substantial increase in *collagen I* and *fibronectin 1* expression and collagen staining. In contrast, in PSTI-I transgenic mice, there was either no increase in pancreatic MPO activity and fibrosis-related gene expression such as *collagen I* and *fibronectin 1* or reduction in other parameters of chronic pancreatitis.

PSTI is normally expressed in acinar cells where it is packaged with enzymes in zymogen granules [24]. It has the ability to bind irreversibly with trypsin to form an inactive complex [12, 13]. Even under physiological conditions, trypsinogen may become inadvertently activated and it is believed that endogenous trypsin inhibitors can bind trypsin and prevent further activation of trypsin and other zymogens. In this manner, endogenous trypsin inhibitors may protect the pancreas against spontaneous pancreatitis. It has been estimated that the amount of endogenous trypsin inhibitor present in the normal pancreas is sufficient to inactivate approximately 20% of the total amount of trypsin [25]. We have previously demonstrated that trypsin inhibitor capacity is 190% higher in PSTI-I transgenic mice compared to non-transgenic animals [18]. Although this may seem like a modest increase in trypsin inhibitor, it is sufficient to protect against acute pancreatitis, and suggests that the ratio of trypsin inhibitor to potentially activatable trypsin is finely regulated. In addition, PSTI-I overexpression in transgenic mice was shown to be sufficient to reduce trypsin activity when activated *in vitro* [18]. These findings are consistent with other studies demonstrating that exogenous trypsin inhibitors including PSTI analogs may reduce the severity of pancreatitis and even improve survival in experimental models of pancreatitis. Administration of the synthetic trypsin inhibitor gabexate was even shown to reduce the incidence of pancreatitis following endoscopic retrograde pancreatography in humans [26]. Further, the protease inhibitor camostat was shown to reduce pancreatic fibrosis [27]. Trypsin inhibitors, by directly reducing trypsin activity, are believed to prevent deleterious effects that result from widespread intrapancreatic enzyme activation that lead to pancreatitis. Induction of pancreatic fibrosis likely occurs through activation of pancreatic stellate cells [3]. However, it is unknown whether trypsin directly activates stellate cells or involves extrapancreatic inflammatory mediators.

Caerulein administered at supraphysiologic doses has been used as a reproducible model of both acute and chronic pancreatitis [28]. Within 10 minutes of supramaximal caerulein administration, trypsinogen is activated to trypsin [29]. Trypsin activation occurs within zymogen granules, and in this location would have the ability to autoactivate more trypsin. Previous data demonstrated that conversion of trypsinogen to trypsin as determined by measurement of trypsinogen activation peptide was not reduced in PSTI-I mice indicating that the protection conferred by PSTI-I in transgenic mice was due to inhibition of trypsin activity and not reduction of trypsinogen activation. However, it remains to be determined whether the primary damage from trypsin that results in pancreatitis comes from an intracellular or extracellular site of action [30].

Depending upon the duration of treatment, both acute and chronic pancreatitis can be produced by repeated administration of supramaximal doses of caerulein. This observation indicates that chronic pancreatitis can result from repeated episodes of acute pancreatitis. Considerable support for a similar mechanism contributing to human chronic pancreatitis came about with identification of the most common genetic defect in hereditary pancreatitis [31]. Patients with certain mutations of the cationic trypsinogen gene experience episodes of pancreatitis that are believed to be triggered when trypsin levels occurring through the spontaneous activation of trypsinogen exceed the inhibitory capacity of endogenous trypsin inhibitor. Repeated episodes of clinical acute pancreatitis ultimately result in chronic pancreatitis with all of the expected complications of chronic inflammation, pain, fibrosis, and pancreatic exocrine and endocrine insufficiency. In this regard, the use of caerulein to cause repeated injury seems to be a reasonable approach as a model to study pathophysiologic events in chronic pancreatitis.

Interestingly, we did not observe an increase in smooth muscle alpha-actin (α-SMA) mRNA expression during the fibrogenic response. On one hand, this is surprising since it is believed that myofibroblasts (which express α-SMA after injury, and proliferate) are an important source of extracellular matrix after injury. Since we found dramatic increases in collagen I and fibonectin, it follows that myofibroblasts would be prominent. On the other hand, it is clear that fibrogenic cells in a multitude of wound healing diseases are heterogenous or at different stages of gene expression, and that fibroblasts that express minimal α-SMA may be a prominent source of matrix proteins in the mouse pancreas under our experimental conditions [32, 33]. Furthermore, Perides et. al. found that when mice were subjected to caerulein alone, α-SMA expression was only slightly increased in the pancreas [34]. However, when caeruelin treatement was accompanied by ethanol in the diet, the increase in α-SMA expression was much larger. This raises the possibility that different injury signals stimulate different types of fibrogenic effector cells. In contrast, Van Westerloo et. al. were able to detect a significant increase in cells expressing α-SMA in a caerulein model of chronic pancreatitis using an immunostaining technique [35]. The discrepancy, however, could be attributed to variation of the protocols used by different laboratories.

Mutations in the serine protease inhibitor Kazal type 1 gene (*SPINK1*) which encodes human pancreatic secretory trypsin inhibitor have been associated with chronic pancreatitis. This mutation is believed to confer “loss of function” by reducing the protease inhibitory activity of the trypsin inhibitor. The most frequent *SPINK1* mutation associated with chronic pancreatitis is N34S [15]. It has been proposed that *SPINK1* mutations may predispose individuals to pancreatitis [36] rather than actually cause chronic pancreatitis [15]. Other mutations in *SPINK1* have been identified that would be expected to cause complete loss of the SPINK1 protein and, thereby, may be disease-causing mutations [37]. These associations indicate that alterations in endogenous trypsin inhibitor activity predispose individuals to pancreatitis and imply that endogenous trypsin inhibitor is necessary to limit protease activity within the pancreas under physiological conditions.

In the clinical setting, pancreatic injury often leads to pancreatitis, however, most often, these episodes are sporadic and no effective therapies have been developed that halt pancreatitis once it has been initiated. Nevertheless, a few causes of pancreatitis are predictable, such as following endoscopic retrograde chlolangiopancreatography (ERCP), surgical manipulation of the gland, or in patients with hereditary pancreatitis. In these circumstances, it is not unreasonable to envision therapies that may effectively prevent the disease or reduce its severity. The current experimental model suggests that limiting inflammation following repeated pancreatic injury may prevent pancreatic fibrosis. If similar findings are found in humans, it is possible that strategies to increase trypsin inhibitor activity within the pancreas could be useful treatments of human chronic pancreatitis.

Acknowledgments

This work was supported by NIH grants DK-38626, DK-073908 and DK-064213 (R.A.L.), DK-10106 (J.D.N.), DK-50574 (DCR), the Department of Veterans Affairs, and the Transgenic Mouse Facility Shared Resources of the Duke Comprehensive Cancer Center.

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Abbreviations

PSTI	Pancreatic secretory trypsin inhibitor

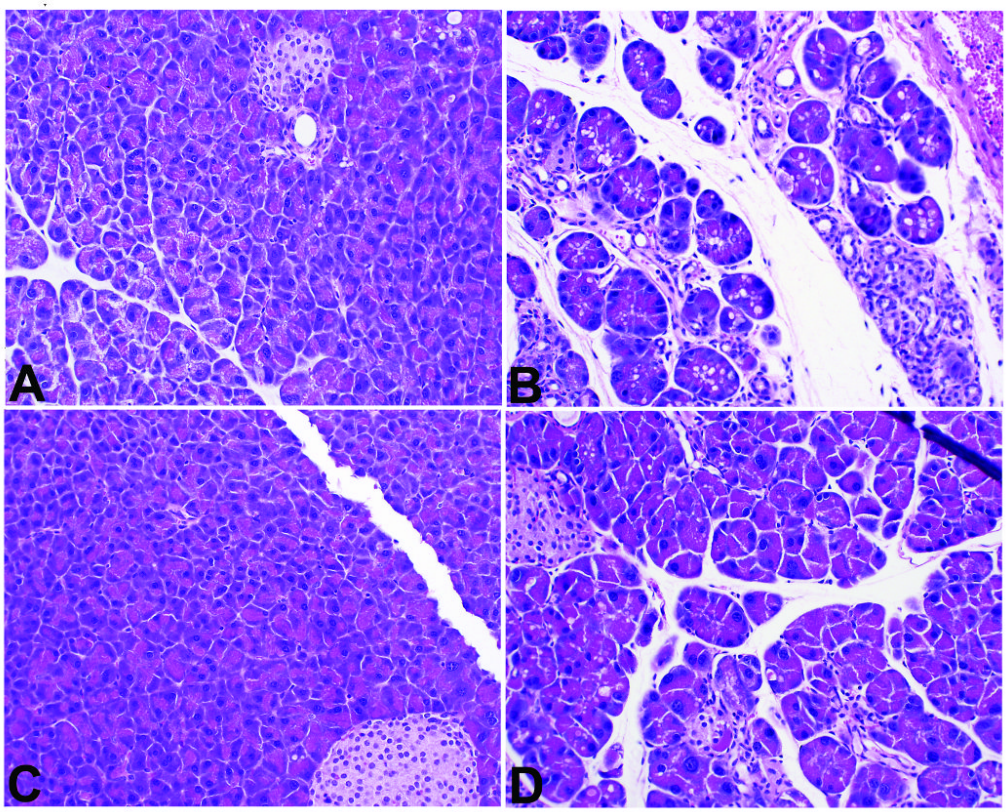

Figure 1. The effects of caerulein and PSTI-I over-expression on pancreatic histoarchitecture

Representative histological sections of mouse pancreas stained with hematoxylin and eosin from vehicle-treated nontransgenic (*A*), caerulein-treated nontransgenic (*B*), vehicle-treated PSTI-I transgenic (*C*), and caerulein-treated PSTI-I transgenic (*D*) mice. Caerulein administration caused inflammatory infiltration, atrophy, pancreatic fibrosis in nontransgenic mice. PSTI-I over-expression inhibited the effects of caerulein on pancreatic histoarchitecture. Magnification: × 250.

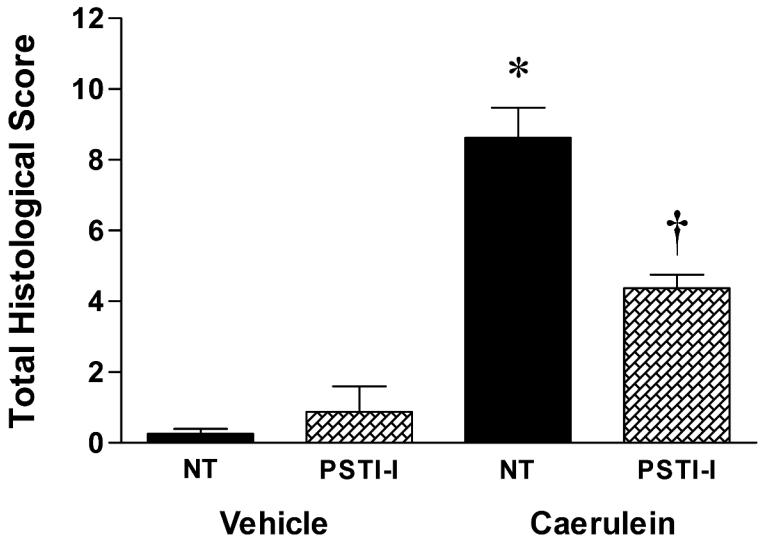

Figure 2. The effects of caerulein and PSTI-I over-expression on total histological score of chronic pancreatitis

Caerulein administration increased the total histological severity score of chronic pancreatitis in nontransgenic mice, and PSTI-I over-expression significantly inhibited this effect. Results are expressed as mean ± SEM (n = 4). *$p < 0.001$ vs. NT, vehicle; $^{\dagger}p < 0.01$ vs. NT, caerulein. NT, nontransgenic; PSTI-I, PSTI-I transgenic.

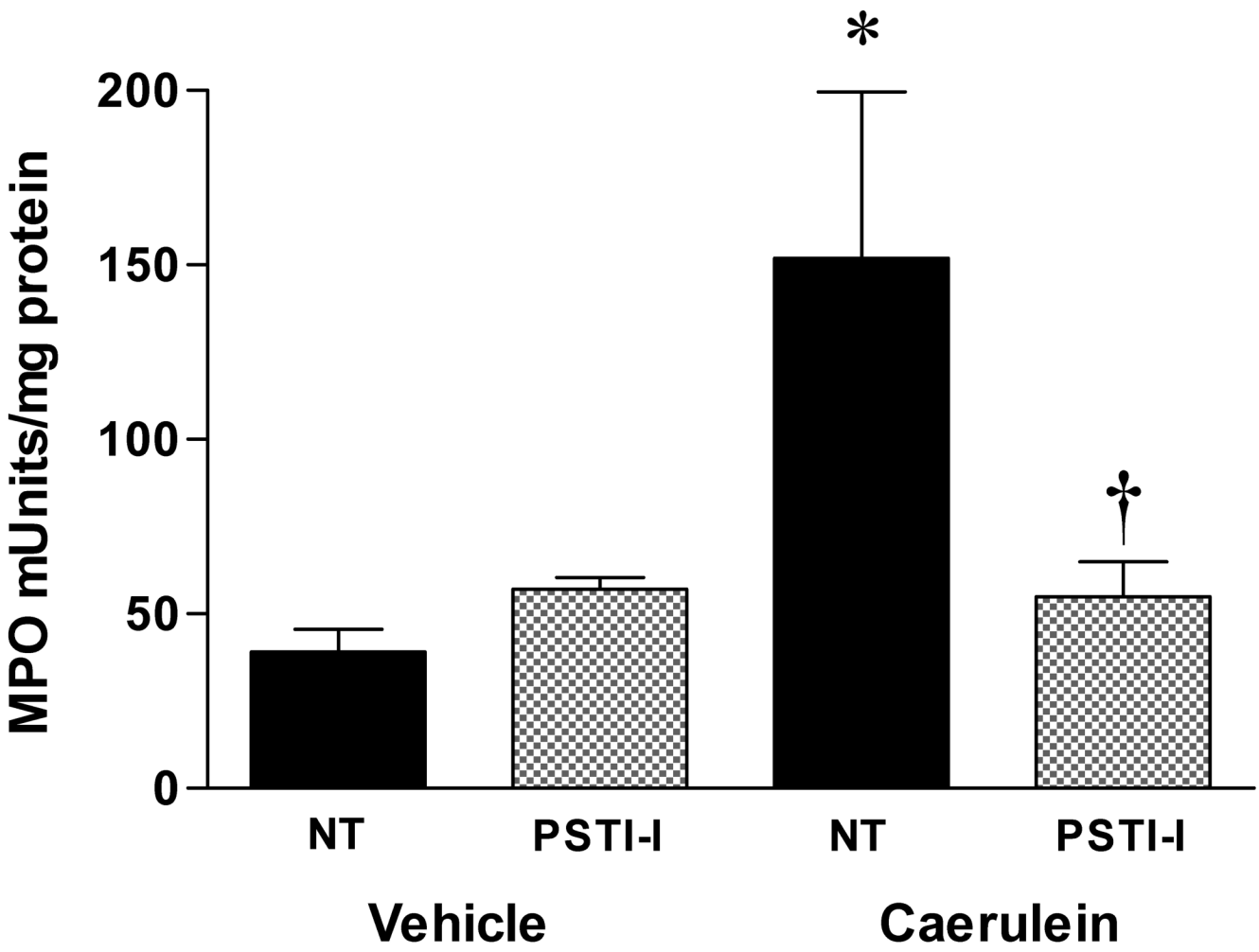

Figure 3. The effect of caerulein and PSTI-I overexpression on pancreatic myeloperoxidase (MPO) activity

Results are expressed as mean ± SEM (n=5-8), *$p < 0.05$ versus NT, vehicle.

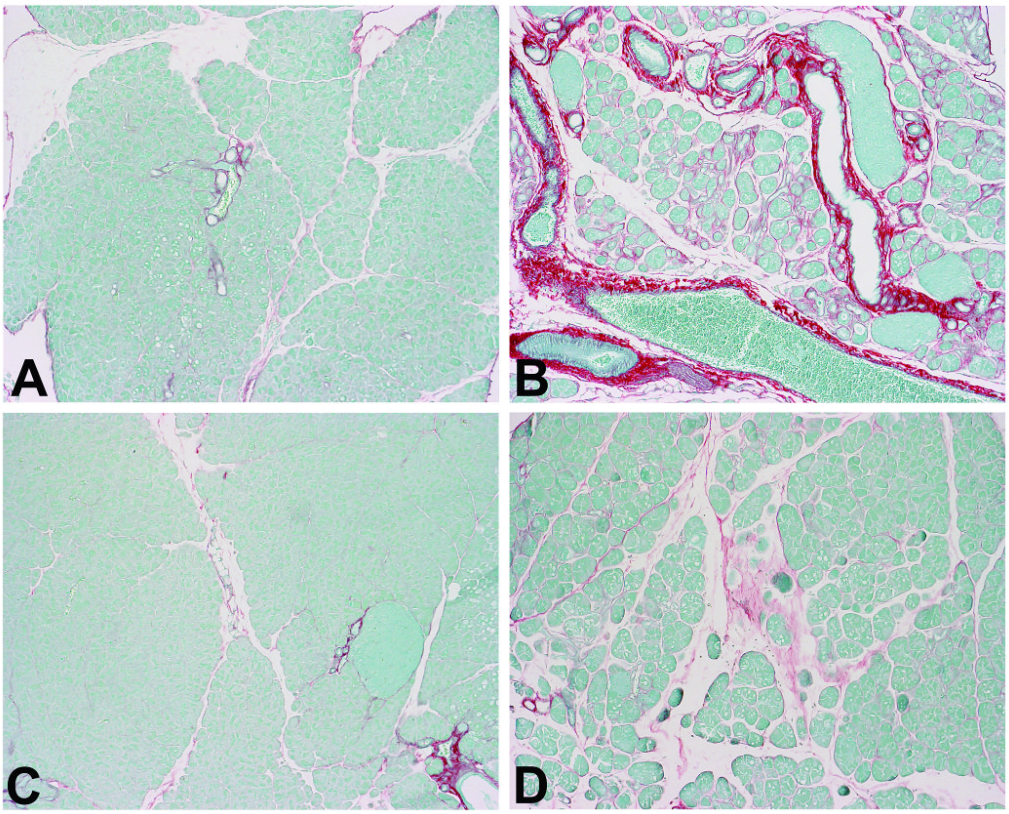

Figure 4. The effects of caerulein and PSTI-I over-expression on collagen production

Pancreatic injury was induced in nontransgenic and transgenic mice as in Figure 1. Mouse pancreas was fixed, sectioned, and stained with picrosirius red as in Methods. Representative sections from vehicle-treated nontransgenic (*A*), caerulein-treated nontransgenic (*B*), vehicle-treated PSTI-I transgenic (*C*), and caerulein-treated PSTI-I transgenic (*D*) mice are shown. Caerulein administration caused a marked increase in collagen content in nontransgenic mice. PSTI-I over-expression inhibited the effect of caerulein. Magnification: × 160.

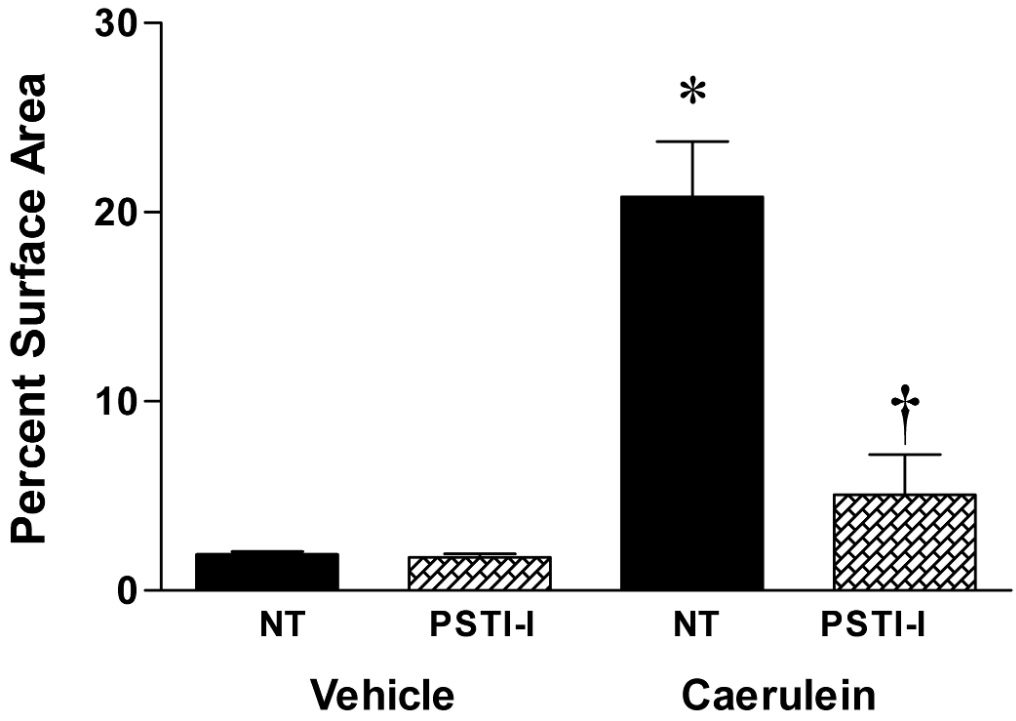

Figure 5. The effects of caerulein and PSTI-I over-expression on total collagen expression

Mice were subjected to repeated caerulein administration as in Figure 1, and pancreas sections were stained with sirius red and histomorphometric analysis was performed as in Methods. Aggregate data from each mouse pancreas is presented graphically ($n = 4$). *$p < 0.001$ vs. NT, vehicle; $^{\dagger}p < 0.001$ vs. NT, caerulein. NT, nontransgenic; PSTI-I, PSTI-I transgenic.

Table I
Histologic grading criteria for chronic pancreatitis

Histologic parameter	Possible score (increments of 0.5)
Inflammatory infiltrate	0 to 3 (none to profuse infiltration)
Atrophy	0 to 3 (none to extensive atrophy)
Fibrosis	
Intralobular	0 to 3 (none to extensive fibrosis)
Perilobular	0 to 3 (none to extensive fibrosis)
Interlobular	0 to 3 (none to extensive fibrosis)

Total histological score is combined scores of inflammatory infiltrate, atrophy, and fibrosis.

Table II
Effects of PSTI-I over-expression and caerulein on pancreatic histology

	Inflammatory Infiltrate	Atrophy	Fibrosis
Nontransgenic, vehicle	0.2 ± 0.1	0.00 ± 0.00	0.00 ± 0.00
PSTI-I transgenic, vehicle	0.00 ± 0.00	0.2 ± 0.2	0.6 ± 0.5
Nontransgenic, caerulein	1.8 ± 0.5[A]	2.6 ± 0.2[B]	4.2 ± 0.5[B]
PSTI-I transgenic, caerulein	0.6 ± 0.1[C]	1.9 ± 0.1	1.9 ± 0.4[D]

Results are expressed as mean ± SEM (n = 4). Histological parameters were scored by a pathologist blinded to the experimental design.

[A] $p < 0.01$ vs. nontransgenic, vehicle;

[B] $p < 0.001$ vs. nontransgenic, vehicle;

[C] $p < 0.05$ vs. nontransgenic, caerulein;

[D] $p < 0.01$ vs. nontransgenic, caerulein.

Table III

Gene expression of smooth muscle alpha-actin, collagen, fibronectin, and TGF-B1 in pancreas

	Acta 2	Collagen	Fibronectin	TGF-β1
Nontransgenic, vehicle	1 (0.2-6.0)	1 (0.3-3.0)	1 (0.4-2.2)	1 (0.3-3.5)
PSTI-I, vehicle	1.2 (0.6-2.4)	0.2 (0.1-0.3)	0.6 (0.2-2.0)	0.4 (0.1-2.1)
Nontransgenic, caerulein	2.6 (1.4-5.0)	12.8* (12.7-12.9)	17.5* (5.2-58.9)	5.8 (1.5-22.3)
PSTI-I, caerulein	2.5 (0.2-27.1)	0.5 (0.1-1.5)	1.2 (0.6-2.4)	0.2 (0.1-0.8)

The fold increases and ranges of mRNA expression are shown relative to the reference gene β-actin, (n=3).

* $p<0.05$ nontransgenic, caerulein vs PSTI-I, vehicle and caerulein.