BASIC AND TRANSLATIONAL—PANCREAS

Fibrosis Reduces Severity of Acute-on-Chronic Pancreatitis in Humans

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BACKGROUND & AIMS: Acute pancreatitis (AP) and chronic pancreatitis (CP) share etiologies, but AP can be more severe and is associated with a higher rate of mortality. We investigated features of CP that protect against severe disease. The amount of intrapancreatic fat (IPF) is increased in obese patients and fibrosis is increased in patients with CP, so we studied whether fibrosis or fat regulate severity of AP attacks in patients with CP. **METHODS:** We reviewed records from the University of Pittsburgh Medical Center/Presbyterian Hospital Autopsy Database (1998–2008) for patients with a diagnosis of AP (n = 23), CP (n = 35), or both (AP-on-CP; n = 15). Pancreatic histology samples from these patients and 50 randomly selected controls (no pancreatic disease) were analyzed, and IPF data were correlated with computed tomography data. An adipocyte and acinar cell Transwell coculture system, with or without collagen type I, was used to study the effects of fibrosis on acinar-adipocyte interactions. We studied the effects of nonesterified fatty acids (NEFAs) and adipokines on acinar cells in culture. **RESULTS:** Levels of IPF were significantly higher in nonobese patients with CP than in nonobese controls. In patients with CP or AP-on-CP, areas of IPF were surrounded by significantly more fibrosis than in controls or patients with AP. Fat necrosis–associated peri-fat acinar necrosis (PFAN, indicated by NEFA spillage) contributed to most of the necrosis observed in samples from patients with AP; however, findings of peri-fat acinar necrosis and total necrosis were significantly lower in samples from patients with CP or AP-on-CP. Fibrosis appeared to wall off the fat necrosis and limit peri-fat acinar necrosis, reducing acinar necrosis. In vitro, collagen I limited the lipolytic flux between acinar cells and adipocytes and prevented increases in adipokines in the acinar compartment. This was associated with reduced acinar cell necrosis. However, NEFAs, but not adipokines, caused acinar cell necrosis. **CONCLUSIONS: Based on analysis of pancreatic samples from patients with CP, AP, or AP-on-CP and in vitro studies, fibrosis reduces the severity of acute exacerbations of CP by reducing lipolytic flux between adipocytes and acinar cells.**

Keywords: Pancreas; Inflammation; Necrosis; Mechanism.

Several studies have shown that the risk of severe acute pancreatitis (SAP) and mortality increases in obese patients[1–5] (body mass index [BMI] $\geq$30 kg/m^2). Additionally, various studies[4,6,7] have shown that visceral fat depots (ie, intrapancreatic fat [IPF]) increase with body weight or BMI in children[8,9] and adults and that fat abutting acinar cells is involved in parenchymal damage in patients with acute pancreatitis (AP).[4,10,11] Fatty pancreas replacement may also occur in patients with chronic pancreatitis (CP)[12–15]; however, SAP is a rare outcome or cause of mortality in patients with CP.[16–18] Thus, the mechanisms of IPF differentially affecting the outcomes of patients with AP in the context of obesity or CP (ie, AP-on-CP) require further investigation.

AP and CP share some common etiologies such as alcohol consumption,[19] genetic mutations (eg, CFTR, PRSS1),[20–22] obstructive lesions (eg, a mass),[23–25] and metabolic causes (eg, hyperlipidemia).[26,27] Recent studies suggest AP, recurrent AP, and CP are part of a continuum.[18,19,28] However, although the first couple of attacks of AP may cause extensive pancreatic necrosis within weeks,[4,18] patients with CP have a prolonged disease course with recurrent attacks of AP and atrophy, extensive fibrosis, and fatty replacement developing over months to years. Evidence for large pancreatic areas necrotizing acutely in CP is scarce, despite the insult (eg, alcohol consumption, tumor, metabolic cause, or genetic cause) persisting over this time. Similarly, although AP may result in significant mortality over days,[29–31] mortality over the several years of the disease course of CP is rarely attributed to AP.[16–18]

The reasons for these different outcomes remain unclear. It has been variously argued that this may be due to a lower acinar cell mass in patients with CP, but studies in children

Abbreviations used in this paper: AP, acute pancreatitis; ATP, adenosine triphosphate; BMI, body mass index; CP, chronic pancreatitis; CT, computed tomography; FN, fat necrosis; IPF, intrapancreatic fat; NEFA, nonesterified fatty acid; PFAN, peri-fat acinar necrosis; PI, propidium iodide; SAP, severe acute pancreatitis; UFA, unsaturated fatty acid.

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and adolescents, who have smaller pancreata than adults older than 30 years of age[6] and lower serum amylase levels than adults[32] (which is relevant because lower serum enzyme levels in patients with CP are often attributed to lower acinar mass), experience no difference in severity compared with adults.[33–37] Rather, children with SAP have higher BMI[36] and children with a higher weight at admission are more prone to SAP.[35] Additionally, a large study of children treated for acute lymphoblastic leukemia mentioned that children "who had grade 3 or 4 pancreas/glucose toxicity during induction also had higher BMIs throughout treatment."[38] Pancreatic toxicities are based on criteria for pancreatitis. Grade 3 or 4 is severe compared with grade 1 or 2, according the Children's Cancer Group common toxicity criteria (version 2.0) used in the study (http://www.eortc.be/services/doc/ctc/ctcv20_4-30-992.pdf).

Therefore, other phenomena such as fibrosis, which increases in patients with CP,[39] may be considered to reduce the severity of AP-on-CP. We thus studied different pancreatic disease states (including controls, AP, CP, and AP-on-CP) for differences in the amount of IPF and its relationship to BMI, fibrosis, and the pancreatic parenchyma. Because the pancreas is not usually sampled in live humans without pancreatic disease and samples removed during an invasive procedure are small (eg, using fine-needle aspiration or a Tru-Cut biopsy) or altered by disease (eg, cancer removed during a Whipple procedure), we chose to examine autopsy tissue in control patients (ie, no evidence of pancreatic disease) and those with a clinical or autopsy diagnosis of AP or CP at the time of death. We have previously shown that postmortem changes, such as limited autolysis, do not significantly affect the interpretation of findings using this approach.[4]

We compared IPF and acinar injury among the various groups quantitatively and morphologically, verifying critical conclusions using external controls (eg, computed tomography [CT] scan), and mechanistically in vitro using adipocytes and acinar cells. Our findings suggest that differences in the patterns of IPF and fibrosis in patients with CP compared with obese patients may explain the differences in outcomes in these 2 groups.

Patients and Methods

The medical records of all patients in the University of Pittsburgh Medical Center/Presbyterian Hospital Autopsy Database (1998–2008) with a diagnosis of AP (n = 23), CP (n = 35), or AP-on-CP (total, n = 15; clinical AP-on-CP, n = 10) were reviewed. Pancreatic histology slides of these patients and 50 randomly selected controls were scored for IPF, fat necrosis (FN), peri-fat acinar necrosis (PFAN), and fibrotic area (as percent pancreatic area). The percentage of fat with >50% of its immediate perimeter surrounded by fibrosis was measured. For further details, please refer to Supplementary Patients and Methods.

Results

There was no significant difference in age ($F[3,119] = 0.790$, $P = .502$) or BMI ($F[3,119] = 0.845$, $P = 0.472$) (Supplementary Table 1) between the groups.

BMI Correlates Positively With IPF in Controls, Patients With AP, and Patients With AP-on-CP but not Patients With CP

Because IPF may increase with BMI[4,6] or CP,[40,41] we studied the correlation of BMI to histologically measured %IPF. Significantly positive correlations were found in controls ($r[48] = 0.592$, $P < .001$), patients with AP ($r[21] = 0.506$, $P = .014$),[4,6] and patients with AP-on-CP ($r[13] = 0.729$, $P = .002$) (Figure 1*A*–*C*) but not in patients with CP ($r[33] = 0.168$, $P = .334$) (Figure 1*A*). A sensitivity analysis after excluding a possible influential observation (a patient with a BMI >60 kg/m^2) in the CP group showed a correlation coefficient of 0.170 ($P = .337$). Two-way analysis of variance showed significant main effects for group ($F[3,115] = 3.462$, $P = .019$) and BMI ($F[1,115] = 29.548$, $P < .001$) and a significant interaction (group × BMI, $F[3,115] = 3.724$, $P = .013$) for %IPF. Subsequent post hoc tests showed that the %IPF for controls and patients with CP, AP, or AP-on-CP did not differ in the obese group; however, for those with a BMI <30 kg/m^2, patients with CP had a significantly greater IPF when compared with controls. Post hoc tests also showed that obese patients in the control, AP, and AP-on-CP groups, but not the CP group, had a significantly greater IPF than those with a BMI <30 kg/m^2 (Figure 1*C*).

Of the 35 patients with CP, 14 had CT scans with no evidence of AP in the 45 days preceding death. IPF measured on these CT scans by a blinded radiologist using either of the previously validated attenuation[4,6] or thresholding methods[4,6] showed a strong correlation with histology (Figure 1*D* and *E*). The κ value for histologic quantification of IPF by 2 independent blinded observers was 0.951, signifying strong interobserver agreement.

Patients With CP Have a Lower Amount of FN Compared With Patients With AP

Patients with AP had significantly more FN than those with CP ($P < .001$) or controls ($P < .001$) (Figure 2*A*). FN was visible as adipocytes with a bluish, amorphous look on H&E staining (Figures 3*C*, 4*C*, and 4*D*) and stained brown on von Kossa staining, suggesting saponification (Figure 4*C2* and *D2*). This FN seemed to be pathogenically relevant because nonesterified fatty acids (NEFAs) induce cell death,[4] and the FN in patients with AP was associated with surrounding parenchymal necrosis (*dotted area* in Figure 4*C* and *C2*).

Patients With AP Have More PFAN and Necrosis Than Patients With CP or AP-on-CP

We quantified parenchymal necrosis adjacent to the FN mentioned as adjacent to the FN (ie, PFAN). On H&E staining, this was notable for areas with loss of cell outlines bordering FN (*dotted area* in Figure 4*C*). On von Kossa staining, PFAN showed brown staining (Figure 4*C2*) that was most intense adjacent to the FN and decreased with increasing distance, suggesting leakage of NEFAs