The Role of Acetaldehyde in the Pathogenesis of Acute Alcoholic Pancreatitis

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Acetaldehyde (AA), the first product of ethanol metabolism, has been suggested as an important mediator in alcoholic pancreatitis, but experimental evidence has not been convincing. Prior work using the isolated perfused canine pancreas preparation has suggested that toxic oxygen metabolites generated by xanthine oxidase (XO) may mediate the early injury in pancreatitis. Xanthine oxidase is capable of oxidizing AA, and during this oxidation free radicals are released. The hypothesis that acute alcoholic pancreatitis may be initiated by AA in the presence of active XO (converted from xanthine dehydrogenase [XD]) was tested in the authors' experimental preparation by converting XD to XO by a period of ischemia, and infusing AA. Control preparations remained normal throughout the 4-hour perfusion (weight gain, 7 ± 4 g; amylase activity, 1162 ± 202 U/dL). One hour of ischemia, or infusion of AA at 25 mg/hr or at 50 mg/hr without ischemia did not induce changes in the preparation. Acetaldehyde at 250 mg/hr induced minimal edema and weight gain (16 ± 4 g; $p < 0.05$), but not significant hyperamylasemia. Changes also were not observed when 1-hour ischemia was followed by a bolus of ethanol (1.5 g) or sodium acetate (3.0 g), or by infusion of 25 mg/hr of AA. One hour of ischemia followed by infusion of AA at 50 mg/hr or at 250 mg/hr induced edema, hemorrhage, weight gain (22 ± 7 g [$p < 0.05$] and 26 ± 17 g [$p < 0.05$]) and hyperamylasemia (2249 ± 1034 U/dL [$p < 0.05$] and 2602 ± 1412 U/dL [$p < 0.05$]). Moreover infusion of AA at 250 mg/hr after 2 hours of ischemia potentiated the weight gain (62 ± 20 g *versus* 30 ± 14 g [$p < 0.05$]), but not the hyperamylasemia (3404 ± 589 U/dL *versus* 2862 ± 1525 U/dL) as compared with 2 hours of ischemia alone. Pancreatitis induced by 1 hour of ischemia followed by AA at 50 mg/hr could be inhibited by pretreatment with the free radical scavengers superoxide dismutase and catalase and ameliorated with the XO inhibitor allopurinol. The authors conclude that AA, in the presence of active XO, can initiate acute pancreatitis in the isolated canine pancreas preparation and may be important in the initiation of acute alcoholic pancreatitis in man. Toxic oxygen metabolites appear to play an important intermediary role.

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THE ASSOCIATION BETWEEN alcohol and acute pancreatitis has been known for over one hundred years,[1] but the mechanisms by which alcohol initiates acute pancreatitis have remained obscure. Several pathogenetic mechanisms have been suggested, including a direct toxic effect of alcohol on the pancreas, and mediation by alcohol induced hyperlipidemia.[2] It has been suggested by some investigators that acetaldehyde, the first stable product of ethanol oxidation, may play a role in the pathogenesis of acute alcoholic pancreatitis,[3-5] although this has not been supported by others.[6] Acetaldehyde is considered one of the major factors in alcohol-induced liver injury,[7] perhaps mediated by free radicals.[7,8] Prior work from this laboratory using the isolated *ex vivo* perfused canine pancreas preparation has suggested that oxygen-derived free radicals generated by xanthine oxidase play an important role in the initiation of experimental pancreatitis.[9,10] Acetaldehyde is an excellent substrate for the enzyme xanthine oxidase, and during its oxidation free radicals are released.[11] We hypothesized that acute alcoholic pancreatitis may be initiated by free radicals from acetaldehyde, generated by xanthine oxidase. To test this hypothesis in our experimental preparation, acetaldehyde was infused after the conversion of xanthine dehydrogenase to xanthine oxidase by a period of ischemia.

Methods

Under general anesthesia (Nembutal, Steris Inc., Chicago, IL) the pancreas with a cuff of duodenum was removed from adult mongrel dogs weighing between 18 and 23 kg. The pancreas was placed in a perfusion circuit in a humified chamber, as previously described.[12] The preparation was perfused through catheters in the splenic and

superior mesenteric arteries at a constant flow (17 mL/100 g tissue/min). The perfusate was collected in a venous reservoir through a cannula in the portal vein and returned to a Harvey® infant oxygenator (C. R. Bard Inc., Billerica, MA), from where the perfusate was pumped back into the preparation by a roller pump (Watson-Marlow Ltd., England). The temperature of the perfusate was maintained at 37 C with a heat exchanger. The perfusate consisted of 400 mL autologous dog blood, 100 mL Ringer's lactate solution, 0.5 g glucose, 2.5 g human albumin, and 15 mEq sodium bicarbonate. An additional 0.25 g glucose was given after 2 hours of perfusion to maintain the glucose level in the perfusate over 70 mg/dL. An additional 1 to 2 mEq sodium bicarbonate was given when needed to maintain the pH within the physiologic range (7.37 to 7.54).

The perfusion consisted of an initial 30-minute stabilization period, followed by a 4-hour study period. In the ischemic preparations, the perfusion circuit was adjusted to bypass the preparation for either 1 or 2 hours after the stabilization period, but before the 4-hour study period. The preparation was monitored continuously for gross appearance (edema and hemorrhage estimated in the scale none, mild, moderate, or severe), weight, and arterial blood pressure, and measurements were recorded hourly. The perfusate was sampled from the venous line at the beginning of the 4-hour perfusion period (time 0), and hourly thereafter to assay for hematocrit, glucose concentration (automatized blood sugar analyzer, Beckman 2, Beckman, Fullerton, CA) and amylase activity (Amylo-Tube, Pharmacia Diagnostic Inc., Fairfield, NJ). The perfusate was sampled from the arterial line at the same intervals to determine hemoglobin, pH, and blood gases (OSM 2 Hemoximeter and Acid-Base Analyzer, Radiometer, Copenhagen, Denmark). Pancreatic juice was collected with a 16-gauge cannula in the pancreatic duct, and the volume was recorded hourly.

In 10 experiments the perfusate was sampled hourly from both the arterial and the venous lines for assaying acetaldehyde. The sample (0.5 mL) was injected into cooled (0 C), sealed vials containing 0.25 mL 3.0 mol/L perchloric acid. The vials were shaken vigorously, and placed into ice until acetaldehyde was assayed by headspace gas chromatography during the same day. Fresh standards were prepared for every group of experiments.

Student's t test was used to compare the results between groups.

Acetaldehyde—Groups I Through X

Group I.

Controls (6 Preparations). These preparations were perfused for 4 hours after the initial 30-minute stabilization period and monitored as described above. In one preparation the perfusate was sampled for acetaldehyde.

Group II.

Acetaldehyde 25 mg/hr (AA 25) (3 Preparations). These preparations were managed identically to those in group I (controls), except that acetaldehyde (Morton Thiokol Inc., Danvers, MA) was infused at 25 mg/hr into the arterial line over the 4-hour perfusion period starting at time 0. Fresh 5% solution of acetaldehyde in 0.1 mol/L phosphate buffer was prepared at 0 C for every experiment. The infusion syringe was packed in ice to avoid escape of acetaldehyde from the solution (boiling point, 21 C). In three preparations the perfusate was sampled for acetaldehyde.

Group III.

Acetaldehyde 50 mg/hr (AA 50) (8 Preparations). These preparations were managed identically to those in group II, except that acetaldehyde was infused at 50 mg/hr. In three preparations the perfusate was sampled for acetaldehyde.

Group IV.

Acetaldehyde 250 mg/hr (AA 250) (8 Preparations). These preparations were managed identically to those in group II, except that acetaldehyde was infused at 250 mg/hr. Fresh 10% solution of acetaldehyde in 0.1 mol/L phosphate buffer was prepared at 0 C for every experiment. In three preparations the perfusate was sampled for acetaldehyde.

Group V.

One-hour Ischemia (ISCH 1) (5 Preparations). These preparations were managed identically to those in group I (controls), except for a 60-minute period of ischemia between the 30-minute stabilization period and the 4-hour perfusion period.

Group VI.

One-hour Ischemia Followed by Acetaldehyde 25 mg/hr (ISCH 1 + AA 25) (3 Preparations). These preparations were managed identically to those in group V (ISCH 1), except that acetaldehyde was infused over the 4-hour perfusion period at the rate of 25 mg/hr.

Group VII.

One-hour Ischemia Followed by Acetaldehyde 50 mg/hr (ISCH 1 + AA 50) (10 Preparations). These preparations were managed identically to those in group VI (ISCH 1 + AA 25), except that acetaldehyde was infused over the 4-hour perfusion period at the rate of 50 mg/hr.

Group VIII.

One-hour Ischemia Followed by Acetaldehyde 250 mg/hr (ISCH 1 + AA 250) (5 Preparations). These preparations were managed identically to those in group VI (ISCH 1 + AA 25), except that acetaldehyde was infused over the 4-hour perfusion period at the rate of 250 mg/hr.

Group IX.

Two-hour Ischemia (ISCH 2) (5 Preparations). These preparations were managed identically to those in group I (controls), except for a 120-minute period of ischemia

between the 30-minute stabilization period and the 4-hour perfusion period.

Group X.

Two-hour Ischemia Followed by Acetaldehyde 250 mg/hr (ISCH 2 + AA 250) (5 Preparations). These preparations were managed identically to those in group IX (ISCH 2), except that acetaldehyde was infused over the 4-hour perfusion period at the rate of 250 mg/hr.

Ethanol and Acetate—Groups XI Through XIV

Group XI.

Ethanol (ETOH) (3 Preparations). These preparations were managed identically to those in group I (controls), except that 1.5 g ethanol as a 10% solution in saline was added to the perfusate at time 0.

Group XII.

One-hour Ischemia Followed by Ethanol (ISCH 1 + ETOH) (4 Preparations). These preparations were managed identically to those in group V (ISCH 1), except that ethanol was added to the perfusate as in group XV (ETOH).

Group XIII.

Acetate (ACE) (3 Preparations). These preparations were managed identically to those in group I (controls), except that 3.0 g sodium acetate (Baker & Adamson, Phillipsburg, NJ; containing three molecules crystallized water in each molecule of sodium acetate) in 10 mL saline was added to the perfusate at time 0.

Group XIV.

One-hour Ischemia Followed by Acetate (ISCH 1 + ACE) (5 Preparations). These preparations were managed identically to those in group V (ISCH 1), except that sodium acetate was added to the perfusate as in group XVII (ACE).

Treatment of Acetaldehyde-induced Injury—Groups XV Through XVII

Group XV.

ISCH 1 + AA 50 + SOD + CAT (5 Preparations). These preparations were managed identically to those in group VII (ISCH 1 + AA 50), except that 50 mg bovine erythrocyte superoxide dismutase (SOD) (Sigma Chemicals Co., St. Louis, MO) and 50 mg bovine liver catalase (CAT) (Calbiochem Corp., La Jolla, CA) were added to the perfusate at the beginning of the stabilization period.

Group XVI.

ISCH 1 + AA 50 + ALLO (5 Preparations). These preparations were managed identically to those in group VII (ISCH 1 + AA 50), except that 100 mg allopurinol (Sigma) were added to the perfusate at the beginning of the stabilization period.

Group XVII.

ISCH 2 + AA 250 + SOD + CAT (4 Preparations). These preparations were managed identically to those in group X (ISCH 2 + AA 250), except that 50 mg SOD and 50 mg CAT were added to the perfusate at the beginning of the stabilization period.

Results

The control preparations (group I) remained normal in gross appearance throughout the 4-hour perfusion period. Weight gain was minimal, and amylase activity in the perfusate remained normal (Tables 1 and 2).

Acetaldehyde infusion at 25 mg/hr (group II) and at 50 mg/hr (group III) did not induce changes in the gross appearance of the pancreas. No significant differences in weight gain or in amylase activity were observed when compared with controls (Tables 1 and 2). When acetaldehyde was infused at 250 mg/hr (group IV), the preparations became mildly edematous, gained weight when compared with the controls, but the amylase activity in the perfusate did not increase significantly (Tables 1 and 2).

One hour of ischemia alone (group V) did not result in changes in gross appearance, weight gain, or amylase activity (Tables 1 and 2). One-hour ischemia followed by acetaldehyde infusion at 25 mg/hr (group VI) also did not induce changes in the preparation (Tables 1 and 2). When 1 hour of ischemia was followed by acetaldehyde at 50 mg/hr (group VII) or at 250 mg/hr (groups VIII), pancreatitis developed with moderate edema and hemorrhage, significant weight gain (Table 1), and hyperamylasemia (Table 2).

Two hours of ischemia alone (group IX) induced pancreatitis with moderate edema, hemorrhage, significant weight gain (Table 1), and an increase in amylase activity (Table 2). Two hours of ischemia followed by acetaldehyde at 250 mg/hr (group X) induced severe pancreatitis with marked edema, severe hemorrhage, high amylase activity (Table 2), and weight gain, which was significantly increased over that induced by a 2-hour period of ischemia alone (Table 1).

Ethanol or acetate alone, or in combination with ischemia (groups XI through XIV), did not induce edema, hemorrhage, weight gain, or significant hyperamylasemia (Tables 3 and 4).

Treatment with SOD and CAT of the pancreatitis induced by 1 hour of ischemia followed by acetaldehyde infusion at 50 mg/hr (group XV) prevented edema, hemorrhage, weight gain, and increase in the amylase activity (Tables 5 and 6). Treatment with SOD and CAT of the more severe pancreatitis induced by 2 hours of ischemia followed by acetaldehyde infusion at 250 mg/hr (group XVII) also significantly ameliorated the weight gain and increase in the amylase activity (Tables 5 and 6). Similarly allopurinol treatment (group XVI) inhibited edema, hemorrhage, and weight gain (Table 5), and ameliorated

TABLE 1. *Weight Gain (g) of* Ex Vivo *Perfused Canine Pancreas Preparations*

Group	n	Time (hr) 0	1	2	3	4
I Control	6	0	2 ± 2	3 ± 3	5 ± 3	7 ± 4
II AA 25	5	0	1 ± 1	2 ± 2	3 ± 5	3 ± 5
III AA 50	8	0	2 ± 3	6 ± 4	6 ± 5	9 ± 6
IV AA 250	8	0	7 ± 6	10 ± 7*	11 ± 4*	16 ± 4*
V ISCH 1	5	0	6 ± 3	8 ± 2	10 ± 4	11 ± 4
VI ISCH 1 + AA 25	3	0	1 ± 1	1 ± 2	3 ± 2	5 ± 3
VII ISCH 1 + AA 50	10	0	5 ± 3	11 ± 3*	17 ± 6*	22 ± 7*
VIII ISCH 1 + AA 250	5	0	6 ± 4	14 ± 11*	20 ± 13*	26 ± 17*
IX ISCH 2	5	0	10 ± 8*	15 ± 10*	20 ± 12*	30 ± 14*
X ISCH 2 + AA 250	5	0	18 ± 6*	34 ± 14*	44 ± 15*	62 ± 20*

Mean ± SD.
Acetaldehyde stimulus with or without preceding ischemia.
* $p < 0.05$ as compared with the control group (I).
Other differences are not significant.
AA, acetaldehyde; ISCH, ischemia.

TABLE 2. *Amylase Activity (U/dL) in the Perfusate of* Ex Vivo *Perfused Canine Pancreas Preparations*

Group	n	Time (hr) 0	1	2	3	4
I Control	6	570 ± 117	705 ± 137	833 ± 111	1031 ± 175	1162 ± 202
II AA 25	5	411 ± 83	498 ± 109	654 ± 239	650 ± 233	831 ± 280
III AA 50	8	599 ± 121	835 ± 356	731 ± 302	1010 ± 224	1365 ± 608
IV AA 250	8	647 ± 194	896 ± 439	1057 ± 542	1368 ± 793	1828 ± 1119
V ISCH 1	5	474 ± 67	911 ± 183	1086 ± 282	1425 ± 181	1445 ± 224
VI ISCH 1 + AA 25	3	624 ± 232	737 ± 351	924 ± 401	838 ± 609	1344 ± 672
VII ISCH 1 + AA 50	10	664 ± 204	1155 ± 515*	1648 ± 798*	1980 ± 1143*	2249 ± 1034*
VIII ISCH 1 + AA 250	5	721 ± 186	1465 ± 814*	2124 ± 1270*	2350 ± 1408*	2602 ± 1412*
IX ISCH 2	5	796 ± 129	1694 ± 227*	2349 ± 970*	3170 ± 2292*	2862 ± 1525*
X ISCH 2 + AA 250	5	929 ± 175	1771 ± 405*	1776 ± 398*	2630 ± 449*	3404 ± 589*

Mean ± SD.
Acetaldehyde stimulus with or without preceding ischemia.
* $p < 0.05$ as compared with the control group (I).
Other differences are not significant.
AA, acetaldehyde; ISCH, ischemia.

TABLE 3. *Weight Gain (g) of* Ex Vivo *Perfused Canine Pancreas Preparations*

Group	n	Time (hr) 0	1	2	3	4
I Control	6	0	2 ± 2	3 ± 3	5 ± 3	7 ± 4
XI ETOH	3	0	5 ± 3	6 ± 3	7 ± 5	9 ± 6
XII ISCH 1 + ETOH	4	0	4 ± 2	7 ± 3	7 ± 5	9 ± 7
XIII ACE	3	0	5 ± 6	5 ± 6	3 ± 1	7 ± 4
XIV ISCH 1 + ACE	5	0	4 ± 4	5 ± 2	8 ± 3	10 ± 4

Mean ± SD.
Ethanol and acetate stimuli with and without preceding ischemia.
Differences are not significant.
ETOH, ethanol; ISCH, ischemia; ACE, acetate.

the increase in amylase activity in the perfusate (Table 6).

In the control preparation, the perfusate contained no detectable acetaldehyde. The acetaldehyde concentration after 4 hours of infusion varied between 320 and 2015 μmol/L in the arterial sample, and between 95 and 1740 μmol/L in the venous sample, depending on the infusion rate (Fig. 1).

In all preparations the arterial pH ranged between 7.36 and 7.53, the Pa_{O_2} between 280 and 550 mm Hg, and the Pa_{CO_2} between 28 and 45 mm Hg during the 4-hour perfusion period. Plasma glucose remained over 70 mg/

TABLE 4. *Amylase Activity (U/dL) in the Perfusate of* Ex Vivo *Perfused Canine Pancreas Preparations*

Group	n	Time (hr) 0	1	2	3	4
I Control	6	570 ± 117	705 ± 137	833 ± 111	1031 ± 175	1162 ± 202
XI ETOH	3	709 ± 127	858 ± 186	834 ± 134	1038 ± 185	1181 ± 176
XII ISCH 1 + ETOH	4	555 ± 144	760 ± 221	982 ± 404	1248 ± 378	1421 ± 374
XIII ACE	3	541 ± 93	730 ± 258	970 ± 300	1327 ± 563	1611 ± 723
XIV ISCH 1 + ACE	5	775 ± 259	773 ± 387	1002 ± 477	1275 ± 518	1554 ± 577

Mean ± SD.
Ethanol and acetate stimuli with and without preceding ischemia.
Differences are not significant.
ETOH, ethanol; ISCH, ischemia; ACE, acetate.

TABLE 5. *Weight Gain (g) of* Ex Vivo *Perfused Canine Pancreas Preparations*

Group	n	Time (hr) 0	1	2	3	4
I Control	6	0	2 ± 2	3 ± 3	5 ± 3	7 ± 4
VII ISCH 1 + AA 50	10	0	5 ± 3	11 ± 3*	17 ± 6*	22 ± 7*
XV ISCH 1 + AA 50 + SOD + CAT	5	0	2 ± 2	3 ± 3†	5 ± 2†	5 ± 3†
XVI ISCH 1 + AA 50 + ALLO	5	0	3 ± 4	4 ± 3†	6 ± 3†	8 ± 4†
X ISCH 2 + AA 250	5	0	18 ± 6*	34 ± 14*	44 ± 15*	62 ± 20*
XVII ISCH 2 + AA 250 + SOD + CAT	4	0	14 ± 11*	20 ± 14*	23 ± 16‡	31 ± 11‡

Mean ± SD.
Pancreatitis was induced by a combination of ischemia and acetaldehyde infusion, and was treated with free radical scavengers SOD and CAT, or xanthine oxidase inhibitor allopurinol.
* $p < 0.05$ as compared with the control group (I).
† $p < 0.05$ as compared with the respective untreated group (VII).
‡ $p < 0.05$ as compared with the respective untreated group (X).
Other differences are not significant.
ISCH, ischemia; AA, acetaldehyde; SOD, superoxide dismutase; CAT, catalase; ALLO, allopurinol.

TABLE 6. *Amylase Activity (U/dL) in the Perfusate of* Ex Vivo *Perfused Canine Pancreas Preparations*

Group	n	Time (hr) 0	1	2	3	4
I Control	6	570 ± 117	705 ± 137	833 ± 111	1031 ± 175	1162 ± 202
VII ISCH 1 + AA 50	10	664 ± 204	1155 ± 515*	1648 ± 798*	1980 ± 1143*	2249 ± 1034*
XV ISCH 1 + AA 50 + SOD + CAT	5	571 ± 99	693 ± 165†	812 ± 234†	1017 ± 418†	1087 ± 507†
XVI ISCH 1 + AA 50 + ALLO	5	662 ± 140	853 ± 185	1230 ± 350	1311 ± 307	1480 ± 363
X ISCH 2 + AA 250	5	929 ± 175	1771 ± 405*	1776 ± 398*	2630 ± 449*	3404 ± 589*
XVII ISCH 2 + AA 250 + SOD + CAT	5	837 ± 166	1121 ± 278*‡	1306 ± 225*	1594 ± 222‡	1608 ± 133‡

Mean ± SD.
Pancreatitis was induced by a combination of ischemia and acetaldehyde infusion, and was treated with free radical scavengers SOD and CAT, or xanthine oxidase inhibitor allopurinol.
* $p < 0.05$ as compared with the control group (I).
† $p < 0.05$ as compared with the respective untreated group (VII).
‡ $p < 0.05$ as compared with the respective untreated group (X).
Other differences not significant.
ISCH, ischemia; AA, acetaldehyde; SOD, superoxide dismutase; CAT, catalase; ALLO, allopurinol.

dL throughout the perfusion in all the preparations. In these parameters there were no differences between the various groups. Hemoconcentration was observed in the preparations developing pancreatitis, reaching borderline significance in some of the groups. Arterial pressure declined gradually during the perfusion as peripheral resistance decreased (constant flow) in all the preparations, except in the ISCH 2 + AA 250 group, where the pressure did not decline with the most severe pancreatitis present. The secretory volume of the pancreas was changed significantly from the controls only in the preparations treated with allopurinol, where secretion increased fourfold ($p < 0.02$).

Discussion

Despite decades of investigation, the pathogenesis of acute alcoholic pancreatitis remains obscure. Various hypotheses have been proposed, including biliary-pancreatic

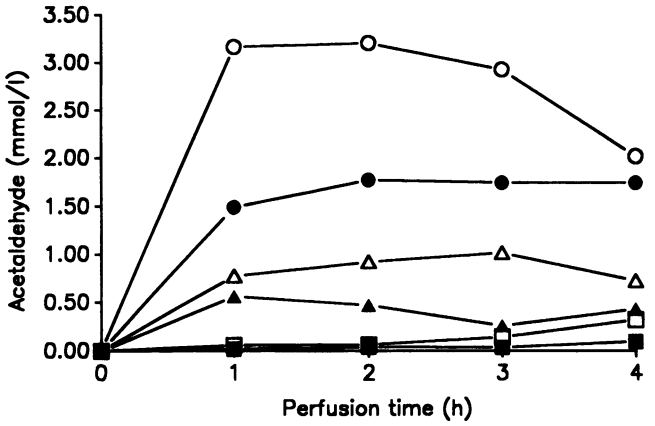

FIG. 1. Acetaldehyde concentration in the perfusate, as sampled in the arterial or venous line, at various rates of acetaldehyde infusion. Mean of three experiments in each group. ○ —— ○, acetaldehyde infusion at 250 mg/hr, arterial sample; ● —— ●, acetaldehyde infusion at 250 mg/hr, venous sample; △ —— △, acetaldehyde infusion at 50 mg/hr, arterial sample; ▲ —— ▲, acetaldehyde infusion at 50 mg/hr, venous sample; □ —— □, acetaldehyde infusion at 25 mg/hr, arterial sample; ■ —— ■, acetaldehyde infusion at 25 mg/hr, venous sample.

reflux, duodenopancreatic reflux, and sphincter of Oddi obstruction with hypersecretion.[2,3] There is little evidence to support these theories, however. The obstruction of pancreatic ductules by protein plugs comes closer to explaining the pathogenesis of chronic pancreatitis than of acute pancreatitis.[3] Previous studies from this institution have provided evidence that hypertriglyceridemia, induced by alcohol abuse, may serve as an intermediary in the initiation of acute pancreatitis.[12] Although this may explain the development of pancreatitis in some alcoholics, it certainly is not the cause in many others.[13] More recently interest has focused on the effects of ethanol on acinar cell metabolism, creating a "toxic metabolic hypothesis."[2] This includes a decrease of trypsin-inhibiting capacity in pancreatic tissue and pancreatic juice, an increase in intracellular ionized calcium, an increase in specific activity of lysosomal enzymes, and an increase in both trypsinogen content and trypsin activity.[2] None of the hypotheses presented thus far is supported by convincing clinical and laboratory data. Experimental acute pancreatitis can not be initiated by giving ethanol to an animal,[14,15] and thus there has been a lack of a suitable experimental model for studying the pathogenesis of acute alcoholic pancreatitis.

In an effort to develop models of acute pancreatitis that simulate the disease as seen in the human, the isolated canine pancreas preparation was adopted.[12,16] Models were developed that simulate acute pancreatitis caused by hyperlipemia,[13] gallstones,[17] and ischemia.[18] An increase in capillary permeability was demonstrated to be the first measurable physiologic response in each of the models studied.[19] Subsequent studies demonstrated this capillary injury to be mediated by toxic oxygen metabolites (free radicals), because the free radical scavengers SOD and CAT inhibited or significantly ameliorated the injury.[9] It was demonstrated that the toxic oxygen metabolites were generated by the enzyme xanthine oxidase, because allopurinol, a specific inhibitor of xanthine oxidase, also inhibited or significantly ameliorated the injury.[10] Because acetaldehyde is known to be an excellent substrate for xanthine oxidase, and during its oxidation toxic oxygen metabolites are generated,[11] we hypothesized that acetaldehyde may initiate acute pancreatitis in many alcoholics in the presence of activated xanthine oxidase.

Xanthine oxidase occurs in tissues primarily as the inactive enzyme xanthine dehydrogenase.[20] Several stimuli are able to induce the conversion of xanthine dehydrogenase to active xanthine oxidase. These include ischemia,[21] hyperthermia,[22] chymotrypsin,[23] trypsin,[24] sulfhydryl oxidase,[25] complement C5,[26] and also acetaldehyde in a dose-dependent manner.[27] In the present experiments, a period of ischemia was used to induce this conversion. Although a 2-hour period of ischemia will by itself initiate acute pancreatitis in the preparation, a 1-hour period of ischemia will not. This has been previously studied and documented in detail.[18] A 1-hour period of ischemia should be enough, however, to induce the conversion of xanthine dehydrogenase to xanthine oxidase.[28]

Infusion of acetaldehyde at 25 mg/hr or at 50 mg/hr, without preceding ischemia, did not induce injury to the isolated pancreas preparation. When acetaldehyde was infused at 50 mg/hr after a 1-hour period of ischemia, however, pancreatitis developed. Pretreatment with SOD and CAT inhibited this injury. In addition acetaldehyde at 250 mg/hr after 2 hours of ischemia potentiated the injury seen after 2 hours of ischemia alone. This injury also could be ameliorated with SOD and CAT. These findings suggest that toxic oxygen metabolites mediated the injury. Moreover because pretreatment with allopurinol also ameliorated the injury response, this suggests that the toxic oxygen metabolites are generated by xanthine oxidase. This supports our hypothesis that acetaldehyde together with xanthine oxidase may initiate acute pancreatitis in our experimental model. The mild edema and minor weight gain after infusion of acetaldehyde at 250 mg/hr without preceding ischemia may be explained by the ability of high acetaldehyde concentrations to induce the conversion of xanthine dehydrogenase to xanthine oxidase.[27]

Acetaldehyde is the first metabolite of ethanol oxidation catalyzed by alcohol dehydrogenase. Ethanol was not injurious to the preparation at concentrations of about 60 mmol/L (calculated), even when administered after 1 hour of ischemia. This suggests that ethanol itself is not metabolized to acetaldehyde in significant amounts in this

preparation. This is in agreement with the finding that dog pancreas contains negligible amounts of alcohol dehydrogenase.[29] Interestingly human pancreas has been demonstrated to contain alcohol dehydrogenase.[30]

Acetaldehyde is metabolized to acetate by aldehyde dehydrogenase. Acetate may stimulate the formation of purines, which again may serve as a substrate for xanthine oxidase in the generation of toxic oxygen metabolites.[6] Acetate at concentrations of about 45 mmol/L (calculated), however, was not injurious to the pancreas preparation after 4 hours of perfusion, whether administered with or without preceding ischemia. This suggests that it is acetaldehyde and not its precursor or metabolite that is responsible for the injury.

Previously others have demonstrated that acetaldehyde can be injurious to the pancreas. Intraperitoneal injections of acetaldehyde in the rat increased serum amylase activity and induced structural changes in the acinar cells.[31] Another group demonstrated that when acetaldehyde was infused in the isolated canine pancreas preparation together with secretin stimulation, the pancreas became edematous and hemorrhagic, and secretory volume decreased.[4] In contrast, according to other investigators infusion of acetaldehyde into the splenic artery in the pig did not induce macroscopic or microscopic changes in the pancreas, nor did it affect the amylase activity in the serum.[6] Because acetaldehyde boils at room temperature, and is readily metabolized by several mammalian tissues,[32] including red blood cells,[33] inadequate experimental exposure of the pancreas to acetaldehyde may easily occur. In our studies the presence of acetaldehyde in the perfusate was confirmed by assaying acetaldehyde concentrations in perfusate sampled both in the arterial and venous line. Pancreatitis was observed to develop after 1 hour of ischemia, when acetaldehyde was infused at 50 mg/hr. This infusion rate exposed the pancreas to acetaldehyde at concentrations in the range of 500 μmol/L. In healthy individuals acetaldehyde levels in the peripheral blood are below 10 μmol/L after a moderate test dose of ethanol.[34] The blood acetaldehyde level may exceed 100 μmol/L,[34] however, and may reach even 525 μmol/L,[35] after ethanol abuse. In addition even a low test dose of ethanol may result in blood acetaldehyde concentrations of over 100 μmol/L if an inhibitor for aldehyde dehydrogenase is simultaneously administered.[36] Thus the levels of acetaldehyde achieved in our experimental preparation are what one might expect to find clinically in patients with acute pancreatitis. In humans most of the alcohol dehydrogenase is located in the liver, but some also is found in the pancreas.[30] In addition to blood acetaldehyde originating from liver, some might be produced in the pancreas, possibly resulting in very high local exposure of acinar cells to acetaldehyde. In conclusion these studies suggest that acetaldehyde may play an important role in the pathogenesis of acute alcoholic pancreatitis.

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