The Role of Oxygen-derived Free Radicals in the Pathogenesis of Acute Pancreatitis

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Acute pancreatitis may be initiated in the *ex vivo*, perfused canine pancreas preparation by a variety of stimuli. These include 1) oleic acid infusion (FFA), 2) partial duct obstruction with secretin stimulation (POSS), and 3) a 2-hour period of ischemia (ISCH). In each model, pancreatitis is characterized by weight gain, edema, and hyperamylasemia. Oxygen-derived free radicals such as superoxide, hydrogen peroxide, and the hydroxyl radical are highly reactive toxic substances that are normally produced in small amounts during oxidative metabolism. Ordinarily, these substances are detoxified by endogenous intracellular enzymes called free radical scavengers (FRS), such as superoxide dismutase (SOD) and catalase (CAT). These studies were undertaken to evaluate the possible role of oxygen-derived free radicals in the initiation of acute pancreatitis in the isolated canine model. All preparations were perfused for 4 hours with autologous blood. Controls (N = 6): these glands remained normal in appearance, gained minimal weight (6 ± 1 g), and serum amylase remained normal (<1000 u/dl). FFA pancreatitis, FFA alone (N = 6): these glands became edematous, gained weight (113.5 ± 27.0 g), and developed hyperamylasemia (2087 ± 387 u/dl). FFA + FRS (N = 6), SOD (50 mg) and CAT (50 mg) were added to the perfusate at time zero: these glands became only minimally edematous, gained less weight (31.8 ± 10.1 g, $p < 0.05$), and amylase remained normal ($p < 0.05$). POSS pancreatitis, POSS alone (N = 8): these glands became edematous, gained weight (38.6 ± 4.6 g), and developed marked hyperamylasemia (9522 ± 3226 u/dl). POSS + FRS (N = 6): these glands did not develop edema, gained less weight (15.1 ± 2.6 g, $p < 0.05$), and serum amylase only increased to 1815 ± 343 u/dl, ($p < 0.05$). ISCH pancreatitis, ISCH alone (N = 6): these glands became edematous, gained weight (75.8 ± 25 g), and developed hyperamylasemia (1679 ± 439 u/dl). ISCH + FRS (N = 6): these glands did not develop edema, gained only 18.3 ± 9.0 g ($p < 0.005$), and serum amylase remained normal ($p < 0.05$). These studies demonstrate that, in this canine preparation, acute pancreatitis is significantly ameliorated by oxygen-free radical scavengers. Since this was true whether the pancreatitis was produced by FFA infusion, POSS, or ischemia, it suggests that oxygen-derived free radicals may mediate a common essential step in the pathogenesis of all forms of pancreatitis.

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ACUTE PANCREATITIS may be initiated by a variety of stimuli, including alcohol-induced hyperlipemia, a migrating gallstone, and a period of ischemia such as that associated with shock. Each of these clinical situations has been previously simulated in the *ex vivo* isolated, perfused canine pancreas model. Alcohol-induced hyperlipemic pancreatitis has been simulated by free fatty acid (FFA) infusion,[1,2] a migrating gallstone by partially obstructing the pancreatic duct while stimulating secretion with secretin (POSS),[3] and ischemic pancreatitis by a 2-hour period of total ischemia.[4] In each of these models, the isolated organ develops gross edema, gains weight, and leaks amylase into the perfusate (hyperamylasemia). Previous studies from this laboratory have suggested that capillary injury manifested as an increase in capillary permeability is an early step in the pathogenesis of each of these forms of experimental pancreatitis.[1,3,5,6] In other forms of tissue injury, similar changes in capillary permeability have been demonstrated to be mediated by oxygen-derived free radicals. These highly reactive molecular species are produced endogenously from the univalent reduction of molecular oxygen. These highly charged molecules, which contain an unpaired electron in their outer shell, include the superoxide free radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^\cdot$). Normally, only small amounts of these toxic intermediates are produced as a byproduct of oxidative metabolism, and these are detoxified by endogenous free radical scavengers, including intracellular superoxide dismutase (SOD) and catalase (CAT). In a variety of pathologic conditions, oxygen-derived free radical production may exceed this scavenging capability, and tissue injury is produced, largely through the peroxidation of structural lipids in the membranes of cells and organelles, the denaturation of hyaluronic acid and collagen in the interstitium, and the destruction of nucleic acids. This injury is often manifested by endothelial cell damage, which is reflected by an increase

in capillary permeability. The current study was designed to evaluate the possible role of oxygen-derived free radicals in the pathogenesis of acute pancreatitis in the isolated perfused canine model.

Materials and Methods

Experimental Preparation

The *ex vivo* isolated, perfused canine pancreas preparation was prepared as previously described.[2] Healthy mongrel dogs (18–22 kg) were anesthetized with sodium pentobarbital (30 mg/kg), and the pancreas was mobilized with a short segment of adjacent duodenum. Following heparinization, perfusion catheters were placed in the splenic artery, superior mesentery artery, and portal vein. The pancreatic duct was cannulated with a 16-gauge cannula through a small duodenotomy. The preparation was then removed from the dog and placed in a perfusion circuit where it was perfused with 400 ml of autologous blood and 100 ml of Ringer's lactate. Glucose (0.5 g), albumin (2.5 g), and sodium bicarbonate (20 ml) were added to the perfusate at the outset of perfusion. Additional sodium bicarbonate was added to the perfusate throughout the experiment in order to maintain the pH within the physiologic range. Blood was perfused retrograde through the splenic and superior mesenteric arteries using a Watson Marlow pump. Portal venous blood was collected in a reservoir and passed through a Harvey infant oxygenator equilibrated with 95% O_2 and 5% CO_2. A heat exchanger was used to maintain perfusate temperature at 37°C. Blood was perfused through the gland at a rate of 1 ml/min/6 g of preparation weight. The preparation was suspended on a perforated platform attached to a calibrated strain gauge transducer to allow pancreatic weight to be monitored continuously. Arterial pressure was also monitored with a conventional pressure transducer. Aliquots of perfusate were withdrawn at Time 0 and hourly thereafter for glucose, amylase, and hematocrit determinations. Arterial blood gases were measured at 2-hour intervals. The volume of pancreatic secretion was measured hourly. All preparations were allowed to stabilize for 30 minutes prior to selection for one of the study groups. Based upon this selection, specific agents were then added to the perfusate. The perfusions were then continued for an additional 4 hours.

Experimental Protocol

I. FFA-induced pancreatitis: Group 1. Controls (N = 6). The preparations in this group were perfused for 4 hours following the 30-minute stabilization period and monitored as described above.

Group 2. SOD/CAT controls (N = 3). Following the 30-minute stabilization period, at a point defined as Time 0, 50 mg of SOD and 50 mg of CAT were added to the perfusate. The preparations were then perfused for 4 hours and monitored as previously described.

Group 3. FFA controls (N = 6). Following the 30-minute stabilization period, at Time 0, 0.3 ml of oleic acid was infused into the arterial line over the first hour of the 4-hour perfusion using a Harvard infusion pump. The perfusion was continued for 4 hours and monitored as previously described.

Group 4. FFA/SOD (N = 3). Following the 30-minute stabilization period, at Time 0, FFA infusion was commenced as outlined above. SOD (50 mg) was added to the perfusate 15 minutes prior to FFA infusion. The preparations were perfused for 4 hours and monitored as previously described.

Group 5. FFA/CAT (N = 3). Following the 30-minute stabilization period, at Time 0, FFA infusion was commenced as outlined above. Catalase (50 mg) was added to the perfusate 15 minutes prior to FFA infusion. The preparations were perfused for 4 hours and monitored as previously described.

Group 6. FFA/SOD/CAT (N = 6). Following the 30-minute stabilization period, at Time 0, FFA infusion was commenced as outlined above. SOD (50 mg) and CAT (50 mg) were added together to the perfusate 15 minutes prior to commencing FFA infusion. The preparations were perfused for 4 hours and monitored as previously described.

II. Gallstone pancreatitis (POSS): Group 1. Controls (N = 6). All preparations were perfused for 4 hours following the 30-minute stabilization period. The glands were monitored as previously described.

Group 2. SOD/CAT controls (N = 3). These glands were prepared in a manner identical to that used in Group 1. Following the 30-minute stabilization period, at Time 0, SOD (50 mg) and CAT (50 mg) were added to the perfusate. The preparations were perfused for 4 hours and monitored as previously described.

Group 3. POSS controls (N = 8). In these preparations the pancreatic duct was cannulated with a 25-gauge cannula to produce a high resistance to excretion. Following the 30-minute stabilization period, at Time 0, 30 units of secretin were added to the perfusate. An additional 10 units of secretin were added at 1, 2, and 3 hours. The preparations were perfused for 4 hours and monitored as previously described.

Group 4. POSS/SOD (N = 3). These glands were prepared in a manner identical to that used in Group 3. Each preparation was allowed to stabilize for 30 minutes and then perfused for 4 hours. SOD (50 mg) was added to the perfusate 15 minutes prior to the administration of the first dose of secretin. The preparations were monitored as previously described.

TABLE 1. *Mean Weight Gain (gm) in the FFA-induced Pancreatitis Study Group*

Time	Group 1 Control	Group 2 SOD/CAT	Group 3 FFA	Group 4 FFA/SOD	Group 5 FFA/CAT	Group 6 FFA/SOD/CAT
1 hour	0	0.6 ± 1.2	12.6 ± 12.8*	17.3 ± 20	8.3 ± 2.8	1.0 ± 1.6†
2 hours	1.2 ± 0.98	3.7 ± 1.5	42.6 ± 28.3*	33.3 ± 25	31.6 ± 17.5	12.2 ± 6.2†
3 hours	2.3 ± 1.5	6.3 ± 3.2	71.0 ± 26*	61.6 ± 34	60.0 ± 36.0	20.3 ± 8.8†
4 hours	3.8 ± 1.9	8.0 ± 1.7	113.5 ± 27*	111.6 ± 40	110.0 ± 17.3	31.8 ± 10.1†

* $p < 0.05$ compared with Group 1 (Control).
† $p < 0.05$ compared with Group 3 (FFA alone).

See text for abbreviations.

Group 5. POSS/CAT (N = 3). These glands were prepared in a manner identical to that used in Group 3. Each preparation was allowed to stabilize for 30 minutes and then perfused for 4 hours. Catalase (50 mg) was added to the perfusate 15 minutes prior to administering the first dose of secretin. The preparations were monitored as previously described.

Group 6. POSS/SOD/CAT (N = 8). These glands were prepared in a manner identical to that used in Group 3. Each preparation was allowed to stabilize for 30 minutes and then perfused for 4 hours. SOD (50 mg) and CAT (50 mg) were added together to the perfusate 15 minutes prior to administering the first dose of secretin. The preparations were monitored as previously described.

III. Ischemic pancreatitis: Group 1. Controls (N = 6). The preparations in this group were perfused for 4 hours following a 30-minute stabilization period. The preparations were monitored as previously described.

Group 2. SOD/CAT controls (N = 3). Following a 30-minute stabilization period, these preparations were perfused for 4 hours. At Time 0, SOD (50 mg) and CAT (50 mg) were added to the perfusate. The preparations were perfused for 4 hours and monitored as previously described.

Group 3. ISCH controls (N = 6). Two hours of ischemia were allowed to elapse after connecting the pancreas to the perfusion system before perfusion was commenced. An initial period of 30 minutes was then allowed for stabilization. The preparations were perfused for 4 hours and monitored as previously described.

Group 4. ISCH/SOD (N = 6). These preparations were subjected to a 2-hour period of total ischemia. SOD (50 mg) was added to the perfusate 15 minutes prior to starting perfusion. Following a 30-minute stabilization period, all preparations were perfused for 4 hours and monitored as previously described.

Group 5. ISCH/CAT (N = 6). These preparations were subjected to a 2-hour period of total ischemia. Catalase (50 mg) was added to the perfusate 15 minutes prior to starting perfusion. Following a 30-minute stabilization period, all preparations were perfused for 4 hours and monitored as previously described.

Group 6. ISCH/SOD/CAT (N = 6). These preparations were subjected to a 2-hour period of total ischemia. SOD (50 mg) and CAT (50 mg) were added to the perfusate 15 minutes prior to starting perfusion. Following a 30-minute stabilization period, all preparations were perfused for 4 hours and monitored as previously described.

Results

I. FFA-induced Pancreatitis

The preparations in Group 1 (Controls) remained normal in appearance, gained only minimal weight, and the amylase level in the perfusate remained normal (Tables 1 and 2). The same pattern was seen in Group 2 (SOD/CAT controls). Only three SOD/CAT controls were performed. The results from these three preparations were used in Sections I, II, and III (FFA-induced pancreatitis, gallstone pancreatitis, and ischemic pancreatitis). The arterial pressure was high at the start of perfusion and then decreased, as peripheral resistance fell, as is characteristic of this preparation.[7] The hematocrit (Hct) remained constant over the 4 hours of perfusion. The glucose concentration steadily decreased over the 4 hours of perfusion, but remained within the normal range. The mean hourly output of pancreatic secretion was within the usual range for this preparation (Table 3).

In contrast, following the infusion of FFA into the arterial line, the preparations in Group 3 became edematous, gained weight, and developed hyperamylasemia (Tables 1 and 2). As the preparations became edematous, peripheral resistance increased, resulting in an increase in arterial pressure. The Hct increased over the course of the experiment as interstitial edema developed, and fluid was lost from the perfusate.

Neither one of the free radical scavengers, SOD (Group 4) or CAT (Group 5), added separately to the perfusate, reduced the injury response induced by the infusion of FFA. These preparations gained weight, became edematous, and developed hyperamylasemia (Tables 1 and 2). The arterial pressure remained elevated in both groups, and the Hct level increased over the 4

TABLE 2. *Mean Serum Amylase Concentration (u/dl) in the FFA-induced Pancreatitis Study Group*

Time	Group 1 Control	Group 2 SOD/CAT	Group 3 FFA	Group 4 FFA/SOD	Group 5 FFA/CAT	Group 6 FFA/SOD/CAT
0	584 ± 230	817 ± 27	733 ± 178	592 ± 212	723 ± 193	704 ± 142
1 hour	668 ± 122	625 ± 61	900 ± 249	797 ± 269	1070 ± 364	538 ± 241
2 hours	709 ± 198	662 ± 94	1417 ± 263*	1137 ± 310	1231 ± 271	493 ± 191†
3 hours	825 ± 214	709 ± 125	1868 ± 468*	1530 ± 565	1271 ± 395	704 ± 240†
4 hours	875 ± 165	816 ± 163	2087 ± 387*	1794 ± 435	1532 ± 294	812 ± 187†

* $p < 0.001$ compared with group 1 (Control).
† $p > 0.001$ compared with Group 3 (Untreated FFA preparations).
See text for abbreviations.

hours of perfusion. All other parameters were similar to those observed in Group 3.

However, when both free radical scavengers SOD and CAT were added together to the perfusate (Group 6), significantly less edema and weight gain developed and the amylase level in the perfusate remained within the normal range (Tables 1 and 2). The arterial pressure was elevated compared with the Group 1 controls at the end of 4 hours, but was less than that seen in the untreated FFA-induced pancreatitis preparations in Group 3. The Hct increased slightly towards the end of the experiment but not to the degree seen in Group 3. All other parameters were similar to those observed in the control groups.

II. Gallstone Pancreatitis

The control preparations remained normal in gross appearance, did not become edematous, did not gain weight, and did not develop hyperamylasemia (Tables 4 and 5). The mean hourly volume of pancreatic secretion was within the normal range for this preparation (Table 3). The untreated POSS preparations in Group 3 became edematous, gained weight, and developed marked hyperamylasemia (Tables 4 and 5). The mean arterial pressure was similar to that of the controls. The Hct increased due to loss of fluid into the interstitial space. As would be expected, the mean volume of pancreatic secretion increased following the administration of secretin (Table 3).

When the free radical scavengers SOD (Group 4) or CAT (Group 5) were added alone to the perfusate, the injury response was unchanged from that seen in the untreated POSS preparations (Group 3). The glands became edematous, gained weight, and developed hyperamylasemia (Tables 4 and 5). When both free radical scavengers SOD and CAT were added together to the perfusate (Group 6), the glands became edematous, but to a much lesser extent than was observed in the untreated POSS preparations in Group 3. Weight gain and the level of amylase in the perfusate were both significantly decreased (Tables 4 and 5). All other parameters were similar to those observed in the control groups.

III. Ischemic Pancreatitis

The control glands remained normal in appearance, gained only minimal weight, and the serum amylase was less than 1000 u/dl after 4 hours of perfusion

TABLE 3. *Mean Volume of Pancreatic Secretion (ml/hour)*

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
FFA-induced pancreatitis group						
	Control	SOD/CAT	FFA	FFA/SOD	FFA/CAT	FFA/SOD/CAT
Mean hourly volume	0.54 ± 0.12	0.21 ± 0.03	0.22 ± 0.03	0.23 ± 0.03	0.15 ± 0.07	0.42 ± 0.1
POSS-induced pancreatitis group						
	Control	SOD/CAT	POSS	POSS/SOD	POSS/CAT	POSS/SOD/CAT
Mean hourly volume	0.4 ± 0.06	0.21 ± 0.03	4.52 ± 1.08*	4.08 ± 1.3	3.83 ± 0.45	3.56 ± 0.89
Ischemic pancreatitis group						
	Control	SOD/CAT	ISCH	ISCH/SOD	ISCH/CAT	ISCH/SOD/CAT
Mean hourly volume	0.43 ± 0.03	0.21 ± 0.03	0.60 ± 0.20	0.42 ± 0.27	0.37 ± 0.14	0.26 ± 0.19

* $p < 0.01$ compared with Group 1 (Control).
See text for abbreviations.

TABLE 4. *Mean Weight Gain (gm) in the Gallstone Pancreatitis (POSS) Study Group*

Time	Group 1 Control	Group 2 SOD/CAT	Group 3 POSS	Group 4 POSS/SOD	Group 5 POSS/CAT	Group 6 POSS/SOD/CAT
1 hour	0	0.6 ± 1.2	8.5 ± 3.8*	8.8 ± 2.5	6.3 ± 3.2	3.4 ± 1.9†
2 hours	1.7 ± 0.8	3.7 ± 1.5	21.1 ± 4.3*	13.0 ± 2.4	16.7 ± 7.6	7.7 ± 3.7†
3 hours	3.8 ± 0.75	6.3 ± 3.2	28.5 ± 3.5*	18.7 ± 2.5	22.3 ± 4.0	12.0 ± 2.3†
4 hours	6.0 ± 1.0	8.0 ± 1.7	38.6 ± 4.6*	32.5 ± 8.6	38.3 ± 7.6	15.1 ± 2.6†

* $p < 0.01$ compared with Group 1 (Control).
† $p < 0.001$ compared with Group 3 (POSS alone).

See text for abbreviations.

(Tables 6 and 7). Following a 2-hour period of total ischemia, the glands in Group 3 became edematous, gained weight (Table 6), and developed hyperamylasemia (Table 7) during the 4-hour perfusion. The arterial pressure remained elevated, rather than decreasing as is characteristic of a normal preparation. The Hct increased, reflecting the loss of fluid into the interstitial space. The mean hourly volume of pancreatic secretion remained within the usual range for control preparations (Table 3).

When the free radical scavenger SOD was added alone to the perfusate of the ischemic preparations in Group 4, edema and weight gain were less than that seen in the untreated ischemic glands, and the serum amylase remained normal. All other parameters were similar to those observed in the control groups.

When the free radical scavenger CAT was added to the perfusate of the preparations in Group 5, the glands became edematous and gained weight (Tables 6 and 7). The mean serum amylase became minimally elevated. All other parameters were similar to those observed in the control groups. When both free radical scavengers SOD and catalase were added to the perfusate of the preparations in Group 6, the response was similar to that observed when the preparations were treated with SOD alone (Group 4): the glands became minimally edematous, gained very little weight, and the serum amylase remained normal.

Discussion

A free radical is a molecule containing a single unpaired electron occupying its outer shell.[8] Oxygen-derived free radicals are potent oxidizing and reducing agents, far more reactive than molecular oxygen and as such present a severe threat to cellular integrity. Oxygen-derived free radicals can cause tissue injury by degrading hyaluronic acid and collagen in the extracellular matrix.[9] They may directly damage cell membranes through the peroxidation of structurally important lipids within the phospholipid structure of the membrane itself,[10] and/or may cause disruption of lysosomes and mitochondria by injuring the membrane surrounding these organelles. They also attack nucleic acids within the nucleus and cytoplasm. Under normal conditions most molecular oxygen is reduced tetravalently by efficient intracellular reduction systems, the best known of which is the cytochrome oxidase system within the mitochondria. However, a small amount of oxygen (1%–2%) "leaks" from this pathway to undergo univalent reduction in the cytoplasm,[11–13] as illustrated in Figure 1. During this process of univalent reduction, several toxic intermediates may be generated. These include the superoxide free radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^\cdot$). Ordinarily, the small quantities of these species produced by the univalent "leak" are detoxified inside the cell by endogenously produced scavenger enzymes. Superoxide dismutase detoxifies the superoxide radical by catalysing its dismutation to oxygen and hydrogen peroxide.[13] Hydrogen peroxide is reduced by intracellular catalases and peroxidases to water and oxygen.[8]

Under a variety of pathologic conditions, oxygen-free radical production may exceed this scavenging capability and tissue injury is produced. One manifestation of the

TABLE 5. *Mean Serum Amylase Concentration (u/dl) in the Gallstone Pancreatitis (POSS) Study Group*

Time	Group 1 Control	Group 2 SOD/CAT	Group 3 POSS	Group 4 POSS/SOD	Group 5 POSS/CAT	Group 6 POSS/SOD/CAT
0	665 ± 228	817 ± 27	778 ± 178	417 ± 93	655 ± 193	513 ± 102
1 hour	542 ± 187	625 ± 61	2174 ± 1578*	1963 ± 670	1765 ± 764	604 ± 184†
2 hours	563 ± 196	662 ± 94	3959 ± 2467*	4105 ± 2755	3791 ± 1947	902 ± 284†
3 hours	614 ± 218	709 ± 125	7045 ± 2980*	6087 ± 2245	5410 ± 2399	1372 ± 331†
4 hours	663 ± 282	816 ± 163	9522 ± 3226*	9386 ± 2325	7514 ± 1079	1815 ± 343†

* $p < 0.05$ compared with Group 1 (Control).
† $p < 0.05$ compared with Group 3 (Untreated POSS preparations).

See text for abbreviations.

TABLE 6. *Mean Weight Gain (gm) in the Ischemic Pancreatitis Study Group*

Time	Group 1 Control	Group 2 SOD/CAT	Group 3 ISCH	Group 4 ISCH/SOD	Group 5 ISCH/CAT	Group 6 ISCH/SOD/CAT
1 hour	0.3 ± 0.8	0.6 ± 1.2	10.5 ± 7.7*	0.6 ± 4.8†	5.2 ± 7.4	1.3 ± 3.9†
2 hours	−0.2 ± 2.8	3.7 ± 1.5	24.5 ± 11.2*	8.4 ± 4.3†	20.0 ± 17.0	7.0 ± 4.8†
3 hours	1.8 ± 4.5	6.3 ± 3.2	43.3 ± 15.0*	15.7 ± 6.4	32.7 ± 18.0	11.6 ± 6.8†
4 hours	5.7 ± 4.5	8.0 ± 1.7	75.8 ± 25.2*	23.5 ± 5.5§	43.8 ± 21.6	18.3 ± 9.1†

* $p < 0.05$ compared with Group 1 (Control).
† $p < 0.01$ compared with Group 3 (Ischemia alone).
See text for abbreviations.

injury often seen is an increase in capillary permeability, presumably due to endothelial cell disruption. This has been demonstrated by other investigators in the heart,[14] lung,[15] and intestine[16,17] and other organs. Previous work from our laboratory has demonstrated that capillary injury manifested as an increase in capillary permeability is an early common step in the pathogenesis of experimental acute pancreatitis in the isolated perfused canine pancreas preparation.[1,3,5,6] The current studies suggest that oxygen-derived free radicals play a primary role in this injury.

The isolated, perfused canine pancreas model was used for these studies. A great deal of experience has accumulated with this model.[1-6,18-21] It is known to remain stable for perfusion periods of up to 6 or 7 hours.[4] Weight gain, which is probably the most sensitive indicator of the stability of an isolated perfused organ, remains constant in control preparations. Previous clinical[22-24] data have suggested that elevated serum triglyceride levels play an important role in the pathogenesis of some cases of alcoholic pancreatitis. Experimental studies utilizing the isolated, perfused canine pancreas preparation have confirmed the ability of elevated serum tryglicerides to initiate pancreatitis.[2] The mechanism of injury is believed to be via free fatty acid release.[2] Infusion of oleic acid into the arterial line of the isolated pancreas over the first hour of perfusion leads to a marked and reproducible injury response. The preparations become edematous, gain weight, and develop hyperamylasemia. This model is used to simulate alcohol-induced hyperlipemic pancreatitis.

In recent years, two clinical studies have demonstrated what has been long suspected: that migration of a gallstone through the ampulla, causing partial obstruction of the pancreatic duct, is an important step in the pathogenesis of gallstone pancreatitis.[25,26] Since gallstone pancreatitis often follows ingestion of a heavy meal, stimulation of pancreatic secretion may also be an important etiologic factor. The combination of a stimulated pancreas and a partially obstructed ampulla is thought to result in acute pancreatitis. This clinical situation can be simulated in the isolated canine pancreas by partially obstructing the pancreatic duct with a small-diameter, high-resistance cannula, while maximally stimulating the gland with secretin.[3] This results in edema formation, weight gain, and marked hyperamylasemia and has served as a reproducible model for the study of gallstone pancreatitis. The ability of ischemia to initiate acute pancreatitis has been known for some time.[27-30] Ischemic pancreatitis can be simulated experimentally by subjecting the isolated canine pancreas to a 2-hour period of total ischemia. After reperfusion, the preparation becomes edematous, gains weight, and develops hyperamylasemia.

In all these models, the untreated preparations became edematous to gross inspection, gained significant weight, and developed a significantly elevated level of amylase in the perfusate, thus fulfilling our objective experimental criteria for acute pancreatitis. In all three models this injury response was significantly reduced when both free radical scavengers SOD and CAT were added together to the perfusate. Edema and weight gain were significantly less than in the corresponding untreated preparations. Serum amylase remained within the normal range in the FFA-induced and ischemic preparations and was significantly reduced in the POSS glands. These findings

TABLE 7. *Mean Serum Amylase Concentration (u/dl) in the Ischemic Pancreatitis Study Group*

Time	Group 1 Control	Group 2 SOD/CAT	Group 3 ISCH	Group 4 ISCH/SOD	Group 5 ISCH/CAT	Group 6 ISCH/SOD/CAT
0	548 ± 278	817 ± 27	765 ± 128	484 ± 209	606 ± 195	480 ± 196
1 hour	615 ± 202	625 ± 61	631 ± 271	535 ± 155	610 ± 312	328 ± 178
2 hours	637 ± 215	662 ± 94	1033 ± 404	610 ± 247†	734 ± 315	400 ± 55†
3 hours	688 ± 213	709 ± 125	1280 ± 461*	689 ± 218†	926 ± 532	533 ± 119†
4 hours	749 ± 180	816 ± 163	1679 ± 439*	838 ± 241†	1076 ± 603	555 ± 89†

* $p < 0.02$ compared with Group 1 (Controls).
† $p < 0.05$ compared with Group 3 (Untreated ischemic preparations).
See text for abbreviations.

indicate an important role for some form of oxygen-derived free radicals in the pathogenesis of pancreatitis.

In order to more specifically define which radicals are involved, the experiments were also conducted in all three preparations with each of the specific scavengers, SOD, and CAT, given individually. In the ischemic preparations, SOD alone provided a level of protection that was equal to the combination of the two agents, while CAT alone provided no significant protection. This suggests that in the pancreas, as in the intestine[16,17] and the heart,[31] reperfusion following ischemia produces tissue injury primarily by means of the superoxide radical. Any toxic radical species produced by other means must play a relatively minor role, if any, in the process. In the intestine,[17] the superoxide radicals are generated from the reaction between the substrate hypoxanthine, which accumulates in the absence of oxygen from the breakdown of ATP, and the enzyme xanthine oxidase, which is activated by ischemia. When reperfusion is initiated, the reduction of hypoxanthine to xanthine by xanthine oxidase proceeds rapidly, and an excess of highly toxic superoxide radicals are produced. The production of the superoxide radical from xanthine oxidase by reperfusion following ischemia is one of the best understood mechanisms of free radical injury. Because the experiments reported here were not designed to define the source of the free radical generation, it is not possible to know the role of xanthine oxidase in their production in these preparations. In all other ways, however, the isolated canine pancreas behaves like other organs that have been studied with respect to its response to ischemia/reperfusion.

It is somewhat more surprising to find that pancreatic injury initiated by stimuli other than ischemia also seems to be produced by a free radical process. A possible mechanism for FFA injury to the pancreas is the peroxidation of structural membrane lipids by these acids themselves. However, the results of the current studies suggest either that oxygen-derived free radicals act as intermediates in this process, or that the free radical scavengers used may be able to act by scavenging the FFAs directly. This latter possibility seems quite unlikely, as both SOD and CAT are highly specific enzymes that are thought to have very specific, hence limited functions (Fig. 1). Indeed, it is the presumption of that specificity that is the basis for most of our knowledge about the role of free radicals in tissue injury: it is not possible, at present, to measure these toxic radical species directly, and we must rely upon the ablation of their effects by specific scavengers to define their roles.[12]

Free radical scavengers appear to play a similar intermediary role in the development of pancreatitis secondary to secretin stimulation against a partial duct obstruction, simulating gallstone pancreatitis. As with the injury produced by FFA, however, the precise role of each radical is less clear. In both of these latter preparations, neither scavenger, administered alone, provided significant protection, while both together markedly decreased injury. This suggests that, unlike the situation created by ischemia/reperfusion, both O_2^- and $OH^\cdot$ appear to play a role in the injury. Since free radicals often act by initiating a cascade of reactions, each producing a new radical in turn by adding the extra electron to the next molecule, they may therefore produce a whole series of radicals other than those primary radical species cited above.[16] It is therefore possible that both O_2^- and $OH^\cdot$ may be produced by a pathway or pathways other than that shown in Figure 1, and that either species alone is sufficient to create a maximal injury.

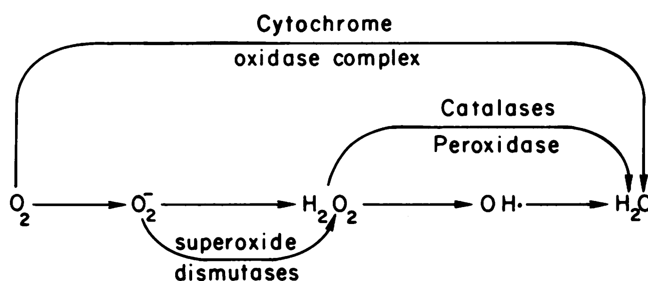

FIG. 1. Under normal conditions most molecular oxygen is reduced by intracellular systems such as the cytochrome oxidase complex. However, even under normal conditions, a small amount of oxygen leaks from this pathway to undergo univalent reduction, during which minimal amounts of toxic-free radicals are produced. These intermediates are usually detoxified by endogenous free radical scavengers such as intracellular superoxide dismutase and catalase.

Despite these possible differences in the sources of free radical production, the most significant finding of this study is that all three forms of experimental pancreatitis appear to be produced through some form of free radical intermediary. It seems likely in light of our previous studies,[1,3,5,6] that these agents act at least in part by injuring endothelial cells, resulting in an increase in capillary permeability. Therefore, acute pancreatitis, although it can be initiated by widely disparate stimuli, develops by means of a final common pathway, a pathway in which oxygen derived free radicals play a central role. This finding not only helps elucidate the pathogenesis of this common disease, but provides a significant potential for therapeutic exploitation in the future.

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Discussion

Dr. R. Scott Jones (Charlottesville, Virginia): First I want to compliment these workers on an excellent experimental study. This is one of several outstanding experiments from Dr. Cameron and his associates over the last few years that have provided new insights into the etiology and pathogenesis of acute pancreatitis.

I have a few comments and basically want to ask a couple of questions to elicit further discussion.

The first question is to ask if the discussants would mention and describe both the similarities as well as the differences that occur in the lipids in the perfusate, as contrasted to clinical hyperlipidemia. In other words, how is infusion of oleic acid similar to clinical hyperlipidemia, and how does it differ from clinical hyperlipidemia?

The other point that I think would merit discussion is whether the infused enzymes produce their changes, their improvement in the pancreatitis, by the mechanism that is assumed. Is there any evidence that infusion of catalase or superoxide dismutase truly produces alterations in the free radicals in the tissue? I know that these are questions that the investigators have given considerable thought to, and I would be interested in their discussion.

This is an excellent study. I think that the observations on the role of lipids and the other mechanisms in the pathogenesis and etiology of pancreatitis are truly important hypotheses to be pursued further.

Dr. Charles F. Frey (Sacramento, California): The authors have found a common mechanism of injury among three forms of experimental pancreatitis. They found the pancreatic injuries to be similar and mediated by one or more free radicals. The result of these agents' action is endothelial cell injury and increased capillary permeability, as measured by edema and increased weight of the pancreas. Recognition that there is a common mechanism and pathway of injury among these three diverse causes of pancreatitis is, I believe, of fundamental importance. This report provides hope that the enzymes superoxide dismutase, catalase, and peroxidase may have a therapeutic role in abating pancreatitis.

The authors are to be complimented for their well-conceived and -executed experiment, and the compelling logic in the interpretation of their experimental data. As with any good piece of work, more questions are raised than answered. Some of these questions I would like the authors to address.

How generally applicable is this free radical injury mechanism in the initiation of acute pancreatic injury, other than in the three examples studied? For instance, hyperlipidemia, as a cause of pancreatitis in alcoholics, might account for about 20% of the total patients. Do you feel free radicals have a role in the development of other forms of alcoholic-initiated pancreatitis, aside from those associated with hyperlipoproteinemia? An if so, what is the mechanism?

My second question relates to the role of these free radicals in hemorrhagic pancreatitis. Dr. Bulkley, one of the co-authors of this report, suggested that in the small bowel the free radicals may play an important role in altering capillary permeability and producing mucosal lesions from ischemic injury following infusion of hypoxanthine/xanthine oxidase. He reported these mucosal lesions can be attenuated by superoxidase, superoxide dismutase, or DMSO. However, these same agents, superoxide dismutase, DMSO, play virtually no role in tissue damage necrosis produced by total arterial occlusion in the small bowel. Can the authors tell us if we are justified in making any deductions from what happens in the small bowel that will have application to the pancreas? Specifically, do you know if superoxide dismutase, catalase, or peroxidase have any effect in ameliorating hemorrhagic pancreatitis?

If we can apply the findings from the small bowel, they would not. This is important, because most mortality of acute pancreatitis is