Brief report

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Preservation-induced pancreatitis in an isolated perfused pancreas model in the dog

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Abstract. In order to investigate pancreatitis caused by cold ischemic damage to pancreatic grafts, an isolated, normothermic, ex vivo perfusion model was employed. Canine pancreases were subjected to 24 and 48 h of cold ischemia and then reperfused. The results showed that cold ischemia results in pancreatitis as measured by weight gain (tissue edema) and elevated leakage of amylase into the perfusate. The addition of allopurinol to the perfusion system did not prevent the signs of pancreatitis. From the results it can be concluded that the isolated, perfused pancreas model in the dog is useful for studying preservation-induced pancreatitis. The absence of any effect of allopurinol treatment suggests that oxygen-free radicals mediated by the xanthine oxidase system is of minor importance for the pathogenesis of postischemic pancreatitis.

Key words: Pancreas preservation - Preservation solution - Preservation-induced pancreatitis.

Pancreatitis is a common occurrence following pancreas transplantation. It can vary in severity from a mild to a fulminant disease, causing morbidity and even graft loss. The major cause is thought to be ischemic injury resulting from the period of preservation. One reason for limiting cold ischemia in clinical pancreas transplantation is to avoid postoperative pancreatitis.

Lundgren et al. [2] showed a highly significant correlation between serum amylase levels in pancreas graft recipients and preservation time. Similar findings have been reported by Tydén et al. [4]. The recent introduction of the University of Wisconsin (UW) preservation solution has extended the safe preservation time for the pancreas to 72 h in dogs [5]. In a clinical setting, pancreases have been stored for over 20 h and successfully transplanted. However, postoperative pancreatitis is still significant, and methods to prevent it would be a major advance in clinical pancreas transplantation.

Post-transplant pancreatitis is difficult to study in dog transplantation models, and in our earlier studies we were unable to demonstrate pancreatitis (elevated serum amylase) even with preservation times of 72 h. For this study, we adapted a method of normothermic, ex vivo, isolated perfusion of the dog pancreas in order to determine how preservation affects pancreatitis. This approach has been used extensively by Sanfey et al. [3]. In their studies, pancreatitis was induced by a variety of methods, including exposing the pancreas to 2 h of normothermic ischemia. After 2 h of ischemia and reperfusion the pancreas became edematous, and pancreatitis was assessed by the leakage of amylase into the perfusion fluid. In these studies, pancreatitis induced by warm ischemia could, in essence, be eliminated by the addition of allopurinol to the perfusion fluid. It was concluded that oxygen-free

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radical damage played a major role in the pathogenesis of acute pancreatitis caused by warm ischemia.

The purpose of the present study was to investigate the relationship between duration of preservation time and pancreatitis using blood-free, isolated reperfusion of the pancreas. We also investigated the effects of xanthine oxidase inhibition on preservation-induced pancreatitis by adding allopurinol to the perfusate.

Materials and methods

The left segment of the pancreas was harvested from adult mongrel dogs (15-25 kg body wt.) as previously described [3]. After removal the grafts were flushed with 200-300 ml cold (0°-4°C) UW solution [6] at a pressure of about 70 cm water. (In these experiments the UW solution did not contain allopurinol.) The pancreatic duct was cannulated with a plastic tube and the cut surface of the pancreatic segment tied off to avoid leakage of perfusate and pancreatic juices. Freshly isolated pancreases (controls) were immediately connected to the isolated perfusion system and evaluated. Pancreases were also cold stored (5°C) for 24 or 48 h.

The ex vivo isolated perfusion system is similar to that described by Sanfey et al. [3] and consisted of a roller pump set to deliver the perfusate at a rate of 1 ml/min per gram of tissue (at a pressure of about 30 mm Hg) to the pancreas. The perfusate (final volume 350 ml) was initially passed through a heat exchanger (37°C) and membrane oxygenator (95% O_2/5% CO_2, pO_2 = 350-450 mm Hg) prior to entering the pancreas. The perfusate was recirculated and was similar in composition to that described by Iversen et al. [1] with dextran (5 g%) as a colloid. Perfusion was through the splenic artery and the portal vein was cannulated. The venous outflow was collected in the reservoir. The pancreatic juice from the cannulated pancreatic duct was diverted to outside the system and collected in tubes. Perfusion was for 2 h and amylase in the perfusate was determined after 1 and 2 h of perfusion using an amylase kit (Amylase Phadebase kit, Pharmacia, Uppsala, Sweden). Pancreatic edema was determined by comparing the weight of the organ prior to isolated perfusion and following 2 h of perfusion.

The following groups of pancreases were studied:

Group 1 (controls; $n = 6$) Grafts were freshly harvested and perfused immediately after flushout

Group 2A ($n = 4$) Grafts were preserved for 24 h and reperfused without allopurinol in the reperfusion medium

Group 2B ($n = 6$) The same as for group 2A but with allopurinol (250 mg/l) added to the reperfusion medium

Group 3A ($n = 4$) The same as for group 2A but with 48 h of preservation

Group 3B ($n = 6$) The same as for group 3A but with allopurinol added to the reperfusion medium.

Statistical comparisons between groups were done using Student's *t*-test.

Results

Control pancreases gained, on the average, 35 ± 7 % during 2 h of isolated perfusion (Table 1). Pancreases stored for 24 or 48 h gained significantly more weight (76 ± 13 % and 81 ± 9 %, respectively; $P < 0.05$), and the weight gain was not affected by the presence of allopurinol in the perfusion fluid. This result indicates that our experimental model demonstrated one sign of pancreatitis - namely, tissue edema - that was not affected by allopurinol-induced suppression of oxygen-free radical production.

Table 1. Percent weight gain of pancreatic grafts cold stored (c.s.) for 24 or 48 h after 2 h of isolated normothermic perfusion with or without allopurinol in the perfusate. * $P < 0.05$ compared with cold-stored pancreatic grafts

	Without allopurinol	With allopurinol
Control	35 ± 7*	–
24 h c.s.	61 ± 8	76 ± 13
48 h c.s.	80 ± 8	81 ± 9

Table 2. Amylase concentration (IU/dl) in the perfusate after 1 and 2 h of isolated normothermic perfusion with or without allopurinol in the perfusate of pancreatic grafts cold stored (c.s.) for 24 or 48 h. * $P < 0.05$ compared with cold-stored pancreatic grafts

	Without allopurinol	With allopurinol
Control - 1 h perfusion	1358 ± 258*	–
Control - 2 h perfusion	2215 ± 330	–
24 h c.s. - 1 h perfusion	1826 ± 227	2419 ± 312
24 h c.s. - 2 h perfusion	4373 ± 622	4130 ± 665
48 h c.s. - 1 h perfusion	4447 ± 821	4050 ± 1136
48 h c.s. - 2 h perfusion	6260 ± 1120	5420 ± 1181

Another parameter of pancreatitis is the accumulation of amylase in the perfusate. In the control group, amylase activity reached 1358 ± 258 IU/dl after 1 h of perfusion. A significant increase in perfusate amylase activity occurred in pancreases stored for 24 or 48 h (1826 ± 227 IU/dl and 4447 ± 821 IU/dl, respectively; $P < 0.05$) and perfused for 1 h. After 2 h of perfusion (Table 2), control amylase increased to 2215 ± 330 IU/dl. In preserved pancreases, 2 h of reperfusion induced a further increase in perfusate amylase to 4373 ± 622 IU/dl and 6260 ± 1120 IU/dl after 24 and 48 h of cold storage, respectively. Allopurinol had no significant effect on the amount of amylase released from the preserved pancreases.

Discussion

These results demonstrate that cold storage of the pancreas results in pancreatitis as measured by weight gain of the organ (tissue edema) and elevated leakage of amylase into the perfusion fluid. Although allopurinol has been shown to be effective in suppressing the development of pancreatitis in

the warm ischemic model, it does not appear to be effective in the cold ischemic (preservation) model. This suggests that the generation of oxygen-free radicals mediated by the xanthine oxidase enzyme system is not a factor in pancreatitis induced by cold ischemia. However, oxygen-free, radical-mediated damage following reperfusion of a cold-preserved pancreas could occur through other mechanisms, including mitochondrial generation of oxygen-free radicals.

In conclusion, the isolated, perfused pancreas model in the dog provides a simple method for studying how preservation conditions affect the function of the organ. Using this model, methods for suppressing pancreatitis and techniques that effectively reduce reperfusion-induced edema and leakage of amylase can be tested both in the transplant model and in the clinical situation. Such methods would significantly improve pancreas transplantation in the diabetic patient.

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