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Abdominal organ cluster transplantation in pigs and FK506*

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Abstract. Using a swine abdominal organ cluster transplantation model, we investigated the postoperative function and immunological reactions of a cluster graft and evaluated the immunosuppressive activity of FK506. The animals were divided into two groups. Group I ($n = 6$) served as controls, while in group II ($n = 6$) a daily dose of 0.1 mg/kg FK506 was given intramuscularly. Postoperative pancreatitis was the most important factor influencing the early outcome in both groups. In group I, the cause of late death was cachexia due to diabetes mellitus induced by pancreatic rejection. In group II, emaciation despite a well-functioning graft was the principal cause of late death. Histologically, in group I the grade of rejection in the pancreas was more severe than in the liver, and no sign of rejection was observed in group II. In conclusion, the pancreas suffered more severe rejection than the liver, and FK506 could significantly prevent cluster allograft rejection in this model.

Key words: Abdominal organ cluster transplantation – Postoperative pancreatitis – FK506 – Diabetes mellitus – pancreatic rejection

Since 1988, abdominal organ cluster transplantation (AOCTX) has been introduced into surgical practice to treat malignant tumors of the biliary tract, pancreas, or duodenum with secondary involvement of the liver [7]. AOCTX has created new surgical, physiological, and immunological problems which were not usually seen in the transplantation of a single organ. It is clear that the future development of AOCTX will depend upon more specific and less toxic forms of immunosuppression. FK506 (FK) is a recently developed agent with potent immunosuppressive activity, as shown by in vitro and vivo studies [2, 5]. In this study, we characterized the postoperative function and immunological reactions of a cluster graft and also evaluated the immunosuppressive activity of FK in swine AOCTX.

Materials and methods

Dulock-Jersy pigs and Large-White pigs weighing 20–25 kg were used as the donors and recipients, respectively.

Operative procedure. The transplantation procedure has been described in detail previously [3]. Briefly, the recipients underwent en bloc removal of the liver, pancreas, nearly all of the stomach, duodenum, and spleen under general anesthesia. The void upper abdomen was filled with an en bloc cluster graft consisting of the liver, pancreas, duodenum, spleen, and aortic conduit under venovenous bypass. Gastrointestinal reconstruction was performed by interposition of the duodenal graft between the recipient stomach and jejunum. Harvesting and transplantation procedures are shown in Fig. 1.

Experimental groups. Experimental animals were divided into two groups. Group I ($n = 6$) served as controls and underwent no special treatment. In group II ($n = 6$), a daily intramuscular injection of 0.1 mg/kg FK was commenced from the immediate postoperative period.

Biochemical and histopathological studies. Blood chemistry tests were performed regularly to monitor the function of the transplanted graft, including determinations of the serum total bilirubin (T. Bil), serum glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (ALP), serum amylase (sAMY), and blood glucose. An autopsy was performed in each animal, and the cluster graft as well as other organs were histologically examined to determine the cause of death. Tissues were fixed with formalin and stained with H&E.

Statistical analysis. Differences between the mean values were assessed for significance by Student's unpaired t-test.

Results

As shown in Table 1, survival times ranged from 7 to 42 days in group I and from 7 to 112 days in group II. The

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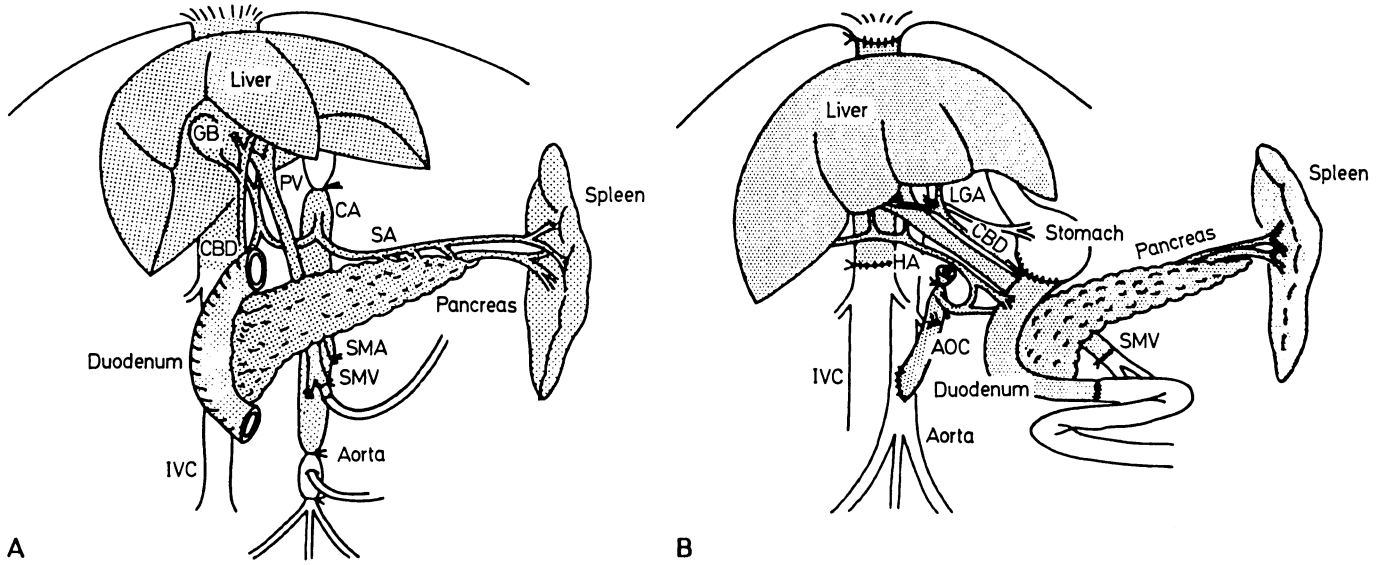

Fig. 1 A, B. The procedure for swine abdominal organ cluster transplantation (AOCTX). **A** Harvesting procedure, **B** transplantation procedure. The *shaded area* indicates the cluster graft. *AOC*, aortic conduit; *CA*, celiac artery; *CBD*, common bile duct; *HA*, hepatic artery; *IVC*, inferior vena cava; *LGA*, left gastric artery; *PV*, portal vein; *RRV*, right renal vein; *SA*, splenic artery; *SMA*, superior mesenteric artery; *SMV*, superior mesenteric vein

causes of early death in both groups were severe pancreatitis and complications following gastrointestinal reconstruction. In group I, the cause of late death was cachexia due to diabetes mellitus induced by pancreatic allograft rejection, and emaciation despite a well-functioning graft was the principal cause in group II.

In group I, in all animals, the T. Bil content increased rapidly on about day 4 after surgery, peaked on day 7 or 8, and gradually decreased thereafter. Furthermore, the GOT and ALP levels began to rise on about day 5. The sAMY level began to increase on day 6, while the blood glucose level began to rise on about day 10, and hyperglycemia persisted until death. In group II, the T. Bil value remained below 1.0 mg/dl throughout the postoperative course, and mild hyperglycemia was observed in only 1 pig (Fig. 2). In group I, the mean T. Bil level on day 7 was significantly higher than that in group II (3.55 ± 1.19 mg/dl vs. 0.32 ± 0.18 mg/dl; $P < 0.01$), and the mean blood glucose level on day 10 was significantly higher than in group II (317 ± 32 mg/dl vs. 176 ± 73 mg/dl; $P < 0.02$). Histopathologically, in group I the grade of rejection in the pancreas was more severe than in the liver (Fig. 3). No clinical sign of rejection was observed, and the reduction in histological evidence of rejection was dramatic in group II, as shown in Fig. 4. GVH reaction was not observed in any case.

Table 1. Summary of 12 pigs that survived for 7 days or more after AOCTX (mean ± SD)

Pig number	AHT (min)	TIT (min)	OPT (h)	Survival time (days)	Cause of death[a]
Group I (control group)					
1	41	101	5.5	7	Pancreatitis
2	36	93	5.8	10	Sacrificed
3	40	88	4.9	13	Pancreatitis
4	35	76	5.1	14	Pancreatitis/necrosis of the duodenal graft
5	43	107	5.9	41	Diabetes mellitus (rejection)
6	37	85	5.4	42	Diabetes mellitus (rejection)
Total	38.7 ± 3.1[b]	91.7 ± 11.2[b]	5.4 ± 0.4[b]		
Group II (FK506-treated group)					
1	42	101	5.4	7	Sacrificed
2	34	79	4.8	10	Sacrificed
3	38	89	5.1	10	Pancreatitis
4	40	102	5.5	10	Necrosis of the residual stomach
5	37	86	5.3	16	Pancreatitis
6	41	92	4.9	112	Emaciation
Total	38.6 ± 2.9[b]	91.5 ± 8.9[b]	5.2 ± 0.3[b]		

AHT, anhepatic time; TIT, total ischemic time; OPT, operation time

[a] Five out of 12 pigs (42%) died due to severe pancreatitis or pancreatic necrosis

[b] Group I vs. II, not significant

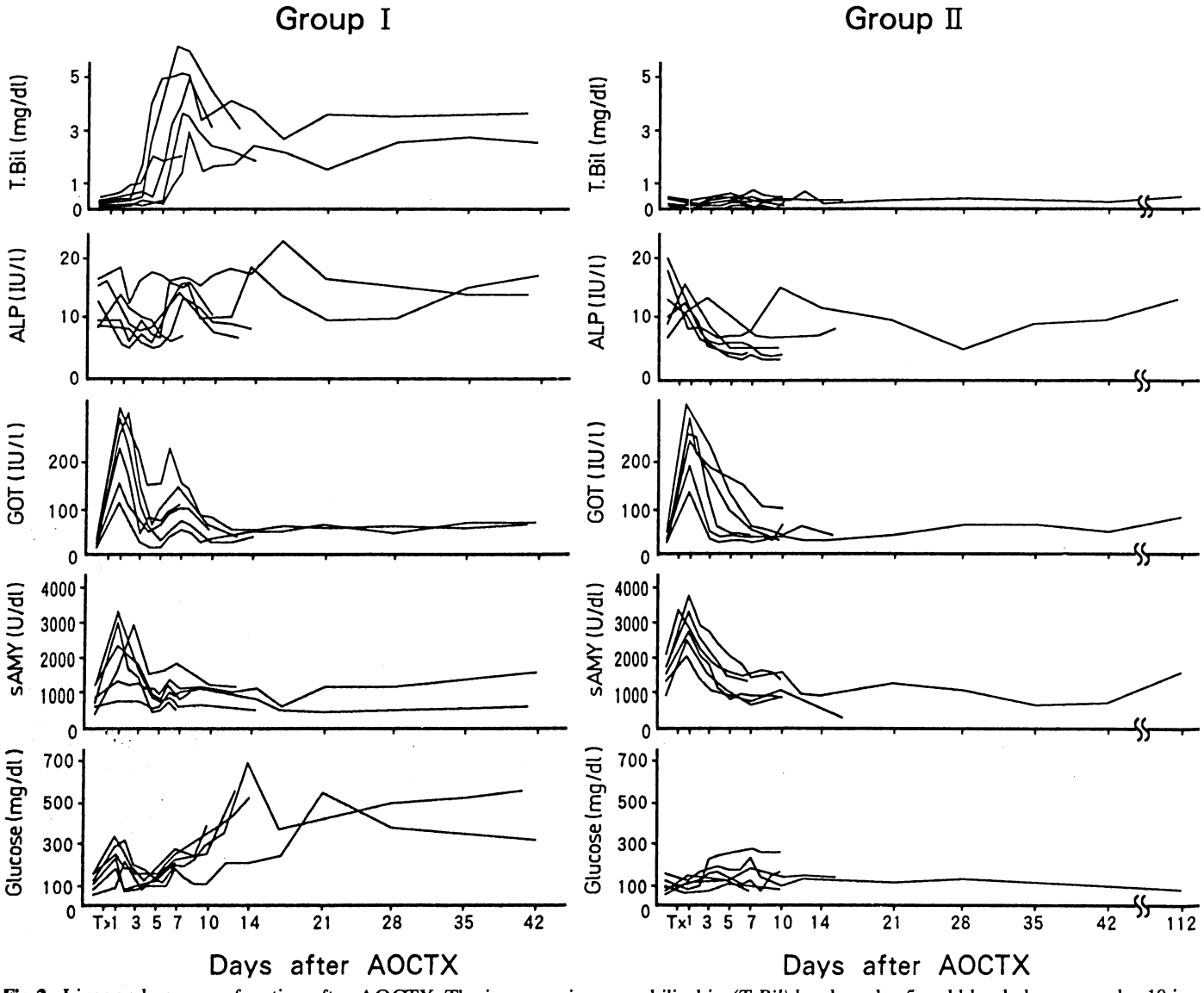

Fig. 2. Liver and pancreas function after AOCTX. The increases in serum bilirubin *(T. Bil)* level on day 5 and blood glucose on day 10 in group I were not observed in group II. *ALP,* alkaline phosphatase; *sAMY,* serum amylase; *GOT,* glutamic oxaloacetic transaminase

Discussion

In the postoperative course in our swine model, acute rejection could be detected in both the liver and pancreas. Acute rejection of the liver seemed mild and subsided without immunosuppressants and was equivalent to transient rejection.

However, acute rejection of the pancreas progressed to graft deterioration. The cause of late death was not hepatic failure but cachexia due to diabetes mellitus induced by pancreatic rejection. These findings suggest that there is a difference in severity between acute rejection of the liver and that of the pancreas. This may be explained by the fact that liver grafts are protected from rejection in comparison with grafts of other organs [1].

Postoperative pancreatitis was the most important factor influencing the early outcome of our swine AOCTX model as well as clinical AOCTX [8]. The prevention of postoperative pancreatitis seems to be extremely important for long-term AOCTX survival. Since marked morbidity and mortality are associated with the transplanted pancreas, Tzakis et al. have proposed a modified cluster procedure with resection of the pancreas and intrahepatic islet allotransplantation. According to their report, all 10 patients demonstrated significant C-peptide production, and prolonged insulin independence was observed in 6 cases [9]. Recently, our group developed a new split-cluster transplantation technique in which the hepatic graft was located orthotopically and a pancreaticoduodenal graft with the spleen was transplanted auxiliarly with urinary bladder drainage to prevent lethal postoperative pancreatitis and to monitor exocrine pancreatic secretions [6].

There are some variations in the reconstruction procedure of the gastrointestinal tract in clinical AOCTX [4, 7]. In cases in which the proximal stomach was saved with an intact left gastric arterial supply, the duodenum was placed in continuity with the gastrointestinal tract so that ingested food passed through the duodenal graft. In this model, we used this simple physiological procedure for ga-

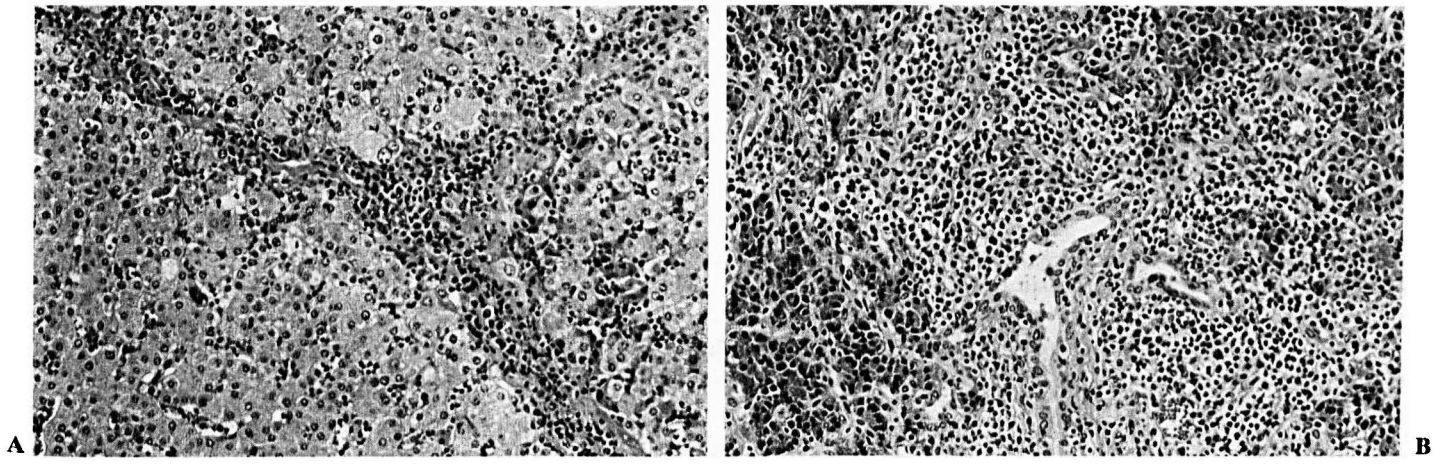

Fig. 3. **A** Liver and **B** pancreas from pig no. 6 (group I) on day 42 after AOCTX. The pancreas suffered more severe rejection than the liver

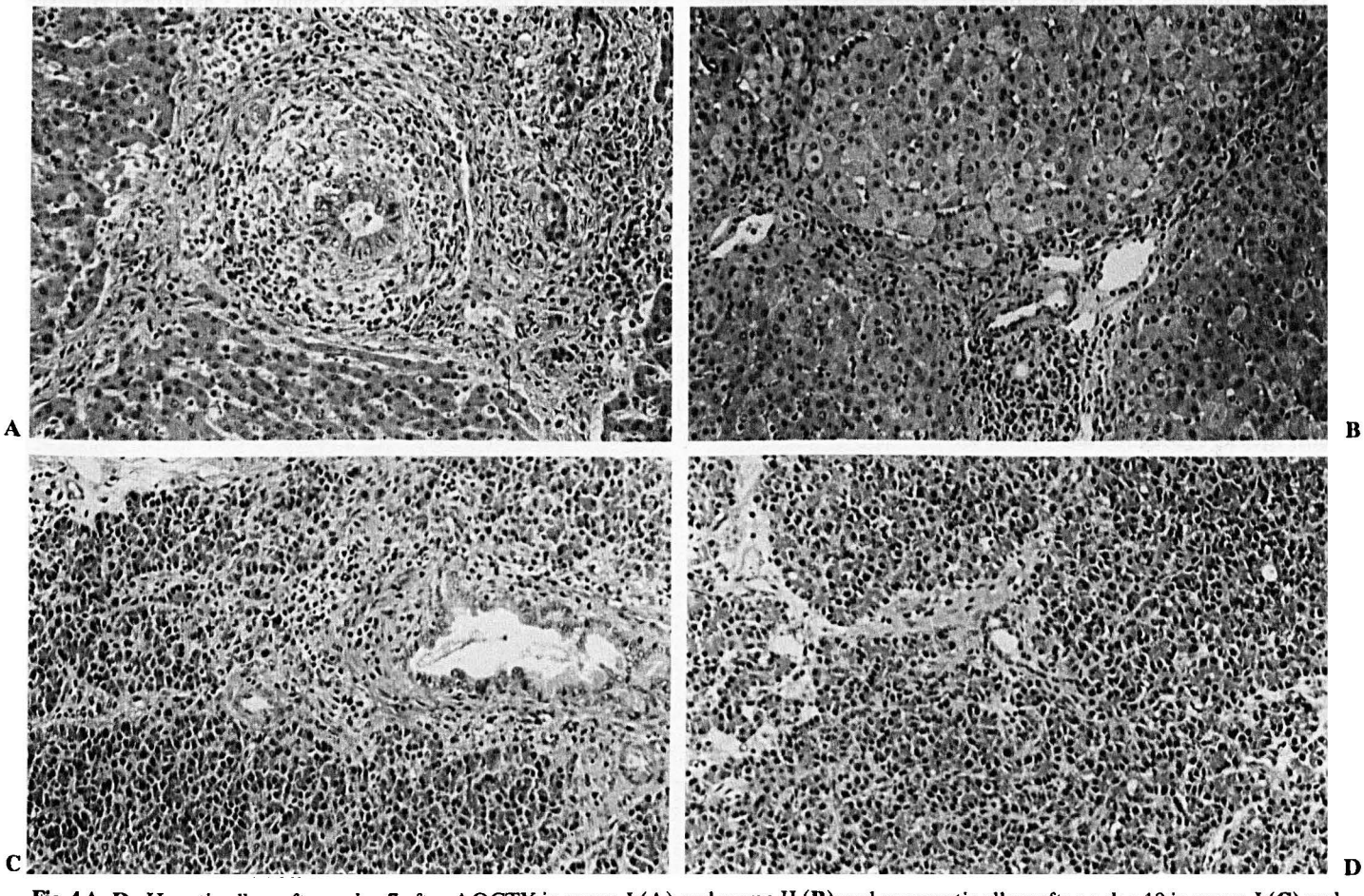

Fig. 4 A–D. Hepatic allografts on day 7 after AOCTX in group I (**A**) and group II (**B**) and pancreatic allografts on day 10 in group I (**C**) and group II (**D**). In group I, each organ exhibited marked rejection in contrast with almost normal organs in group II

strointestinal reconstruction. However, the other cause of early death involved complications following gastrointestinal reconstruction, such as necrosis of the residual stomach.

This study clearly shows that FK is a potent immunosuppressive agent in swine AOCTX. Rejection of the transplanted cluster graft was effectively suppressed by daily intramuscular administration of 0.1 mg/kg FK. There are several reports concerning the major side effects of FK, such as nephrotoxicity and diabetogenesis. In this study, mild hyperglycemia due to generalized peritonitis was observed in only 1 pig, and the other animals were normoglycemic when administered with FK. Histologically, almost normal structures in pancreatic allografts were maintained in FK-treated pigs. According to the postmortem examinations, no side effects were observed except for emaciation, which may have been induced by long-term posttransplant administration of FK.

In conclusion, this study suggests that the pancreas suffers more severe rejection than the liver and that the prevention of postoperative pancreatitis is extremely important to obtain long-term survival in the swine AOCTX model. Daily intramuscular administration of 0.1 mg/kg FK can significantly prevent cluster allograft rejection.

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