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CORRELATION BETWEEN BLOOD FLOW AND PROSTAGLANDIN PRODUCTION IN SEGMENTAL PANCREATIC AUTOGRAFT —SPECIAL REFERENCE TO THEIR INFLUENCE TO VASCULAR THROMBOSIS AFTER TRANSPLANTATION

(segmental pancreatic transplantation／preservation time／vascular thrombosis／prostaglandin／blood flow)

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This experiment was aimed to investigate the changes in the blood flow (splenic arterial blood flow; S.B.F and pancreatic tissue blood flow; P.T.B.F) and the changes of production of prostaglandins (prostacyclin and thromboxane A_2) in canine segmental pancreatic graft before and after transplantation. Furthermore, we investigated correlation between the blood flow of the transplanted pancreas (S.B.F and P.T.B.F) and the changes of production of these two prostaglandins in it according to preservation time after transplantation.

This experiment consisted of 17 dogs divided into two groups; short-term (3 hours) preserved group (group I, N = 12), long-term (24 hours) preserved group (group II, N = 5). In group I, after transplantation, both S.B.F and P.T.B.F increased significantly with enhanced production of prostacyclin. While, in group II, they decresed significantly with enhanced production of thromboxane A_2 and reduced production of prostacyclin. Graft function was monitered by daily fasting blood glucose level in each 5 dogs of both group. Four dogs in group I and only one dog in group II survived with functioning grafts for two weeks. But in group I, one graft was lost at 4 days from vascular thrombosis.

On the other hand, in group II, two grafts were subsequently lost at 1 and 8 days from vascular thrombosis and the other two grafts were never functioning after transplantation.

These results suggested that there was a significant correlation between the productive system of prostacyclin & thromboxane A_2 and the blood flow of the transplanted pancreas in canine segmental pancreatic transplantation, and enhanced production of thromboxane A_2 might be responsible for vascular thrombosis after transplatation. For this reason, good function of the graft after segmental pancreatic transplantation may be possible by inhibiting the production of thromboxane A_2, even if preservation time is long.

Segmental pancreatic transplantation has been recommended in selected patient with type I diabetes by the majority of surgeones in an attempt to normalize metabolism sufficiently to halt the progression of secondary complications of diabetes. But clinical results of this operation are less ideal for several reasons. Especially vascular thrombosis is one of the major problems which lower graft success rates (1,2). And it has been adovocated that vascular thrombosis after segmental pancreatic transplantation had a significant connection with the inadequate or reduced blood flow of the transplanted pancreas after splenectomy (1,3). While it has been demonstrated that, in several organs such as kidney, liver and heart, ischemia of these organs provoked the synthesis of the vasodilating prostaglandins, prostacyclin (PGI_2) and the vasoconstrictive prostaglandin, thromboxane A_2 (TxA_2), and imbalance of prostacyclin - thromboxane system might play an important role in vascular thrombosis after ischemia (4 - 6). It is likely possible that, in segmental pancreatic transplantation, these two prostaglandins are produced in the transplanted pancreas after revascularization and productive system of these two prostaglandins influence vascular thrombosis after transplantation.

So this study was aimed to investigate experimentally

the changes of production of these two prostaglandins in the transplanted pancreas and its blood flow after revascularization according to preservation time, and correlation between these two factors and graft success rate in canine segmental pancreatic autograft.

MATERIAL AND METHODS

Seventeen mongrel dogs of either sex (7 - 20 kg) were anesthetized with sodium pentobarbitone (25 mg/kg) intravenously and then intubated. A cannula was passed into the left femoral artery to record blood pressure and heart rate continously. In all dogs, the pancreas was exposed through a midline skin incision, and the left limb of the pancreas was isolated with splenic vessels in preparation for grafting. And two catheters (7Fr) were inserted into the splenic artery and vein through their distal branches in order to collect blood sample (5ml) to assay 6 - keto - prostaglandin $F_1\alpha$ (6keto - $PGF_1\alpha$), the stable hydrolysis product of PGI_2, and thromboxane B_2 (TxB_2), the stable hydrolysis of TxA_2, by radioimmunoassay. As regards prostaglandins, based on the levels of 6keto - $PGF_1\alpha$ and TxB_2 in the splenic artery and vein, we calculated the total amount of these two prostaglandins (Σ 6keto - $PGF_1\alpha$, Σ TxB_2) produced in the pancreatic segment for one minute per one gram by the calculative formula as follows:

$\underline{\Sigma \text{ 6keto} - PGF_1\alpha \text{ or } \Sigma\, TxB_2}$ = (the level of 6keto - $PGF_1\alpha$ or TxB_2 in the splenic vein - the level of 6keto - $PGF_1\alpha$ or TxB_2 in the splenic artery) × splenic arterial blood flow (S.B.F) ÷ weight of pancreatic segment (unit: pg/min/g).

And its ratio (Σ 6keto - $PGF_1\alpha$ / Σ TxB_2) was also calculated. A splenectomy was performed. After these procedures were performed, splenic arterial blood flow (S.B.F) was assesed with an electromagnetic flowmeter using probe 2, 2.5 or 3 mm in diameter around the origin of the splenic artery, and pancreatic tissue blood flow (P.T.B.F) was measured by hydrogen gas clearance method (7). The graft was then flushed out with 50

-60ml of cold Euro-Collins' (E-C) solution. The graft was resected and measured its weight (g). And it was immediately placed in a beaker with 200 ml of cold E-C solution and stored in a refrigerator. The remainder of the pancreas was removed, preserving the blood supply to the duodenum.

We divided all dogs into two groups according to preservation time. In group I (N=12), the graft was preserved with cold E-C solution for 3hr. In group II (N=5), it was preserved with the same solution for 24hr. After preservation all dogs were anesthetized again, and stored pancreatic grafts were autotransplanted heterotopically to the right iliac fossa with vessels' anastomosis. The main pancreatic duct was left freely into the peritoneal cavity. Immediately after transplantation, S.B.F and P.T.B.F of the transplanted pancreas were measured, and Σ 6keto-$PGF_1\alpha$ and ΣTxB_2 were calculated.

All dogs received intravenous administration of Lactate Ringers' solution (1,000mg/day) and antibiotics for 3 postoperative days and thereafter allowed to drink and eat ad libitum. No anticoagulant was used postoperatively. Graft function was monitered daily by measuring plasma glucose level in each 5 dogs of both group for 14 postoperative days. And when plasma glucose level was more than 200 mg/dl for 2 consecutive days, it was suggested that the graft failure was developed and we sacrificed the dog in order to examine the exact cause of the graft failure pathohistologically. In group I, we measured S.B.F in all 12 dogs and P.T.B.F in 9 dogs, and calculated Σ 6keto-$PGF_1\alpha$ & ΣTxB_2 in 7 dogs. In group II, we measured and calculated them in all 5 dogs.

Statistical significance was determined by Student' t test and values are shown by the mean ± SEM.

RESULTS

1) Splenic arterial blood flow (S.B.F) and pancreatic tissue blood flow (P.T.B.F) before and after transplantation (Fig 1, Fig 2)

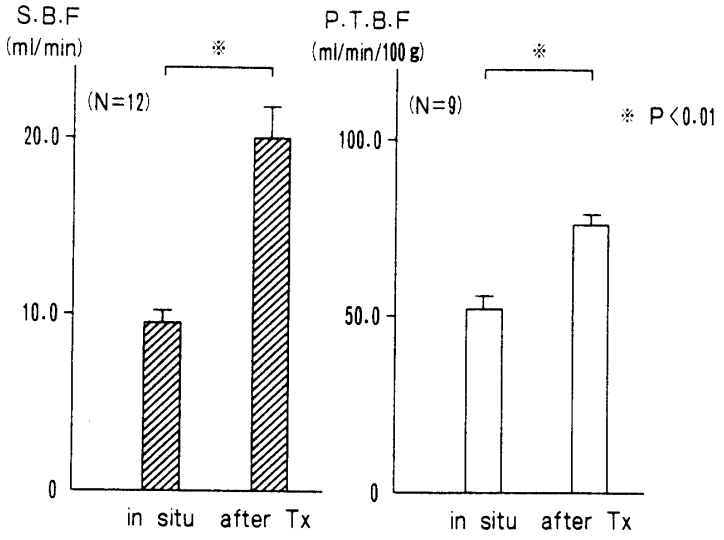

Fig 1 : Splenic arterial blood flow (S.B.F) and pancreatic tissue blood flow (P.T.B.F) before transplantation (in situ) and after transplantation (after Tx) in group I. Values are expressed as Mean ± SEM.

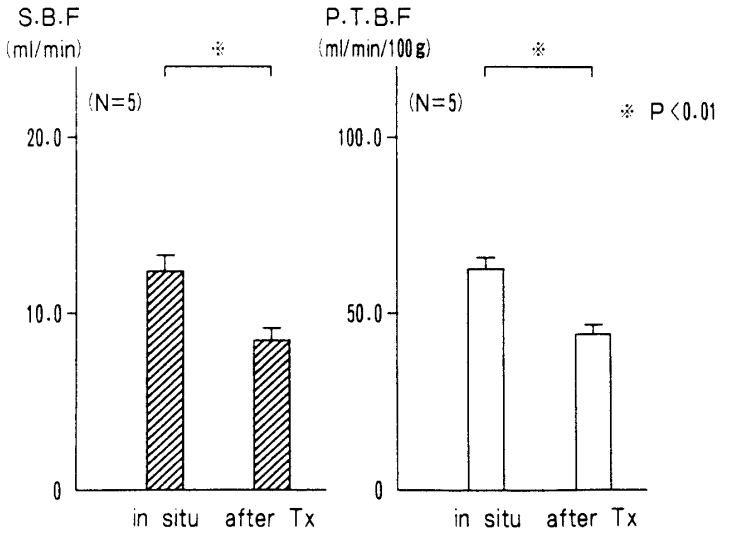

Fig 2 : S.B.F and P.T.B.F in situ and after Tx in group II. Values are expressed as Mean ± SEM.

In group I, S.B.F before and after transplantation were 9.58 ± 0.49 ml/min and 20.00 ± 2.64 ml/min respectively and S.B.F increased significantly ($P < 0.05$) after transplantation. P.T.B.F before and after transplantation were 54.01 ± 3.05 ml/min/100g and 75.69 ± 2.64 ml/min/100g respectively and P.T.B.F increased significantly ($P <$

0.01) after transplantation.

In group II, S.B.F before and after transplantation were 12.04 ± 0.96 ml/min and 8.04 ± 0.73 ml/min respectively and S.B.F decreased significantly ($P < 0.05$) after transplantation. P.T.B.F before and after transplantation were 62.30 ± 2.72 ml/min/100g and 43.08 ± 2.65 ml/min/100g respectively and P.T.B.F decreased significantly ($P < 0.01$) after transplantation.

2) Σ 6keto - $PGF_1\alpha$ & Σ TxB_2, and Σ 6keto - $PGF_1\alpha$ / Σ TxB_2 ratio before and after transplantation (Fig 3, Fig 4)

In group I, Σ 6keto - $PGF_1\alpha$ & Σ TxB_2 before transplantation were 532.3 ± 107.8 pg/min/g and 273.4 ± 58.8 pg/min/g respectively. After transplantation Σ 6keto - $PGF_1\alpha$ and Σ TxB_2 were 3484.7 ± 384.7 pg/min/g and 668.7 ± 97.5 pg/min/g respectively. Both Σ 6keto - $PGF_1\alpha$ and Σ TxB_2 increased significantly ($P < 0.05$) after transplantation. Furthermore, Σ 6keto - $PGF_1\alpha$ / Σ TxB_2 ratio before and after transplantation were 2.45 ± 0.44 and 6.23 ± 1.77 respectively and the ratio increased significantly ($P < 0.05$) after transplantation.

In group II, Σ 6keto - $PGF_1\alpha$ & Σ TxB_2 before transplantation were 862.3 ± 60.0 pg/min/g and 273.5 ± 32.2 pg/min/g respectively. After transplantation Σ 6keto - $PGF_1\alpha$ and Σ TxB_2 were 842.2 ± 165.5 pg/min/g and 831.4 ± 319.9 pg/min/g respectively. Σ 6keto - $PGF_1\alpha$ after transplantation was not changed compared to that before transplantation. On the other hand, Σ TxB_2 increased significantly ($P < 0.01$) after transplantation. And Σ 6keto - $PGF_1\alpha$ / Σ TxB_2 ratio before and after transplantation were 3.31 ± 0.30 and 1.67 ± 0.37 respectively and the ratio decreased significantly ($P < 0.01$) after transplantation.

3) Correlation between S.B.F & P.T.B.F and Σ 6keto - $PGF_1\alpha$ / Σ TxB_2 ratio

Fig 5 and Fig 6 show the plottings of S.B.

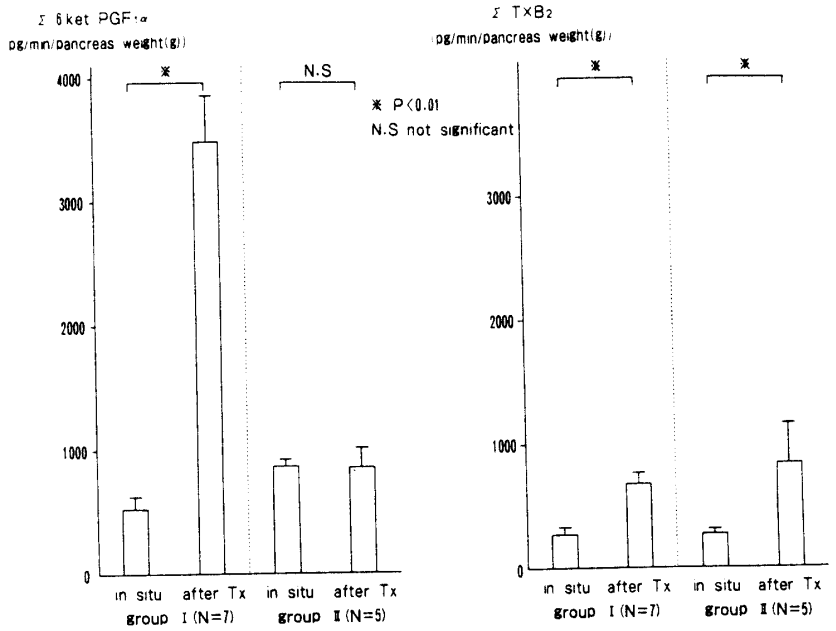

Fig 3 : Σ 6keto－PGF$_{1}\alpha$ in situ and after Tx in both groups (left side) and Σ TxB$_2$ in situ and after Tx in both groups (right side). Values are expressed as Mean ± SEM.

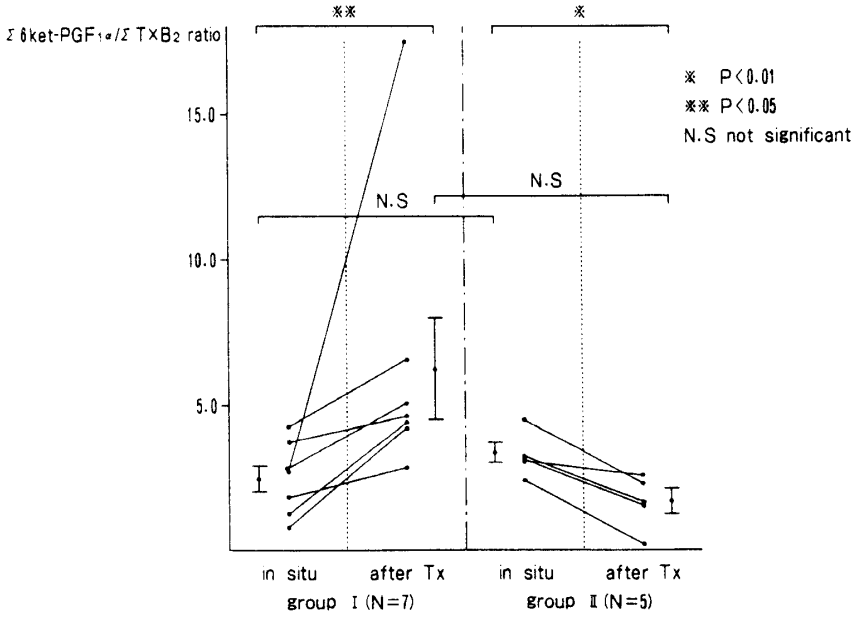

Fig 4 : Σ 6keto－PGF$_{1}\alpha$ / Σ TxB$_2$ ratio in situ and after Tx in both groups. Values are expressed as Mean ± SEM.

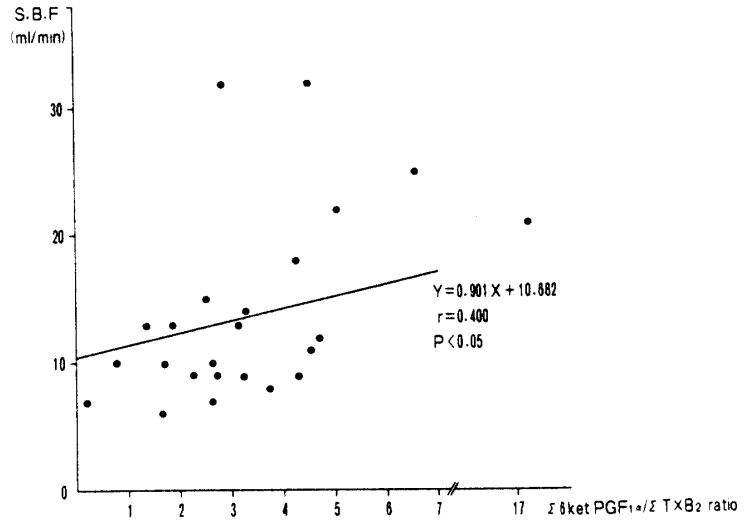

Fig 5 : Correlation between Σ 6keto－PGF$_1$ α ／ Σ TxB$_2$ ratio and S.B.F before and after transplantation in both groups.

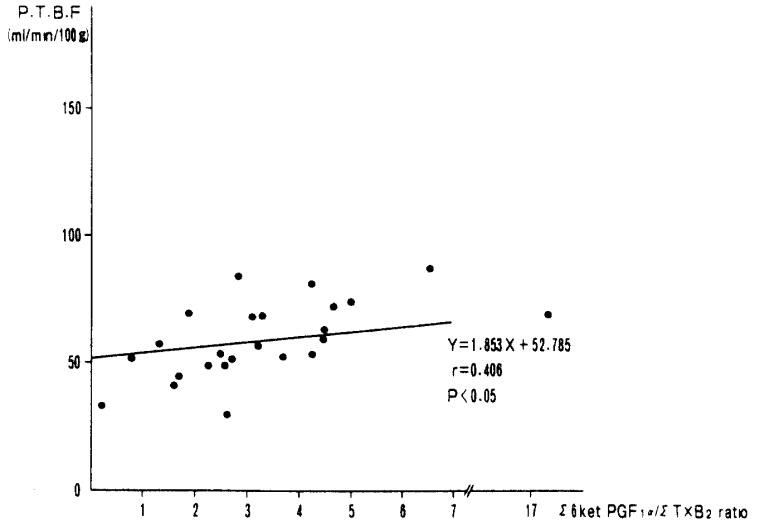

Fig 6 : Correlation between Σ 6keto－PGF$_1$ α ／ Σ TxB$_2$ ratio and P.T.B.F before and after transplantation in both groups.

F vs. Σ 6keto－PGF$_1$ α ／ Σ TxB$_2$ ratio and P.T.B.F vs. Σ 6keto－PGF$_1$ α ／ Σ TxB$_2$ ratio before and after transplantation in both groups. They revealed a high degree of correlation between these variables (the former : $r = 0.400$, $P < 0.05$, $y = 0.901x + 10.682$; the latter : $r = 0.406$, $P < 0.05$, $y = 1.853x + 52.785$).

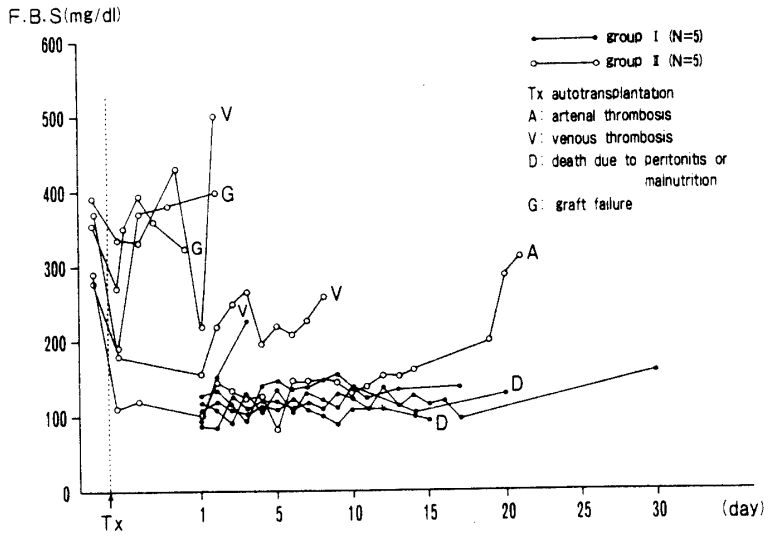

Fig 7 : Postoperative daily plasma glucose level and graft success rates after transplantation, indicating the cause of graft failure in both groups.

4) Postoperative daily plasma glucose level and graft success rates after transplantation, indicating the cause of graft failure in both groups (Fig 7)

In group I, four dogs survived with functioning grafts for 2 - 4 weeks, but one dog lost its graft on 3 day after transplantation by venous thrombosis. On the contrary, in group II, although one dog survived with functioning graft for 2 weeks, two dogs lost their grafts at 1 and 8 day by venous thrombosis and in two dogs grafts never functioned because of preservation failure.

DISCUSSION

Graft success rates after segmental pancreatic transplantation, although improving somewhat in the past year, remain inferior to the results achieved in kidney and liver transplantation. It is reported that technical factors rather than immunologic factors may explain this difference (1,2). Two major technical problems in segmental pancreatic transplantation are

early developing thrombosis and failure of exocrine drainage procedure (1,2). Especially, as the cause of vascular thrombosis, several investigators emphasized that pancreas was a low-flow organ and caine pancreas as a whole occupied 10% of the celiac blood flow (1, 3). It is well known that developing of vascular thrombosis after segmental pancreatic transplantation has a significant connection with inadequate or reduced blood flow of the transplante pancreas after splenectomy (3).

In general, the blood flow of the pancreas is thought to depend on both neurological and humoral control. But, in the case of segmental pancreatic transplantation, since the graft is completely denervated, it is possible that the blood flow of the transplanted pancreas may be controlled simply by humoral substances. Humoral substances, namely vasoactive substances, known to control blood flow of the pancreas are dopamine, bradykinin, secretin, vasoactive intestinal peptide (VIP), somatostatin,peptide YY (PYY), gastrin releasing peptide (GRP) and prostaglandins such as PGE_1, PGE_2, PGI_2, TxA_2 (8-10). And it was already demonstrated that dopamine, bradykinin and secretin had potent abilityto increase the blood flow of the pancreas and their actions were provoked by enhanced production of intrinsic prostaglandins in the pancreas (11). From these facts, it is strongly suspected that prostaglandins may have an important role in modulating or regulating the blood flow of the pancreas.

Among prostaglandins, viewed in the light of smooth muscles' relaxation or constriction and platelet aggregation, PGI_2 and TxA_2 have the most powerful actions (12,13). It was already adovocated that those two prostaglandins were produced in ischemic kidney and its balance of productive system should influence either microcirculation in kidney or renal blood flow after revascularization (4-6). Therefore, if transplanted pancreas produce PGI_2 and TxA_2 after ischemia for preserving organ, productive system of these two substances may influence either blood flow dynamics of the transplanted pancreas or vascular thrombosis formation after transplantation.

So this study was aimed to investigate productive system of PGI_2 and TxA_2 in the transplanted pancreas, especially accroding to preservation time, and correlation between its productive system and blood flow of the transplanted pancreas. In the present study, an autotransplanted model was chosen so that the results could be interpreted without the influence of immunologic factors.

The present study revealed that in group I, both S.B.F and P.T.B.F of the transplanted pancreas increased significantly after transplantation, and in group II, on the contrary, they decreased significantly after transplantation. These results suggested that a degree of the blood flow of the transplanted pancreas correlate with preservation time of organ to be transplanted. The same phenomenon, change of the blood flow of the transplanted organ associated with preservation time, was reported by Klintmalm and his associates in kidney transplantation (6). In general, it is speculated that change of the blood flow of the transplanted pancreas between two groups are related to several factors such as hemodynamic state, edema formation of the pancreas, vasoconstriction caused by operative manipulation and vascular resistence by vasoactive substances. Among these factors, the influence by hemodynamic state can be excluded since arterial blood pressure in this study was maintained constant during operative procedure and the influence of vasoconstriction caused by operative manipulation also can be excluded. As regards the influence by edema, in group II, edema formation of the preserved pancreas was more prominent macroscopically and microscopically compared to that in group I and it was possible that this edema might cause impaired microcirculation in the transplanted pancreas by vessels' compression and eventually make its blood flow decreased. While the influence by change of the vascular resistence was thought to be an important factor which caused different blood flow of the transplanted pancreas between two groups. Our present study demonstrated that, in group I, production of PGI_2 in the transplanted pancreas after revascularization was enhanced significantly. In group II, on the contrary, reduced production of

PGI_2 and enhanced production of TxA_2 were observed. These findings suggested that, in group I, increased blood flow of the transplanted pancreas was associated with enhanced production of PGI_2 and high gain of Σ 6keto $-PGF_1\alpha$ / Σ TxB_2 ratio and in group II, decreased blood flow of it was associated with reduced production of PGI_2, and enhanced production of TxA_2 and low gain of Σ 6keto $-PGF_1\alpha$ / Σ TxB_2 ratio. And our present study also demonstrated that there was a significant correlation between the blood flow (S.B.F & P.T.B.F) of the transplanted pancreas and Σ 6keto $-PGF_1\alpha$ / Σ TxB_2 ratio before and after transplantation in two groups. From these facts, it was speculated that productive system of PGI_2 and TxA_2 in the pancreas might play an important role in regulating the blood flow of it and also its balance of productive system had a significant connection with the blood flow of the transplanted pancreas.

Then we must tentatively identify why this different production of PGI_2 and TxA_2 occured in two groups. Firstly, from pathohistological findings, it was observed that endothelial damage in the preserved pancreas was mimic, indicating the functional capacity to produce PGI_2 was completely maintained in group I and therefore after revascularization vascular production of PGI_2 was initiated and enhanced to a level sufficient to induce vascular relaxation in the transplanted pancreas, and TxA_2 production by platelet adherent to the intimal surface was mild which was shown in group I. On the contrary, in group II, the functional capacity to produce PGI_2 in the endothelial cells was low or absent because of its long ischemic time. Therefore vascular production of PGI_2 after ravascularization was heavily impaired and TxA_2 production by platelet adherent to the injured intimal surface was enhanced after revascularization.

In conclusion, if preservation time was short in segmental pancreatic transplantation represented by group I, enhanced production of PGI_2 in the transplanted pancreas after revascularization could not only inhibit intravascular platelet aggregation but also induce improvement in the microcirculation and consequently protect cellular (endothelial cells, pancreatic endocrine

and exocrine cells) injury after ischemia in the transplanted pancreas. And successful maintenance of graft function in group I found to be strongly correlate to enhanced production of PGI_2. While if preservation time was long represented by group II, enhanced production of TxA_2 and reduced production of PGI_2 should be observed. Under these circumstances it was speculated that vasospasm and platelet aggregation in the graft might be acceleratic. Vasospasm and platelet aggregation in the transplanted pancreas increase tissue anoxia and acinar cell injury, and eventually cause gland necrosis. And enhanced production of TxA_2 and decreased blood flow of the transplanted pancreas in group II should contribute to vascular thrombosis formation after transplantation. Accordingly, we would like to emphasize that, in segmental pancreatic transplantation, productive system of PGI_2 and TxA_2 in the transplanted pancreas associated with preservation time directly relate to the blood flow of the transplanted pancreas and eventually graft function, and inhibition of enhanced production of TxA_2 by TxA_2 synthetase inhibitor in the transplanted pancreas may be very important in obtaining success graft function, even if preservation time is long.

REFERENCES

1) Florack, G., Sutherland, D.E.R., Cavallini, M., Najaian, J.S. (1983) Technical aspects of segmental pancreatic autotransplantation in dogs. Am.J.Surgery., 146, 565 - 573

2) Helling, T.S., Christ, D.A., Reinhardt, M,A., Murphy, P.J. (1983) Segmental pancreas transplantation in the canine model. Am.J.Surgery., 146, 838 - 843

3) Kin, S., Tamura, K., Nagami, H., Teramoto, M., Nakase, A. Transplant.Proc., 21, 2812 - 2814

4) Mcgiff, J.C., Crowshaw, K., Terragno, N.A., Lonigro, A.J., Strand, J.C., Williamson, M.A., Lee, J.B., Kevin, K.F. (1970) Prostaglandin - like substances appearing in renal venous blood during renal ischemia. Circ. Res., 27, 765 - 782

5) Leluck, S., Alexander, F., Kobzik, L., Valeri, C.R.,

Shepro, D., Hechtmann, H.B. (1985) Prostacyclin and thromboxane A_2 moderate postischemic renal failure. Surgery., 98, 207 - 212

6) Klintmalm, G.B.G., Cronestrand, R., Wennmalm, A., Lundgren, G., Groth, C.G. (1984) Human renal allograft blood flow, oxygen extraction, and prostaglandin release: their bearing on graft function. Surgery., 95, 427 - 432

7) Aukland, K., Bower, B.F., Berliner, R.W (1964) Measurement of local blood flow with hydrogen gas. Circ.Res., 14, 164 - 187

8) Iwatsuki, K., Homma, K., Malik, K.U (1985) Contribution of prostaglandins to dopamine actiones in the pancreas of anesthetized dogs. Am.J.Physiol., 248, 110 - 117

9) Iwatsuki, K., Homma, K., Malik, K.U (1985) Permmissive role of prostaglandins in the action of bradykinin on the pancreas of anesthetized doga. J.Pharmacol. Exp.Ther., 233,700 - 706

10) Daniel, R.C., Goldie, D., Richard, A.P (1988) The effect of somatostatin analogue (SMA 201 - 995) on the pancreatic blood flow. Surgery., 104, 1024 - 1030

11) Homma, K., Malik, K.U (1983) Effect of secretin and caerulein in canine pancreas: relation to prostaglandins. Am.J.Physiol., 244, 660 - 667

12) Hamberg, M., Svenson, J., Samueisson, B (1975) A new group of biologically active compounds derived from prostaglandin endoperoxides. Proc.Nat.Acad.Sci., 72, 2994 - 2998

13) Moncada, S., Gryglewski, R., Bunting, S., Vane (1976) An enzyme isolated arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature., 263, 663 - 665