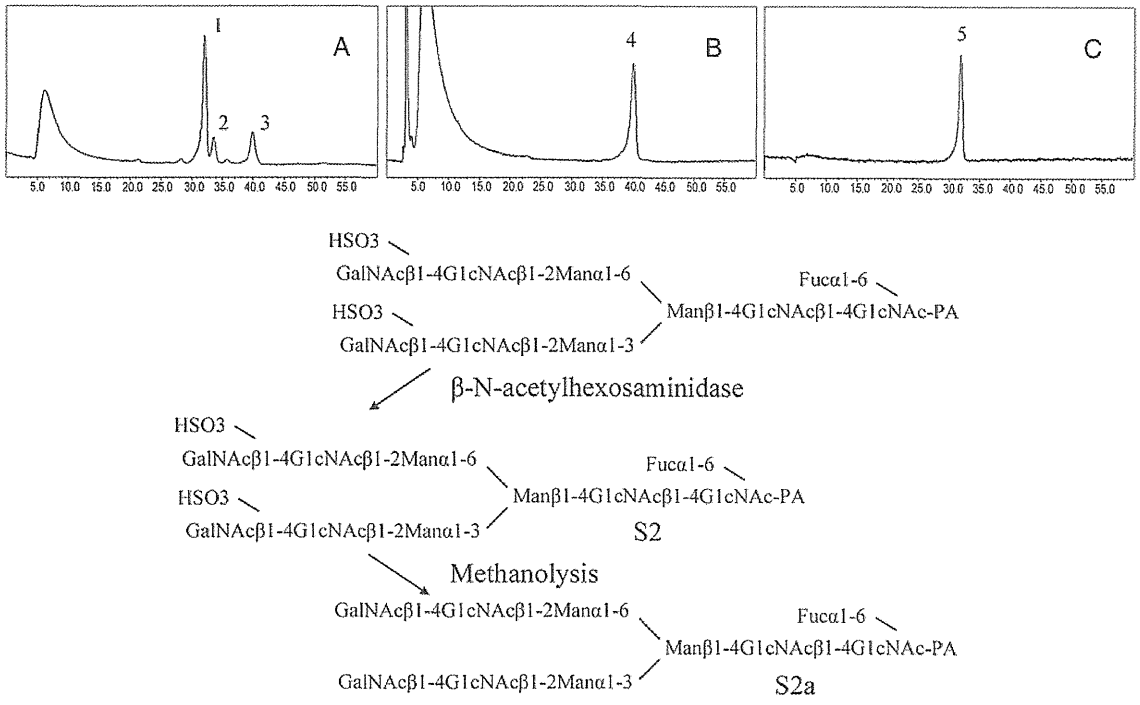


Fig. 7. Structural analysis of S2. (**A**) ODS peak after methanolysis treatment to S2. Peak 1 was the nonreacted sample, S2 (12.7 GU, 2110 Da). Peak 2 lacked one sulfate residue from S2, 13.2 GU and 2029 Da. Peak 2 lacked two sulfate residues from S2, corresponding to S2a (15.1 GU and 1948 Da). (**B**) ODS peak after co-chromatography of the samples of S2a and210.4b. S2a was the same structure as the 210.4b in GALAXY. (**C**) ODS peak after β-*N*-acetylhexosaminidase treatment of S2. Peak 5 was identical to S2 in GU and molecular weight.

Materials for analyses

Glycoamidase A from sweet almond, α-manosidase, β-galactosidase and β-*N*-acetylhexosaminidase from jack bean were purchased from Seikagaku Kogyo Co. (Tokyo, Japan). α-Gal from coffee bean was purchased from Oxford GlycoSciences, Inc. (Oxford, UK). Trypsin and chymotrypsin were obtained from Sigma (St. Louis, MO). Pronase protease from *Streptomyces griseus* was from Calbiochem (San Diego, CA). The PA derivatives of isomalto-oligosaccharides 4–20 (indicating the degree of polymerization of glucose residues) and reference PA-oligosaccharides were purchased from Seikagaku Kogyo Co.

Characterization of N*-glycan derived from islets*

The residue after extracting each islet with a chloroform–methanol solution was used as the starting material. All experimental procedures used, including the chromatographic conditions and glycosidase treatments, have been described previously (Takahashi et al. 2001). The extract was proteolyzed with chymotrypsin and trypsin mixture and further digested with glycoamidase A to release *N*-glycans. After the removal of the peptide materials, the reducing ends of the *N*-glycans were derivatized with 2-aminopyridine (Wako, Osaka, Japan). This mixture was applied to a DEAE column (Tosoh, Tokyo, Japan) or a TSK-gel Amide-80 column (Tosoh), and each fraction that was separated on the amide column was applied to a Shim-pack HRC-ODS column (Shimadzu, Kyoto, Japan). The elution times of the individual peaks onto the amide-silica and ODS columns were normalized with respect to a PA-derivatized isomalto-oligosaccharide with a known degree of polymerization, and are represented in units of glucose unit (GU). Thus, a given compound from these two columns provided a unique set of GU values, which corresponded to the coordinates of the two dimension HPLC map. The PA-oligosaccharides were identified by comparison with the coordinates of <500 reference PA-oligosaccharides in a homemade web application, GALAXY (http://www.glycoanalysis.info/) (Takahashi and Kato 2003). The calculated HPLC map based on the unit contribution values was used to estimate some high-mannose type PA-oligosaccharides. The PA-oligosaccharides were co-chromatographed with the reference to PA-oligosaccharides on the columns to confirm their identities.

MS analyses of PA-glycans

PA-oligosaccharides were subjected to MALDI-TOF-MS analysis. The matrix solution was prepared as follows: 10 mg of 2,5-dihydroxybenzoic acid (Sigma) was dissolved in 1:1 (v/v) of acetonitrile/water (1 mL). Stock solutions of PA-glycans were prepared by dissolving them in pure water. One microliter of sample solution was mixed on the target spot of a plate with 1 μL of matrix solution and then allowed to air-dry. MALDI-TOF-MS data were acquired in the positive modes using AXIMA-CFR (Shimadzu) operated in the linear mode.

Single islet cell preparation

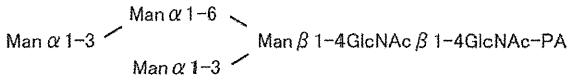
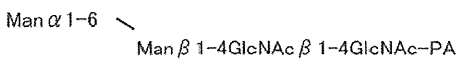
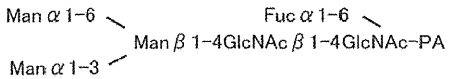
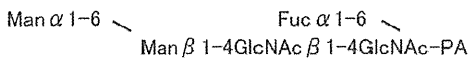
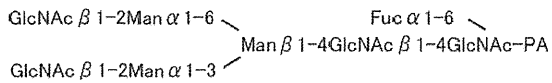
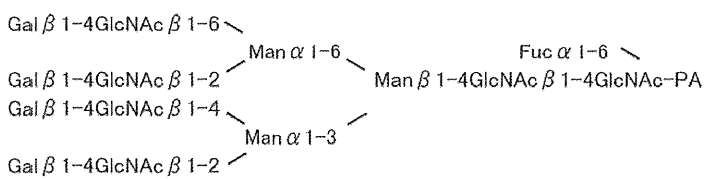
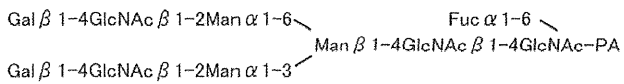
Single-cell suspensions were prepared by the method described by Ono et al. (1977). Isolated islets were exposed to 0.04% ethylenediaminetetraacetic acid for 5 min at room temperature

Table I. Structures and relative quantities of neutral, mono-sialyl, di-sialyl or mono-sulfate, mono-sialyl-mono-sulfate and di-sulfate PA-oligosaccharides derived from human and porcine islets

Peak code number	GU[a] ODS (Amid)	Molecular[b] mass (Da)	Structure[c]	Relative quantity (%)[d]	
					Pig
Neutral glycan					
N1	4.9 (8.8)	1800	Man α 1–2Man α 1–6 Man α 1–6 Man α 1–3 Man β 1–4GlcNAc β 1–4GlcNAc–PA Man α 1–2Man α 1–2Man α 1–3	11.6	24.9
N2-1	5.3 (7.9)	1638	Man α 1–2Man α 1–6 Man α 1–6 Man α 1–3 Man β 1–4GlcNAc β 1–4GlcNAc–PA Man α 1–2Man α 1–3	5.8	3.5
N2-2	5.3 (9.5)	1962	Man α 1–2Man α 1–6 Man α 1–6 Man α 1–2Man α 1–3 Man β 1–4GlcNAc β 1–4GlcNAc–PA Man α 1–2Man α 1–2Man α 1–3	7.4	9.5
N3	6.0 (7.9)	1638	Man α 1–6 Man α 1–6 Man α 1–3 Man β 1–4GlcNAc β 1–4GlcNAc–PA Man α 1–2Man α 1–2Man α 1–3	3.0	1.9
N4	6.2 (7.0)	1475	Man α 1–6 Man α 1–6 Man α 1–3 Man β 1–4GlcNAc β 1–4GlcNAc–PA Man α 1–2Man α 1–2Man α 1–3	16.7	10.1
pN5 = hN7	7.3 (6.1)	1313	Man α 1–6 Man α 1–6 Man α 1–3 Man β 1–4GlcNAc β 1–4GlcNAc–PA Man α 1–3	24.0	11.3
pN6-1 = hN8	7.5 (4.2)	989	Man α 1–6 Man β 1–4GlcNAc β 1–4GlcNAc–PA Man α 1–3	2.3	6.2

Continued

Table I. (Continued)

Peak code number	GU[a] ODS (Amid)	Molecular[b] mass (Da)	Structure[c]	Relative quantity (%)[d]	
				Pig	
pN6-2	7.5 (5.1)	1151		1.7	-
pN7 = hN9	7.7 (3.3)	827		2.4	2.2
pN8 = hN10	10.3 (4.6)	1135		3.9	4.1
pN9 = hN11	10.5 (3.7)	973		2.2	3.0
hN12-1	12.8 (5.4)	1541		–	1.8
hN12-2	12.8 (6.5)	1948		–	2.9
hN13	14.2 (7.4)	1866		–	3.1
hN5-1	6.6 (7.4)	1558	(Hexose)4(HexNAc)4(PA)1[e]	–	2.7
hN5-2	6.6 (7.9)	1720	(Hexose)5(HexNAc)4(PA)1[e]	–	2.0
hN6-1	6.9 (8.1)	1720	(Hexose)5(HexNAc)4(PA)1[e]	–	1.5
hN6-2	6.9 (8.5)	1882	(Hexose)6(HexNAc)4(PA)1[e]	–	1.2

[a]Units of GU were calculated from the elution times of the peaks obtained from the ODS column in Figure 2 and the Amide column in Figure 3.
[b]Average mass calculated from the *m/z* values of $[M + Na]^+$ or $[M + H]^+$ ion for neural, $[M - H]^-$ ion for mono-sialyl and mono-sulfated and $[M + Na - 2H]^-$ ions for mono-sialyl-mono-sulfated and di-sulfated PA-oligosaccharides (Supplementary data, Figure S1).
[c]Structures of PA-oligosaccharides are represented.
[d]Molecular percentage of was calculated from the peak area in Figure 2 by comparison with total *N*-glycan content in each islet tissue.
[e]*N*-glycans did not coincide with those of known references in the GALAXY database.

Table II. Structures and relative quantities of neutral, mono-sialyl, di-sialyl or mono-sulfate, mono-sialyl-mono-sulfate and di-sulfate PA-oligosaccharides derived from human and porcine islets

Peak code number	GU[a] ODS (Amid)	Molecular[b] mass (Da)	Structure[c]	Relative quantity (%)[d]	
				Pig	Human
Mono-sialyl glycan					
pM2-1	9.0 (5.4)	1646	Man α 1-6 Man β 1-4GlcNAc β 1-4GlcNAc-PA Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-2Man α 1-3	0.2	–
pM3-1	11.9 (5.9)	1792	Man α 1-6 Fuc α 1-6 Man β 1-4GlcNAc β 1-4GlcNAc-PA Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-2Man α 1-3	0.3	–
hM2-1	7.9 (6.0)	1646	Man α 1-6 Man β 1-4GlcNAc β 1-4GlcNAc-PA Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3	–	0.15
pM1 = hM3	8.6 (7.1)	1970	Man α 1-6 Man α 1-6 Man α 1-3 Man β 1-4GlcNAc β 1-4GlcNAc-PA Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-2Man α 1-3	0.6	0.2
pM2-2	9.0 (6.2)	1808	Man α 1-6 Man α 1-3 Man β 1-4GlcNAc β 1-4GlcNAc-PA Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-2Man α 1-3	0.3	–
pM3-2	11.9 (6.7)	1954	Man α 1-6 Fuc α 1-6 Man α 1-3 Man β 1-4GlcNAc β 1-4GlcNAc-PA Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-2Man α 1-3	0.3	–
hM1	7.6 (7.7)	1970	(Hexose)6(HexNAc)3(NEuAc) 1(PA)1[e]	–	0.2
hM2-2	7.9 (6.8)	2255	(Hexose)4(HexNAc)6(NeuAc)1(PA)1[e]	–	0.15
hM4-1	11.2 (6.4)	1792	Man α 1-6 Fuc α 1-6 Man β 1-4GlcNAc β 1-4GlcNAc-PA Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3	–	0.1
hM4-2	11.2 (6.7)	2011	Gal β 1-4GlcNAc β 1-2Man α 1-6 Man β 1-4GlcNAc β 1-4GlcNAc-PA Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-2Man α 1-3	–	0.4
pM4 = hM5	13.5 (7.6)	2157	Gal β 1-4GlcNAc β 1-2Man α 1-6 Fuc α 1-6 Man β 1-4GlcNAc β 1-4GlcNAc-PA Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3	0.5	0.5
pM5	14.4 (6.2)	1995	GlcNAc β 1-2Man α 1-6 Fuc α 1-6 Man β 1-4GlcNAc β 1-4GlcNAc-PA Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-2Man α 1-3	0.6	–
M6	15.1 (7.1)	2157	Gal β 1-4GlcNAc β 1-2Man α 1-6 Fuc α 1-6 Man β 1-4GlcNAc β 1-4GlcNAc-PA Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-2Man α 1-3	0.6	2.1

[a]Units of GU were calculated from the elution times of the peaks obtained from the ODS column in Figure 2 and the Amide column in Figure 3.
[b]Average mass calculated from the *m/z* values of $[M+Na]^+$ or $[M+H]^+$ ion for neural, $[M-H]^-$ ion for mono-sialyl and mono-sulfated and $[M+Na-2H]^-$ ions for mono-sialyl-mono-sulfated and di-sulfated PA-oligosaccharides (Supplementary data, Figure S1).
[c]Structures of PA-oligosaccharides are represented.
[d]Molecular percentage of was calculated from the peak area in Figure 2 by comparison with total *N*-glycan content in each islet tissue.
[e]*N*-glycans did not coincide with those of known references in the GALAXY database.

Table III. Structures and relative quantities of neutral, mono-sialyl, di-sialyl or mono-sulfate, mono-sialyl-mono-sulfate and di-sulfate PA-oligosaccharides derived from human and porcine islets

Peak code number	GU[a] ODS (Amid)	Molecular[b] mass (Da)	Structure[c]	Relative quantity (%)[d]	
				Pig	Human
Di-sialyl glycan					
D1	10.6 (7.5)	2302	Neu5Ac α2-6Gal β1-4GlcNAc β1-2Man α1-6 \ Man β1-4GlcNAc β1-4GlcNAc-PA / Neu5Ac α2-6Gal β1-4GlcNAc β1-2Man α1-3	0.2	0.4
hD2	12.1 (6.5)	2302	Neu5Ac α2-3Gal β1-4GlcNAc β1-2Man α1-6 \ Man β1-4GlcNAc β1-4GlcNAc-PA / Neu5Ac α2-3Gal β1-4GlcNAc β1-2Man α1-3	–	0.3
pD2 = hD3	13.5 (7.9)	2448	Neu5Ac α2-6Gal β1-4GlcNAc β1-2Man α1-6 \ Fuc α1-6 \ Man β1-4GlcNAc β1-4GlcNAc-PA / Neu5Ac α2-6Gal β1-4GlcNAc β1-2Man α1-3	0.8	0.2
pD3 = hD4	15.8 (6.9)	2448	Neu5Ac α2-3Gal β1-4GlcNAc β1-2Man α1-6 \ Fuc α1-6 \ Man β1-4GlcNAc β1-4GlcNAc-PA / Neu5Ac α2-3Gal β1-4GlcNAc β1-2Man α1-3	0.5	0.9

[a]Units of GU were calculated from the elution times of the peaks obtained from the ODS column in Figure 2 and the Amide column in Figure 3.
[b]Average mass calculated from the *m/z* values of $[M + Na]^+$ or $[M + H]^+$ ion for neural, $[M - H]^-$ ion for mono-sialyl and mono-sulfated and $[M + Na\text{-}2H]^-$ ions for mono-sialyl-mono-sulfated and di-sulfated PA-oligosaccharides (Supplementary data, Figure S1).
[c]Structures of PA-oligosaccharides are represented.
[d]Molecular percentage of was calculated from the peak area in Figure 2 by comparison with total *N*-glycan content in each islet tissue.
[e]*N*-glycans did not coincide with those of known references in the GALAXY database.

Table IV. Structures and relative quantities of neutral, mono-sialyl, di-sialyl or mono-sulfate, mono-sialyl-mono-sulfate and di-sulfate PA-oligosaccharides derived from human and porcine islets

Peak code number	GU[a] ODS (Amid)	Molecular[b] mass (Da)	Structure[c]	Relative quantity (%)[d]	
				Pig	Human
Mono-sulfated glycan					
S1-1	7.3 (3.8)	1478	(Hexose)3(HexNAc)4(HSO3)1(PA)1[e]	0.2	–
S1-2	7.3 (4.5)	1641	Man α1-6 \ SHO3 — Man α1-3 / Man β1-4GlcNAc β1-4GlcNAc-PA; GalNAc β1-4GlcNAc β1-2Man α1-3	0.6	–

[a]Units of GU were calculated from the elution times of the peaks obtained from the ODS column in Figure 2 and the Amide column in Figure 3.
[b]Average mass calculated from the *m/z* values of $[M + Na]^+$ or $[M + H]^+$ ion for neural, $[M - H]^-$ ion for mono-sialyl and mono-sulfated and $[M + Na - 2H]^-$ ions for mono-sialyl-mono-sulfated and di-sulfated PA-oligosaccharides (Supplementary data, Figure S1).
[c]Structures of PA-oligosaccharides are represented.
[d]Molecular percentage of was calculated from the peak area in Figure 2 by comparison with total *N*-glycan content in each islet tissue.
[e]*N*-glycans did not coincide with those of known references in the GALAXY database.

and collected by centrifugation at 400 × *g* for 1 min. The islets were then suspended in 4 mL of 1000 PU/mL Dispase-II (Godo-Shusei Co. Tokyo, Japan) and treated at 37°C for 15 min. Cell aggregates were allowed to settle and the supernatant was transferred to a conical tube. The pooled harvests were centrifuged at 400 × *g* for 3 min. The cell pellet was washed twice with phosphate buffer saline (PBS) and re-suspended in PBS.

Flowcytometry

The islets were incubated with a 10% solution of normal human pooled serum (NHS) at 4°C for 1 h, washed and then incubated with 1.25 μg of fluorescein isothiocynate-conjugated anti-human IgG and IgM (Cappel, West Chester, PA) as a second antibody for 1 h at 4°C. The stained cells were analyzed with a FACS Calibur flow cytometer (Nippon Becton Dickinson, Tokyo, Japan).

Sulfate-depleted cells

Islets were starved for 24 h in sulfate-free RPMI1640 medium containing 1% of fetal cow serum supplemented with fresh 10 mM sodium chlorate (Nakarai Tesque, Kyoto, Japan).

Supplementary data

Supplementary data for this article are available online at http://glycob.oxfordjournals.org/.

Table V. Structures and relative quantities of neutral, mono-sialyl, di-sialyl or mono-sulfate, mono-sialyl-mono-sulfate and di-sulfate PA-oligosaccharides derived from human and porcine islets

Peak code number	GU[a] ODS (Amid)	Molecular[b] mass (Da)	Structure[c]	Relative quantity (%)[d]	
				Pig	Human
Mono-sialyl-mono-sulfated glycan					
MS1	9.8 (5.0)	2133	(Hexose)4(HexNAc)5(NeuAc)1(HSO3)1(PA)1[e]	0.3	–
MS2	12.7 (5.3)	2279	Neu5Ac α2-6Gal β1-4GlcNAc β1-2Man α1-6 \ Fuc α1-6 \ SHO3 \ Man β1-4GlcNAc β1-4GlcNAc-PA / GalNAc β1-4GlcNAc β1-2Man α1-3 /	1.3	–
MS3	15.9 (5.4)	2279	(Hexose)4(HexNAc)5(Deoxyhexose)1(NeuAc)1(HSO3)1(PA)1*	0.4	–

[a]Units of GU were calculated from the elution times of the peaks obtained from the ODS column in Figure 2 and the Amide column in Figure 3.
[b]Average mass calculated from the *m/z* values of $[M+Na]^+$ or $[M+H]^+$ ion for neural, $[M-H]^-$ ion for mono-sialyl and mono-sulfated and $[M+Na-2H]^-$ ions for mono-sialyl-mono-sulfated and di-sulfated PA-oligosaccharides (Supplementary data, Figure S1).
[c]Structures of PA-oligosaccharides are represented.
[d]Molecular percentage of was calculated from the peak area in Figure 2 by comparison with total *N*-glycan content in each islet tissue.
[e]*N*-glycans did not coincide with those of known references in the GALAXY database.

Table VI. Structures and relative quantities of neutral, mono-sialyl, di-sialyl or mono-sulfate, mono-sialyl-mono-sulfate and di-sulfate PA-oligosaccharides derived from human and porcine islets

Peak code number	GU[a] ODS (Amid)	Molecular[b] mass (Da)	Structure[c]	Relative quantity (%)[d]	
				Pig	Human
Di-sulfated glycan					
S2	12.7 (3.9)	2110	SHO3 \ GalNAc β1-4GlcNAc β1-2Man α1-6 \ Fuc α1-6 \ SHO3 \ Man β1-4GlcNAc β1-4GlcNAc-PA / GalNAc β1-4GlcNAc β1-2Man α1-3 /	7.0	–

[a]Units of GU were calculated from the elution times of the peaks obtained from the ODS column in Figure 2 and the Amide column in Figure 3.
[b]Average mass calculated from the *m/z* values of $[M+Na]^+$ or $[M+H]^+$ ion for neural, $[M-H]^-$ ion for mono-sialyl and mono-sulfated and $[M+Na-2H]^-$ ions for mono-sialyl-mono-sulfated and di-sulfated PA-oligosaccharides (Supplementary data, Figure S1).
[c]Structures of PA-oligosaccharides are represented.
[d]Molecular percentage of was calculated from the peak area in Figure 2 by comparison with total *N*-glycan content in each islet tissue.
[e]*N*-glycans did not coincide with those of known references in the GALAXY database.

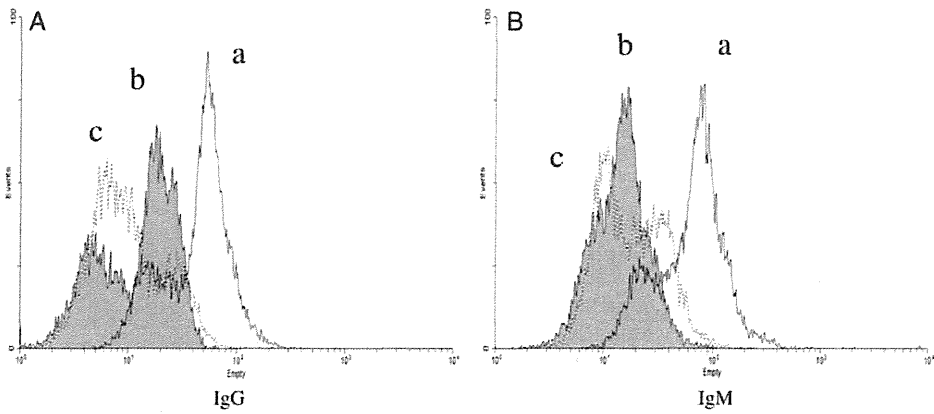


Fig. 8. FACS analysis for the antigenicity of sulfate structures. Islets from adult pigs were treated with 10% NHS as the first antibody and anti-human immmunoglobulins as the second antibodies. Typical FACS profiles of human IgG (**A**) and IgM (**B**) deposition on islets are shown. The effect of removal of sulfate structures by sodium chlorate and sulfate-free medium on the antigenicity of pig islet cells was next investigated. The presence of sodium chlorate led to a reduction in the reactivity of islets to a natural antibody, suggesting that the sulfate structures of islets contain a considerable amount of natural antibody epitopes; a, Normal line: API in usual medium; b, painted out: Sulfate depleted API and c, dotted line: Second antibody control.

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Conflict of interest

None declared.

Abbreviations

2D, two dimension; API, adult pig islets; ATP, adenosine triphosphate; DEAE, diethylaminoethyl; FCS, fetal cow serum; FITC, fluorescein isothiocynate; GALAXY, glycoanalysis by the three axes of MS and chromatography; GalNAc, *N*-acetylgalactosamine; GKO, α1-3-galactosyltransferase knockout; GlcNAc, *N*-acetylglucosamine; GU, glucose unit; Hex, hexose; HexNAc, *N*-acetylhexosamine; HPLC, high-performance liquid chromatography; Lew^x, Lewis x; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometric; Man, mannose; MS2, mono-sialyl-mono-sulfate; NeuAc, neuranimic acid; NeuGc, N-glycolylneuraminic acid; NHS, normal human pooled serum; ODS, octa decyl silyl; PA, pyridylamino; PBS, phosphate buffer saline; S2, di-sulfate; α-Gal, α-galactosidase.

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A Novel Resting Strategy for Improving Islet Engraftment in the Liver

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Background. Several studies have revealed that posttransplant insulin treatment is beneficial to rest the islet grafts. However, insulin infusion per se is not enough to completely suppress the heavy workload arising caused by postprandial hyperglycemia. Therefore, the present study examined whether short-term fasting combined with insulin treatment could effectively prevent graft exhaustion after intraportal islet transplantation.

Methods. A marginal dose of syngeneic rat islet grafts were transplanted intraportally into the control, insulin-treated, and insulin+rest groups of streptozotocin-induced diabetic rats. The control group fed freely without insulin treatment, and the other groups were continuously treated with an optimal amount of insulin to maintain normoglycemia. In addition, the insulin+rest group fasted and received total parenteral nutrition during the 2 weeks after transplantation.

Results. The curative rate was significantly higher in both the insulin and insulin+rest groups than the control group ($P<0.0001$). The glucose tolerance, residual graft mass, and graft function were significantly ameliorated in the insulin+rest group, but not in the insulin group, compared to the control group ($P<0.01$, $P=0.03$, $P=0.001$).

Conclusions. These data suggest that short-term fasting combined with insulin treatment, especially during the avascular period of the grafts, could therefore be a promising regimen for improving pancreatic islet engraftment in the liver.

Keywords: Islets, Transplantation, Engraftment, Insulin, Resting, Parenteral nutrition.

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Owing to recent advances, islet transplantation is becoming one of the most attractive treatments for type 1 diabetic patients. However, there are still many issues to be resolved regarding this treatment. One of the current drawbacks to islet transplantation is the necessity of multiple donor organs to effectively cure one diabetic patient. For islet transplantation to become more widespread, diabetes reversal must be achieved with a single donor to reduce the risks and costs, and to increase the availability of cells for transplantation.

One of the possible explanations for the requirement of multiple donor organs in islet transplantation is poor engraftment. Unlike whole pancreas transplantation, pancreatic islet grafts are rendered avascular after enzymatic isolation and must become revascularized after transplantation (*1, 2*). Several studies have shown that it takes approximately 2 weeks until angiogenesis and revascularization of the transplanted islets can be completed (*1–3*). Therefore, the poor engraftment during islet transplantation may at least in part be attributable to islet exhaustion resulting from a high workload during the avascular period following transplantation.

Several studies have reported that exogenous insulin treatment after transplantation could effectively prevent islet exhaustion (*4–9*). In support of these findings, Koh et al. recently demonstrated that peritransplant infusions of insulin and heparin were positively correlated with the clinical outcome of islet transplantation (*10*). On the other hand, Dafoe et al. (*11*) and Keymeulen et al. (*12*) have shown that exogenous insulin treatment after transplantation did not improve subsequent endocrine function.

Most likely, the beneficial effects of exogenous insulin administration could be explained by the avoidance of

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glucotoxicity (*13–16*), a protective and trophic action on islet grafts (*17–20*), and a reduction of the heavy workload resulting from postprandial hyperglycemia (*21, 22*). However, it may be speculated that exogenous insulin infusions per se are insufficient to completely suppress the deleterious effects of a heavy workload caused by postprandial hyperglycemia. This may explain the conflicting results obtained following exogenous insulin treatment after transplantation. It was hypothesized that short-term fasting, supported by a total parenteral nutrition during a limited period after transplantation, could be a helpful strategy to avoid overtaxing the islet grafts and allow them to rest during the avascular period.

Hence, in the present study, it was examined whether short-term fasting, combined with insulin treatment during the avascular period, could prevent graft exhaustion after intraportal islet transplantation.

RESULTS

Metabolic Evolution After Transplantation

The control group maintained high blood glucose levels during the whole study period, even after transplantation. In contrast, the insulin+rest and insulin groups remained normoglycemic throughout the study (Fig. 1A). The curative rate was significantly higher in both the insulin+rest and insulin groups than in the control group (100% [7/7] and 100% [5/5] vs. 12.5% [1/8], $P<0.001$) (Fig. 1B). Moreover, both the insulin+rest and insulin groups had a tendency to demonstrate an increase in their body weight than did the control group (Fig. 1C).

Intravenous Glucose Tolerance Test

Although the blood glucose changes and curative rates were similar in the insulin+rest and insulin groups, glucose tolerance was significantly ameliorated only in the insulin+rest group (AUC: 23,587±456 min*mg/dL, Kg: 1.62±0.08%/min; insulin group AUC: 25,289±1,399 min*mg/dL, Kg: 1.34±0.05%/min) compared to the control group (AUC: 32,138±2,673 min*mg/dL, $P<0.01$; Kg: 1.04 ±0.11%/min, $P< 0.001$) (Fig. 2A–C).

The Amount of Insulin in the Liver

The amount of insulin in the liver was higher in the insulin+rest group (13.2±3.8 ng/IEQs) and the insulin group (7.0±1.5 ng/IEQs) than in the control group (3.5±1.1 ng/IEQs), but the increase was only statistically significant in the case of the insulin+rest group ($P=0.03$) (Fig. 3A).

The Secretory Unit of Islet Transplant Objects (SUITO) Index

At 5 weeks after transplantation, the SUITO index (*23*), which reflects the graft function, was again higher in

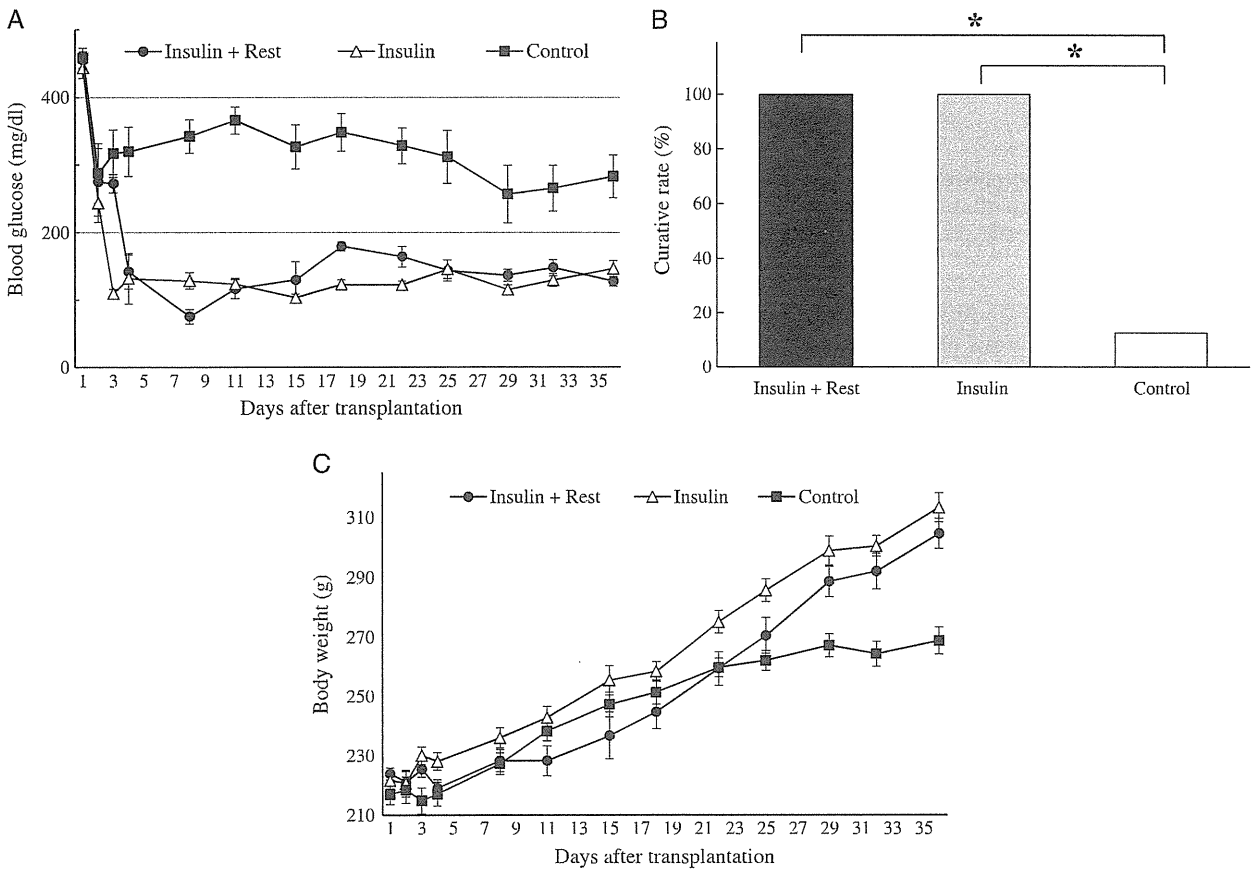

FIGURE 1. Metabolic evolution after islet transplantation. A, the changes in the blood glucose levels after islet transplantation. B, the number of normoglycemic rats at the end of the observation period. *$P<0.001$ between the control group vs. the insulin+rest and insulin groups. C, the changes in body weight after islet transplantation.

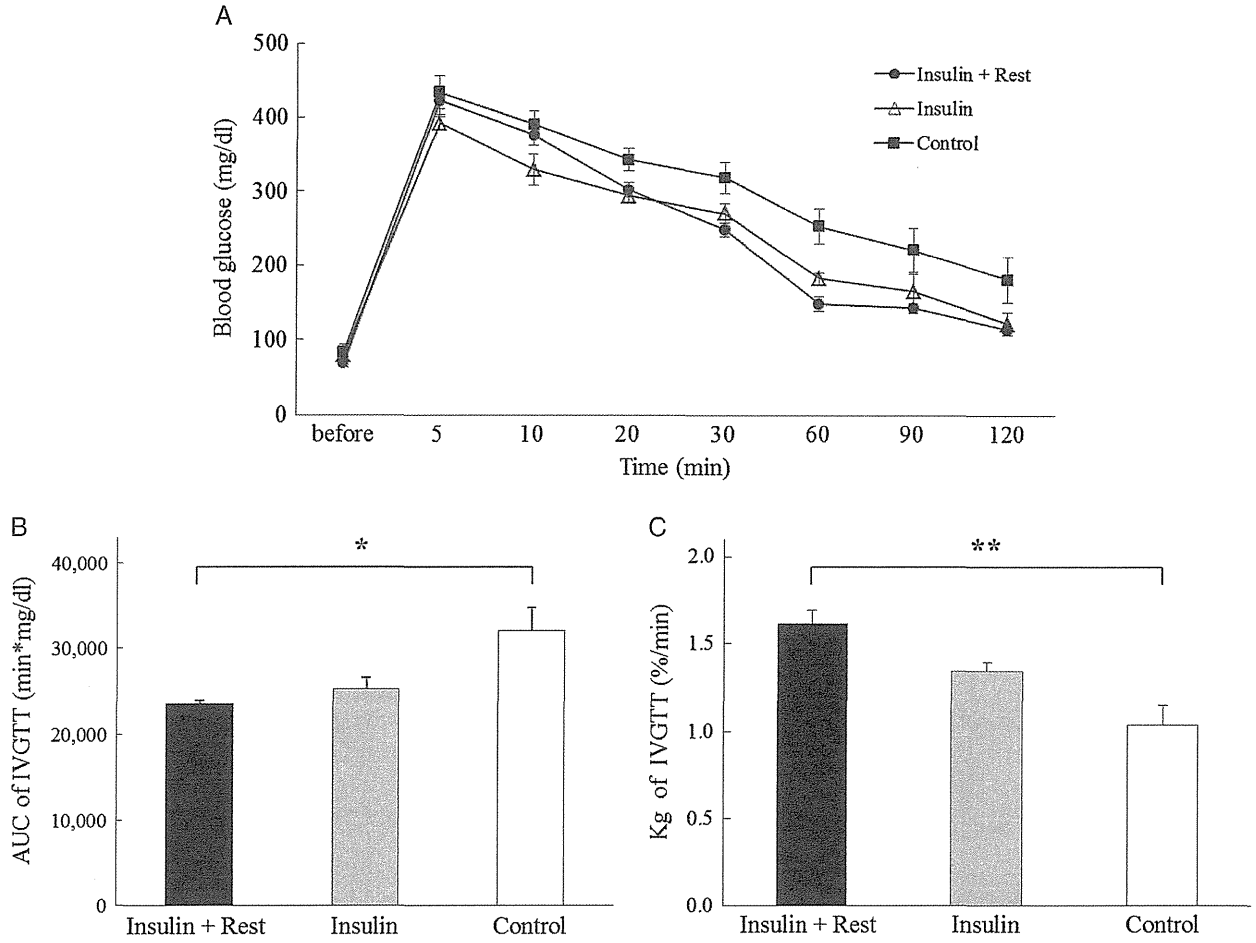

FIGURE 2. Profile of glucose tolerance in each group. A, the results of the intravenous glucose tolerance test (IVGTT) in each group at 5 weeks after transplantation. Glucose tolerance was significantly ameliorated in the insulin+rest group, but not in the insulin group, compared to the control group as indicated by both the AUC (B) (*P<0.01) and Kg values (**P<0.001) (C).

the insulin+rest group (13.2±2.3) and the insulin group (8.1±1.6) than in the control group (3.6±1.1), but the improvement was only statistically significant in the case of the insulin+rest group (P=0.002) (Fig. 3B).

The Expression of Inflammatory Mediators in Each Group

Regarding the expression of inflammatory mediators, such as TNF-α, IL-6, and MCP-1, no significant differences were observed in any of the groups on day 14 after islet transplantation (Table 1).

The Influence of Short-Term Fasting on Oxidative Stress in the Transplant Recipients

The serum levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) were measured to analyze the extent of oxidative DNA damage in each group (Table 1). Although the serum levels of 8-OHdG in the insulin+rest group were considerably lower than those of the insulin and control groups, the differences did not reach significance (P=0.08).

The Influence of Short-Term Fasting on the Glucagon-Like Peptide-1 (GLP-1) Levels

The serum active GLP-1 levels were measured in all three groups at 14 days after transplantation (Table 1). Significantly lower levels of GLP-1 were observed in the insulin+rest group compared to the insulin and the control groups (P =0.0001).

The State of Apoptosis and Revascularization of the Islet Grafts

Using the terminal deoxynucleotide transferase-mediated dUTP nick-end labeling (TUNEL) assay, the number of apoptotic islet cells in the liver sections was examined. Representative examples are shown in Figure 4A. No significant differences were observed among the groups (P=0.28) (Fig. 4C). The number of vWF-positive vessels around the islets was also counted to examine the state of revascularization of the islet grafts (Fig. 4B). The counts of the vWF-positive vessels in the insulin+rest (375±35/mm^2) and insulin groups (564±71/mm^2) were remarkably lower than those in the control group (923±227/mm^2) (P=0.08) (Fig. 4D).

DISCUSSION

A considerable number of studies to date, including a recent clinical report (*10*), have revealed that posttransplant glycemic control is crucial for successful islet transplantation (*4–9*). Most of these studies concluded that exogenous insulin treatment is beneficial to allow the transplanted islet grafts to

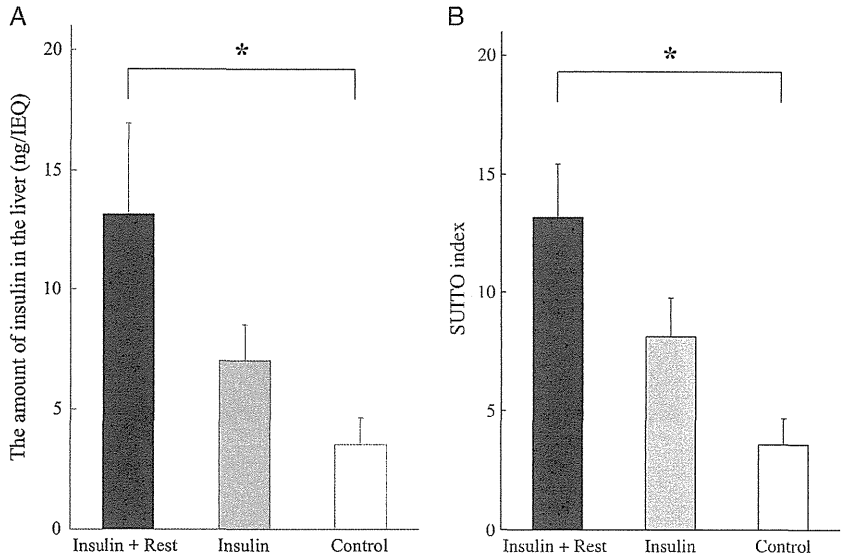

FIGURE 3. Evaluation of graft function. A, the amount of insulin in the liver. After the intravenous glucose tolerance test, the recipient livers were harvested, and the amount of insulin in the liver per transplanted islet equivalents (IEQs) was evaluated. The level was significantly higher in the insulin+rest group compared to that in the control group (*P=0.03). B, the secretory unit of islet transplant objects (SUITO) index at 5 weeks after islet transplantation. The SUITO index, which is used as an index of graft function in clinical islet transplantation, was significantly improved in the insulin+rest group compared to the control group (*P=0.002).

rest, particularly during the avascular period. Because it can minimize the workload experienced by the transplanted grafts, the regulation of postprandial hyperglycemia is therefore considered to be of great importance. However, the current manner of insulin infusion may not be sufficient to effectively prevent the development of postprandial hyperglycemia. It was therefore assumed that short-term fasting, supported by total parenteral nutrition, during a limited period after transplantation could be a helpful strategy to more effectively rest the islet grafts during the avascular period.

In the present study, normoglycemia in both the insulin+rest and insulin groups was maintained even after discontinuing insulin treatment, which did not occur in the control group. The cure rate at 5 weeks after transplantation was also significantly improved in both the insulin+rest and insulin groups compared to the control group. These results are consistent with the previous reports indicating that exogenous insulin treatment during the early stage of engraftment improved the outcome of islet transplantation (*4–10*). Furthermore, it was demonstrated that the glucose tolerance, the function of transplanted islet grafts indicated by the SUITO index (*23*), and the amount of insulin in the transplanted livers were significantly improved in the insulin+rest group, but not in the insulin group, compared to the control group. These data clearly show that short-term fasting, combined with insulin treatment during the avascular period, can better preserve transplanted islet grafts in the liver.

Although exogeneous insulin was administered subcutaneously in the previous studies (*5, 6, 12*), it was infused intravenously to more precisely control the blood glucose level in the present study. In fact, such intensive insulin treatment during the early stage of engraftment would likely be performed intravenously in the clinical setting. Moreover, in the present study, the short-term fasting was performed in conjunction with total parenteral nutrition via a central venous catheter to mimic the clinical condition. The experimental models used in the present study demonstrate approaches that could be applied clinically.

TABLE 1. The influence of short-term fasting on the expression of inflammatory mediators, oxidative stress, and glucagon-like peptide-1 (GLP-1) levels

	Insulin+rest (n=4)	Insulin (n=5)	Control (n=8)
TNFα, pg/mL	50.9±11.6	39.8±3.1	59.7±6.6
IL-6, pg/mL	3,199±752	2,651±412	4,812±933
MCP-1, pg/mL	823±182	752±53	1,018±97
8-OHdG, ng/mL	0.22±0.07	0.89±0.40	0.59±0.23
GLP-1 levels, pmol/L[a]	1.0±1.0[b] (n = 6)	9.0±1.2 (n = 6)	11.6±1.6 (n = 8)

[a] Values are obtained in the other series of experiments.
[b] P=0.0001 vs. the insulin and the control groups.
TNFα, tumor necrosis factor-alpha; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; GLP-1, glucagon-like peptide-1; 8-OHdG; 8-hydroxy-2′-deoxyguanosine.
Values are means±SE.

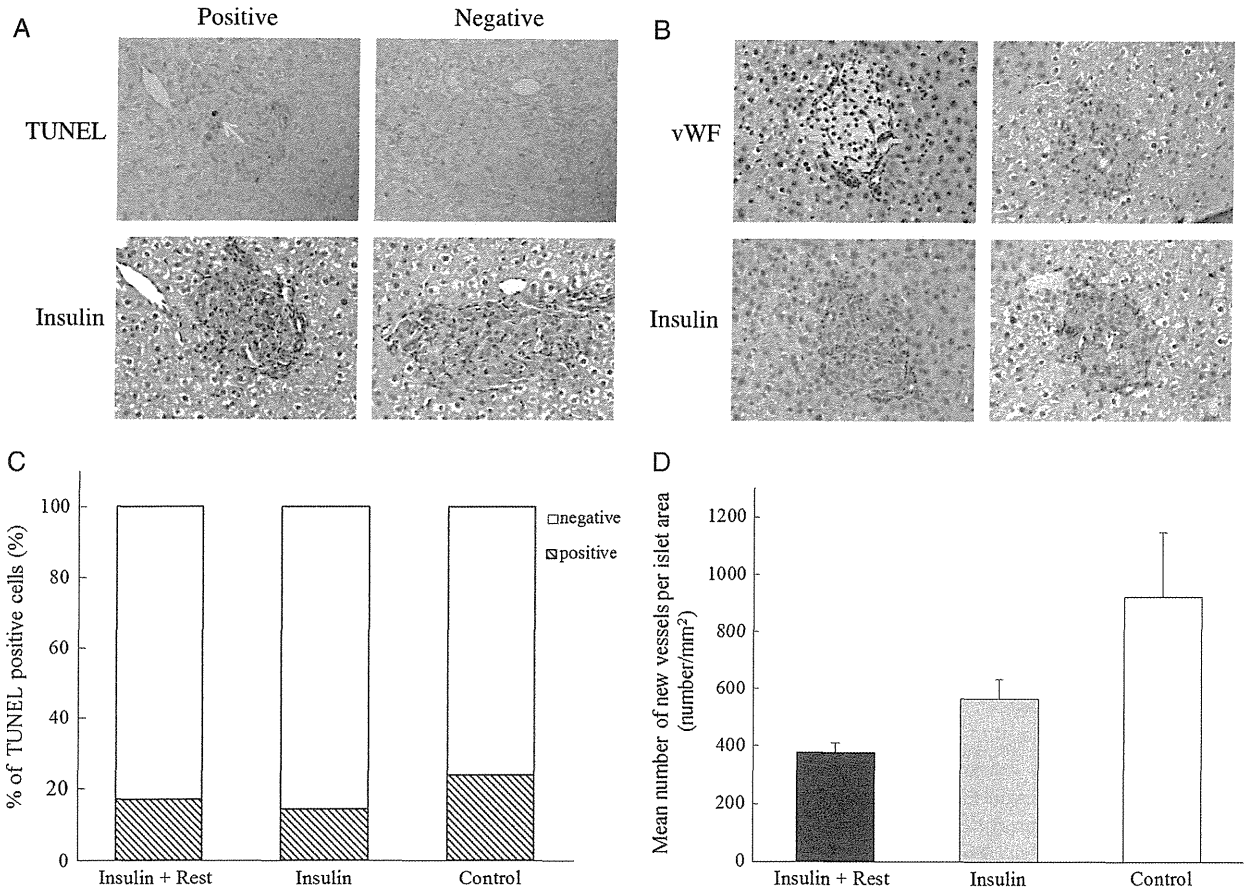

FIGURE 4. Immunohistochemical analyses. A, upper panel: TUNEL staining. Lower panel: insulin staining of the same islets. "Positive" represents TUNEL-positive cells in the islets, and "Negative" represents TUNEL-negative cells in the islets. B, upper panel: vWF staining. Lower panel: insulin staining of the same islets. C, the percentage of TUNEL-positive cells. D, Mean number of new vessels per islet area. The vWF-positive vessels in the insulin+rest (375±35/mm^2) and insulin groups (564±71/mm^2) were remarkably lower than those in the control group (923±227/mm^2) (P=0.08).

Moreover, the current rat model enabled us to achieve almost the same background, including the insulin dose, the rate of the increase in body weight, and the inflammatory status between the resting and insulin groups. Therefore, it is believed that the differences between them may be more accurately attributed to the stressful workload on the islet grafts resulting from the postprandial hyperglycemia in the non-fasting group. In this study, postprandial blood glucose fluctuation in the insulin group was not directly measured because of a technical limitation. However, postprandial hyperglycemia under continuous insulin administration has already been reported (*24, 25*) even when CSII (continuous subcutaneous insulin infusion) was introduced. Therefore, it is speculated that there was not enough regulation of postprandial hyperglycemia in the insulin group in the present study as well. Corroborating the findings of this study, Sato et al. reported that isolated islets with a high mitochondrial workload can become hypoxic, especially when the oxygen supply is limited (*22*). In that report, the authors also observed that decreasing the mitochondrial workload rescued the islet cells from becoming hypoxic. Likewise, Yanjun et al. showed that blood glucose fluctuations substantially damaged the pancreatic islets by enhancing oxidative stress (*21*). Although the difference did not reach statistical significance, it was observed that there is a tendency toward reduction of oxidative stress in the insulin+rest group in this study compared with the other groups. Because the revascularization was pronounced in the control group compared with the insulin+rest group, it seems likely that the avoidance of oxidative stress may be one of the crucial targets for preventing or overcoming islet exhaustion.

Unexpectedly, the newly formed vessels surrounding the grafts were markedly sparse in the insulin+rest and insulin groups compared with the control group. Considering that the islets subjected to the various stresses are well known to release a potent angiogenesis factor (*26*), it may be speculated that enhanced revascularization in the control group is attributed to angiogenesis factors released from the stressful islets exposed to hyperglycemia and a heavy workload. In other words, this novel finding also suggests that the grafts in the resting group appear to be free from several types of stress, and therefore the resting protocol is most likely highly effective.

In this study, both insulin treatment and fasting were performed throughout the initial 2 weeks after islet transplantation because it was previously demonstrated that the vascularization process is completed after approximately 14 days (*1, 3*). Indeed, Merino et al. reported that the beneficial effect of insulin treatment was maximal when it was maintained

throughout the 14-day revascularization period after transplantation (*5*). Considering that insulin per se seems to be a strong trophic factor for islet grafts (*19, 27–30*) and an effective inhibitor of glucotoxicity (*13–16*), it may be speculated that the optimal duration of insulin treatment would be no less than 14 days. This would be feasible in view of practical aspects as well because diabetic patients are often already being treated with insulin. On the other hand, further investigations of the optimal duration for short-term fasting are required because parenteral feeding may be associated with a risk of bacterial translocation and down-regulation of endogenous incretin production.

In the present study, the influence of short-term fasting on the serum concentration of GLP-1, one of the crucial incretins, was examined and found that the GLP-1 levels were significantly suppressed in the insulin+rest group compared to the other groups. In general, stimulation of proliferation and inhibition of the apoptosis of beta cells in vivo or in vitro studies are induced by a pharmacological level of GLP-1 analogues with a longer half-life or continuous infusion of GLP-1 (*31, 32*). Considering that the serum levels of GLP-1 in all experimental groups are within physiological level, and might not be enough to effectively circumvent apoptosis of β cells, it is believed that the advantages of graft preservation by the resting protocol outweigh the disadvantages of GLP-1 down-regulation, at least during the initial avascular period following transplantation. Of particular interest, the resting protocol, used in combination with GLP-1 analog administration, represents a promising regimen for further improving the graft function in the liver.

In summary, the present study demonstrates that short-term fasting combined with insulin treatment during the initial avascular period after transplantation could be a promising strategy for improving islet engraftment in the liver. Further optimization of the present resting protocol, especially with regard to the minimum duration of fasting, would be facilitated by a prospective clinical study.

MATERIALS AND METHODS

Animals

All animals used in the present study were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (*33*). Male Lewis rats were used as both donors (weighing 280–350 g, 10–12 weeks of age) and recipients (weighing 220–260 g, 8 weeks of age) (Japan SLC Inc., Shizuoka, Japan).

Islet Isolation and Transplantation

Islet isolation and culture were performed as previously described (*34*). Diabetic Lewis rats underwent intraportal islet transplantation after receiving isoflurane (Abbot Japan Co., Ltd., Tokyo, Japan) for anesthesia. Rat islets were infused at a total volume of 1 mL into the recipient liver through the portal vein using a 25-gauge insulin syringe.

Induction and Diagnosis of Diabetes in the Recipients

Diabetes was induced by intravenous injection of streptozotocin (65 mg/kg) 7 days before surgery. Rats whose non-fasting blood glucose levels were ≥400 mg/dL on two consecutive measurements were considered diabetic. Serial blood glucose levels were determined, and recipients whose non-fasting blood glucose was <200 mg/dL on two consecutive measurements were considered to be cured.

Experimental Groups

Four islet equivalents (IEQs) per gram of syngeneic rat islet grafts were transplanted intraportally into three groups of streptozotocin-induced diabetic rats: the control, insulin-treated, and insulin+rest groups (868±14, 886±11, and 895±8 IEQs/rat, respectively). The control group (n=8) fed freely without insulin treatment during the study period. Both the insulin (n=5) and insulin+rest (n=7) groups received continuous insulin (Eli Lilly Japan Corp., Kobe, Japan) infusion intravenously from days 1 to 14 after transplantation by the following method. At day 1, in both groups, a small-gauge catheter was inserted into the right jugular vein under isoflurane anesthesia. The proximal end of the catheter was tunneled subcutaneously, exited between the shoulders and connected to a harness (Quick Connect Infusion System with Harness; Strategic Applications Inc., Lake Villa, IL). The catheter was then passed through a flexible and protective coil and attached via a freely rotating swivel (Strategic Applications Inc.) to an infusion pump (REGRO Digital; Ismatec SA, Glattbrugg, Switzerland).

The insulin dose was adjusted daily so that blood glucose levels were maintained between 80 and 150 mg/dL (mean dose: insulin group 1.87±0.24 U/day, resting group 1.89±0.23 U/day, P=0.91).

In addition, the insulin+rest group fasted while receiving total parenteral nutrition (TPN) from days 1 to 14 after transplantation. Nutritional support was prepared and infused under sterile conditions. The TPN solution (FULCALIQ No. 3; Tanabe Seiyaku Co., Ltd., Osaka, Japan) was composed of amino acids, dextrose, vitamins, and electrolytes. One liter contained 36.3 g of amino acids and 226.7 g of dextrose. Rats in the insulin+rest group received approximately 300 kcal/kg per day infused at 1.05 kcal/mL × 2.7 mL/h (*35–39*).

Blood Analyses

Blood samples were collected from anesthetized rats via a tail incision on days 0, 14, and 35. These samples ware centrifuged immediately for 10 min at 2,200 *g*, and the resulting serum was frozen at −80°C until the analyses. The serum levels of interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-alpha (TNF-α) were determined using a MILLIPLEX MAP Kit Rat (Millipore Corp., Billerica, MA). The serum levels of 8-OHdG were determined using a Highly Sensitive ELISA kit for 8-OHdG (NIKKEN SEIL Corp., Shizuoka, Japan) to analyze the oxidative stress in the recipients. For the glucagon-like peptide-1 (GLP-1) analysis, blood samples obtained on days 0 and 14 were collected into microtubes containing a dipeptidyl peptidase 4 (DPP-4) inhibitor and centrifuged immediately for 10 min at 1,000 *g*, and then the serum was frozen at −80°C. The blood samples from the resting group on day 14 were collected under fasting conditions. The serum levels of GLP-1 were determined using a GLP-1 (Active) ELISA Kit (Shibayagi, Gunma, Japan).

Intravenous Glucose Tolerance Testing (IVGTT)

The IVGTT was performed 5 weeks after islet infusion. After a 14-hr fast, D-glucose (1.0 g/kg) was infused intravenously as a single bolus, and the blood glucose concentrations were determined before and at 5, 10, 20, 30, 60, 90, and 120 min after the glucose injection. The results of the IVGTT were evaluated by area under the curve (AUC) and Kg values.

Quantitation of Insulin in the Recipient Livers

Recipient livers were retrieved and homogenized in 5 mL of deionized water at 4°C. After adding 25 mL of deionized water and 75 mL of 0.18 M HCl in 96% ethanol, the homogenate was stored at 4°C for 24 hr and was then centrifuged at 2,150 *g* for 10 min. The resulting supernatant was stored at −80°C. The insulin concentration in the supernatant was evaluated using a commercial ELISA kit (Mercodia, Uppsala, Sweden).

Immunohistochemical Staining

The recipient livers with islet grafts were harvested and fixed with 4% paraformaldehyde overnight, and embedded in paraffin for immunohistochemical staining 14 days after transplantation. Immunohistochemical staining was performed using an In Situ Apoptosis Detection Kit (Trevigen, Inc. Gaithersburg, MD) for TUNEL staining, and an anti-von Willebrand Factor (vWF) antibody (Millipore) and Envision kit (Dako, Glostrup,

Denmark) for vWF staining. At least 35 sections from each experimental group (n=3, respectively) were evaluated for islet apoptosis by counting the TUNEL-positive cells. For the evaluation of revascularization, the number of new vessels around the grafts that consisted of vWF-positive cells was compensated by graft size. The mean number of new vessels per islet area from three individual experiments (at least 10 sections from one experiment) was compared among the three groups. The count was performed among triple-blind evaluations.

Statistical Analysis

All data are expressed as the means±SEM and were compared using a one-way factorial analysis of variance (ANOVA). The Bonferroni correction was used as a post hoc test when the data were determined to be significant by ANOVA. Differences were considered to be significant when $P<0.05$.

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Review

A Review of Autologous Islet Transplantation

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Autologous islet transplantation after total or semitotal pancreatectomy aims to preserve insulin secretory function and prevent the onset of diabetes. The major indication for pancreatectomy is chronic pancreatitis with severe abdominal pain, a benign pancreatic tumor, and trauma. The metabolic outcome of autologous islet transplantation is better than that of allogeneic transplantation and depends on the number of transplanted islets. Achieving islet isolation from a fibrous or damaged pancreas is one of the biggest challenges of autologous islet transplantation; a major complication is portal vein thrombosis after crude islet infusion. However, the incidence of portal vein thrombosis has decreased as islet preparation techniques have improved over time.

Key words: Total pancreatectomy; Islet; Autologous transplantation

INTRODUCTION

Autologous islet transplantation may prevent the onset of postoperative diabetes. Even if some exogenous insulin is required, diabetic control is simplified because the transplanted islets produce insulin in the long term. The major indication of pancreatectomy is chronic pancreatitis with severe abdominal pain. The first total pancreatectomy with autologous islet transplantation was carried out in 1977 at the University of Minnesota (28). Recently, some institutions have reported high numbers of autologous islet transplantation, including the University of Alberta (15 cases in 2012) (12), the University of South Carolina (33 cases in 2012) (19), the Baylor Research Institute (17 cases in 2010) (29), the University of Alabama (27 cases in 2009) (3), the University of Minnesota (86 cases in 2009) (2), the University of Leicester (46 cases in 2008) (32), and the University of Cincinnati (45 cases in 2005) (1). Here we review the current status of autologous islet transplantation.

Indications

Autologous islet transplantation is chiefly applied after a pancreatectomy to relieve pain due to chronic pancreatitis. However, the indications of total pancreatectomy are strict (5). The first step in managing pain due to chronic pancreatitis is to confirm its diagnosis. The next step is to search for complications related to the diagnosis, including pancreatic cancer and gastroparesis, which should be treated first. Abstinence from tobacco and alcohol prior to and during the autologous islet transplantation process is essential (6). Medical therapy includes the use of nonsteroidal anti-inflammatory drugs, narcotic analgesic agents, antidepressant agents, and pancreatic enzymes. Endoscopic therapy, including ductal decompression, is limited to the dilated main pancreatic duct and requires the highest level of experience. The most commonly performed procedure for chronic pancreatitis with a dilated pancreatic duct is lateral pancreaticojejunostomy (27). A part of the pancreas is also resected. The final surgical component is total pancreatectomy with autologous islet transplantation.

Autologous islet transplantation is applied after partial pancreatectomy in cases of insulinoma (20), neuroendocrine tumors, and cystic neoplasms of the pancreas (11,23). We have also experienced two cases of autologous islet transplantation following a distal pancreatectomy. In these cases, tumors such as microcystic serous cystadenoma and intraductal papillary mucinous adenoma were present in the center of the pancreas; the tail part of the pancreas was digested and infused into the patient. In addition, autologous islet transplantation has been performed for trauma (10).

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Islet Preparation and Transplantation

In cases of chronic pancreatitis, islet isolation is difficult because of the presence of fibrous tissue in pancreatic parenchyma. The method of islet isolation for autologous transplantation is not different from that for allogeneic transplantation. Islets are isolated using Ricordi's method (22) with modifications by each faculty. Collagenase, which is used to digest the pancreas, is a critical factor affecting isolation results. Liberase HI (Roche Diagnostics, Indianapolis, IN, USA) was widely used for clinical islet isolation for more than 10 years. However, this enzyme was disqualified from clinical use because of the potential risk of transmissible spongiform encephalopathy. Currently, the SERVA/Nordmark Collagenase NB1 and Neutral Protease NB Blend (SERVA Electrophoresis, Heidelberg, Germany) and Liberase MTF (mammalian tissue free) are used for clinical islet isolation (2,15). The development of new enzyme blends containing purified collagenases from *Clostridium histolyticum* and a neutral protease from *Bacillus thermoproteolyticus rokko* or *C. histolyticum* has also progressed (4).

Islets are generally transplanted into the portal vein immediately after isolation. Heparin is infused intravenously or with islet suspension to prevent clot formation around islets. Portal vein pressure is monitored during infusion. If portal hypertension (>25–28 mm H_2O) is observed, infusion into the portal vein is abandoned, and the remaining islets are transplanted into other sites.

Preventing hyperglycemia during the operative period is important because it can damage islet cells as a result of islet exhaustion (24). Hyperglycemia is reported to decrease blood flow in the transplanted islets and inhibit their vascularization (7). Glucose-containing solutions should not be administered to patients before islet cell infusion. Moreover, IV exogenous insulin administration is essential after transplantation (14).

Complications

Portal vein thrombosis can occur after autologous islet transplantation. The Leicester group reported their experiences with 24 patients who underwent this process (33), in which one patient (4.2%) developed portal vein thrombosis and was subsequently treated with anticoagulant therapy. The University of Cincinnati group reported portal vein thrombosis in 1 (0.9%) of 107 cases (30) that was treated with a combination of mechanical thrombectomy and thrombolytics. The Baylor group reported that major portal vein thrombosis with radiological intervention was required in 1 (3.8%) of 26 autologous islet transplantation procedures (15). The University of Minnesota (31) and University of Alabama (3) groups have reported 48 and 26 cases of autologous islet transplantation, respectively; neither group reported any patient who developed portal vein thrombosis. The crude preparation increased thrombogenicity due to elevated thromboplastin activity (8,30). The University of Alberta group reported that nonpurified islet autologous transplantation increases the risk of acute portal hypertension compared to allogeneic islet transplantation; in addition, they have reported that portal hypertension is associated with the packed cell volume and number of transplanted cells (12).

Several cases of disseminated intravascular coagulation (DIC) after transplantation have been reported (8,16). Severe postoperative hemorrhage was observed in some cases. The presence of tissue factor from the mincing of the pancreas is suggested to be an initiating factor for the development of thrombosis and DIC. Heparin is infused before autologous islet transplantation to prevent clot formation. One case of heparin-induced thrombocytopenia has been reported (21). The incidence of portal vein thrombosis has decreased over time as the technique of islet preparation has improved.

Postoperative infectious complications require attention because pancreatic fluid is frequently reported to be infected (18). The University of Cincinnati group reported that 25 (89.3%) of 28 patients had bacterial culture-positive media solution (transport or transplantation solution); however, only four (14.3%) patients had an infectious complication (35).

The Leicester group reported splenic infarction after spleen-preserved total pancreatectomy and autologous islet transplantation into the spleen (34). They mention that if the splenic artery and vein are ligated, the spleen should be used for islet transplantation with caution.

Transplantation Site

Most programs apply intraportal infusion to the liver because of its large capacity to receive transplanted islets as well as the relative ease of transplantation with minimal side effects. However, the liver may not be the optimal transplantation site (25,26). The liver presents unstable environments for the islets including variable oxygen availability and angiogenic activity. Postprandial hyperglycemia in the liver may affect islet β-cells. Insulin and glucagon from the islets transplanted in the liver drain into the systemic vein and not into the portal vein. The spleen and intra-abdominal cavity, especially the omental pouch, are suggested to be optimal autologous islet transplantation sites (9). Theoretically, the spleen provides an environment similar to the native pancreas for the transplanted islets. Moreover, insulin naturally drains into the portal vein. However, as described above, islet transplantation into the spleen confers risks of splenic infarction and portal vein thrombosis. The intraperitoneal and omental pouch sites are suitable for transplanting unpurified autologous islets because they do not limit the amount of transplanted tissue (13). Furthermore, the omentum has a relatively high blood content and a number

of blood vessels, which increases the vascular supply to the transplanted islets. However, a large number of islets are required to actually reverse hyperglycemia (17).

At present, comparatively pure islets should be transplanted via the portal vein while monitoring the portal vein pressure; if the portal vein pressure is elevated, relatively crude islets should be transplanted into the omental pouch.

Metabolic Outcome

The University of Minnesota, University of Alabama, University of Cincinnati, and University of Leicester have reported on the metabolic outcomes of autologous islet transplantation. The Minnesota group used two different enzyme blends: Liberase HI (LH) and SERVA/Nordmark (SN). In the LH group ($n=33$), 5 patients (15%) were insulin independent, 21 (64%) had partial function, and 7 had graft failure. In the SN group ($n=26$), 6 patients (23%) were insulin independent, 19 (73%) had partial function, and 1 had graft failure. If >5,000 IEQ/kg islets were transplanted, the probability of graft function was 100% (2). The Alabama group reported that insulin independence was not achieved in any of their patients ($n=27$); however, the total number of transplanted islets was very low (1,331±304 IEQ/kg, mean±SEM) (3). Meanwhile, the Cincinnati group divided 45 patients into insulin-dependent (27 patients, 60%) and insulin-independent (18 patients, 40%) groups. The number of transplanted islets was significantly greater in the insulin-independent group than in the insulin-dependent group (6,635±229 vs. 3,799±629). Interestingly, only 1 of 15 male patients achieved insulin independence, in contrast to 17 of 30 female patients. The authors reported that the possible reason of higher islet yields in women was that they were 10 kg lighter than men, on average (1). The Leicester group reported the long-term assessment of graft function. Twelve of 46 patients (26%) showed periods of insulin independence for 2–63 months. Over a 10-year follow-up period, notable increases in insulin requirements and the percentage of glycosylated hemoglobin levels were observed. However, all tested patients were C-peptide positive, and high fasting and stimulated C-peptide values were recorded 10 years after transplantation (32).

CONCLUSIONS

Total pancreatectomy may provide pain relief for patients with chronic pancreatitis when other therapies have failed. Autologous islet transplantation is performed to prevent or minimize postsurgical diabetes. In addition, it is performed after total or partial pancreatectomy for benign pancreatic tumors and trauma. The major complication is portal hypertension and portal thrombosis. Improvements in islet preparation have decreased the incidence of portal vein thrombosis.

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膵・膵島移植における免疫抑制療法

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REVIEW ARTICLE

Immunosuppressive therapy for pancreas and islet transplantation

Immunosuppressive regimen for pancreas transplantation is currently almost the same as that for kidney transplantation in Japan. A number of clinical trials, such as avoidance or withdrawal of steroids, use of mTOR inhibitor, etc, have been carried out abroad. T-cell depleting antibody has been commonly used as an induction therapy. For islet transplantation, various clinical trials in immunosuppressive therapy have been carried out after Edmonton protocol. Minnesota group reported long-term graft survival using antithymocyte globulin (rATG), TNF-α inhibitor, cyclosporine and everolimus. In Japan, a new clinical trial has started using calcineurin inhibitor, MMF, rATG and TNF-α inhibitor.

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***key words*：** steroid withdrawal, T-cell depleting antibody, TNF-α inhibitor

2010年の改正臓器移植法の施行により脳死膵臓移植件数は急増し，多くの1型糖尿病患者に大きな恩恵をもたらしている．わが国における脳死膵臓移植の免疫抑制療法はほぼ腎移植と同様に行われており，従来の欧米での成績と遜色ない[1)]．

膵島移植では，2000年に発表されたエドモントンプロトコール[2)]がその高いインスリン離脱率からセンセーショナルを巻き起こしたが，長期成績は良好ではなかった[3)]．そこで，現在まで数々の免疫抑制療法が開発されている．わが国の臨床膵島移植は5年にわたる中断ののち[4)]，2010年秋にようやく先進医療として再開した．

本稿では，現在わが国で行われている生体および脳死膵臓移植の免疫抑制療法，海外でのトピック，膵島移植の免疫抑制療法の歴史，わが国で行われている先進医療における免疫抑制療法につき概説する．

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生体膵臓移植

国立病院機構千葉東病院では，2004～12年までに18例の生体膵臓移植(膵腎同時移植16例，膵単独移植1例，腎移植後膵移植1例)を施行している．その免疫抑制療法は基本的に生体腎移植と同様で，導入にバシリキシマブ(BXM)を用い，維持はタクロリムス(Tac)またはシクロスポリン(CyA)，ミコフェノール酸モフェチル(MMF)，プレドニゾロン(PSL)の3剤である．ABO血液型不適合移植では腎移植と同様，移植の28日前からMMF，10日前からPSLおよびTacまたはCyAを先行投与し，抗体除去として3回の二重膜血漿濾過と，1回の血漿交換を行っている．

生体膵臓移植は保険適応となっておらず(2013年1月現在)，周術期の免疫抑制剤が保険適応の規制にないため，導入にBXMに替えてサイモグロブリン(rATG)を投与している施設もあるようである．

千葉東病院における生体膵臓移植の成績は，18例中1例がprimary non-functionであったが，他の全例はインスリンを離脱した．1例が脳梗塞にて

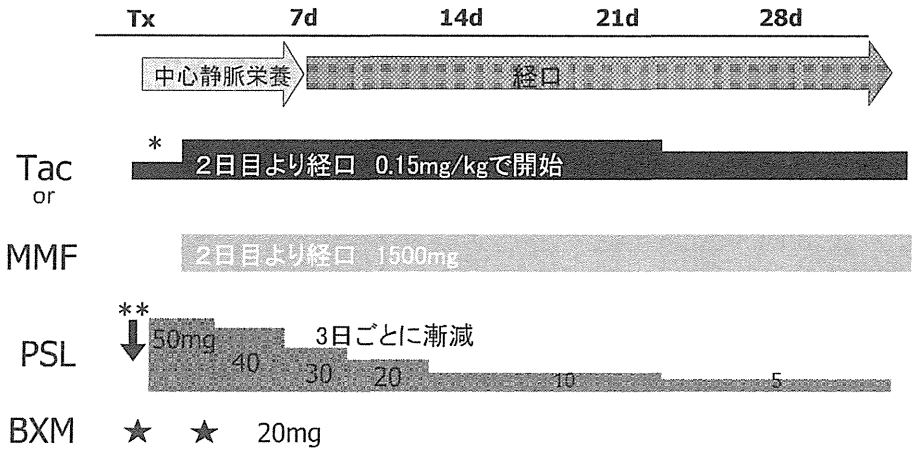

図1 脳死膵臓移植時の免疫抑制療法

*移植当日～移植後1日目はタクロリムスを0.05 mg/kg/dayで持続静注する.

**術中にメチルプレドニゾロンを250 mg経静脈的に投与する.

死亡，1例が急性期に血栓症にて膵グラフト摘出，2例がインスリン再導入となっている．14例(78%)がインスリン離脱中である．膵腎同時移植の腎機能については，全例が透析を離脱したが，1例が透析再導入となっている．

脳死膵臓移植

脳死膵臓移植も死体腎移植と同様の免疫抑制療法で行っている(**図1**)．TacまたはCyAは可能であれば移植前1回経口投与をしている．移植後1日目まで静脈投与し，2日目からMMFとともに経口投与を行う．

導入はBXMであるが，膵単独移植の場合は成績向上を期して(後述)，rATGを使用する場合もある．

千葉東病院および藤田保健衛生大学にて，筆者らが経験した脳死膵臓移植は19例(腎移植後膵移植2例，膵単独移植3例，膵腎同時移植14例)である．移植膵機能は，1例がインスリンを離脱できず，少量のインスリンを必要としているほかは，全例がインスリンを離脱した．1例が心不全にて死亡し，2例がインスリン再導入となっている．膵腎同時移植の移植腎機能については，心不全で亡くなった1例を除く13例が現在も透析を離脱している．

膵臓移植における免疫抑制剤の今後の展望

MMFに替わりmTOR(mammalian target of rapamycin)阻害剤であるrapamycinを用いる臨床治験の報告が増えている．マイアミ大学のCiancioらの報告[5]による170例の10年にわたる単施設ランダム化比較試験では，膵グラフト生着率では有意差がみられなかったものの，biopsy proven acute rejection発生率は移植1年後，10年後ともrapamycin群で有意に低かったとしている．またMMF群において，消化器障害や骨髄抑制にてMMFを減量することが拒絶反応発症のリスクとしている．HbA1cと血中脂質(総コレステロール，中性脂肪)レベルは，両群とも正常範囲であったものの，rapamycin群で有意に高値であった．

膵臓移植におけるステロイドの中止や回避するプロトコールは数多く報告されており，The International Pancreas Transplant Registry(IPTR)によると，膵腎同時移植後の約1/3の患者が退院時にステロイドを投与されていないとのことである[6]．Malheiroらは，rATG，Tac，MMF，PSLで，移植後6カ月以降にステロイドを中止するレジメを行い，54例中42例(78%)でステロイドの中止に成功している[7]．膵グラフト生着率はステロイド中止群で良好(5年90%)であったが，これはステロイドの中止ができなかった理由が拒絶反応の既往や感染症にてMMFを減量したためとしている．Axelrodらは，rATGまたはalemtuzumab，MMFま