Fodero-Tavoletti MT, Mulligan RS, Okamura N, Furumoto S, Rowe CC, **Kudo Y,** Masters CL, Cappai R, Yanai K, Villemagne VL	In vitro characterisation of BF227 binding to α-synuclein/Lewy bodies	Eur J Pharmacol	617	54-58	2009
幡谷速昭、**木村芳孝**、伊藤拓哉、辛島彰洋、片山統裕、八重樫伸生、中尾光之	1計測信号参照系独立成分分析法のシミュレーション研究	IEICE Technical Report			2009
松田尚美、**木村芳孝**,他	母体葉酸欠乏が胎仔胎盤発育に及ぼす影響とそのエピジェネティック制御機構解析	British Journal of Nutrition		In press	2010
Tomita H, Sugano E, Isago H, Hiroi T, Wang Z, Ohta E, Tamai M	Channelrhodopsin-2 Gene Transduced into Retinal Ganglion Cells Restores Functional Vision in Genetically Blind Rats	Exp Eye Res	90	426-436	2010
Tomita H, Sugano E, Isago H, **Tamai M**	Channelrhodopsins provide a breakthrough insight into strategies for curing blindness	J Genetics	88	409-415	2009
Tomita H, Sugano E, Fukazawa Y, Isago H, Sugiyama Y, Hiroi T, **Tamai M**,et al	Visual Properties of Transgenic Rats Harboring the Channelrhodopsin-2 Gene regulated by the Thy-1.2 Promoter.	PLoS ONE	4(11)	e7679	2009
Murakawa H., Aono N., Tanaka T., Kikuchi H., Yoshida H., Yoshida H., Yokoo M., **Abe H.**	Morphological evaluation and measurement of the respiration activity of cumulus-oocyte complexes to assess oocyte quality	J. Mamm. Ova Res.	Vol. 26	32-41	2009
Yamashiro H., Toyomizu M., Kikusato M., Toyama N., Sugimura S., Hoshino Y., **Abe H.**, Moisyadi S., Sato E.	Lactate and adenosine triphosphate in the extender enhance the cryosurvival of rat epididymal sperm	J. Am. Assoc. Lab. Anim. Sci.	Vol. 49	160-166	2010

Kyono K., Nakajo Y., Nishinaka C., Hattori H. Kyoya T., Ishikawa T., **Abe H.**, Araki Y.	A birth from the transfer of a single vitrified-warmed blastocyst using ICSI with calcium ionophore oocyte activation in a globozoospermic patient	Fertil. Steril.	Vol. 91	e7-e11	2009
H.Shiku, T.Yamakawa,, Y.Nashimoto, Y.Takahashi,Y.Torisawa, **H.Abe**, et al.	A microfluidic dual capillary probe to collect messenger RNA from adherent cells and spheroids	Analytical Biochemistry	385	138-142	2009
T.S.Matsui, S.Deguchi, N.Sakamoto, T.Ohashi, **M.Sato**	A Versatile micro-mechanical tester for actinstress fibers isolated from cells	Biorheology 46	46	401-415	2009
Y. Ueki, N. Sakamoto, T. Ohashi and **M. Sato**	Morphological responses of vascular endothelial cells induced by local stretch transmitted through intercellular junctions	Experimental Mechanics	49-1	125-134	2009
S. Sugita, N. Sakamoto, T. Ohashi and **M. Sato**	Novel image analysis for trajectory of microtubules gliding on kinesins with tip detection	Journal of Biomechanical Science and Engineering	4-3	404-414	2009
S. Deguchi, and **M. Sato**	Biomechanical properties of actin stress fibers of non-motile cells	Biorheology	46-2	93-105	2009
Y. Ueki, N. Sakamoto, and **M. Sato**	Direct measurement of shear strain in adherent vascular endothelial cells exposed to fluid shear stress	Biochemical and Biophysical Research Communications	394-1	94-99	2010
Toyohara T., Suzuki T., **Abe T.**, et al	SLCO4C1 Transporter Eliminates UremicToxins and Attenuates Hypertension andRenal Inflammation	J. Am. Soc. Nephrol.	20	2546-2555	2009
Tanemoto M., Suzuki T., Abe M., **Abe T.**,and Ito S.	Physiologic variance of corticotropin affects diagnosis in adrenal vein sampling	Eur. J. Endocrinol.	160	459-463	2009
Tanemoto M., Suzuki T., **Abe T.**, and Ito S.	Hemodynamic Index of Atheromatous Renal Artery Stenosis for Angioplasty.	Clin. J. Am. Soc. Nephrol.	4	651-655	2009

Yusuke Ono,Viola F. Gnocchi, Peter S.Zammit, and **Rhoichi Nagatomi**	Presenilin-1 acts via Id1 to regulate the function of muscle satellite cells in a γ-secretase-independent mannar	Journal of Cell Science	122(24)	4427-4438	2009
Y. Gondoh, M. Tashiro,M Itoh, Mohammad M.Masud,H.Sensui,**R.Nagatomi** and T.Fujimoto,et al	Evaluation of individual skeletal muscle activity by glucose uptakeduring pedaling exercise at different workloads using positron emission tomography	J.Appl Physiol	107	599-604	2009
Kaori Matsuo, Xiumin Zhang, Yusuke Ono, **Ryoichi Nagatomi**	Acute stress-induced colonic tissue HSP70 expression requires commensal bacterial components and intrinsic glucocorticoid	Brain, Behavoir, and Immunity	23	108-115	2009
H.Abe, A.Michimata, K.Sugawara, N.Sugaya and **S. Izumi**	Improving Gait Stability in Stroke Hemiplegic Patients with a Plastic Ankle-Foot Orthosis	Tohoku J.Exp. Med.	218	193-199	2009
T.FURUKAWA, **S.IZUMI**, M.TOYOKURA,and Y.MASAKADO	Effects of Low-frequency Repetitive Transcranial Magnetic Stimulation in Parkinson's Disease	Tokai J Exp Clin Med.	34(3)	63-71	2009
Hany Eldawoody, H.Shimizu, N.Kimura, A.Saito, T.Nakayama, **A.Takahashi**, T.Tominaga	Simplified experimental cerebral aneurysm model in rats:Comprehensive evaluation of induced aneurysms and arterial changes in the circle of Willis	Brain Research	1300	159-168	2009
N.Kimura, H.Shimizu, H.Eldawoody, T.Narayama, A.Saito,T.Tominaga, **A.Takahashi**	Effect of olmesartan and pravastatin on expermental cerebral aneurysms in rats	Brain Research	1322	144-152	2010
H.Eldawoody, H.Shimizu, N.Kimura, A.Saito,T.Nakayama, **A.Takahashi,** T.Tominaga	Fasudil, a Rho-kinase inhibitor, attenuates induction and progression of cerebral aneurysms:Experimental study in rats using vascular corrosion casts	Neuroscience Letters	470	76-80	2010
A.Kohyama, H.Ogawa, Y.Funayama, K.Takahashi,**K. Fukushima**, at al	Bacterial population moves toward a colon-like community in the pouch after total proctocolectomy	SURGERY	145(4)	435-447	2009

Ⅳ. 研究成果の刊行物・別刷

ELSEVIER

The Influence of Brain Death on Tissue Factor Expression in the Pancreatic Tissues and Isolated Islets in Rats

Y. Saito, M. Goto, K. Maya, N. Ogawa, K. Fujimori, Y. Kurokawa, and S. Satomi

ABSTRACT

Introduction. Tissue factor (TF) in islets has been identified as the main trigger of the instant blood-mediated inflammatory reaction. Because the crucial events that directly induce TF remain to be determined, we focused on the influence of brain death (BD) on TF expression in pancreatic tissues and isolated islets.

Materials and Methods. BD was induced in male Lewis rats weighing 250–300 g by inflation of a Fogarty catheter placed intracranially. The rats were mechanically ventilated for 6 hours until removal of the pancreas. The expression of TF protein in pancreatic tissues was examined using Western blotting assay. Messenger RNA (mRNA) expressions of TF in pancreatic tissue and isolated islets were analyzed using real-time polymerase chain reaction (PCR) assay. The influence of BD on the isolation outcome was evaluated by islet yield, purity, viability, and function.

Results. TF protein and mRNA levels in the pancreatic tissues were similar between the groups. However, TF mRNA in the isolated islets of the BD group was significantly greater than that of the control group ($P = .04$). Islet yield was considerably lower, and purity significantly lower in the BD than the control group ($P = .002$). Unexpectedly, ATP/DNA ratio and respiratory activity were comparable between the groups.

Conclusions. Although BD per se was not sufficient to induce TF expression in pancreatic tissues, BD combined with subsequent warm ischemic damage during isolation procedures remarkably up-regulated TF expression in isolated islets, suggesting that BD is of great importance as an initiator of TF induction in the islet grafts. The present study demonstrated that the expression of inflammatory mediators rather than islet viability is more susceptible to BD.

TISSUE factor (TF), a 47-kd transmembrane glycoprotein, acts as the initiator of the extrinsic coagulation system. It is pivotal for activation of the intrinsic pathway as well. Pancreatic islets have thus far been reported to express TF.[1] It has been revealed that TF in islets has been identified as the main trigger of the instant blood-mediated inflammatory reaction.[1,2] Low expression of TF in the graft has been correlated with high C-peptide values after clinical islet transplantation.[3] However, the crucial procedures to directly induce TF remain to be determined.

It is well known that the outcome of organ transplantation is highly influenced by brain death (BD). The success rate of kidney transplantations derived from cadaveric

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This study was supported by grants from Innovation Plaza Miyagi of JST (Japan Science and Technology Agency), the Japanese Grant-in-Aid for Scientific Research (B), and the Nakajima Foundation.

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0041-1345/09/$–see front matter
doi:10.1016/j.transproceed.2008.10.080

donors remains significantly inferior to that from living donors regardless of their genetic relationship to the recipient.[4] Contreras et al presented data that demonstrated BD to reduce isolated pancreatic islet yield and function, as well as up-regulation of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 in the serum and pancreatic tissues from BD donors.[5] In the present study, therefore, we focused on the influence of BD on TF expression in pancreatic tissues and isolated islets.

MATERIALS AND METHODS

Rodent BD Model

BD was induced in male Lewis rats weighing 250–300 g by inflation of a Fogarty catheter placed intracranially. The rats were mechanically ventilated for 6 hours until removal of the pancreas as previously described.[5,6]

Western Blotting Assay of the Pancreatic Tissues

Pieces of pancreatic tissues from BD and control groups snap-frozen in liquid nitrogen were stored at −80°C until use (n = 5 and n = 4, respectively). Approximately 10 mg of pancreatic biopsy specimens prepared on dry ice were immediately transferred into phosphate-buffered saline containing 5 mmol/L EDTA, 10 mmol/L benzamidine (Merck-Schuchardt, Hohenbrunn, Germany), 0.1 g/L soybean trypsin inhibitor (Sigma-Aldrich, Steinheim, Germany), and 1 mmol/L phenyl methyl sulfonyl fluoride (Sigma). The samples were then homogenized using Polytron PT 1300D (Kinematica AG, Littau-Lucerne, Switzerland) and Vibra-Cell (Sonics & Materials Inc, Newtown, Conn, USA) for 30 seconds each. Thereafter the samples were centrifuged at 4°C at 10,000*g* for 30 minutes to collect the supernate. The samples, containing 2.5 mg/mL of protein measured by BCA Protein Assay kit (Thermo Prod, Rockford, Ill, USA), were separated using SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Invitrogen, Carlsbad, Calif, USA). Membranes were incubated with rabbit anti-rat TF polyclonal antibody (Hokudo, Sapporo, Japan) at 4°C overnight and subsequently with goat anti-rabbit immunoglobulin (Ig)G-horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, Calif) antibody for 1 hour at room temperature. TF antigen was visualized using enhanced chemiluminescence Western Blotting Detection Reagents (GE Healthcare, Buckinghamshire, UK).

Determination of TF mRNA in the Pancreatic Tissues

Pieces of pancreatic tissues from BD and control groups snap-frozen in liquid nitrogen were stored at −80°C until use (n = 6 and n = 4, respectively). Total RNA was prepared using the RNeasy Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's protocol. RNA concentrations were estimated from absorbance at 260 nm. First-strand complementary DNA (cDNA) was synthesized from 2500 ng total RNA using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Indianapolis, Ind, USA). The cDNAs were amplified by PCR using rat TF primer probe set (Nihon Gene Research Laboratories Inc., Sendai, Japan) and rat GAPDH primer probe set (Nihon Gene Research Laboratories Inc.) with a Lightcycler (Roche Diagnostics).

Islet Isolation and Culture

Before removal of the pancreas, the cannulated bile duct was injected with 10 mL of cold Hanks' Balanced Salt Solutions (HBSS) containing 1 mg/mL Coragenase (Sigma type V; Sigma Chemicals, St. Louis, Mo). After addition of 10 mL HBSS the pancreas was digested at 37°C for 14 minutes. Thereafter, density-gradient centrifugation was performed using Histopaque-1119 (Sigma Diagnostics) and Lymphoprep (Nycomed Pharma AS, Oslo, Norway) to isolate pancreatic islets. The islet count was performed as islet equivalents (IEQ) under a scaled microscope using diphenylthiocarbazone (Wako, Osaka, Japan) staining (BD, n = 8; control, n = 7). One IEQ was the islet tissue mass equivalent to a spherical islet of 150 μm in diameter. Islets were cultured in RPMI-1640 containing 5.5 mmol/L glucose and 10% FBS at 37°C in 5% CO_2 and humidified air before examination.

Islet Viability and Function

ATP/DNA ratio was measured to evaluate the energy status of isolated islets. Eighty islet equivalents of islets with overnight culture were used in both BD and control groups (BD, n = 6; control, n = 5). The ApoGlow kit (Lonza Rockland Inc, Rockland, ME, USA) was used for ATP measurement as described previously.[7] Using the same sample, the DNA content was measured using DNA Quantify kit (Primary Cell, Sapporo, Japan) as described previously.[8] We evaluated the respiratory activity of isolated islets with overnight culture using scanning electrochemical microscopy (BD, n = 6; control, n = 5). The stimulation index of the respiratory activity, defined as the ratio of the respiratory activity in high glucose (16.7 mmol/L) against that in basal glucose (1.67 mmol/L), is a novel marker that was applied as a rapid, potent predictor for the outcome of clinical islet transplantation.

Determination of TF mRNA in the Isolated Islets

Total RNA extracted from the 40 islets after 3-hour culture was prepared using RNeasy Micro Kit (Qiagen) according to the manufacturer's protocol (BD, n = 5; control, n = 8). RNA concentration was estimated from absorbance at 260 nm. First-strand cDNA was synthesized from 100 ng total RNA using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics). The cDNAs were amplified by PCR, using rat TF primer probe set (Nihon Gene Research Laboratories Inc.) and rat GAPDH primer probe set (Nihon Gene Research Laboratories Inc.) with a Lightcycler (Roche Diagnostics).

Statistical Analysis

All data are expressed as mean values ± SD. Comparisons between groups were performed by student *t* test using Statcel 2nd Edition (Oms Publishing, Osaka, Japan). Statistical significance was established at $P < .05$.

RESULTS

Tissue factor protein and mRNA levels in the pancreatic tissues were similar between the groups (Fig 1 and 2). However, TF mRNA in the isolated islets of the BD group was significantly greater than that of the control group (TF/GAPDH BD, 0.169 ± 0.033; control, 0.119 ± 0.041; $P = .04$) (Fig 3). Islet yield was considerably lower (BD, 2110 ± 231 IEQs; control, 2390 ± 528 IEQs; $P = .19$), and purity was significantly lower in the BD than the control group (BD, 87.7 ± 7.5%; control, 97.0 ± 2.6%; $P = .002$). Unexpectedly, the ATP/DNA ratio and respiratory activity were comparable between the groups (ATP/DNA BD, 51.6 ± 12.8; control, 59.1 ± 3.47; $P = .20$; and BD, 2.39 ± 0.55; control, 2.58 ± 0.19; $P = .45$).

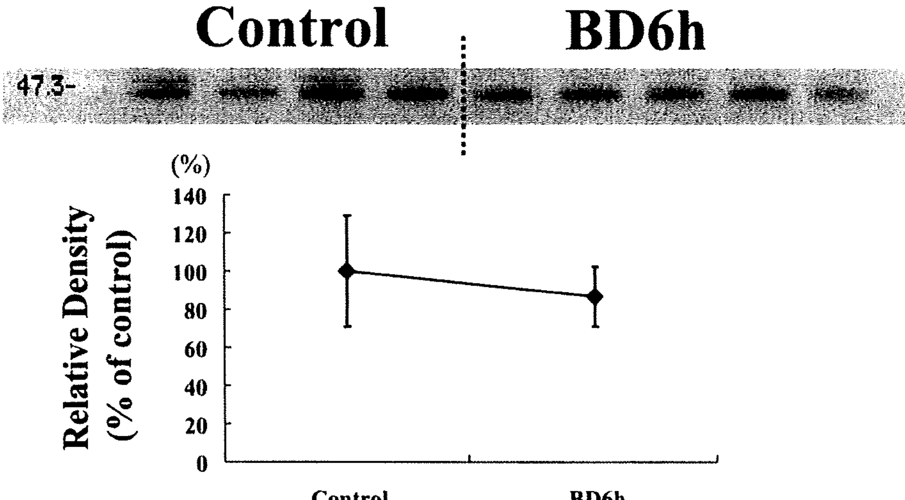

Fig 1. Protein expression of TF in the pancreatic tissues from the donors with/without BD was analyzed using Western blotting assay.

DISCUSSION

In the present study, our data showed that BD influenced TF expression in isolated islets but not in pancreatic tissues prior to the digestion procedure. It may be speculated that the difference was attributable to warm ischemic damage during the digestion procedure. In islet transplantation, unlike other organ transplantations, islet grafts are placed at 37°C during whole digestion procedure. This period could theoretically be considered as one kind of "warm ischemia," a concept that is supported by many investigators in the field of islet transplantation.[9–11] As shown in the present study, TF was not up-regulated in the isolated islets from the donors without BD, suggesting that warm ischemic damage during digestion procedure per se was not sufficient to induce TF in isolated islets. We therefore believe that the induction of TF from BD was accelerated by warm ischemic damage during the digestion procedure.

In the present study, islet yield and purity were certainly affected by BD. However, the difference was extremely small compared with a previous report,[5] moreover, almost no influence was observed in terms of islet viability. One possible explanation for this discrepancy is a difference in isolation procedures. In our isolation procedures, pancreatic tissues were kept on ice except during the digestion phase. Furthermore, at the density-gradient centrifugation phase, we applied Histopaque-1119 and Lymphoprep, in contrast, a dextran gradient separation was performed in the previous report.[5] Hence, the important message from our present study is that the expression of inflammatory mediators rather than islet viability is more susceptible to BD.

In conclusion, although BD per se was not sufficient to induce TF expression in pancreatic tissues, BD combined with warm ischemic damage during isolation procedures remarkably up-regulated TF expression in isolated islets, suggesting that BD is of great importance as an initiator of TF induction in islet grafts.

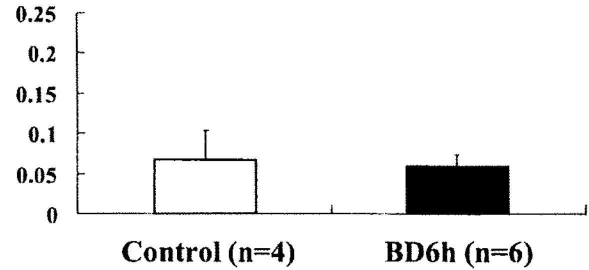

Fig 2. mRNA expression of TF in the pancreatic tissues from the donors with/without BD was analyzed using real-time PCR assay.

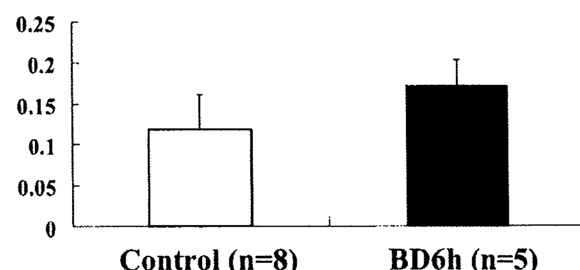

Fig 3. mRNA expression of TF in the isolated islets from the donors with/without BD was analyzed using real-time PCR assay.

ACKNOWLEDGMENTS

We thank Takehiro Imura and Megumi Goto for their excellent technical assistance.

REFERENCES

1. Moberg L, Johansson H, Lukinius A, et al: Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. Lancet 360: 2039, 2002

2. Berman DM, Caberera O, Kenyon NM, et al: Interference with tissue factor prolongs intrahepatic islet allograft survival in a nonhuman primate marginal mass model. Transplantation 84:308, 2007

3. Goto M, Johansson U, Eich TM, et al: Key factors for human islet isolation and clinical transplantation. Transplant Proc 37:1315, 2005

4. Terasaki PI, Cecka JM, Gjertson DW, et al: High survival rates of kidney transplants from spousal and living unrelated donors. N Engl J Med 333:333, 1995

5. Contreras JL, Eckstein C, Smyth CA, et al: Brain death significantly reduces isolated pancreatic islet yields and functionality in vitro and in vivo after transplantation in rats. Diabetes 52:2935, 2003

6. Pratschke J, Wilhelm MJ, Kusaka M, et al: A model of gradual onset brain death for transplant-associated studies in rats. Transplantation 69:427, 2000

7. Goto M, Holgersson J, Kumagai-Braesch M, et al: The ADP/ATP ratio: a novel predictive assay for quality assessment of isolated pancreatic islets. Am J Transplant 6:2483, 2006

8. Wennberg L, Song Z, Bennet W, et al: Diabetic rats transplanted with adult porcine islets and immunosuppressed with cyclosporineA, mycophenolate mofetil, and leflunomide remain normoglycemic for up to 100 days. Transplantation 71:1024, 2001

9. Goto T, Tanioka Y, Sakai T, et al: Application of the two-layer method on pancreas digestion results in improved islet yield and maintained viability of isolated islets. Transplantation 83:754, 2007

10. Avila JG, Wang Y, Barbaro B, et al: Improved outcomes in islet isolation and transplantation by the use of a novel hemoglobin-based O_2 carrier. Am J Transplant 6:2861, 2006

11. Swift S, Kin T, Mirbolooki M, et al: Comparison of cooling systems during islet purification. Cell Transplant 15:175, 2006

A Novel Predictive Method for Assessing the Quality of Isolated Pancreatic Islets Using Scanning Electrochemical Microscopy

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ABSTRACT

Introduction. The current methods for evaluating islet potency are not useful in clinical transplantation. Therefore, we need reliable, rapid methods enabling accurate prediction of islet quality.

Materials and Methods. We evaluated respiratory activity using scanning electrochemical microscopy (SECM), glucose-stimulated respiratory activity, glucose-stimulated insulin release, ADP/ATP assays, insulin/DNA levels, and Trypan blue exclusion tests as predictive methods for the ability of isolated rat islets to cure syngeneic diabetic rats.

Results. Although glucose-stimulated respiratory activity, basal respiratory activity, ADP/ATP ratio, and glucose-stimulated insulin release were significantly correlated with the outcome of transplantation into diabetic rats, there was no correlation between outcomes, insulin/DNA ratios, and Trypan blue exclusion tests. The glucose-stimulated respiratory activity in islet preparations that could cure diabetic rats was significantly greater than those unable to cure diabetes. Rat islets with >1.5-fold glucose-stimulated respiratory activity consistently cured diabetic rats, whereas those with a value <1.5 hardly cured any rats.

Conclusion. Measurement of the glucose-stimulated respiratory activity using SECM technique is a novel method that may be useful as a rapid, potent predictor of the outcome of clinical islet transplantation.

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This study has been supported by grants from Innovation Plaza Miyagi of JST (Japan Science and Technology Agency) and the Japanese Grant-in-Aid for Scientific Research (A).

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0041-1345/09/$–see front matter
doi:10.1016/j.transproceed.2008.10.075

THE CURRENT standard methods to evaluate islet potency are not useful in clinical islet transplantation. Furthermore, most tests are relatively subjective and time-consuming.[1] We have thus far shown that the ADP/ATP ratio correlated with in vivo viability of isolated islets.[2] However, insulin release from isolated islets is not entirely related to the ADP/ATP ratio. Moreover, it is difficult to continuously measure the ADP/ATP ratio of the same islets. Therefore, we sought to establish a reliable, rapid method enabling accurate prediction of both islet viability and insulin release. Scanning electrochemical microscopy (SECM) is a technique in which the tip of a microelectrode monitors the local distribution of electro-active species near the sample surface. SECM has been used to investigate numerous biological molecules, including DNA,[3] enzymes,[4] and antigen-antibody interactions.[5] This technique noninvasively measures respiratory activity of isolated islets under physiological conditions. We have used SECM to examine islet viability and potency of insulin release.

MATERIALS AND METHODS

In the present study, we evaluated respiratory activity using SECM, glucose-stimulated respiratory activity, glucose-stimulated insulin release, ADP/ATP assays, insulin/DNA levels, and Trypan blue exclusion tests as predictive methods to evaluate the ability of isolated rat islets exposed to various degrees of heat shock stress (0, 40, 50, 60 or 80 seconds) to cure syngeneic Streptozotocin-induced diabetic rats (n = 7, 6, 6, 7, and 7, respectively). SECM was programmed to automatically measure the reduction current of far and near points of samples based on spherical diffusion theory.[6] The respiratory activity of 10 islets in each group was calculated by evaluating the difference of the reduction current around the samples using 2–4 μm platinum-coated microelectrode. The glucose-stimulated respiratory activity was indicated by the stimulation index of the respiratory activity, defined as the ratio of the respiratory activity in high-glucose concentration (16.7 mmol/L) against that in basal glucose concentration (1.67 mmol/L). The ADP/ATP assay, insulin/DNA levels, and Trypan blue exclusion tests were performed as previously described.[2,7] In islet transplantation, 6 islet equivalents/g of body weight were transplanted into recipient livers via the portal vein using a 24-gauge butterfly needle using the previously described method.[8] Heat shock stress was induced by placing the isolated islets at 60°C for 0, 40, 50, 60, or 80 seconds.

RESULTS

On the one hand, significant correlations with the outcome of transplantation into diabetic rats were observed for glucose-stimulated respiratory activity (heat shock stress; 0 seconds; 2.39 ± 0.08; 40 seconds, 1.85 ± 0.17; 50 seconds, 0.86 ± 0.08; 60 seconds, 0.49 ± 0.03; 80 seconds, 0.37 ± 0.07; cured group: 1.94 ± 0.18; noncured group: 0.57 ± 0.07, respectively), basal respiratory activity (heat shock stress: 0 seconds, 5.65 ± 0.15, 40 seconds, 5.31 ± 0.51, 50 seconds, 4.18 ± 0.58, 60 seconds, 1.83 ± 0.27, 80 seconds, 0.31 ± 0.05; cured group: 5.27 ± 0.26; noncured group: 1.98 ± 0.46, respectively), ADP/ATP ratio (heat shock stress; 0 seconds, 0.003 ± 0.003, 40 seconds, 0.05 ± 0.03, 50 seconds, 0.21 ± 0.05, 60 seconds, 0.30 ± 0.07, 80 seconds, 0.42 ± 0.05, cured group: 0.05 ± 0.03; noncured group: 0.30 ± 0.04, respectively), and glucose-stimulated insulin release (heat shock stress; 0 seconds, 11.0 ± 2.6, 40 seconds, 2.51 ± 0.76, 50 seconds, 1.12 ± 0.14, 60 seconds, 1.13 ± 0.21, 80 seconds, 1.40 ± 0.41; cured group: 6.59 ± 1.78; noncured group: 1.35 ± 0.18, respectively) P <.0001, <.0001, <.0001, and .002: ρ = .80, .71, −.66, and .53, respectively. On the other hand, there was no correlation between islet transplantation outcome and insulin/DNA ratio (heat shock stress: 0 seconds, 0.73 ± 0.05, 40 seconds, 0.99 ± 0.13, 50 seconds, 0.86 ± 0.10, 60 seconds, 0.91 ± 0.06, 80 seconds, 1.12 ± 0.06; cured group: 0.78 ± 0.04; noncured group: 1.03 ± 0.05, respectively), and Trypan blue exclusion test (heat shock stress: 0 seconds, 100.0 ± 0.0, 40 seconds, 98.8 ± 0.6, 50 seconds, 99.3 ± 0.5, 60 seconds, 99.9 ± 0.1, 80 seconds, 94.9 ± 1.8; cured group: 99.5 ± 0.3; noncured group: 97.8 ± 0.8, respectively). The glucose-stimulated respiratory activity in islet preparations that could cure diabetic rats was significantly higher than in those unable to cure diabetes (P < .0001). Rat islets with glucose-stimulated respiratory activity more than 1.5 consistently cured diabetic rats, whereas rat islets with a value <1.5 hardly cured any rats (P < .0001) (Fig 1). Notably, the predictive rate for curing diabetic rats was 91% when glucose-stimulated respiratory activity was used.

DISCUSSION

It is well known that unexpectedly poor effects of grafts are still seen in the field of islet transplantation even using the current refined procedures. Most likely, this is attributed to suboptimal quality of the isolated islets.

It has been reported that the current methods of islet quality assessment have only a limited ability to predict

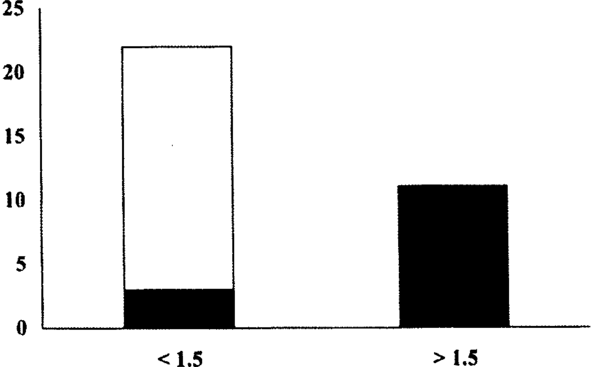


Fig 1. Streptozotocin-induced diabetic rats underwent intraportal transplantation with syngeneic islets that were exposed to various degrees of heat shock stress (0, 40, 50, 60, and 80 seconds). The X-axis indicates the glucose-stimulated respiratory activity, and the Y-axis indicates the number of animals. The black bar shows cured animals; the white bar shows noncured diabetic animals. Rat islets with glucose-stimulated respiratory activity >1.5 consistently cured diabetic rats, whereas rat islets with a value <1.5 hardly cured any rats (P < .0001).

outcomes after clinical transplantation.[1,9,10] In vivo bioassay has thus far been regarded as the most reliable assessment.[11,12] However, it is not clinically useful because several days are needed for evaluation. Therefore, we need establishment of reliable, rapid methods enabling accurate prediction of islet potency. This issue is crucial for Japan because only marginal organs from non–heart-beating donors are currently available for islet isolation.

In 2006, we reported that the ADP/ATP ratio was a useful predictive assay for isolated islets.[2] Although the ADP/ATP assay has many advantages as islet quality assessment, its limitation is the absence of a correlation with insulin release from the isolated islets, suggesting that it reflects islet viability rather than function.

As shown in the present study, glucose-stimulated respiratory activity strongly correlated with islet quality. This highly sensitive, noninvasive method made it possible to distinguish respiratory activity even in one islet by visualizing the reduction current in a simple form. Notably, the glucose-stimulated respiratory activity is expected to reflect not only islet viability but also function.

Taken together, measurement of the glucose-stimulated respiratory activity using SECM technique is a novel rapid, potent predictor of the outcome of clinical islet transplantation.

ACKNOWLEDGMENTS

We thank Takehiro Imura and Kozue Maya for their excellent technical assistance.

REFERENCES

1. Barnett MJ, McGhee-Wilson D, Shapiro AM, et al: Variation in human islet viability based on different membrane integrity stains. Cell Transplant 13:481, 2004

2. Goto M, Holgersson J, Kumagai-Braesch M, et al: The ADP/ATP ratio: a novel predictive assay for quality assessment of isolated pancreatic islets. Am J Transplant 6:2483, 2006

3. Yamashita K, Takagi M, Uchida K, et al: Visualization of DNA microarrays by scanning electrochemical microscopy (SECM). Analyst 126:1210, 2001

4. Oyamatsu D, Hirano Y, Kanaya N, et al: Imaging of enzyme activity by scanning electrochemical microscope equipped with a feedback control for substrate-probe distance. Bioelectrochemistry 60:115, 2003

5. Kasai S, Yokota A, Zhou H, et al: Immunoassay of the MRSA-related toxic protein, leukocidin, with scanning electrochemical microscopy. Anal Chem 72:5761, 2000

6. Yasukawa T, Uchida I, Matsue T: Microamperometric measurements of photosynthetic activity in a single algal protoplast. Biophys J 76:1129, 1999

7. Wennberg L, Song Z, Bennet W, et al: Diabetic rats transplanted with adult porcine islets and immunosuppressed with cyclosporine A, mycophenolate mofetil, and leflunomide remain normoglycemic for up to 100 days. Transplantation 71:1024, 2001

8. Brandhorst D, Brandhorst H, Zwolinski A, et al: Prevention of early islet graft failure by selective inducible nitric oxide synthase inhibitors after pig to nude rat intraportal islet transplantation. Transplantation 71:179, 2001

9. Eckhard M, Brandhorst D, Winter D, et al: The role of current product release criteria for identification of human islet preparations suitable for clinical transplantation. Transplant Proc 36:1528, 2004

10. Gerling IC, Kotb M, Fraga D, et al: No correlation between in vitro and in vivo function of human islets. Transplant Proc 30:587, 1998

11. Hering BJ, Kandaswamy R, Harmon JV, et al: Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. Am J Transplant 4:390, 2004

12. Ricordi C, Scharp DW, Lacy PE: Reversal of diabetes in nude mice after transplantation of fresh and 7-day-cultured (24 degrees C) human pancreatic islets. Transplantation 45:994, 1988

Current status of pancreatic islet transplantation

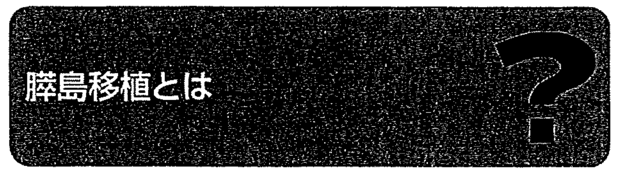
ここまで進んだ膵島移植

後藤 昌史

Key Words：①膵島 ②膵島移植 ③重症1型糖尿病 ④グラフト生着

□はじめに

2000年に新しい膵島移植療法としていわゆるエドモントンプロトコール(Tips 1)が報告されたことにより，膵島移植は世界中で大きな社会的関心を集め，1型糖尿病患者に対するひとつの選択肢としてその第一歩を踏み出した．わが国においても2004年4月より膵島移植が開始され，すでに現在までに28例が行われている．現在の膵島移植はエドモントンプロトコールの長期成績を受け，“血糖を安定化させるための低侵襲治療”と位置付けられている．本稿では，膵島移植の現状・課題について分かりやすく説明し，それに対する筆者らの取り組みについても併せて述べる．



□膵島移植を要する患者

1型糖尿病患者は，現在世界中に400万人以上いると言われている．さまざまな理由で患者数は現在も増加し続けており，2025年にはおよそ3,000万人に達するものと推測されている．これらのうち多くは頻回の自己血糖測定とインスリン療法によって血糖コントロールが可能であるが，一部の患者は深刻な低血糖性昏睡による致死の危険に常に曝されている．さらに近年に至り，インスリン療法ではその厳格な使用にもかかわらず，血管病変に基づく腎不全，失明，神経障害，心筋梗塞，脳卒中といった糖尿病長期合併症の併発を完全には阻止できないことが明らかとなってきた．わが国においても，現在約10万人の1型糖尿病患者が存在している．昨今の人口高齢化に伴い，透析導入患者に占める糖尿病患者の割合は年々増加しており，またその予後が他疾患を原因とするものよりはるかに不良であることが報告されている．したがって，医療的側面および長期的な医療費削減を踏まえた社会的側面からも，これら糖尿病患者を治癒へ向かわせる根治療法，すなわち膵β細胞の置換療法の確立が急務であると考えられている．

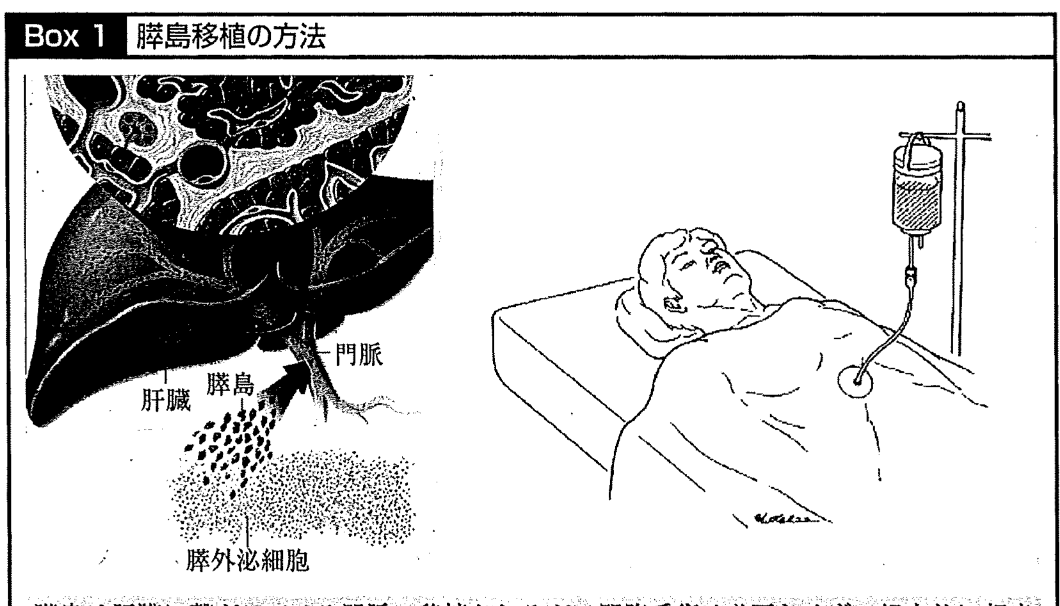
□膵島移植と膵臓移植

これまでそういった置換療法として膵臓の臓器移植が臨床的に確立されてきたが，手術侵襲が大きいこと，必要ではない外分泌組織の付随移植が合併症の原因となること，また拒絶反応時に移植グラフト(Tips 2)の除去が必要であることなどから，それに代わる置換療法として低侵襲で合理的な膵島移植の確立が強く望まれてきた．このよ

エドモントンプロトコール

カナダのアルバータ大学グループにより導入された，ステロイド剤を用いない新しい膵島移植方法．

Box 1　膵島移植の方法



膵島は肝臓に繋がっている門脈へ移植されるが，開腹手術は必要とせず，経皮的に超音波ガイド下にカテーテルを挿入し，点滴の要領で約20〜30分で治療は終了する．

うな状況下で，2000年に新しい膵島移植療法としてエドモントンプロトコールが報告され[1]，欧米を中心に広く普及し，現在まさに1型糖尿病患者に対する理想的治療法として確立されようとしている．

□膵島移植の目的

膵島移植の第一義的目的は，インスリンの強化療法によっても血糖コントロールが困難である重症1型糖尿病患者の血糖値を安定化させ，それにより低血糖発作を解消することである．それにより副次的に糖尿病合併症を阻止し，究極的には患者の生活の質を向上させるためインスリンからの離脱を目指す．膵島移植の適応となる患者は膵臓のインスリン分泌能が枯渇しており，糖尿病学会専門医などのエキスパートによる治療によっても血糖のコントロールが困難であるインスリン依存性の重症1型糖尿病患者である．膵島移植の禁忌としては，重症感染症や悪性腫瘍の治療中が挙げられる．また，アルコール中毒や重症肥満の患者も適応外となる．

Tips 2　グラフト

移植治療で，機能を回復させることを目的に移植される臓器や組織のこと．

□膵島移植の手続き

わが国で膵島移植を希望する場合は，必要検査データを揃えたうえで，膵・膵島移植研究会が設置する膵島移植適応検討委員会に主治医より申請を行う必要がある．適応があると認められた場合には，膵・膵島移植研究会にレシピエント登録を行い膵島の提供を待つことになる．膵・膵島移植研究会が認定し，膵島移植を行うことが可能な施設が現在全国に6つ(東北大学，福島医科大学，千葉東病院，大阪大学，京都大学，福岡大学)存在するが，現行のルールでは患者が複数の施設に登録を行うことも可能である．

□膵島移植の手技

膵島移植は膵臓移植と異なり，局所麻酔下で行うことが可能である(Box 1)．膵臓より分離した膵島を，約200 mLの溶液とともに輸液バックに入れて移植に備える．超音波検査で肝臓内の状況を確認したうえで，肝臓内の血管である門脈へカテーテルを穿刺し，X線透視で確認しながらカテーテルを通して膵島浮遊液を注入する．注入終了後，穿刺したカテーテルを抜去し，止血操作を

行って移植操作が終了する．全工程に要する時間は約 20～40 分である．膵島移植手技は現在の医療技術では簡易な技術であり，移植手術の際の合併症は臓器移植手術に比較して極めて少ないと言える．また，同様の手技は門脈の採血や血管の造影などで頻繁に行われている方法であるため，多くの経験が積まれている．膵島移植においては，拒絶反応が起こった場合でも移植した膵島は自然に消滅するため，膵臓移植と異なり改めてグラフトを摘出する必要がない点が大きな魅力のひとつである．

膵島移植の現状

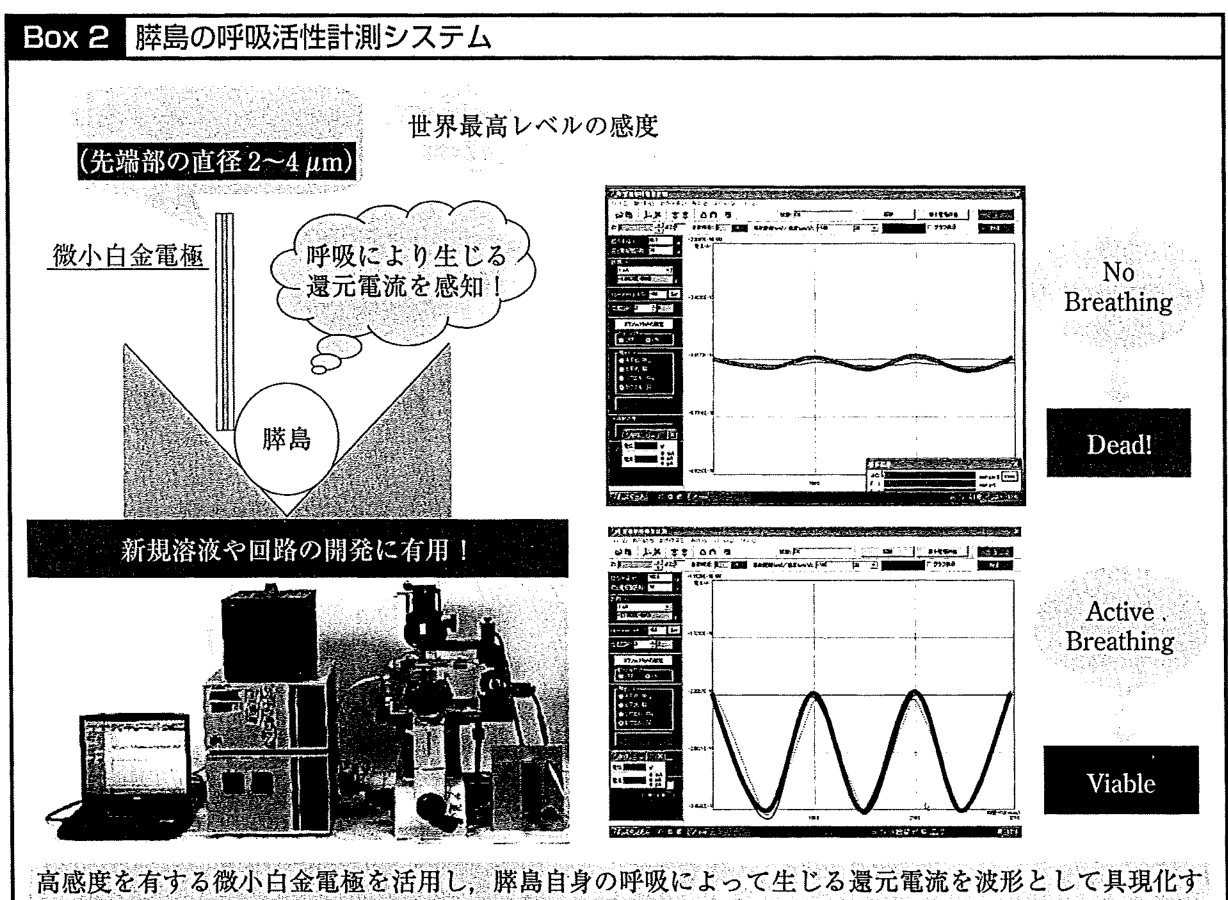
これまで膵島移植推進の原動力となってきたのは，アルバータ大学，マイアミ大学，ミネソタ大学を中心とする北米の研究グループである．一方，ヨーロッパにおいてもギッセン，ミラン，GRAGIL，Nordic Network を中心とし，膵島移植を医療として確立することができるよう試行錯誤が繰り返されてきた．とくに近年，その地理的および財政的不利な条件を補うべく，国家の枠を越えた大規模な膵島移植統合プロジェクトが推進される傾向が見られ，その結果ヨーロッパにおいても膵島移植は急激に普及し始めている．ヨーロッパによく見られる膵島移植統合プロジェクトの試みは，ドナーおよびレシピエントのプールを増やし臓器を有効利用するといった利点をもたらすばかりではなく，短期間での大規模臨床試験を可能にするため，今後も一層進み，この分野全体にさまざまな恩恵をもたらすものと思われる．

エドモントンプロトコールの世界規模の追試における長期経過により，エドモントンプロトコールでは脳死ドナーからの膵島移植を数回重ねることによって長期に及ぶ血糖安定化が可能であるが，インスリン離脱の長期維持は難しいことが判明した[2]．しかし，現在重症の 1 型糖尿病患者が膵島移植を受ける目的は，インスリン注射が面倒であるからではなく，インスリンでコントロールできない血糖状態を安定化させるためである．したがってその本来の目的を達成するうえでは，リスクの少ない低侵襲療法である膵島移植は合目的な治療法と言える．近年では，移植時の免疫抑制導入薬として，抗胸腺細胞抗体と抗 TNFα 製剤を併用することにより，グラフト生着が大幅に促進され，インスリン離脱期間も延長するという報告がなされている[3]．現在，わが国における膵島移植は，欧州より供給されていた膵島分離用酵素剤にウシ成分が混入されていたことが発覚したため，一時停止へ追い込まれている．しかし，ようやくウシ成分を含有しない酵素剤が開発されたため，厚労省の定める高度医療制度(Tips 3)を活用し，欧米の新規免疫抑制プロトコールに準拠するかたちで 2010 年春頃より再開する予定である．わが国の膵島移植の大きな特徴のひとつとして，心停止ドナーからの膵臓使用を余儀なくされている点が挙げられる．わが国における膵島移植が，法律上組織移植の範疇に組み入れられていることがその原因である．これは世界的に見ても極めてまれであり，これまで心停止ドナーを使用する膵島移植は不可能であると言われていたことを考慮すれば，ひとつの大きな臨床的チャレンジであると言える．膵島移植が一時停止となる 2007 年 3 月までに計 57 回の膵島分離が行われ，そのうち 28 回において，延べ 17 人の患者へ移植が行われている．心停止ドナーからの膵臓提供に限られるというわが国独特の厳しい環境が，短期間に飛躍的な膵島分離技術の革新をもたらしているため，今後脳死ドナーからの状態のよい膵臓を使用するこ

Tips 3　高度医療制度

医学・医療の高度化やこれらの医療技術を受けたいという患者のニーズなどに対応するために作られた制度．薬事法の承認などが得られていない医薬品・医療機器の使用を伴う先進的な医療技術を，一定の要件のもとに，「高度医療」として認め，保険診療と併用できる制度．薬事法上の承認申請などに繋がる科学的に評価可能なデータの収集の迅速化も目的にされている．

Box 2　膵島の呼吸活性計測システム



高感度を有する微小白金電極を活用し，膵島自身の呼吸によって生じる還元電流を波形として具現化するシステムを構築した．呼吸活性値は，膵島のバイアビリティーと強く相関することが，筆者らのこれまでの研究により判明している．

とにより，わが国の膵島移植は世界に類を見ない素晴らしい発展を遂げる可能性を有していると思われる．

膵島移植の課題とその対策

膵島移植が今後より広く普及していくためには，患者の要求を満たすのはもちろんであるが，それに加え臓器という限りある貴重な社会資源を有効利用するため，1つの臓器によって1人の患者を治療できるようにする必要がある．われわれは，これを妨げる主たる原因が①膵島分離技術の未成熟，②有用な移植前膵島評価法の欠如，③移植後早期における膵島グラフトの生着不全，に集約されるものと考え，こういった諸問題に対応すべく多角的アプローチを積極的に導入し，独自の膵島移植法を考案するに至っている．具体的には，これまでの基礎検討の結果に基づき，膵管への酵素注入圧を低圧(約 60 mmHg)とし，その代わりに酵素溶液濃度を世界標準の約 4 倍(6 mg/mL)に保つというユニークな膵島分離法を確立した[4]．世界標準として広まったエドモントンプロトコールでは，酵素注入圧は高圧(180 mmHg)であり，酵素溶液濃度は低値(1.5 mg/mL)に設定されていたが，膵島へのダメージを考慮して，近年われわれの方式に切り替える施設も出始めている．また，膵島分離の成否を分ける最大の要因は，膵管を通し，いかに酵素を効率よく外分泌組織内へ注入できるかという点であるが，ケースによっては膵尾部まで酵素を充填することが困難であるため，膵管内へカテーテルを導入する手技も確立した[4]．

さらに近年われわれは，動物成分を完全に含ま

Tips 4　リコンビナント

目的とするタンパク質を産生するための設計図となる遺伝子を菌に人為的に組み込む手法のこと.

ない安全なリコンビナント(Tips 4)タイプの国産新規酵素剤の開発に乗り出している. この新規膵島分離用酵素剤の開発により，これまでの酵素剤に共通する課題であったロット間の格差問題を完全に解消できると考えている.

現行の膵島移植におけるもうひとつの大きな課題として，有用な移植前膵島評価法の欠如が挙げられる. われわれはこれまでに分離膵島のバイアビリティーを簡便かつ正確に計測する手法としてADP/ATPアッセイを確立し提唱してきたが[5]，近年医工連携を導入し，膵島自身の呼吸によって生じる還元電位を波形として具現化する呼吸活性測定システムの確立に成功している(Box 2). このシステムはこれまでのバイアビリティー評価法とは根本的に異なり，非侵襲的であるため，膵島そのもののバイアビリティーを経時的に観察することが初めて可能となった. 今後，東北大学に設立された未来医工学治療開発センターを活用し，この開発システムの臨床応用を行っていく予定である.

膵島移植の今後の展望

膵島移植は，血糖コントロールに苦しむ重症1型糖尿病患者にとり，まさに理想的な患者に優しい治療法である. カテーテル手術や内視鏡手術をより好むわが国においては，今後技術の進展に伴って膵島移植は一層広く普及していくものと考えられる. しかし，極端にドナーが少ないわが国の特殊事情を考慮すると，今後の膵島移植の方向性として，異種膵島を使用するバイオ人工膵島移植や再生医療といった選択肢も十分視野に入れておく必要があると思われる. Ⓜ

文　献

1) Shapiro AM, Lakey JR, Ryan EA, et al (2000) Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 343 : 230-238
2) Shapiro AM, Ricordi C, Hering BJ, et al (2006) International trial of the Edmonton protocol for islet transplantation. N Engl J Med 355 : 1318-1330
3) Bellin MD, Kandaswamy R, Parkey J, et al (2008) Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. Am J Transplant 8 : 2463-2470
4) Goto M, Eich TM, Felldin M, et al (2004) Refinement of the automated method for human islet isolation and presentation of a closed system for in vitro islet culture. Transplantation 78 : 1367-1375
5) Goto M, Holgersson J, Kumagai-Braesch M, et al (2006) The ADP/ATP ratio : A novel predictive assay for quality assessment of isolated pancreatic islets. Am J Transplant 6 : 2483-2487

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Eur J Nucl Med Mol Imaging (2010) 37:934–941
DOI 10.1007/s00259-009-1314-7

ORIGINAL ARTICLE

In vivo detection of prion amyloid plaques using [11C]BF-227 PET

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Received: 7 August 2009 / Accepted: 21 October 2009 / Published online: 17 December 2009
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Abstract

Purpose In vivo detection of pathological prion protein (PrP) in the brain is potentially useful for the diagnosis of transmissible spongiform encephalopathies (TSEs). However, there are no non-invasive ante-mortem means for detection of pathological PrP deposition in the brain. The purpose of this study is to evaluate the amyloid imaging tracer BF-227 with positron emission tomography (PET) for the non-invasive detection of PrP amyloid in the brain.
Methods The binding ability of BF-227 to PrP amyloid was investigated using autoradiography and fluorescence microscopy. Five patients with TSEs, including three patients with Gerstmann-Sträussler-Scheinker disease (GSS) and two patients with sporadic Creutzfeldt-Jakob disease (CJD), underwent [11C]BF-227 PET scans. Results were compared with data from 10 normal controls and 17 patients with Alzheimer's disease (AD). The regional to pons standardized uptake value ratio was calculated as an index of BF-227 retention.
Results Binding of BF-227 to PrP plaques was confirmed using brain samples from autopsy-confirmed GSS cases. In clinical PET study, significantly higher retention of BF-227 was detected in the cerebellum, thalamus and lateral temporal cortex of GSS patients compared to that in the corresponding tissues of normal controls. GSS patients also showed higher retention of BF-227 in the cerebellum, thalamus and medial temporal cortex compared to AD patients. In contrast, the two CJD patients showed no obvious retention of BF-227 in the brain.
Conclusion Although [11C]BF-227 is a non-specific imaging marker of cerebral amyloidosis, it is useful for in vivo detection of PrP plaques in the human brain in GSS, based on the regional distribution of the tracer. PET amyloid imaging might provide a means for both early diagnosis and non-invasive disease monitoring of certain forms of TSEs.

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Keywords Prion · PET · Amyloid · Creutzfeldt-Jakob disease

Introduction

Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a group of fatal neurodegenerative disorders, including Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS) and kuru [1–3]. TSEs are characterized by progressive deposition of abnormal prion protein (PrP) in the brain. CJD is the most common type of human TSE and is classified into sporadic, genetic and infectious forms according to the aetiology of illness. GSS is a familial neurodegenerative disorder associated with mutations of the PrP gene and is clinically recognized by cerebellar ataxia combined with postural abnormalities and cognitive decline [1–3]. Two major types of abnormal PrP deposition, synaptic and plaque types, have been described in the brain of people with TSEs [1]. The synaptic type of PrP deposition, which does not have tinctorial properties of amyloid in tissue sections, is most commonly observed in sporadic CJD, whereas the plaque type, which frequently forms congophilic amyloid plaques, is a hallmark of such TSEs as GSS, variant CJD (vCJD) and iatrogenic dura CJD with plaques [1, 4]. Abnormal PrP deposition in the brain is suggested to start before the occurrence of clinical symptoms [5–7]. Thus, preclinical diagnosis and, when available, early disease-specific therapeutic interventions, can be beneficial for people predisposed to or affected by TSEs.

Several positron emission tomography (PET) imaging agents have been recently developed and used for in vivo detection of brain amyloid-β (Aβ) plaques in patients with Alzheimer's disease (AD) [8–12]. Most of these β-sheet binding agents show high binding affinity to PrP amyloid because PrP aggregates in TSEs form β-pleated sheet structures and share a common secondary structure with Aβ deposits in AD brains [13–16]. Therefore, these agents would be useful for the in vivo detection of PrP amyloid in the brain. Two clinical PET studies were performed using [^{18}F]FDDNP and/or [^{11}C]PIB in sporadic and familial CJD patients [17, 18]. The results indicated moderate retention of FDDNP and no obvious retention of PIB in the brain [17, 18]. Therefore, agents that can sensitively detect abnormal PrP deposits should be further explored for the diagnosis of TSEs. We have demonstrated in vitro and in vivo binding of benzoxazole derivatives to both Aβ and PrP amyloids [19, 20]. One of these derivatives, BF-227, was used for a clinical PET study where it successfully visualized amyloid deposits in the brain of AD patients in vivo [12, 21]. Therefore, [^{11}C]BF-227 appears to be a promising candidate for PET imaging of PrP deposits. The purpose of this study was to evaluate the clinical utility of [^{11}C]BF-227 PET for the non-invasive detection of abnormal PrP deposits in patients with TSEs.

Methods

Preparation of compounds

BF-227 and its 2-tosyloxyethoxy and *N*-desmethylated derivatives were custom synthesized by Tanabe R&D Service Co. (Osaka, Japan). [^{18}F]BF-227 was synthesized for autoradiography of brain sections, as described previously [22]. For the clinical studies, [^{11}C]BF-227 was synthesized as described previously [12]. Radiochemical yields were greater than 50% based on [^{11}C]methyl triflate, and specific radioactivities were 119–138 GBq/μmol at the end of synthesis. Radiochemical purities were greater than 95%.

Histopathological staining and in vitro autoradiography

Autopsy-diagnosed brain samples from two GSS cases with PrP plaque deposition and two sporadic CJD cases with synaptic PrP deposition were provided by Dr. Toru Iwaki of the Department of Neuropathology, Kyushu University, Japan. The brain sample from an 81-year-old man with autopsy-confirmed physiological aging was obtained from Tohoku University Hospital. The two GSS cases had a proline-to-leucine mutation at codon 102 and methionine homozygosity at codon 129 of the PrP gene, and the two sporadic CJD cases had no mutations and methionine homozygosity at codon 129; they showed type 1 abnormal PrP in immunoblotting of the brain tissues. All of the brain samples were treated with 98% formic acid for 1 h before paraffin embedding to eliminate prion infectivity. Sections from paraffin-embedded blocks of the cerebellum or frontal cortex were then dewaxed in xylene and ethanol. For staining with BF-227, tissue sections were immersed in 100 μM BF-227 solution containing 50% ethanol for 10 min. They were then dipped briefly into water and rinsed in phosphate-buffered saline for 10 min before coverslipping with FluorSave Reagent (Calbiochem, La Jolla, CA, USA). Subsequently, they were examined using an Eclipse E800 microscope (Nikon, Tokyo, Japan) equipped with a V-2A filter set (excitation, 380–420 nm; dichroic mirror, 430 nm; Longpass filter, 450 nm). For autoradiography, the section was incubated with 1.0 MBq/ml of [^{18}F]BF-227 at room temperature for 10 min and then washed briefly with water and 50% ethanol. After drying, the labelled section was exposed to a BAS-III imaging plate (Fuji Film, Tokyo, Japan) overnight. Autoradiographic images were obtained using a BAS-5000 phosphor imaging instrument (Fuji Film, Tokyo, Japan). Neighbouring sec-

tions were immunostained using 3F4 anti-PrP monoclonal antibody (Covance, Princeton, NJ, USA) as described previously [13, 20].

Subjects and patients in the clinical PET study

Five TSE patients, including two sporadic CJD patients [63-year-old woman (CJD1) and 58-year-old man (CJD2)] and three GSS patients [69-year-old woman (GSS1), 61-year-old man (GSS2) and 30-year-old woman (GSS3)], underwent PET scans with [^{11}C]BF-227 (Table 1). For comparison, [^{11}C]BF-227 PET studies were also performed in 17 AD patients [mean age ± standard deviation (SD)= 72.6±6.7; mean Mini-Mental State Examination score ± SD=19.8±4.0] and 10 aged normal controls (mean age ± SD=67.2±2.5). Some of these AD and normal subjects were included in our previous report [12].

CJD1's health was unremarkable until the manifestation of depressive symptoms at the age of 62 years. The patient then developed subacutely progressive dementia, motor disturbances and myoclonus. CJD2 showed subacutely progressive dementia and gait disturbance and then developed psychotic symptoms, dysarthria and myoclonus. Both CJD patients had no mutations and showed methionine homozygosity at codon 129 of the PrP gene. PET studies in CJD1 and CJD2 were performed when they reached grade 4 of the modified Rankin scale at 3 and 4 months after onset of symptoms, respectively. Both patients showed periodic synchronous discharges in electroencephalograms and hyperintensity in the caudate, putamen and cerebral cortex on diffusion-weighted magnetic resonance (MR) images. Diagnosis of probable CJD was made according to the WHO criteria [23].

Each GSS patient was from a different pedigree and had a family history of the same disease, carrying a proline-to-leucine mutation at codon 102 and methionine homozygosity at codon 129 of the PrP gene. GSS1 and GSS2, having a 9- and 20-month clinical duration from the onset, respectively, showed signs of moderate cerebellar ataxia, such as gait disturbance and slurred speech; however, they could walk unassisted and had slight or no cognitive impairment. GSS1 and GSS2 scored 22 and 26 points, respectively, on the Mini-Mental State Examination. GSS3, having a 27-month clinical duration, showed severe gait disturbance and slurred speech and was unable to walk unassisted; however, she had no cognitive impairment (30 points on the Mini-Mental State Examination) at the time of this study.

AD diagnosis was made according to the National Institute of Neurological and Communicative Diseases and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [24]. CJD, GSS and AD patients were recruited from Miyagi National Hospital, Fukuoka University Hospital, Kagoshima University Hospital and Tohoku University Hospital. Normal controls were recruited from volunteers with no cognitive impairment or cerebrovascular lesions on MR images and who were not taking any centrally acting medications. No significant difference in age distribution was apparent between the groups. This study was approved by the Ethics Committee on clinical investigations of Tohoku University School of Medicine and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained after complete description of the study to the patients and subjects.

Image acquisition protocols

PET scans were performed using a SET-2400W (Shimadzu Inc., Kyoto, Japan). After intravenous injection of 211–366 MBq (5.7–9.9 mCi) of [^{11}C]BF-227, dynamic PET images were obtained for 60 min with the subjects' eyes closed. Arterial blood sampling in the TSE patients was not

Table 1 Regional to pons standardized uptake value ratio (SUVRp) values in aged normal controls (Control), Alzheimer's disease patients (AD), Creutzfeldt-Jakob disease patients (CJD) and Gerstmann-Sträussler-Scheinker disease patients (GSS)

	Control (n=10) Mean ± SD	AD (n=17) Mean ± SD	CJD1	CJD2	GSS (n=3) Mean ± SD	GSS1	GSS2	GSS3
Frontal	0.60±0.03	0.64±0.04	0.57	0.61	0.67±0.08	0.74	0.69	0.57
Lateral temporal	0.59±0.03	0.69±0.04*	0.63	0.62	0.67±0.05*	0.71	0.68	0.61
Parietal	0.62±0.02	0.69±0.04*	0.62	0.62	0.67±0.06	0.72	0.68	0.61
Occipital	0.62±0.04	0.65±0.05	0.62	0.69	0.67±0.07	0.74	0.67	0.60
Medial temporal	0.64±0.04	0.62±0.03	0.57	0.65	0.67±0.02**	0.66	0.70	0.67
Striatum	0.71±0.04	0.75±0.04*	0.69	0.72	0.76±0.04	0.80	0.77	0.72
Thalamus	1.00±0.04	1.01±0.04	0.97	1.04	1.08±0.00*, **	1.08	1.07	1.08
Cerebellum	0.58±0.01	0.57±0.02	0.58	0.59	0.62±0.01*, **	0.61	0.63	0.61

*p<0.05 compared to aged normal group

**p < 0.05 compared to AD group

performed because the Committee on Clinical Investigation at Tohoku University School of Medicine did not approve blood sampling during the PET scan, from the standpoint of infection risk management. T_1-weighted MR images were obtained using a Signa 1.5-T machine (General Electric Inc., Milwaukee, WI, USA).

Image analysis

Standardized uptake value (SUV) images of [^{11}C]BF-227 were obtained by normalizing tissue concentration by injected dose and body weight. Average summations of SUV images were created from early frames (0–30 min post-injection) and late frames (40–60 min post-injection) of dynamic PET images. Early frame images were created for co-registration with individual MR images, and late frame images were used for calculation of SUV. Individual MR images were anatomically co-registered with the early frame PET images using statistical parametric mapping software (SPM2, Welcome Department of Imaging Neuroscience, London, UK) [25]. Spatial normalization was performed using an MR T_1 template of SPM2 to transfer PET images into a standard stereotactic space. Regions of interest (ROIs) were placed on a spatially normalized MR image, as described previously [12]. ROI information was then copied onto delayed PET SUV images, and regional SUV images at 40–60 min post-injection were sampled using Dr.View/LINUX software (AJS, Tokyo, Japan). Deposition of PrP plaques is reportedly frequent in the cerebellum but scarce in the pons of GSS brain [26]. Furthermore, BF-227 retention in the pons does not differ between AD patients and normal controls. Therefore, we used the pons as a reference region and calculated the regional to pons SUV ratio (SUVRp) as an index of BF-227 retention.

Statistical analysis

For statistical comparison in each group, we applied one-way analysis of variance, followed by the Bonferroni-Dunn post hoc test. Statistical comparison of age distribution was performed using the Kruskal-Wallis test, followed by Dunn's multiple comparison test. Statistical significance for each analysis was defined as $p<0.05$.

Results

Autoradiography examination indicated binding of a tracer dose of BF-227 to PrP plaque deposits. BF-227 retention was present in brain sections from GSS cases with PrP plaque deposition but not from normal control cases and sporadic CJD cases with synaptic PrP deposition (Fig. 1a–c). The regional distribution of [^{18}F]BF-227 in the autoradiograms co-localized with the immunostained PrP plaques in the cerebellar cortex of GSS cases (Fig. 1d–e). BF-227 binding to PrP plaques was additionally examined using a microscope, because BF-227 is a fluorescent compound. Core regions of the PrP plaques were intensely stained with BF-227 (Fig. 2, arrows), indicating that BF-227 preferentially binds to the fibril-rich core of PrP amyloid plaques.

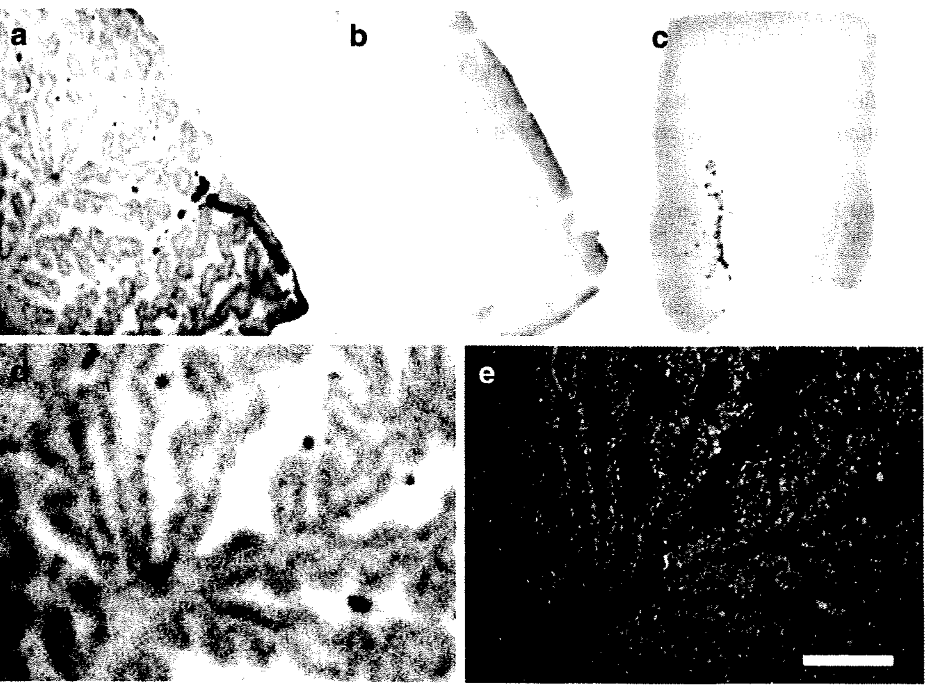

Fig. 1 [^{18}F]BF-227 autoradiograms of a cerebellar section from a Gerstmann-Sträussler-Scheinker (GSS) case (**a**), a cerebellar section from a physiological aging case (**b**) and a frontal cortex section from a sporadic Creutzfeldt-Jakob disease (CJD) case (**c**) are shown, together with a magnified view of **a** (**d**) and prion protein (PrP) immunostaining of the same field as **d** (**e**). BF-227 retention was present in the brain section from a GSS case with PrP plaque deposition, but not from a normal control case and sporadic CJD case with synaptic PrP deposition. *Bar*=200 μm