ATP production by reducing the H^+ gradient across the mitochondrial membrane.

High glucose increased NAD(P)H fluorescence during the initial 5 min, which suggests that increased NADH production overwhelms NADH utilization. After 5 min of high-glucose exposure, it reaches a plateau, suggesting that increased NADH production balances increased NADH utilization, which eventually increases ATP production. During the plateau period, DPH did not affect NADH fluorescence. Thus, DPH might decrease NADH production and utilization simultaneously, resulting in unchanged NADH fluorescence and decreased ATP production. To rule out this possibility, glucose oxidation was measured. Glucose oxidation reflects the velocity of glucose-derived metabolite metabolism in the Krebs cycle that constitutes the major source of NADH production. NH_4^+ (3 mM) did not affect glucose oxidation, which is compatible with a previous result using 10 mM NH_4^+ (27). Because DPH also did not affect glucose oxidation, both NADH production and utilization were unaffected by cellular alkalization, suggesting that cellular alkalization reduces the efficacy of NADH utilization in producing ATP. This may result from the reduction of H^+-gradient generated by NADH utilization due to diffusion of H^+ near the cytoplasmic side of the mitochondrial inner-membrane to a cytoplasmic milieu in which the H^+ concentration is reduced.

The effects of intracellular pH on insulin release in the presence of a basal level of glucose were also reported (28). In the presence of a basal level of glucose, insulin release was not changed by alteration of intracellular pH when the intracellular pH was less than 7.5. In the presence of 2.8 mM glucose, 20 μM DPH also elevated cytoplasmic pH. Consistent with these results, in the present study, insulin release in the presence of a basal level (2.8 mM) of glucose was unaltered by 20 μM DPH, which increased intracellular pH to approximately 7.2.

The effects of increasing cytoplasmic pH on insulin release from islets and pancreatic β-cells have been examined. Glucose-induced, time-dependent potentiation of insulin release is inhibited by increasing intracellular pH (29). The addition of more than 10 mM NH_4^+ to the extracellular medium decreases glucose-induced insulin release (30, 31, 32), increases cytoplasmic pH (30, 31, 33), hyperpolarizes the plasma membrane (31, 34), and decreases $[Ca^{2+}]_i$ (31). Hyperpolarization due to cytoplasmic alkalization has been shown to be derived from augmentation of K_{ATP} channel activity in experiments using intact β-cells (31, 35). However, in experiments using excised patch, alkalization decreases K_{ATP} channel activity at clamped ATP levels (35, 36). This discrepancy might be explained by our present results: cytoplasmic alkalization suppresses mitochondrial ATP production by reducing the H^+ gradient across the inner membrane, and the subsequent decrease in intracellular ATP concentration augments K_{ATP} channel activity. Consistently, in the present study DPH suppressed high glucose-induced Ca^{2+} elevation, but did not suppress K^+-induced Ca^{2+} elevation in the presence of basal glucose.

Ca^{2+} and ATP directly affect the exocytotic system and enhance insulin release synergistically in experiments using single β-cells (37, 38) and permeabilized islets (39). To quan-

tify Ca^{2+} efficacy at clamped concentrations of ATP in the exocytotic process of insulin secretory granules directly, islets were electrically permeabilized to manipulate the intracellular Ca^{2+} and ATP concentration according to the extracellular medium, and insulin release was examined. DPH had no effect on Ca^{2+} efficacy at a clamped concentration of ATP in the exocytotic system. In addition, decreased intracellular ATP reduces Ca^{2+} efficacy in the exocytotic system (37–39). Accordingly, the lower ATP level due to reduced ATP production by cytoplasmic alkalization plays a role in the attenuation of insulin secretion from DPH-treated islets in response to high glucose. Biphasic glucose-induced insulin secretion can be described as the release of distinct pools of granules. The first phase of insulin secretion represents the release of readily releasable (primed/docked) granules located immediately below the plasma membrane. The second slower phase results from the time- and ATP-dependent mobilization of granules situated farther away from the plasma membrane (37). Because both phases are Ca^{2+} dependent, the precise site at which ATP and H^+ modulate Ca^{2+} efficacy in these processes cannot be determined in the present study.

In the presence of 2.8 mM glucose, 20 μM DPH suppressed K^+-induced monophasic insulin release without affecting Ca^{2+} and ATP levels, which is compatible with the finding that increased pH decreased insulin release from permeabilized islets at clamped concentrations of ATP and Ca^{2+}. Consistent with our findings, important roles of secretory granule pH in exocytosis of insulin granules have been proposed recently. Blocking V-type H^+-ATPase by bafilomycin increases secretory granule pH (40) and decreases exocytosis of insulin granules measured by cell capacitance (41) and by insulin release from permeabilized β-cells (42). However, the fact that protein kinase A activation, which potentiates insulin release, increases secretory granule pH (40) indicates that granular pH is not the sole regulatory factor in the exocyotsis of insulin granules.

Our results demonstrate that DPH decreases Ca^{2+} efficacy in the exocytotic system by reducing both the cytoplasmic ATP level and the cytoplasmic H^+ level, and clearly show that the inhibitory effect on insulin release of the agent does not necessarily require reduced $[Ca^{2+}]_i$.

In pancreatic β-cells, Na^+/H^+ exchange and Na^+-dependent Cl^-/HCO_3^- exchange may play an important role in the regulation of cytoplasmic pH (17, 43). In the present study, DPH did not affect intracellular pH in Na^+-deprived and HCO_3^--deprived conditions, in which application of a permeable weak base, NH_4^+, raised cytoplasmic pH. These results suggest that Na^+ and HCO_3^- transport across the plasma membrane is involved in the increase in cytoplasmic pH by DPH. Additional study is necessary to elucidate precise mechanism of cytoplasmic alkalization by DPH.

References

1. Malherbe C, Burrill KC, Levin SR, Karam JH, Forsham PH 1972 Effect of diphenylhydantoin on insulin secretion in man. N Engl J Med 286:339–342
2. Goldberg EM, Sanbar SS 1969 Hyperglycemic, nonketotic coma following administration of dilantin (diphenylhydantoin). Diabetes 18:101–106
3. Hofeldt FD, Dippe SE, Levin SR, Karam JH, Blum MR, Forsham PH 1974 Effects of diphenylhydantoin upon glucose-induced insulin secretion in three patients with insulinoma. Diabetes 23:192–198
4. Pelkonen R, Taskinen MR 1973 Effect of diphenylhydantoin on plasma-insulin in insulinoma. Lancet 1:604–605
5. Levin SR, Booker Jr J, Smith DF, Grodsky GM 1970 Inhibition of insulin secretion by diphenylhydantoin in the isolated perfused pancreas. J Clin Endocrinol Metab 30:400–401
6. Kizer JS, Vargas-Gordon M, Brendel K, Bressler R 1970 The in vitro inhibition of insulin secretion by diphenylhydantoin. J Clin Invest 49:1942–1948
7. Henquin JC 2000 Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 49:1751–1760
8. Matthews EK, Sakamoto Y 1975 Pancreatic islet cells: electrogenic and electrodiffusional control of membrane potential. J Physiol 246:439–457
9. Levin SR, Kasson BG, Driessen JF 1978 Adenosine triphosphatases of rat pancreatic islets: comparison with those of rat kidney. J Clin Invest 62:692–701
10. Herchuelz A, Lebrun P, Sener A, Malaisse WJ 1981 Ionic mechanism of diphenylhydantoin action on glucose-induced insulin release. Eur J Pharmacol 73:189–197
11. Pace CS, Livingston E 1979 Ionic basis of phenytoin sodium inhibition of insulin secretion in pancreatic islets. Diabetes 28:1077–1082
12. Siegel EG, Janjic D, Wollheim CB 1982 Phenytoin inhibition of insulin release. Studies on the involvement of Ca^{2+} fluxes in rat pancreatic islets. Diabetes 31:265–269
13. Fujimoto S, Ishida H, Kato S, Okamoto Y, Tsuji K, Mizuno N, Ueda S, Mukai E, Seino Y 1998 The novel insulinotropic mechanism of pimobendan: direct enhancement of the exocytotic process of insulin secretory granules by increased Ca^{2+} sensitivity in β-cells. Endocrinology 139:1133–1140
14. Fujimoto S, Tsuura Y, Ishida H, Tsuji K, Mukai E, Kajikawa M, Hamamoto Y, Takeda T, Yamada Y, Seino Y 2000 Augmentation of basal insulin release from rat islets by preexposure to a high concentration of glucose. Am J Physiol 279:E927–E940
15. Takehiro M, Fujimoto S, Shimodahira M, Shimono D, Mukai E, Nabe K, Radu RG, Kominato R, Aramaki Y, Seino Y, Yamada Y 2005 Chronic exposure to β-hydroxybutyrate inhibits glucose-induced insulin release from pancreatic islets by decreasing NADH contents. Am J Physiol 288:E365–E371
16. Kajikawa M, Fujimoto S, Tsuura Y, Mukai E, Takeda T, Hamamoto Y, Takehiro M, Fujita J, Yamada Y, Seino Y 2002 Ouabain suppresses glucose-induced mitochondrial ATP production and insulin release by generating reactive oxygen species in pancreatic islets. Diabetes 51:2522–2529
17. Shepherd RM, Henquin JC 1995 The role of metabolism, cytoplasmic Ca^{2+}, and pH-regulating exchangers on glucose-induced rise of cytoplasmic pH in normal mouse pancreatic islets. J Biol Chem 270:7915–7921
18. Juntti-Berggren L, Civelek VN, Berggren PO, Schultz V, Corkey BE, Tornheim K 1994 Glucose-stimulated increase in cytoplasmic pH precedes increase in free Ca^{2+} in pancreatic β-cells. A possible role for pyruvate. J Biol Chem 269:14391–14395
19. Thomas JA, Buchsbaum RN, Zimniak A, Racker E 1979 Intracellular pH measurements in Ehrlich ascites tumor cells utilizing spectroscopic probes generated in situ. Biochemistry 18:2210–2218
20. Ashcroft FM, Ashcroft SJ, Harrison DE 1988 Properties of single potassium channels modulated by glucose in rat pancreatic β-cells. J Physiol 400:501–527
21. Radu RG, Fujimoto S, Mukai E, Takehiro M, Shimono D, Nabe K, Shimodahira M, Kominato R, Aramaki Y, Nishi Y, Funakoshi S, Yamada Y, Seino Y 2005 Tacrolimus suppresses glucose-induced insulin release from pancreatic islets by reducing glucokinase activity. Am J Physiol 288:E372–E380
22. Shimono D, Fujimoto S, Mukai E, Takehiro M, Nabe K, Radu RG, Shimodahira M, Kominato R, Aramaki Y, Nishi Y, Funakoshi S, Yamada Y, Seino Y 2005 ATP enhances exocytosis of insulin secretory granules in pancreatic islets under Ca^{2+}-depleted condition. Diabetes Res Clin Pract 69:216–223
23. Mitchell P 1979 Keilin's respiratory chain concept and its chemiosmotic consequences. Science 206:1148–1159
24. Meglasson MD, Matschinsky FM 1986 Pancreatic islet glucose metabolism and regulation of insulin secretion. Diabetes Metab Rev 2:163–214
25. Patterson GH, Knobel SM, Arkhammar P, Thastrup O, Piston DW 2000 Separation of the glucose-stimulated cytoplasmic and mitochondrial NADPH responses in pancreatic islet β cells. Proc Natl Acad Sci USA 97:5203–5207
26. Hutson SM 1987 pH regulation of mitochondrial branch chain α-keto acid transport and oxidation in rat heart mitochondria. J Biol Chem 262:9629–9635
27. Sener A, Malaisse WJ 1991 Hexose metabolism in pancreatic islets. Regulation of D-[6-^{14}C]glucose oxidation by non-nutrient secretagogues. Mol Cell Endocrinol 76:1–6
28. Lindström P, Sehlin J 1986 Effect of intracellular alkalinization on pancreatic islet calcium uptake and insulin secretion. Biochem J 239:199–204
29. Gunawardana SC, Sharp GW 2002 Intracellular pH plays a critical role in glucose-induced time-dependent potentiation of insulin release in rat islets. Diabetes 51:105–113
30. Best L, Yates AP, Gordon C, Tomlinson S 1988 Modulation by cytosolic pH of calcium and rubidium fluxes in rat pancreatic islets. Biochem Pharmacol 37:4611–4615
31. Juntti-Berggren L, Arkhammar P, Nilsson T, Rorsman P, Berggren PO 1991 Glucose-induced increase in cytoplasmic pH in pancreatic β-cells is mediated by Na$^+$/H$^+$ exchange, an effect not dependent on protein kinase C. J Biol Chem 266:23537–23541
32. Misler S, Barnett DW, Gillis KD, Pressel DM 1992 Electrophysiology of stimulus-secretion coupling in human β-cells. Diabetes 41:1221–1228
33. Juntti-Berggren L, Rorsman P, Siffert W, Berggren PO 1992 Intracellular pH and the stimulus-secretion coupling in insulin-producing RINm5F cells. Biochem J 287:59–66
34. Pace CS, Goldsmith KT 1986 Effect of substitution of a permeable weak acid for the permissive role of glucose in amino acid-induced electrical activity in B-cells. Endocrinology 119:2433–2438
35. Misler S, Gillis K, Tabcharani J 1989 Modulation of gating of a metabolically regulated, ATP-dependent K$^+$ channel by intracellular pH in B cells of the pancreatic islet. J Membr Biol 109:135–143
36. Proks P, Takano M, Ashcroft FM 1994 Effects of intracellular pH on ATP-sensitive K$^+$ channels in mouse pancreatic β-cells. J Physiol 475:33–44
37. Rorsman P 1997 The pancreatic β-cell as a fuel sensor: an electrophysiologist's viewpoint. Diabetologia 40:487–495
38. Takahashi N, Kadowaki T, Yazaki Y, Ellis-Davies GC, Miyashita Y, Kasai H 1999 Post-priming actions of ATP on Ca^{2+}-dependent exocytosis in pancreatic β cells. Proc Natl Acad Sci USA 96:760–765
39. Fujimoto S, Mukai E, Hamamoto Y, Takeda T, Takehiro M, Yamada Y, Seino Y 2002 Prior exposure to high glucose augments depolarization-induced insulin release by mitigating the decline of ATP level in rat islets. Endocrinology 143:213–221
40. Tompkins LS, Nullmeyer KD, Murphy SM, Weber CS, Lynch RM 2002 Regulation of secretory granule pH in insulin-secreting cells. Am J Physiol 283:C429–C437
41. Barg S, Huang P, Eliasson L, Nelson DJ, Obermuller S, Rorsman P, Thevenod F, Renstrom E 2001 Priming of insulin granules for exocytosis by granular Cl⁻ uptake and acidification. J Cell Sci 114:2145–2154
42. Maechler P, Wollheim CB 1999 Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis. Nature 402:685–689
43. Lynch A, Best L 1990 Cytosolic pH and pancreatic β-cell function. Biochem Pharmacol 40:411–416

Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

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A single transplantation of the islets can produce glycemic stability and reduction of basal insulin requirement

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Received 15 September 2005; received in revised form 6 December 2005; accepted 27 January 2006
Available online 5 April 2006

Abstract

We investigated glycemic stability and insulin requirement 1 month after a single transplantation of the islets from non-heart-beating donors or a living donor. Overall blood glucose levels decreased immediately after transplantation. The M-value and mean amplitude of glycemic excursions (MAGE) decreased significantly from 53.0 (range, 8.9–91.0) to 4.2 (0.6–8.8, $P < 0.05$) and from 8.5 mM (4.8–11.7) to 3.3 mM (2.0–4.5, $P < 0.05$), respectively. The values after transplantation were lower than the first quartile of 102 type 2 diabetic control patients. The estimated HbA_{1c} level decreased significantly from 7.9% (5.7–10.9) to 5.4% (4.7–5.9, $P < 0.05$). The supplement of basal insulin decreased 43% from 0.31 units/kg/day (0.16–0.37) to 0.18 units/kg/day (0–0.22, $P < 0.05$), while that of stimulated insulin did not decrease significantly, from 0.28 units/kg/day (0.13–0.51) to 0.21 units/kg/day (0–0.41). Thus, only one islet transplantation can be sufficient to attain metabolic stability, probably by effective supply of basal insulin secretion, sufficient to avoid life-threatening severe hypoglycemia and prevent or delay the progress of secondary complications of diabetes by decreasing the HbA_{1c} level.
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Keywords: A single transplantation of the islets; Non-heart-beating donor; Living donor; Metabolic stability

1. Introduction

Diabetes mellitus (DM) is a clinically and genetically heterogeneous group of disorders classified mainly into type 1 and type 2 diabetes. Type 1 diabetes is caused by β-cell destruction that often results in their complete loss and insulin-dependent diabetes mellitus

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(insulin-dependency). As both basal and stimulated insulin secretion from pancreatic β-cells is completely abolished in the majority of patients with type 1 diabetes, the blood glucose level remains unstable despite all effort to use optimal exogenous insulin.

In diabetic patients, blood glucose levels are considerably high or low in comparison with optimal levels, and the amplitude of glycemic excursions are large. The former is assessed by the M-value [1] and the latter by the mean amplitude of glycemic excursions (MAGE) [2]. In diabetic patients with metabolic instability, the M-value and MAGE are significantly high [1–3]. Such patients are at increased risk of progression of diabetic complications [4], life-threatening hypoglycemia unawareness and even "dead-in-bed syndrome" [5].

Since the success of islet transplantation with the corticosteroid-free protocol from Edmonton [6], islet transplantation has become more common radical therapy for type 1 diabetes mellitus [7]. The Edmonton protocol, in addition to optimizing islet isolation and modifying the immunosuppressive regimen, includes multiple infusions of islets from different donors. This therapy generally aims at insulin independence as well as the avoidance of severe hypoglycemia. Under present conditions, when cadeveric donors are used, multiple injections are required to attain insulin independence except in one report [8]. However, in many countries, the donor pool is extremely small, making it impractical to aim at insulin independence. Accordingly, we have investigated the effect of a single transplantation of the islets on glycemic lability.

Using even a non-heart-beating donor, a single transplantation of the islets can achieve metabolic stability probably by effective supply of basal insulin secretion regardless of the achievement of insulin independence, suggesting the therapeutic value of a single transplantation of the islets for patients with insulin-dependency in cases of donor shortage.

2. Methods

2.1. Subjects

Five patients (three women, two men) (Patients 1–5) (median age, 44 years; range, 36–58) who had had type 1 diabetes mellitus for a median of 21 years (range, 14–29) and a 27-year-old woman (Patient 6) who had had pancreatic diabetes for 12 years underwent islet transplantation in Kyoto University Hospital between April 7, 2004 and January 19, 2005. Diagnostic basis for type 1 diabetes in five of the six recipients was completely depleted insulin secretion (negative C-peptide [<0.1 ng/ml] prestimulation and post glucagon

stimulation) and clinical course. The donors of five patients (Patients 1–5) with type 1 diabetes mellitus were non-heart-beating donors, and a donor of a patient (Patient 6) with pancreatic diabetes was a living donor who was a mother of the patient. Each patient received islet transplantation from one or two pancreases [9,10].

The study was approved by the ethics committee of Kyoto University Graduate School and Faculty of Medicine.

2.2. Assessment of glycemic control

The M-value [1] and the mean amplitude of glycemic excursions (MAGE) [2], the indexes of glycemic lability, of the six patients were measured before and 1 month after a first islet transplantation.

There is a predictable relationship between plasma glucose and HbA$_{1c}$. Thus, the estimated HbA$_{1c}$ levels were calculated by the corresponding seven-point plasma glucose profiles (premeal, postmeal and bedtime) before and 1 month after the first transplantation: estimated HbA$_{1c}$ = mean plasma glucose (mg/dl)/35.6 + 1.87 [11,12].

The M-value, MAGE and HbA$_{1c}$ levels of 52 type 1 diabetic patients (31 women and 21 men) (median age, 43 years; range, 16–79) and 102 type 2 diabetic patients (43 women and 59 men) (median age, 65 years; range, 28–79) admitted to Kyoto University Hospital from January 2002 to June 2005 were also examined.

2.3. Exogenous insulin requirement

We defined the total amount of premeal regular insulin or rapid-acting insulin analogue as the supplemental dose of stimulated insulin and that of neutral protamine Hagedorn (NPH) insulin, long-acting insulin analogue, or basal dose of continuous subcutaneous insulin infusion (CSII) as the supplement of basal insulin. The supplemental dose of basal and stimulated insulin were compared before and 1 month after the first islet transplantation.

2.4. Statistical analyses

Results are expressed as medians and ranges. Wilcoxon signed-rank test was used to compare the M-value, MAGE, the estimated HbA$_{1c}$ levels and the amount of exogenous insulin before and 1 month after islet transplantation. Mann-Whitney U test was used to compare the M-value, MAGE, and the HbA$_{1c}$ levels of type 1 and type 2 diabetic control patients. Significance was taken at a P value of <0.05.

3. Results

3.1. Glycemic lability is higher in type 1 diabetic patients

The median HbA$_{1c}$ level of 102 type 2 diabetic patients who had been admitted to Kyoto University

Hospital was 8.6% (range, 5.3–14.5%). Their M-value and MAGE at discharge was 12.2 (range, 1.2–66.2) and 5.2 mM (range, 0.8–12.7 mM), respectively (Fig. 1A and B). The median HbA$_{1c}$ level of 52 type 1 diabetic patients admitted to Kyoto University Hospital was 8.3% (range, 5.0–16.3%), not significantly different from that of type 2 diabetic patients. The M-value and MAGE of 52 type 1 diabetic patients at discharge was 22.0 (range, 1.3–127.6, $P < 0.05$) and 6.7 mM (range, 2.8–16.6 mM, $P < 0.05$), respectively, significantly higher than those of type 2 patients (Fig. 1A and B).

Glycemic lability was improved after the first islet transplantation. The M-value (Fig. 2A) and MAGE

(Fig. 2B) before islet transplantation was 53.0 (range, 8.9–91.0) and 8.5 mM (range, 4.8–11.7 mM), respectively. These medians were higher than the third quartiles of the 52 type 1 diabetic control patients. The M-value (Fig. 2A) decreased significantly to 4.2 (range, 0.6–8.8, $P < 0.05$), and MAGE (Fig. 2B) also significantly decreased to 3.3 mM (range, 2.0–4.5 mM, $P < 0.05$) 1 month after islet transplantation. These values were lower than the first quartile of the 102 type 2 diabetic control patients.

The estimated HbA$_{1c}$ level before islet transplantation was 7.9% (range, 5.7–10.9%). The estimated HbA$_{1c}$ level 1 month after islet transplantation decreased significantly to 5.4% (range, 4.7–5.9%, $P < 0.05$). There was no worsening of the secondary complications of diabetes due to the decrease of HbA$_{1c}$ levels in all six patients.
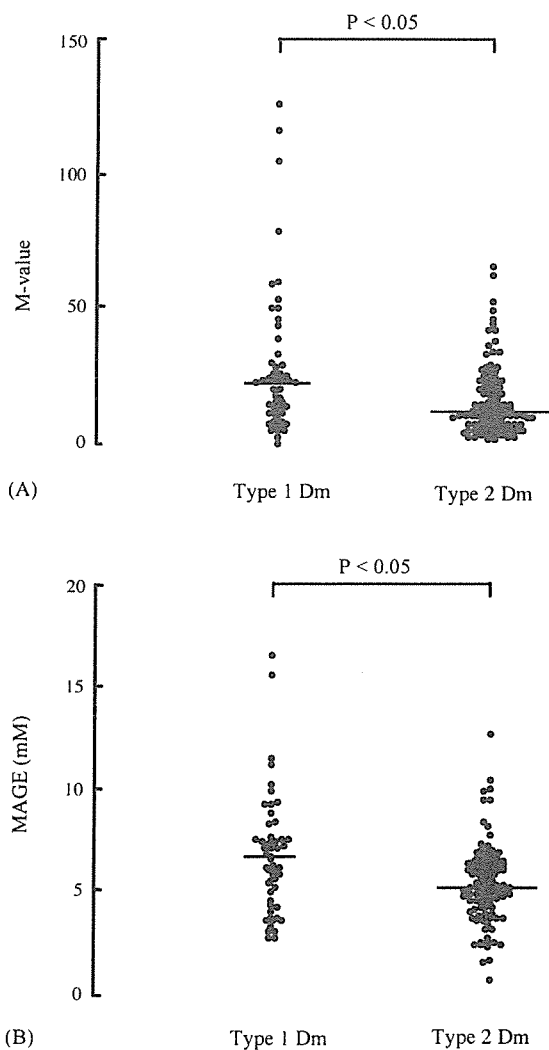

Fig. 1. (A) M-value of 52 type 1 diabetic patients and 102 type 2 diabetic patients at discharge who were admitted to Kyoto University Hospital. Horizontal lines represent medians. (B) MAGE of 52 type 1 diabetic patients and 102 type 2 diabetic patients at discharge who were admitted to Kyoto University Hospital. Horizontal lines represent medians.
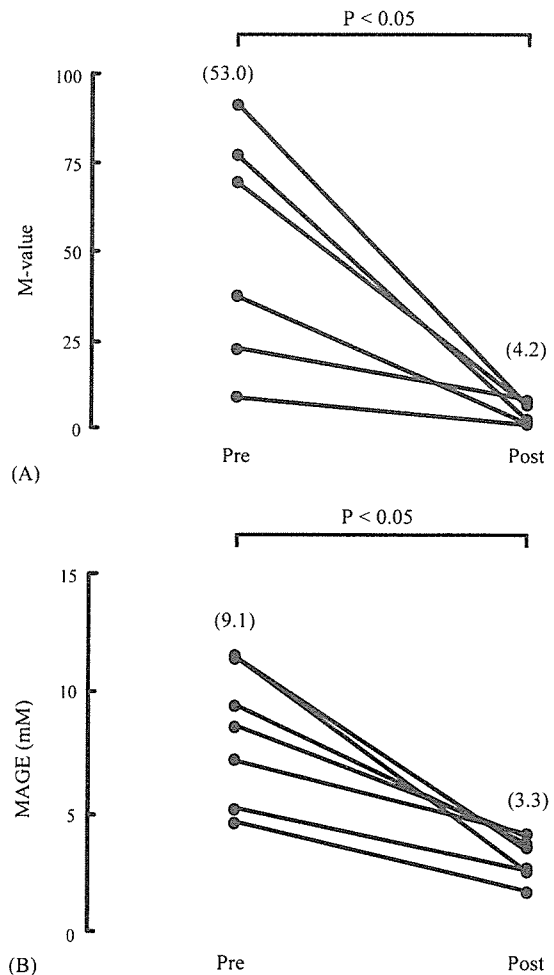
Fig. 2. (A) M-value before and 1 month after islet transplantation. Median values in parentheses. (B) MAGE before and 1 month after islet transplantation. Median values in parentheses.

3.2. Daily exogenous insulin use before and after islet transplantation

The total amount of exogenous insulin decreased gradually in all cases, stabilizing 1 month after transplantation. The supplemental dose of stimulated insulin (Fig. 3A) did not change significantly from 0.28 units/kg/day (range, 0.13–0.51 units/kg/day) to 0.21 units/kg/day (range, 0–0.41 units/kg/day); the supplement of basal insulin (Fig. 3B) decreased significantly by a median of 43% from 0.31 units/kg/day (range, 0.16–0.37 units/kg/day) to 0.18 units/kg/day (range, 0–0.22 units/kg/day, $P < 0.05$).
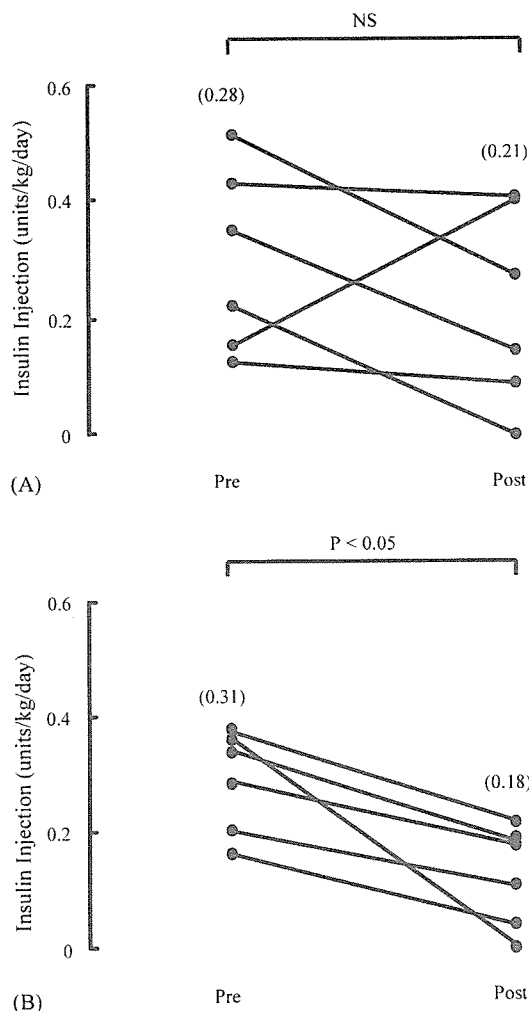

Fig. 3. (A) Daily supplement of stimulated insulin (units/kg/day) before and 1 month after islet transplantation. Median values in parentheses. (B) Daily supplement of basal insulin (units/kg/day) before and 1 month after islet transplantation. Median values in parentheses.

4. Discussion

Intensive insulin therapy can decrease HbA_{1c} and thereby delay the onset and slow progression of secondary complications of diabetes in both type 1 and type 2 diabetic patients [13,14]. However, our results showed that, in many patients with type 1 diabetes, blood glucose levels remained unstable despite optimal insulin therapy in hospital. The levels remained considerably high or low in comparison with the optimal levels, assessed by the M-value, and the amplitude of glycemic excursions remained large, assessed by MAGE, even though the HbA_{1c} levels of type 1 and type 2 diabetic patients on admission were nearly the same. In addition, before islet transplantation, all six patients exhibited hypoglycemia unawareness and life-threatening hypoglycemia despite all effort to optimize insulin therapy. Like this, in type 1 diabetic patients, it is quite difficult to achieve good glycemic control with exogenous insulin even if it is optimized.

Islet transplantation has become an alternative therapy for type 1 diabetes, the Edmonton protocol of multiple transplantations from different donors aiming at insulin independence as well as the avoidance of severe hypoglycemia. However, in some countries, the donor pool is extremely small, and many patients are on waiting lists. We evaluated the effects on glycemic lability in patients with insulin-dependency 1 month after a single transplantation of the islets, when the total amount of exogenous insulin became stable.

Glycemic lability in all six patients was improved significantly 1 month after the first islet transplantation. The M-value and MAGE became better than in most of the type 2 diabetic control patients. This indicates that a gain of endogenous insulin secretion resulted in non-insulin-dependent status. Furthermore, episodes of severe hypoglycemia have not occurred for at least 6 months after islet transplantation [10], even though the overall blood glucose level continues to be considerably lower than that before transplantation.

Only 5 years have passed since the Edmonton protocol was started, and the loss of insulin independence in many patients several years after transplantation has been reported [15]. Thus, the duration of insulin-independent status established by transplantation is uncertain. However, maintaining the blood glucose level close to the normal range for a certain period by transplantation can be beneficial: the Epidemiology of Diabetes Interventions and Complications (EDIC) study [16–20] indicates a reduced risk of onset and progression of diabetic complications for

several years, even after glycemic control becomes fair or poor due to graft failure.

As glycemic lability in type 1 diabetes is due to a complete loss of both basal and stimulated insulin secretion from β-cells, the supplemental dose of basal and stimulated insulin required before and after islet transplantation was compared. The supplement of basal insulin of all six patients decreased significantly 1 month after transplantation, indicating that a single transplantation of the islets is an effective therapy to supply basal insulin secretion.

The Treat-to-Target Trial and related trials have shown that the uniform supplementation of basal insulin throughout the day significantly reduces risk of hypoglycemia and achieves good glycemic control in type 2 diabetic patients [21–23]. Our findings apparently support this. The supplemental dose of stimulated insulin was not changed significantly, partly due to the toxic effects on the islets of the immunosuppressive drugs. Indeed, we have shown that tacrolimus suppresses glucose-induced insulin release from pancreatic islets by reducing glucokinase activity [24]. In addition, sirolimus significantly impairs glucose-induced insulin secretion in islets [25,26]. Accordingly, the development of a new immunosuppressive regimen less toxic to β-cells may be required.

These results demonstrate that only a single transplantation of the islets using even a non-heart-beating donor can be sufficient to achieve metabolic stability, avoid life-threatening severe hypoglycemia, and improve the quality of life in patients with insulin-dependency regardless of the achievement of insulin independence. Thus, a single transplantation of the islets using one donor may enable a greater number of diabetic patients to benefit from islet transplantation therapy where donor shortage is a serious problem.

Acknowledgements

References

[1] J. Schlichtkrull, O. Munck, M. Jersild, The M-value, an index of blood-sugar control in diabetics, Acta Med. Scand. 177 (1965) 95–102.

[2] F.J. Service, G.D. Molnar, J.W. Rosevear, E. Ackerman, L.C. Gatewood, W.F. Taylor, et al., Mean amplitude of glycemic excursions, a measure of diabetic instability, Diabetes 19 (1970) 644–655.

[3] F.J. Service, R.L. Nelson, Characteristics of glycemic stability, Diabetes Care 3 (1) (1980) 58–62.

[4] The Diabetes Control and Complications Trial (DCCT) Research Group, Early worsening of diabetic retinopathy in the Diabetes Control and Complications Trial, Arch. Ophthalmol. 116 (1998) 874–876.

[5] K.V. Allen, B.M. Frier, Nocturnal hypoglycemia: clinical manifestations and therapeutic strategies toward prevention, Endocr. Pract. 9 (6) (2003) 530–543.

[6] A.M.J. Shapiro, J.R.T. Lakey, E.A. Ryan, G.S. Korbutt, E. Toth, G.L. Warnock, et al., Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen, N. Engl. J. Med. 343 (2000) 230–238.

[7] E.A. Ryan, J.R.T. Lakey, B.W. Paty, S. Imes, G.S. Korbutt, N.M. Kneteman, et al., Successful islet transplantation Continued insulin reserve provides long-term glycemic control, Diabetes 51 (2002) 2148–2157.

[8] B.J. Hering, R. Kandaswamy, J.D. Ansite, P.M. Eckman, M. Nakano, T. Sawada, et al., Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes, JAMA 293 (2005) 830–835.

[9] S. Matsumoto, T. Okitsu, Y. Iwanaga, H. Noguchi, H. Nagata, Y. Yonekawa, et al., Insulin independence after living-donor pancreatectomy and islet allotransplantation, Lancet 365 (9741) (2005) 1642–1644.

[10] S. Matsumoto, T. Okitsu, Y. Iwanaga, H. Noguchi, H. Nagata, Y. Yonekawa, et al., Successful islet transplantation from non-heart-beating donor pancreata using modified Ricordi islet isolation method, Transplantation, in press.

[11] C.L. Rohlfing, H.-M. Wiedmeyer, R.R. Little, J.D. England, A. Tennill, D.E. Goldstein, Defining the relationship between plasma glucose and HbA_{1c}, Diabetes Care 25 (2002) 275–278.

[12] M. Tominaga, I. Kobayashi, K. Kuwa, I. Takei, T. Hoshino, G. Yoshino, et al., HbA_{1c} Value of JDS Lot 2 Determined by NSGP, J. Jpn. Diab. Soc. 45 (5) (2002) 385–388.

[13] Diabetes Control and Complications Trial Research Group, The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus, N. Engl. J. Med. 329 (1993) 977–986.

[14] Y. Ohkubo, H. Kishikawa, E. Araki, T. Miyata, S. Isami, S. Motoyoshi, et al., Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study, Diab. Res. Clin. Pract. 28 (1995) 103–117.

[15] A.M.J. Shapiro, J.R.T. Lakey, B.W. Paty, P.A. Senior, D.L. Bigam, E.A. Ryan, Strategic opportunities in clinical islet transplantation, Transplantation 79 (2005) 1304–1307.

[16] Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group, Epidemiology of Diabetes Interventions and Complications (EDIC), Diabetes Care 22 (1999) 99–111.

[17] The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group, Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy, N. Engl. J. Med. 342 (2000) 381–389.

[18] The Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group, Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus, JAMA 287 (2002) 2563–2569.

[19] The Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group, Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy, JAMA 290 (2003) 2159–2167.

[20] The Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications Research Group, Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus, N. Engl. J. Med. 348 (2003) 2294–2303.

[21] M.C. Riddle, J. Rosenstock, J. Gerich, The Treat-to-Target Trial: randomized addition of glargine or human NPH insulin to oral therapy of type 2 diabetic patients, Diabetes Care 26 (2003) 3080–3086.

[22] J. Rosenstock, Basal insulin supplementation in type 2 diabetes: refining the tactics, Am. J. Med. 116 (3A) (2004) 10S–16S.

[23] J. Rosenstock, G. Dailey, M. Massi-Benedetti, A. Fritsche, Z. Lin, A. Salzman, Reduced hypoglycemia risk with insulin glargine: a meta-analysis comparing insulin glargine with human NPH insulin in type 2 diabetes, Diabetes Care 28 (2005) 950–955.

[24] R.G. Radu, S. Fujimoto, E. Mukai, M. Takehiro, D. Shimono, K. Nabe, et al., Tacrolimus suppresses glucose-induced insulin release from pancreatic islets by reducing glucokinase activity, Am. J. Physiol. 288 (2005) E365–E371.

[25] E. Bell, X. Cao, J.A. Moibi, S.R. Greene, R. Young, M. Trucco, et al., Rapamycin has a deleterious effect on MIN-6 cells and rat and human islets, Diabetes 52 (2003) 2731–2739.

[26] B.W. Paty, J.S. Harmon, C.L. Marsh, R.P. Robertson, Inhibitory effects of immunosuppressive drugs on insulin secretion from HIT-T15 cells and Wister rat islets, Transplantation 73 (3) (2002) 353–357.

SUIT, secretory units of islets in transplantation: An index for therapeutic management of islet transplanted patients and its application to type 2 diabetes

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Abstract

Evaluation of a patient's pancreatic β-cell function is important in both diagnosis and treatment of diabetes. We sought to determine β-cell function with a single sampling of blood. Examination of fasting blood glucose (F-BG, mM) and C-peptide (F-CPR, nM) levels in seven post-islet-transplanted states of four patients revealed a linear relationship between F-BG and F-CPR. Assuming that normal subjects aged <40 years have 100% pancreatic β-cell function, we developed the secretory units of islets in transplantation (SUIT) as an index of β-cell function by the formula: $250 \times \text{F-CPR}/(\text{F-BG} - 3.43)$. The SUIT index was correlated with the stimulated C-peptide levels not only in islet-transplanted patients ($R^2 = 0.68$, $P < 0.05$) but also in type 2 patients ($R^2 = 0.34$, $P < 0.001$). Since the SUIT index can be calculated from data obtained at a single fasting blood sampling and predict the pancreatic β-cell function, the formula may be a useful tool in clinical management of diabetes.
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Keywords: Islet transplantation; Insulin; Diabetes

1. Introduction

Diabetes is a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia. As diabetes results from pancreatic β-cell deficiency and/ or insulin resistance, a convenient clinical measure of pancreatic β-cell function and insulin sensitivity should be helpful to achieve the tight control of glycemic levels in diabetes.

Glucose is the most important secretagogue of insulin from pancreatic β-cells. Several tools presently available for measurement of pancreatic β-cell function include the hyperglycemic clamp [1], minimal model method [2], and graded glucose infusion [3], either of which determines secretory response of insulin to

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different glycemic levels and is complicated and time-consuming. In contrast, measurement of pancreatic β-cell mass in human is only possible after autopsy [4] and pancreatic β-cell mass is estimated from in vivo functional tests of pancreatic β-cells.

It has been recently shown that islet transplantation offers a prospect of good glycemic control in labile type 1 diabetes [5], and is used worldwide as a treatment of brittle forms of type 1 diabetes [6]. Islet transplantation in such patients involves extraction of islets from donors with normal glucose tolerance followed by implantation into patients with few islets. Thus, as the patients are under intensive care of blood glucose levels in the postoperative period to avoid gluco-toxicity on islets, each islet transplanted into patients potentially has normal function, and pancreatic β-cell function should be closely related with pancreatic β-cell mass in these patients.

2. Materials and methods

2.1. The proposed SUIT index

Islet isolation was performed using a modification of the Edmonton protocol [5,7,8] from pancreata of non-heart-beating donors [9] or a living donor [10]. Islets were infused into the liver after percutaneous transhepatic cannulation of the main portal vein. The study was approved by the ethics committee of the Kyoto University Graduate School and Faculty of Medicine, Japan.

We examined fasting blood glucose (F-BG) and C-peptide (F-CPR) levels in seven post-transplantation states of four patients and found a linear relationship between F-BG and F-CPR in each state. Since the estimation of the summary blood glucose intercept was the ratio estimation, a simple mean of $-a_i/b_i$ was not a good estimator, where a_i was the individual CPR-intercept and b_i was the individual regression coefficient of the lines in Fig. 1. Instead we estimated the summary F-BG intercept by $-\sum_i a_i / \sum_i b_i$. The associated 95% confidence interval was calculated based on the log transformation and the approximate variance using the delta method. We found the point estimate of the summary F-BG intercept to be 3.43 mM and the 95% confidence interval 3.41–3.45 mM.

Assuming normoglycemic subjects aged <40 years have normal pancreatic β-cell mass, a SUIT index, the secretory units of islets in transplantation, was assessed from F-BG (mM) and F-CPR (nM).

2.2. Application of a SUIT index to type 2 diabetic patients

Type 2 diabetic patients hospitalized in Kyoto University Hospital without renal failure (total 304, male/female 172/132, BMI = 24.0 ± 4.3 (mean ± S.D.), age = 61.0 ± 13.2 years (mean ± S.D.)) were recruited. Serum C-peptide levels were measured 6 min after intravenous injection of 1 mg of glucagon [11].

2.3. Statistical analyses

Statistical evaluation of results was performed using linear regression. P values <0.05 were considered significant.
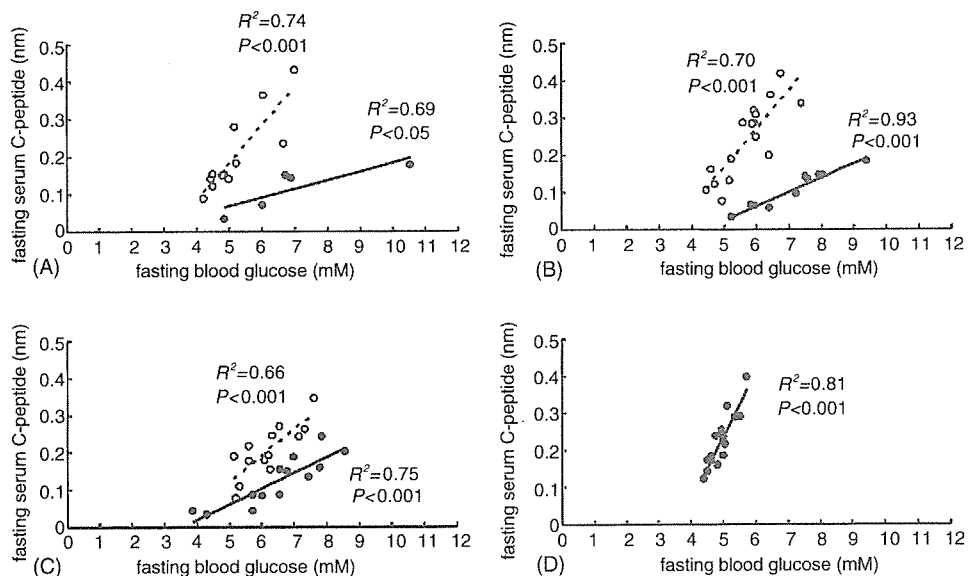

Fig. 1. Linear relationship of fasting blood glucose and C-peptide levels. Seven cases of islet transplantation of four patients (A–D) were plotted. Data from the first transplantation were shown in filled circles and solid lines and data from the second transplantation (A, B and C) were shown in open circles and broken lines. Coefficient of determination (R^2) and P-value (P) are shown.

3. Results

3.1. Correlation of F-BG and F-CPR

Steady state blood glucose and insulin levels are determined by their interaction in a feed-back loop. We examined fasting blood glucose (F-BG) and serum C-peptide (F-CPR) levels in seven post-transplantation states of four patients, and found a linear relationship between F-BG and F-CPR in each state (Fig. 1).

While regression coefficients differ among the states, the slope becomes steeper after sequential islet transplantation (Fig. 1, broken lines compared with solid lines in A, B and C). The most interesting feature is that the F-BG intercept is similar in each case. We found the point estimate of the summary F-BG intercept to be 3.43 mM and the 95% confidence interval 3.41–3.45 mM.

3.2. Formula of the SUIT index

Assuming normoglycemic subjects aged <40 years have normal pancreatic β-cell mass, SUIT, the secretory units of islets in transplantation, can be assessed from F-BG (mM) and F-CPR (nM) by the formula: 250 × F-CPR/(F-BG − 3.43), where SUIT index of normal subjects is 100.0 ± 11.7 (mean ± S.E.).

3.3. The SUIT index in patients receiving exogenous insulin therapy

Measurement of immunoreactive insulin is the standard method for evaluating the pancreatic β-cell

function. However, insulin assays cannot differentiate endogenous insulin from exogenous insulin and the measurement of peripheral concentrations of C-peptide is the most common approach in patients receiving exogenous insulin therapy. We then calculated the SUIT index in a case of living donor islet allo-transplantation [10] requiring 0–17 units of exogenous insulin daily after islet transplantation. The SUIT index of 37.1 ± 1.3 (mean ± S.E.) was independent of the amount of exogenous insulin in this case (Fig. 2) as well as in other cases (data not shown).

3.4. The SUIT index to evaluate the efficacy of islet transplantation

The efficacy of islet transplantation has been evaluated by measuring the stimulated C-peptide levels [12]. We then compared the SUIT index with the results of a glucagon stimulation test of islet secretory capacity in patients after islet transplantation (Fig. 3A). The acute insulin response to glucagon (1 mg) is clearly correlated with the SUIT index ($R^2 = 0.68$, $P < 0.05$).
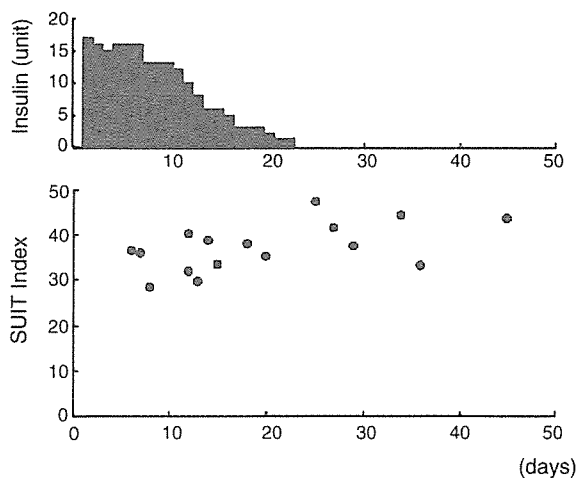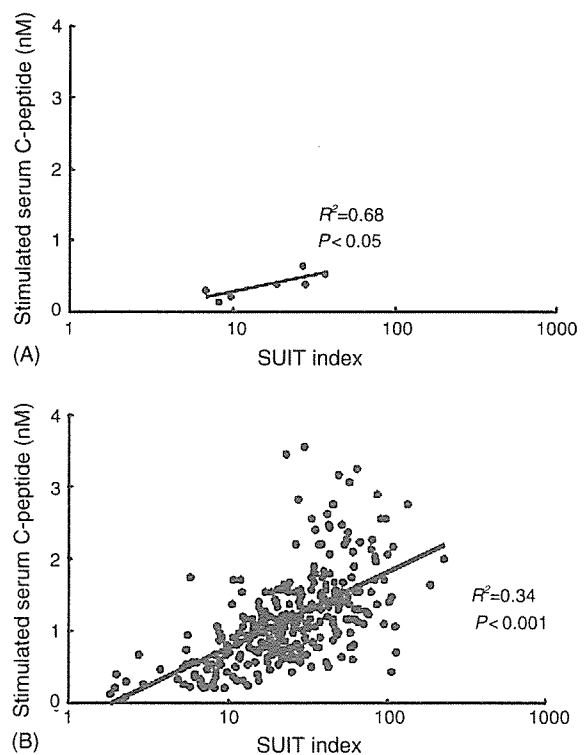
Fig. 2. The SUIT index in a patient receiving exogenous insulin therapy. The clinical course of the SUIT index in a patient after islet transplantation was shown. Amount of exogenous insulin injection and the SUIT index were plotted against post-operative day.

Fig. 3. Validation of the SUIT index as pancreatic β-cell function. Correlation of the SUIT index and the acute insulin response to glucagon were shown in islet-transplanted patients (A) and type 2 diabetic patients (B). Serum C-peptide levels were measured 6 min after intravenous injection of glucagon (1 mg). Coefficient of determination (R^2) and P-value (P) are shown.
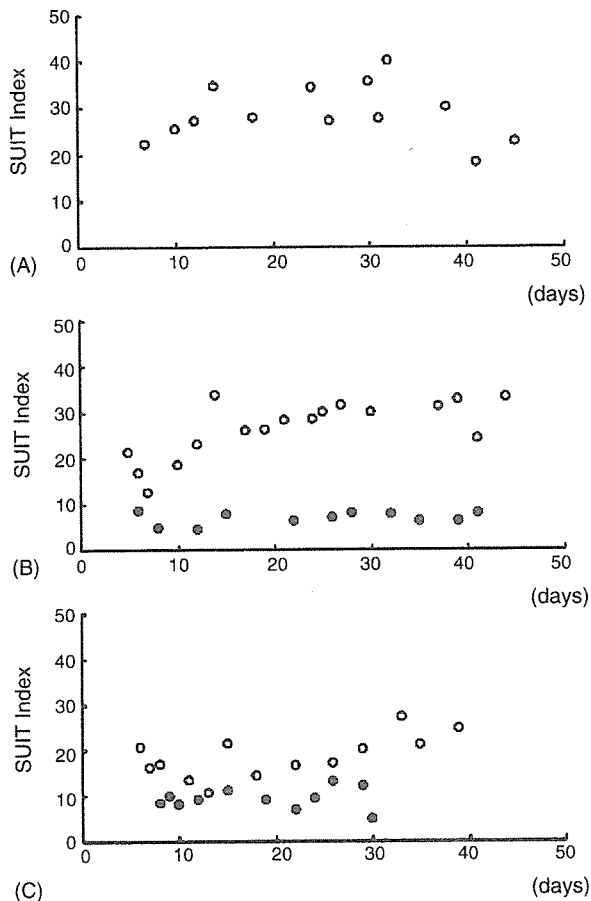
Fig. 4. The SUIT index as early predictor of islet transplantation efficacy. The SUIT indexes were plotted against post-operative day. Data from the first transplantation were shown in filled circle and data from the second transplantation were shown in open circles.

We next compared the SUIT index against the post-operative days (Figs. 2 and 4) and found that the index is similar around the course. These results indicated that the SUIT index was able to predict the efficacy of islet transplantation just after post-operative day 7.

3.5. Application of the SUIT index into type 2 diabetes

The SUIT index was then compared with the results of glucagon stimulation test in type 2 diabetic patients (Fig. 3B). The acute insulin response to glucagon was similarly correlated with the SUIT index ($R^2 = 0.34$, $P < 0.001$).

4. Discussion

The measurement of the pancreatic β-cell function is critical in the management of diabetic patients. In type 1

patients, intensive insulin therapies aimed at preserving or improving endogenous insulin secretion are associated with better metabolic control and lower risk for hypoglycemia and chronic complication [13,14]. In type 2 patients, the United Kingdom Prospective Diabetes Study revealed that the glycemic deterioration is associated with progressive loss of the pancreatic β-cell function [15].

The pancreatic β-cell function is determined by two factors: quantity of pancreatic β-cells and quality of each β-cell. Characterization of maturity-onset diabetes of the young (MODY) shows that each of the factors plays an important role on glucose-induced insulin secretion. Glucokinase, a rate-limiting enzyme of the glycolytic pathway, plays a key role in glucose sensing by the insulin-secreting pancreatic β-cells. In subjects with glucokinase mutations (MODY2), the dose-response curve relating glucose and insulin secretion rate during graded intravenous glucose infusions was shifted to the right, indicating that quality of pancreatic β-cells can determine insulin-secretory capacity in vivo [16]. In contrast, inactivating mutation of the *IPF1* gene leads to MODY4 [17] and partial deficiency of the IPF1, known as PDX-1 in mice, showed that pancreatic β-cell mass was decreased but single β-cells had normal glucose sensing and insulin secretion, indicating that quantity of pancreatic β-cells can also determine insulin-secretory capacity [18]. Therefore, measurement of pancreatic β-cell mass in human is necessary but is only possible after autopsy [4].

In this study, we have shown that in islet-transplanted patients, fasting blood glucose and fasting serum C-peptide levels have a linear relationship and pancreatic β-cell mass can be estimated from the formula by a single sampling of blood after over-night fast. This formula resembles that in the computer-solved model of pancreatic β-cell function, HOMA-β, which is 20 × insulin (mU/L)/(F-BG − 3.5) [19]. However, HOMA-β cannot be used to assess β-cell function in those taking exogenous insulin [20], due to the inability of insulin assays to differentiate endogenous insulin from exogenous insulin. We have not yet determined the range of the linear regression of F-BG and F-CPR. However, the SUIT index is independent of the amount of exogenous insulin. Therefore, it would be possible to know the β-cell function after injection of long-acting insulin.

After islet transplantation, not all of the grafted islets survived in the recipients but some islets were damaged and insulin were released from the eliminated islets especially in the early stage of islet transplantation, resulting in dysregulated elevation of serum C-peptide

levels during a few days of post-transplantation. Our study showed that the SUIT index could predict the efficacy of islet transplantation just after post-operative day 7.

Calculation of the SUIT index by a single sampling of blood after over-night fast can predict pancreatic β-cell function not only in islet-transplanted patients but also in type 2 diabetic patients, and should be a useful tool in the clinical management of diabetes.

Acknowledgements

References

[1] R.A. DeFronzo, J.D. Tobin, R. Andres, Glucose clamp technique: a method for quantifying insulin secretion and resistance, Am. J. Physiol. 237 (1979) E214–E223.

[2] R.N. Bergman, L.S. Phillips, C. Cobelli, Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and β-cell glucose sensitivity from the response to intravenous glucose, J. Clin. Invest. 68 (1981) 1456–1467.

[3] C.N. Jones, D. Pei, P. Staris, K.S. Polonsky, Y.D. Chen, G.M. Reaven, Alterations in the glucose-stimulated insulin secretory dose-response curve and in insulin clearance in nondiabetic insulin-resistant individuals, J. Clin. Endocrinol. Metab. 82 (1997) 1834–1838.

[4] A.E. Butler, J. Janson, S. Bonner-Weir, R. Ritzel, R.A. Rizza, P.C. Butler, β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes, Diabetes 52 (2003) 102–110.

[5] A.M. Shapiro, J.R. Lakey, E.A. Ryan, G.S. Korbutt, E. Toth, G.L. Warnock, et al., Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen, N. Engl. J. Med. 343 (2000) 230–238.

[6] A.M. Shapiro, C. Ricordi, B. Hering, Edmonton's islet success has indeed been replicated elsewhere, Lancet 362 (2003) 1242.

[7] C. Ricordi, Islet transplantation: a brave new world, Diabetes 52 (2003) 1595–1603.

[8] S. Matsumoto, S.A. Qualley, S. Goel, D.K. Hagman, I.R. Sweet, V. Poitout, et al., Effect of the two-layer (University of Wisconsin solution-perfluorochemical plus O_2) method of pancreas preservation on human islet isolation, as assessed by the Edmonton Isolation Protocol, Transplantation 74 (2002) 1414–1419.

[9] S. Matsumoto, T. Okitsu, Y. Iwanaga, H. Noguchi, H. Nagata, Y. Yonekawa, et al., Successful islet transplantation from non-heart-beating donor pancreata using modified Ricordi islet isolation method, Transplantation, in press.

[10] S. Matsumoto, T. Okitsu, Y. Iwanaga, H. Noguchi, H. Nagata, Y. Yonekawa, et al., Insulin independence after living-donor distal pancreatectomy and islet allotransplantation, Lancet 365 (2005) 1642–1644.

[11] E. Samols, J. Tyler, G. Marri, V. Marks, Stimulation of glucagon secretion by oral glucose, Lancet 2 (1965) 1257–1259.

[12] E.A. Ryan, J.R. Lakey, B.W. Paty, S. Imes, G.S. Korbutt, N.M. Kneteman, et al., Successful islet transplantation: continued insulin reserve provides long-term glycemic control, Diabetes 51 (2002) 2148–2157.

[13] The DCCT Research Group, Effects of age, duration and treatment of insulin-dependent diabetes mellitus on residual β-cell function: observations during eligibility testing for the Diabetes Control and Complications Trial (DCCT), J. Clin. Endocrinol. Metab. 65 (1987) 30–36.

[14] The Diabetes Control and Complications Trial Research Group, Effect of intensive therapy on residual β-cell function in patients with type 1 diabetes in the diabetes control and complications trial. A randomized, controlled trial, Ann. Intern. Med. 128 (1998) 517–523.

[15] U.K. Prospective Diabetes Study Group, U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: A progressive disease, Diabetes 44 (1995) 1249–1258.

[16] G.I. Bell, S.J. Pilkis, I.T. Weber, K.S. Polonsky, Glucokinase mutations, insulin secretion, and diabetes mellitus, Annu. Rev. Physiol. 58 (1996) 171–186.

[17] D.A. Stoffers, J. Ferrer, W.L. Clarke, J.F. Habener, Early-onset type-II diabetes mellitus (MODY4) linked to IPF1, Nat. Genet. 17 (1997) 138–139.

[18] J.D. Johnson, N.T. Ahmed, D.S. Luciani, Z. Han, H. Tran, J. Fujita, et al., Increased islet apoptosis in Pdx1$^{+/-}$ mice, J. Clin. Invest. 111 (2003) 1147–1160.

[19] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (1985) 412–419.

[20] T.M. Wallace, J.C. Levy, D.R. Matthews, Use and abuse of HOMA modeling, Diabetes Care 27 (2004) 1487–1495.

Gatifloxacin acutely stimulates insulin secretion and chronically suppresses insulin biosynthesis

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Abstract

Gatifloxacin can cause both hypoglycemia and hyperglycemia in both diabetic and non-diabetic patients. Gatifloxacin recently has been reported to stimulate insulin secretion by inhibition of ATP-sensitive K^+ (K_{ATP}) channels in pancreatic β-cells. Gatifloxacin-induced hypoglycemia is associated with concomitant use of sulfonylureas, and usually occurs immediately after administration of the drug. We find that gatifloxacin acutely stimulates insulin secretion from mouse pancreatic islets and that glibenclamide has additive effects on gatifloxacin-induced insulin secretion. On the other hand, gatifloxacin-induced hyperglycemia often takes several days to develop. We also demonstrate that chronic gatifloxacin treatment decreases islet insulin content by inhibiting insulin biosynthesis, which process may be associated with gatifloxacin-induced hyperglycemia. Moreover, discontinuation of gatifloxacin results in improved insulin secretory response. These data clarify the differing mechanisms of gatifloxacin-induced hyper- and hypoglycemia, and suggest that blood glucose levels should be carefully monitored during gatifloxacin administration, especially in elderly patients with renal insufficiency, unrecognized diabetes, or other metabolic disorders. Because the risk of potentially life-threatening dysglycemia is increased during gatifloxacin therapy, these findings have important implications for clinical practice. © 2006 Elsevier B.V. All rights reserved.

Keywords: Gatifloxacin; Hypoglycemia; Hyperglycemia; Insulin secretion; Islet insulin content; K_{ATP} (ATP-sensitive K^+) channel

1. Introduction

Gatifloxacin is a fourth-generation fluoroquinolone that is active against a broad spectrum of pathogens. Since its introduction in clinical practice in 2000, gatifloxacin use has increased steadily in hospital and community settings. However, gatifloxacin and certain other quinolones have been reported to induce both hypo- and hyperglycemia in diabetic and non-diabetic patients (Baker and Hangii, 2002; Menzies et al., 2002; Biggs, 2003; Donaldson et al., 2004). Most of the hypoglycemic patients were elderly and/or had renal insufficiency, which might increase the serum concentration of gatifloxacin, and they were often diabetic and received concomitant oral hypoglycemic agents such as sulfonylureas (Menzies et al., 2002; Biggs,

2003). The mechanism of such hypoglycemia is thought to involve increased insulin release from pancreatic islets (Maeda et al., 1996), and it has been shown recently that some quinolones such as temafloxacin and gatifloxacin directly stimulate insulin secretion by inhibiting K_{ATP} channel activities in pancreatic β-cells when used at high concentrations (Saraya et al., 2004). Hypoglycemia induced by quinolones usually occurs immediately after the first administration of the drug. In this study, we examined the acute effects of gatifloxacin and the interaction of therapeutically relevant concentrations of gatifloxacin and glibenclamide, one of the most widely used oral hypoglycemic agents, using mouse pancreatic islets.

In contrast to hypoglycemia, gatifloxacin-induced hyperglycemia often takes several days to develop high glucose levels of around 30 mM, and discontinuation of gatifloxacin treatment promptly improves glucose homeostasis in most cases (Arce et al., 2004; Bhatia et al., 2004; Biggs, 2003; Donaldson et al.,

2004). However, little is known of the mechanism of hyperglycemia induced by fluoroquinolones. In this study, we cultured mouse pancreatic islets and mouse insulinoma MIN6 cells with or without various concentrations of gatifloxacin to clarify the chronic effects of gatifloxacin, including changes in islet insulin content and insulin mRNA expression levels. We also determined whether gatifloxacin withdrawal restores insulin secretory response and islet insulin content in gatifloxacin pre-treated islets. In addition to clarifying gatifloxacin-induced glucose abnormalities, these data indicate that blood glucose should be carefully monitored during gatifloxacin administration, especially in patients with impaired glucose tolerance.

2. Materials and methods

2.1. Solutions and drugs

The medium for batch incubation experiments was a bicarbonate-buffered solution containing (in mM): NaCl 120, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.4, $NaHCO_3$ 20, and HEPES 10, supplemented with 0.2% bovine serum albumin and gassed with a mixture of O_2 (95%) and CO_2 (5%) to maintain pH 7.4. Gatifloxacin, norfloxacin, and fleroxacin were from Kyorin Pharmaceutical Co., Ltd., Japan. Levofloxacin and ofloxacin were from Daiichi Pharmaceutical Co., Ltd., Japan. The drugs were dissolved in dimethyl sulphoxide (DMSO) to obtain a stock solution of 200 mM, and further diluted to final concentrations with the medium. Glibenclamide was obtained from Research Biochemicals International, U.S.A., prepared as a stock solution of 1 mM in DMSO, and diluted to the final concentration before use.

2.2. Isolation of mouse pancreatic islets and islet culture

All animal procedures were approved by Kyoto University Animal Care Committees. Pancreatic islets were isolated from fed male C57Bl/6 mice aged 12–16 weeks by collagenase digestion method. For short term exposure, fresh islets were used. For long term exposure, islets were cultured with or without gatifloxacin in RPMI medium containing 10% fetal bovine serum and 11.1 mM glucose, and used after the indicated culture periods for subsequent experiments. In some experiments (Fig. 5), the islets were cultured in the presence of 20 or 100 μM gatifloxacin for one day, washed with gatifloxacin-free RPMI medium three times to remove remaining gatifloxacin in the culture medium, and then cultured for additional two days in gatifloxacin-free medium.

2.3. Batch incubation experiment and islet insulin content analysis

Ten size-matched fresh or cultured islets were collected in each tube and preincubated at 37 °C for 30 min in 1 ml of the medium containing 2.8 mM glucose mentioned above, and incubated for another 30 min in 500 μl of medium containing the indicated concentrations of glucose and test materials. At the

end of the incubation period, islets were pelleted by centrifugation (130 ×g, 1.5 min) and aliquots of the buffer were sampled. The amount of immunoreactive insulin was determined by radioimmunoassay (RIA), using mouse insulin as a standard. To determine insulin content, islets were homogenized in 400 μl acid-ethanol (37% HCl in 75% ethanol, 15:1000 vol/vol) and extracted at 4 °C overnight. The acidic extracts were dried by vacuum, reconstituted, and subjected to insulin measurement. Insulin was measured by RIA.

2.4. Analysis of mouse insulin-2 mRNA from cultured islets and MIN6 cells

After groups of 50 islets were cultured with or without gatifloxacin for 3 days, poly(A)$^+$ RNAs were isolated using a Poly(A)Pure kit (Ambion, U.S.A.) and first strand cDNAs were synthesized by SuperScript™ II Reverse Transcriptase system (Invitrogen, U.S.A.) according to the manufacturer's instructions. TaqMan™ quantitative polymerase chain reaction (PCR) assay for mouse *Insulin-2* (m*Ins-2*) was performed using forward and reverse m*Ins-2*-specific primers and probes in an ABI PRISM™ 7000 Sequence Detection System. The results are expressed as the ratio of m*Ins-2* mRNA to mouse *Glyceraldehydes-3-phosphate dehydrogenase* (*GAPDH*) mRNA.

MIN6 cells were cultured in Dulbecco's Minimal Essential Medium supplemented with 25 mM glucose and 13% fetal bovine serum with or without gatifloxacin for 3 days. Total RNA (10 μg) prepared with TRIzol reagent (Invitrogen, U.S.A.) was used for Northern blot analysis. Mouse *β-actin* mRNA was used for standardization.

Insulin promoter activity was evaluated in MIN6 cells transfected with the human insulin promoter-luciferase reporter gene and cultured for three days with or without 100 μM gatifloxacin, using Dual-Luciferase Reporter Assay System (Promega, U.S.A.) according to manufacture's instructions. Mean values of luciferase activity relative to the gatifloxacin-untreated control were calculated from duplicate wells.

2.5. Statistical analysis

Results are expressed as mean±S.E.M. The overall difference between groups was determined by two-way factorial analysis of variance (ANOVA) (Figs. 1, 3, and 5), repeated measure ANOVA (Fig. 2), or one-way ANOVA (Fig. 4). If the analysis was significant, differences between groups were estimated using Tukey–Kramer multiple comparison post-hoc test.

3. Results

3.1. Acute effect of gatifloxacin and additive effect with glibenclamide on insulin secretion

The acute effect of gatifloxacin on insulin secretion from fresh mouse pancreatic islets was examined at concentrations of 0, 20, 100, and 300 μM in the presence 2.8, 5.5, 11.1, 16.7, and 25 mM glucose (Fig. 1). Insulin secretion was significantly
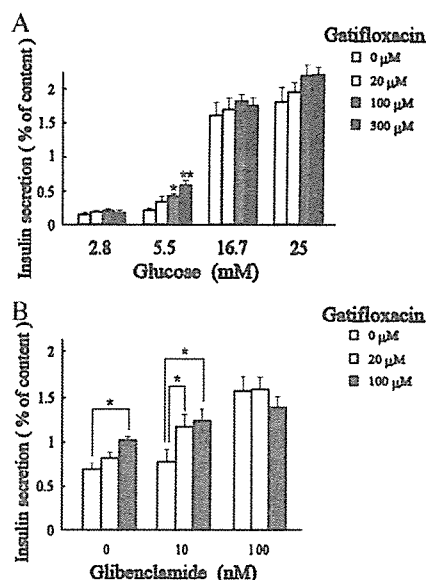
Fig. 1. Effect of various concentrations of gatifloxacin on insulin secretion from pancreatic islets. (A) Batches of 10 islets were preincubated for 30 min in medium containing 2.8 mM glucose, and incubated for 30 min in 2.8 mM, 5.5 mM, 16.7 mM, and 25 mM glucose with 0, 20, 100, and 300 μM gatifloxacin. Values are mean±S.E.M. of eight observations. **$P<0.01$, *$P<0.05$ vs corresponding control (0 μM Gatifloxacin). (B) Additive effect of gatifloxacin on insulin secretion stimulated by glibenclamide. After preincubation for 30 min without glibenclamide in 2.8 mM glucose, batches of 10 islets were incubated for 30 min in medium containing 11.1 mM glucose with 0, 20, and 100 μM gatifloxacin and 0, 10, and 100 nM glibenclamide. Values are mean±S.E.M. of eight observations. *$P<0.05$ vs corresponding control (0 μM Gatifloxacin).

increased by 100 μM and 300 μM gatifloxacin at 5.5 mM glucose (Fig. 1A) and by 100 μM gatifloxacin at 11.1 mM glucose (Fig. 1B). However, it was not significantly increased at low (2.8 mM) or high (16.7 mM and 25 mM) glucose concentrations (Fig. 1A). Addition of 10 nM glibenclamide to 20 μM and 100 μM gatifloxacin augmented insulin release about 1.3 fold and 1.7 fold, respectively, in the presence of 11.1 mM glucose (Fig. 1B). 100 nM glibenclamide had no additional effect on gatifloxacin-induced insulin secretion.

3.2. Effect of gatifloxacin on insulin secretion and islet insulin content during 3-day culture

Cumulative insulin secretion from mouse pancreatic islets into culture medium in the presence of 11.1 mM glucose with or without gatifloxacin was monitored every 24 h for 72 h. In gatifloxacin-untreated (control) islets, the amount of insulin released into culture medium steadily increased during the culture period. On the other hand, insulin secretion from gatifloxacin-treated islets (20 μM and 100 μM gatifloxacin groups) was slightly higher than that of controls at Day 1, possibly due to the stimulating effect of gatifloxacin, but did not increase thereafter (Fig. 2A).

Islet insulin content was observed for 72 h in the same culture conditions (Fig. 2B), and was markedly reduced by 20 μM and 100 μM gatifloxacin during the culture period. Islet insulin content was significantly decreased by gatifloxacin to
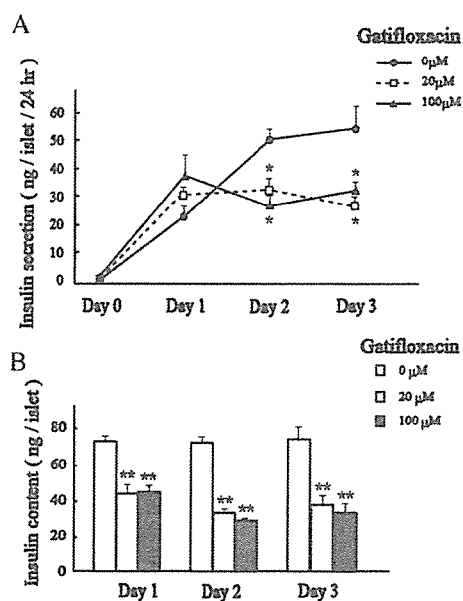

Fig. 2. Chronic effect of gatifloxacin on insulin secretion during a 3-day culture with 0, 20 and 100 μM gatifloxacin in 11.1 mM glucose. (A) The amount of insulin released into culture medium was measured at Day 1, Day 2, and Day 3. Insulin secretion from gatifloxacin-treated islets did not increase after Day 2. Values are mean±S.E.M. of eight observations. *$P<0.05$ vs corresponding control (0 μM Gatifloxacin). (B) Islet insulin content was measured at Day 1, Day 2, and Day 3. Islet insulin content was significantly decreased in gatifloxacin-treated islets. Values are mean±S.E.M. of ten observations. **$P<0.01$ vs corresponding control (0 μM Gatifloxacin).

60% of control islets already at Day 1, and continued to be reduced to 50.1% and 44.7% at Day 3 by 20 μM and 100 μM gatifloxacin, respectively.

3.3. Chronic effect of various new quinolones on islet insulin content

We then compared the effect of gatifloxacin with that of other new quinolones on islet insulin content after 3-day culture in 11.1 mM glucose (Fig. 3). All of the new quinolones tested in this study decreased insulin content at a high dosage (100 μM). Only gatifloxacin decreased islet insulin content at 20 μM, with a half-maximal inhibitory concentration (IC_{50}) of 17.7 μM,
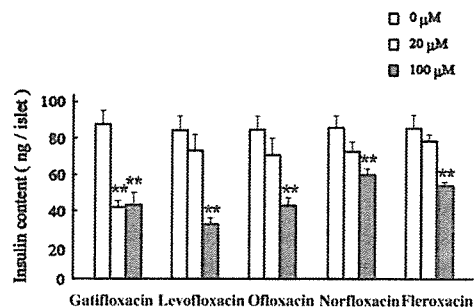

Fig. 3. Chronic effects of various new quinolones on islet insulin content during 3-day culture in 11.1 mM glucose. Gatifloxacin, levofloxacin, ofloxacin, norfloxacin, and fleroxacin were used at concentrations of 0, 20, and 100 μM, and only 20 μM gatifloxacin decreased islet insulin content measured at Day 3 with a half-maximal inhibitory concentration (IC_{50}) of 17.7 μM. Values are mean±S.E.M. of ten observations. **$P<0.01$ vs corresponding control (0 μM Gatifloxacin).
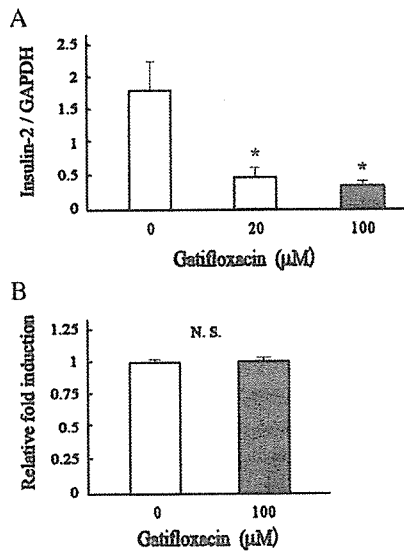
Fig. 4. Chronic effect of gatifloxacin treatment on insulin mRNA levels. (A) mRNA was isolated from islets cultured for 3 days with 0, 20, and 100 μM gatifloxacin in 11.1 mM glucose, and mouse *Insulin-2* expression levels were evaluated by quantitative real-time polymerase chain reaction (PCR) analysis. Values are mean±S.E.M. of six observations. $*P<0.05$ vs corresponding control (0 μM Gatifloxacin). (B) To examine the effects of gatifloxacin on insulin promoter activity, luciferase assay was carried out using MIN6 cells cultured for 3 days with or without 100 μM gatifloxacin ($n=18$ in control, $n=22$ in 100 μM gatifloxacin-treated MIN6 cells).

indicating that among the new quinolones, gatifloxacin is most likely to cause hyperglycemia.

3.4. Chronic effect of gatifloxacin treatment on insulin mRNA level

To investigate the decrease in islet insulin content, we examined insulin mRNA levels as a marker of insulin biosynthesis. mRNA was isolated from islets cultured for 3 days with or without gatifloxacin. Relative mouse *Insulin-2* (m*Ins-2*) mRNA expression levels were significantly decreased to 27.1% in 20 μM gatifloxacin-treated group and to 18.6% in 100 μM group, respectively, compared with that of 0 μM gatifloxacin group (Fig. 4A). We also performed Northern blot analysis of m*Ins-2* mRNA using mouse insulinoma MIN6 cells cultured for 3 days with 0 μM, 20 μM and 100 μM gatifloxacin. We found that relative m*Ins-2* mRNA expression levels were significantly decreased by 20 μM and 100 μM gatifloxacin to 69.4% and 51.9%, respectively, compared with 0 μM gatifloxacin group. In addition, insulin promoter activity in MIN6 cells cultured for three days with 100 μM gatifloxacin was measured using luciferase assay, but was almost identical to that in gatifloxacin-untreated cells with relative fold change of 1.01 (Fig. 4B).

3.5. Effect of gatifloxacin withdrawal on insulin secretion and islet insulin content

Mouse pancreatic islets were cultured in the presence of 20 or 100 μM gatifloxacin for one day, washed thoroughly with

gatifloxacin-free RPMI medium, and cultured for an additional two days in the gatifloxacin-free medium. Glucose-induced insulin secretion was greatly decreased by gatifloxacin treatment, but recovered after removal of gatifloxacin from the culture medium (Fig. 5A). Islet insulin content was decreased by gatifloxacin similarly, while frequently recovering by withdrawal of gatifloxacin in the 20 μM gatifloxacin group (to 77% of control (0 μM Gatifloxacin)) and not at all in the 100 μM gatifloxacin group (Fig. 5B). Since culture in the presence of gatifloxacin lowers islet insulin content, insulin secretion was expressed as % content, and insulin release from islets cultured with both 20 μM gatifloxacin and 100 μM gatifloxacin showed almost complete recovery upon discontinuation of the drug (Fig. 5C).
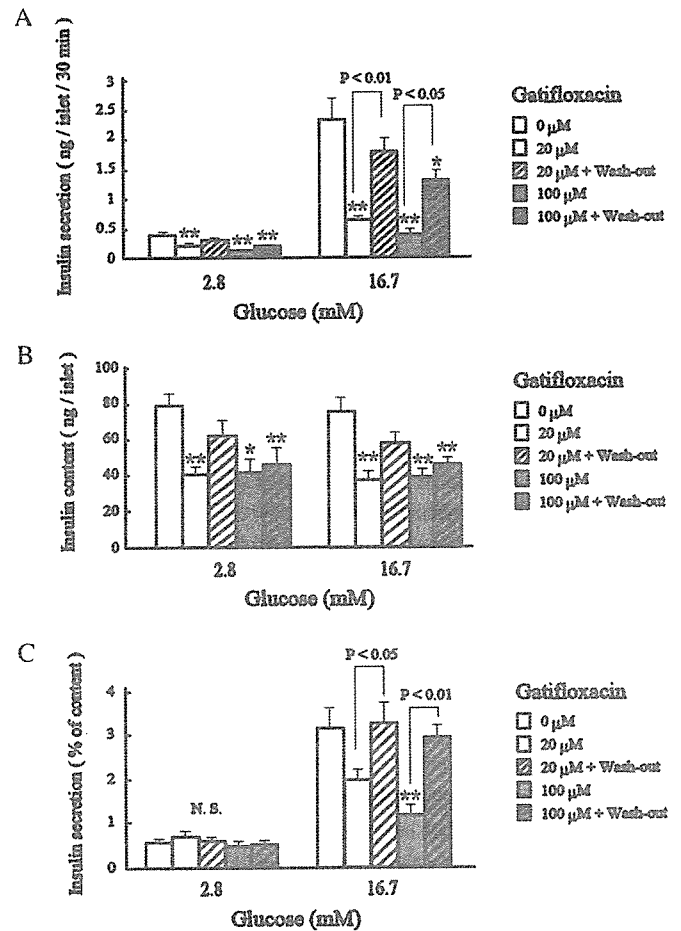

Fig. 5. Effect of gatifloxacin withdrawal on insulin secretion and insulin content from islets cultured in the presence of 20 or 100 μM gatifloxacin for one day, followed by an additional two-day culture in gatifloxacin-free medium. (A) Glucose-induced insulin secretion from each group was measured at Day3. Values are mean±S.E.M. of twelve observations. $**P<0.01$, $*P<0.05$ vs corresponding control (0 μM Gatifloxacin). (B) Islet insulin content was measured at Day 3. Values are mean±S.E.M. of twelve observations. $**P<0.01$, $*P<0.05$ vs corresponding control (0 μM Gatifloxacin). (C) When insulin secretion is expressed as % insulin content, the insulin secretory response impaired by gatifloxacin returned to normal level upon discontinuation of the drug. Values are mean±S.E.M. $**P<0.01$, $*P<0.05$ vs corresponding control (0 μM Gatifloxacin).

4. Discussion

The first report of symptomatic hypoglycemia related to gatifloxacin use in patients with type 2 diabetes mellitus who received oral hypoglycemic agents was published in 2002 (Menzies et al., 2002), and was soon followed by additional studies (Baker and Hangii, 2002; Biggs, 2003; Khovidhunkit and Sunthornyothin, 2004) as well as reports of symptomatic severe hyperglycemia (Biggs, 2003; Arce et al., 2004; Bhatia et al., 2004; Donaldson et al., 2004; Khovidhunkit and Sunthornyothin, 2004). Gatifloxacin is usually administered at 400 mg per day orally, and the maximum serum concentration (C_{max}) for a single oral dose of 400 mg is 8.3 to 10.5 μM in healthy volunteers (Brown, 2000; Grasela, 2000; Mignot et al., 2002). The effective therapeutic concentration range of the other quinolones is around 2–14 μM (Kang et al., 2001; Redfern et al., 2003).

We first examined whether gatifloxacin had an effect at different glucose concentrations, and next the interaction of a therapeutically relevant concentration of gatifloxacin and glibenclamide, because gatifloxacin-induced hypoglycemia is clinically associated with sulfonylureas. Saraya et al. (2004) found that gatifloxacin stimulates insulin secretion at 5.5 mM and 11.1 mM glucose, and we obtained the same results in the present study but found that gatifloxacin had little effect at both low (2.8 mM) and high (16.7 and 25 mM) concentrations of glucose. The reason for the lack of the gatifloxacin-induced insulin secretion at the low glucose concentration is not clear at this stage, although glucose concentration of 2.8 mM is not relevant as the plasma glucose level for the patients. On the other hand, if the glucose or glibenclamide concentration is high enough to fully suppress K_{ATP} channel activity, further suppression by gatifloxacin is not possible. We previously found that less than 10 nM glibenclamide of 11.1 mM glucose could not fully suppress K_{ATP} channel activity (Inagaki et al., 1995; Tsuura et al., 1993), and this may underlie the differing effects of gatifloxacin in the conditions of 5.5 mM glucose, 11.1 mM glucose, or 10 nM glibenclamide from in the conditions of high (16.7 and 25 mM) glucose or high (100 nM) glibenclamide. Thus, the combination of a therapeutically relevant concentration of gatifloxacin and glibenclamide may independently increase the risk of hypoglycemia in diabetic patients.

Hyperglycemia also has been linked to the use of gatifloxacin. In contrast to gatifloxacin-induced hypoglycemia which often occurs within three days of administration of the drug, gatifloxacin-induced hyperglycemia usually takes from 4 to 10 days to develop the high glucose levels of around 30 mM, and discontinuation of gatifloxacin treatment results in improved glucose homeostasis in most cases (Arce et al., 2004; Bhatia et al., 2004; Biggs, 2003; Donaldson et al., 2004; Khovidhunkit and Sunthornyothin, 2004). We also examined the effects of long term exposure of gatifloxacin on pancreatic islets in the present study. The amount of insulin released into the medium from cultured islets was measured, and insulin secretion from gatifloxacin-treated islets was already somewhat greater than in controls at Day1, possibly due to the stimulating effect of gatifloxacin, and not increased thereafter. Under normal physiological conditions,

when glucose stimulates insulin release from pancreatic islets, there is a rapid and corresponding glucose-induced increase in insulin biosynthesis that efficiently replenishes the intracellular insulin stores (Itoh and Okamoto, 1980; Leibowitz et al., 2002; Wicksteed et al., 2003). However, gatifloxacin-treated islets showed a significant decrease in insulin content throughout the culture period. We also compared the chronic effects of several other fluoroquinolones on islet insulin content. Although all of the fluoroquinolones reduced islet insulin content when used at a high dosage, gatifloxacin was shown to have the highest potential, with a half-maximal inhibitory concentration (IC_{50}) of 17.7 μM, which might readily be reached in a patient with renal insufficiency. These results are consistent with clinical experience that gatifloxacin among the fluoroquinolones is associated with the highest risk of hyperglycemia. A recent large-scale study (Park-Wyllie et al., 2006) found an adjusted odds ratio of 16.7 for the association between hyperglycemia-related hospital visits and recent antibiotic use. Accordingly, we examined insulin mRNA levels as a marker of insulin biosynthesis, and found that mouse *Insulin-2* (*mIns-2*) mRNA expression levels were significantly decreased both in islets and MIN6 cells, suggesting that gatifloxacin directly inhibits insulin biosynthesis. However, this was not associated with inhibition of transcriptional activity as shown by luciferase assay. Thus, impaired mRNA stability rather than suppression of transcriptional activity may be the main contributor to the decreased islet insulin content.

Another important clinical feature of gatifloxacin-induced hyperglycemia is that treatment including discontinuation of the drug and blood glucose management readily improves glucose homeostasis in most cases. Insulin secretion was significantly decreased along with the reduction of insulin biosynthesis in gatifloxacin-treated islets, but was improved by withdrawal of the drug, in accordance with gatifloxacin-induced hyperglycemia improving upon discontinuation of the drug.

In conclusion, the present study clearly shows that the combination of a therapeutically relevant concentration of gatifloxacin and glibenclamide increases the risk of hypoglycemia in diabetic patients, while gatifloxacin itself stimulates insulin secretion from pancreatic β-cells when used at high concentrations. Moreover, chronic gatifloxacin treatment directly inhibits insulin biosynthesis, leading to reduced islet insulin content and impaired insulin secretory response, both of which may be associated with gatifloxacin-induced hyperglycemia. These findings have important implications for clinical practice in that the risk of potentially life-threatening dysglycemia may increase during gatifloxacin therapy, especially in elderly patients with renal dysfunction and/or glucose intolerance.

References

Arce, F.C., Bhasin, R.S., Pasmantier, R., 2004. Severe hyperglycemia during gatifloxacin therapy in patients without diabetes. Endocr. Pract. 10, 40–44.

Baker, S.E., Hangii, M.C., 2002. Possible gatifloxacin-induced hypoglycemia. Ann. Pharmacother. 36, 1722–1726.

Bhatia, V., Bhatia, R., Dhindsa, S., 2004. Gatifloxacin-induced hyperglycemia. Endocr. Pract. 10, 86.

Biggs, W.S., 2003. Hypoglycemia and hyperglycemia associated with gatifloxacin use in elderly patients. J. Am. Board Fam. Pract. 16, 455–457.

Brown, W.M., 2000. Gatifloxacin Kyorin Pharmaceutical Co. Curr. Opin. Investig. Drugs 1, 35–44.

Donaldson, A.R., Vandiver, J.R., Finch, C.K., 2004. Possible gatifloxacin-induced hyperglycemia. Ann. Pharmacother. 38, 602–605.

Grasela, D.M., 2000. Clinical pharmacology of gatifloxacin, a new fluoroquinolone. Clin. Infect. Dis. 31 (Suppl 2), S51–S58.

Inagaki, N., Gonoi, T., Clement IV, J.P., Namba, N., Inazawa, J., Gonzalez, G., Aguilar-Bryan, L., Seino, S., Bryan, J., 1995. Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. Science 270 (5239), 1166–1170.

Itoh, N., Okamoto, H., 1980. Translational control of proinsulin synthesis by glucose. Nature 283, 100–102.

Kang, J., Wang, L., Chen, X.L., Triggle, D.J., Rampe, D., 2001. Interactions of a series of fluoroquinolone antibacterial drugs with the human cardiac K^+ channel HERG. Mol. Pharmacol. 59, 122–126.

Khovidhunkit, W., Sunthornyothin, S., 2004. Hypoglycemia, hyperglycemia, and gatifloxacin. Ann. Intern. Med. 141, 969.

Leibowitz, G., Uckaya, G., Oprescu, A.I., Cerasi, E., Gross, D.J., Kaiser, N., 2002. Glucose-regulated proinsulin gene expression is required for adequate insulin production during chronic glucose exposure. Endocrinology 143, 3214–3220.

Maeda, N., Tamagawa, T., Niki, I., Miura, H., Ozawa, K., Watanabe, G., Nonogaki, K., Uemura, K., Iguchi, A., 1996. Increase in insulin release from rat pancreatic islets by quinolone antibiotics. Br. J. Pharmacol. 117, 372–376.

Menzies, D.J., Dorsainvil, P.A., Cunha, B.A., Johnson, D.H., 2002. Severe and persistent hypoglycemia due to gatifloxacin interaction with oral hypoglycemic agents. Am. J. Med. 13, 232–234.

Mignot, A., Guillaume, M., Brault, M., Gualano, V., Millerioux, L., Gohler, K., Stahlberg, H.J., 2002. Multiple-dose pharmacokinetics and excretion balance of gatifloxacin, a new fluoroquinolone antibiotic, following oral administration to healthy Caucasian volunteers. Chemotherapy 48, 116–121.

Park-Wyllie, L.Y., Juurlink, D.N., Kopp, A., Shah, B.R., Stukel, T.A., Stumpo, C., Dresser, L., Low, D.E., Mamdani, M.M., 2006. Outpatient gatifloxacin therapy and dysglycemia in older adults. N. Engl. J. Med. 354, 1352–1361.

Redfern, W.S., Carlsson, L., Davis, A.S., Lynch, W.G., MacKenzie, I., Palethorpe, S., Siegl, P.K., Strang, I., Sullivan, A.T., Wallis, R., Camm, A.J., Hammond, T.G., 2003. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovasc. Res. 58, 32–45.

Saraya, A., Yokokura, M., Gonoi, T., Seino, S., 2004. Effects of fluoroquinolones on insulin secretion and beta-cell ATP-sensitive K^+ channels. Eur. J. Pharmacol. 497, 111–117.

Tsuura, Y., Ishida, H., Okamoto, Y., Kato, S., Sakamoto, K., Horie, M., Ikeda, H., Okada, Y., Seino, Y., 1993. Glucose sensitivity of ATP-sensitive K^+ channels is impaired in beta-cells of the GK rat. A new genetic model of NIDDM. Diabetes 42 (10), 1446–1453.

Wicksteed, B., Alarcon, C., Briaud, I., Lingohr, M.K., Rhodes, C.J., 2003. Glucose induced translational control of proinsulin biosynthesis is proportional to preproinsulin mRNA levels in islet beta-cells but not regulated via a positive feedback of secreted insulin. J. Biol. Chem. 278, 42080–42090.

The spontaneously diabetic Torii rat with gastroenteropathy

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Abstract

The spontaneously diabetic Torii (SDT) rat was recently recognized as a new animal model of non-obese type 2 diabetes. As the severe diabetic ocular complications seen in SDT rats already have been investigated, we examined another common diabetic complication, gastroenteropathy. Male SDT rats developed diabetes at 20 weeks and diarrhea at 28 weeks of age. Gastrointestinal motility was evaluated at 28 weeks by measuring the distance of small intestinal transit by oral administration of the non-absorbed marker, arabic gum. SDT rats exhibited greater intestinal transit distance than control SD rats ($54.1 \pm 2.6\%$ versus $43.0 \pm 1.2\%$). Insulin treatment of SDT rats begun at 20 weeks of age produced improved stool and reduced intestinal transit distance ($41.4 \pm 0.3\%$). Morphologically, the SDT rats exhibited longer villi and heavier weight of intestine compared to control SD rats. These results suggest that the SDT rat may be a useful animal model for studies of diabetic gastroenteropathy.
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Keywords: Diabetes mellitus; Gastroenteropathy; Diarrhea; Intestinal motility

1. Introduction

Diabetic animal models can be made by chemical treatment, pancreas resection, or careful crossbreeding to exhibit spontaneous development, and are especially

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helpful for analysis of the pathogenesis of the complications of human type 2 diabetes. There are several established lines of spontaneously diabetic animal models of the disease, including the GK rat, the Wistar fatty rat, and the OLETF rat [1–3], but recently the spontaneously diabetic Torii (SDT) rat has been recognized as an inbred animal model [4–6]. It was reported that SDT male rats develop hyperglycemia and hypoinsulinemia without obesity at about 20 weeks of age. SDT male rats develop hyperglycemia and hypoinsulinemia at about 20 weeks of age. Interestingly, they also exhibit severe diabetic ocular complications such as cataracts, proliferative retinopathy,