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Effects of Cyclooxygenase-2 Inhibitor and Adenosine Triphosphate–Sensitive Potassium Channel Opener in Syngeneic Mouse Islet Transplantation

J.-H. Juang and C.-H. Kuo

ABSTRACT

In the initial days after transplantation, islet grafts may be attacked by cytokines via cyclooxygenase-2 (COX-2), producing primary nonfunction. In addition, chronic overstimulation of β-cells may impair insulin secretion. To enhance the function of transplanted islets, the present study investigated the effects of rofecoxib, a COX-2 inhibitor, and NN414 (6-chloro-3-[1-methylcyclopropyl]amino-4*H*-thieno[3,2-e]-1,2,4-thiadiazine 1,1-dioxide), an adenosine triphosphate-sensitive potassium channel opener, on islet transplantation. Male inbred C57BL/6 mice were used as donors and recipients. One hundred fifty islets were isolated via collagenase digestion and density gradient, and syngeneically transplanted under the kidney capsule in mice with streptozotocin-induced diabetes. Recipients were treated with or without rofecoxib, 10 mg/kg/d orally, or with or without NN414, 3 mg/kg/d orally, for 4 weeks. After transplantation, recipient body weight, blood glucose concentration, and intraperitoneal glucose tolerance were measured. The grafted kidney was extracted for determination of insulin content at 4 weeks. In the rofecoxib-treated and NN414-treated groups and both control groups, body weight remained stable, and the blood glucose concentration decreased progressively. However, at 4 weeks after transplantation in the groups treated or not treated with rofecoxib or NN414, no significant difference was observed in recipient body weight, blood glucose concentration, and glucose tolerance or in insulin content of the graft. These data indicate that posttransplantation treatment with rofecoxib or NN414 has no beneficial effect on transplantation outcome in diabetic mouse recipients engrafted with a marginal islet mass.

SINCE 1990, islet transplantation has led to insulin-independence in human beings with type 1 diabetes mellitus.[1] Although the initial successful rate markedly improved with the use of Edmonton protocol, 2 or more treatments were usually required to achieve normoglycemia.[2] Previously, we demonstrated a profound decrease in β-cell mass and insulin content of transplanted grafts.[3] Damage to the islets during isolation, technical problems during the transplantation process, insufficient amount of transplanted tissue, hypoxia of transplanted islets, metabolic condition of the recipient, and absence of survival factors in the nonendocrine pancreas have been suggested to have a role in determining the initial fate of transplanted islets.[4–6] Even in syngeneic transplantation, increased β-cell apoptosis and necrosis occur, and lead to reduced β-cell mass in the first few days posttransplantation.[7,8] During this period, the islet graft may be attacked by cytokines such as interleukin-1β (IL-1β), tumor necrosis factor-α, and interferon-γ, and demonstrates primary nonfunction.[9–11] Cytokines mediate β-cell dysfunction and islet degeneration, in part via inducible cyclooxygen-

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ase-2 (COX-2).[12] In addition to inflammation, chronic overstimulation of β-cells (β-cell exhaustion) due to prolonged hyperglycemia may have an important role in decrease of insulin secretion.[13,14] In contrast, induction of β-cell rest by NN414 (6-chloro-3-[1-methylcyclopropyl]amino-4*H*-thieno[3,2-e]-1,2,4-thiadiazine1,1-dioxide), an adenosine triphosphate-sensitive potassium channel opener (K-ATP) has been reported to preserve β-cell insulin stores and insulin secretion in human islets.[15] In addition, NN414 has been noted to also prevent glucose- and IL-1β–induced β-cell secretory dysfunction and apoptosis in human islets.[16] In an effort to improve the survival and function of transplanted islets, the present study investigated the effects of rofecoxib, a COX-2 inhibitor, and NN414, a K-ATP channel opener.

MATERIALS AND METHODS

Animals

Male inbred C57BL/6 mice aged 8 to 12 weeks were used as donors and recipients. Diabetes was induced in the recipients via a single intraperitoneal injection of streptozotocin (Sigma-Aldrich, St. Louis, Missouri), 200 mg/kg of body weight, freshly dissolved in citrate buffer, pH 4.5. Before transplantation, diabetes was confirmed by the presence of hyperglycemia, weight loss, and polyuria. Only mice with a blood glucose concentration greater than 350 mg/dL at 2 weeks after streptozotocin injection underwent transplantation. The glucose concentration of blood obtained from the cut tail was measured using a portable glucose analyzer (One Touch II; LifeScan, Inc, Milpitas, California). The animals were maintained under conventional conditions, with free access to tap water and standard pelleted food.[6]

Islet Isolation

Mice were anesthetized using sodium amobarbital, and the pancreases were distended using 2.5 mL of RPMI-1640 medium (GIBCO BRL, Grand Island, New York) containing 1.5 mg/mL of collagenase (from *Clostridium histolyticum* type XI; Sigma-Aldrich), excised, and incubated in a water bath at 37°C. Islets were separated using a density gradient (Histopaque-1077; Sigma-Aldrich) and those with 75–250 μm in diameter were handpicked under a dissecting microscope for counting.[6]

Islet Transplantation

One hundred fifty C57BL/6 mouse islets were syngeneically transplanted under the left kidney capsule in inbred streptozotocin-induced diabetic mice on the day of islet isolation.[6] Blood glucose concentration and body weight were measured twice a week in the first 2 weeks, and once a week thereafter. Normoglycemia was defined as presence of a nonfasting blood glucose concentration less than 200 mg/dL.

Rofecoxib and NN414 Treatments

Groups were treated with rofecoxib, 10 mg/kg/d orally,[17] and NN414, 3 mg/kg/d orally,[18] for 4 weeks starting from the day of transplantation. Recipients that did not receive rofecoxib or NN414 treatment served as controls.

Intraperitoneal Glucose Tolerance Test

After an overnight fast, a 5% glucose solution, 1.5 g/kg, was injected intraperitoneally, and the blood glucose concentration was measured at 0, 30, 60, 90, and 120 minutes by tail snipping.[3] An intraperitoneal glucose tolerance test (IPGTT) was performed at 2 and 4 weeks.

Insulin Content of Graft

The grafted kidneys were removed, minced, and homogenized in acid ethanol, and samples were extracted overnight at 4°C. On the following day, they were centrifuged at 2400 rpm for 30 minutes, and the supernate was stored at −20°C. The pellets were rehomogenized in acid ethanol and incubated for 2 hours at 4°C. This procedure was repeated for overnight insulin extraction. After centrifugation, the supernate was added to the previous extraction sample and stored at −20°C in a freezer until assay. Insulin was measured via radioimmunoassay (INSI-PR kit; CIS-US, Inc, Bedford, Massachusetts).[6]

Statistical Analysis

Results are given as mean (SEM). Comparisons between the 2 groups were made using the unpaired *t* test. $P < .05$ was considered significant.

RESULTS

In the rofecoxib-treated and control group (Fig 1) and the NN414-treated and control group (Fig. 2), the blood glucose concentration decreased progressively and body weight was maintained after islet transplantation. At 4 weeks, no significant difference was observed in mean (SEM) blood glucose concentration (220 [64] mg/dL vs 273

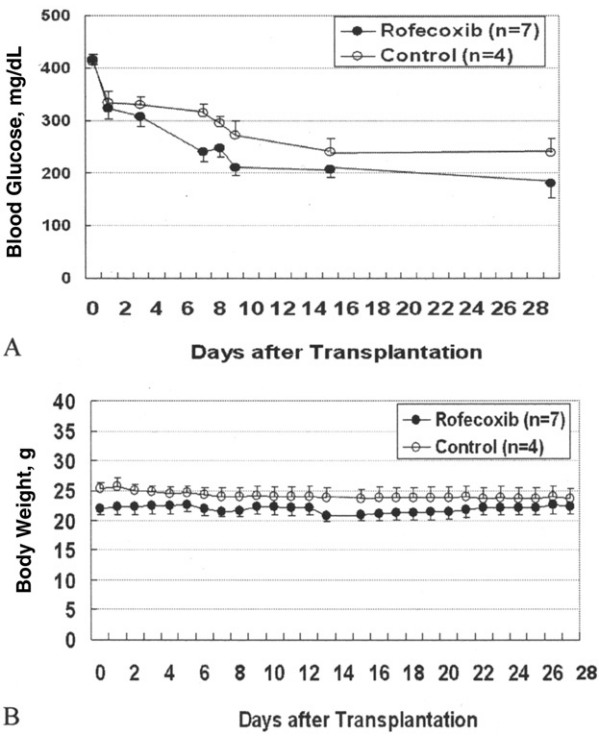

Fig. 1. Blood glucose (A) and body weight (B) changes in recipients treated (solid circles) or not treated (open circle) with rofecoxib.

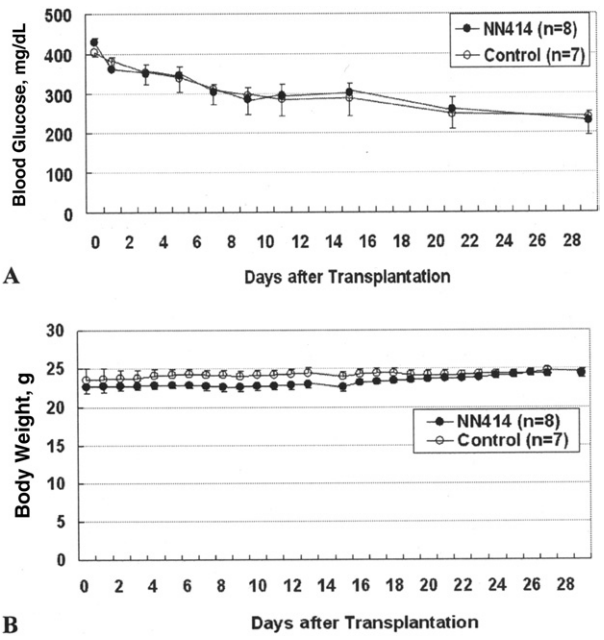

Fig. 2. Blood glucose (A) and body weight (B) changes in recipients treated (solid circle) or not treated (open circle) with NN414.

[21] mg/dL), body weight (19.9 [3.5] g vs 20.4 [0.4] g), IPGTT (area under the curve, 32,509 [2987] mg vs 34,132 [2047] mg), or graft insulin content (7.62 [1.85] μIU vs 7.92 [0.61] μIU) between the rofecoxib-treated group and their controls. Also at 4 weeks, no significant difference was observed in blood glucose concentration (229 [62] mg/dL vs 239 [118] mg/dL), body weight (24.3 [1.1] g vs 24.6 [1.0] g), IPGTT (area under the curve, 31,562 [9259] mg vs 33,928 [10,768] mg), or graft insulin content (6.76 [1.14] μIU vs 6.54 [1.57] μIU) between NN414-treated group and their controls.

DISCUSSION

The present study attempted to improve the survival and function of transplanted islets using a COX-2 inhibitor to inhibit cytokine-mediated inflammation and a K-ATP channel opener to induce β-cell rest. The selective COX-2 inhibitors SC-58236 and celecoxib attenuate cytokine-induced prostaglandin E_2 formation in rat and human islets; however, they failed to prevent cytokine-mediated inhibition of insulin secretion or islet degeneration.[12] Anti-inflammatory agents including acetylsalicylic acid, rofecoxib, transforming growth factor-β, and IL-1 receptor antagonist prolonged mean survival time of islet xenografts in nonobese diabetic mice; however, only acetylsalicylic acid and IL-1 receptor antagonist prevented primary nonfunction.[17] In our syngeneic mouse islet transplantation model with a marginal islet mass, posttransplantation treatment with a COX-2 inhibitor, rofecoxib, did not improve recipient blood glucose concentration, body weight, glucose tolerance, or graft insulin content. These observations indicate that prostaglandin E_2 does not mediate cytokine-induced β-cell dysfunction and islet degeneration during transplantation. Chronic hyperglycemia may produce overstimulation of β-cells (β-cell exhaustion), and lead to decreased insulin secretion.[13,14] Previously, we demonstrated that stimulation of insulin secretion in islet recipients by gliclazide, a sulfonylurea that closes the K-ATP channel in β-cells, did not improve the outcome of transplantation.[19] In contrast, K-ATP channel openers have been shown to be beneficial for β-cells. In vitro, NN414, a K-ATP channel opener, not only preserved β-cell insulin stores and secretion,[15] but prevented glucose- and IL-Iβ-induced β-cell secretory dysfunction and apoptosis[16] in human islets. In islet transplantation in neonatal rats with streptozotocin-induced diabetes with moderate hyperglycemia, both NN414 and diazoxide improved glucose-stimulated insulin secretion of perfused grafts.[18] However, they did not improve recipient blood glucose concentration or graft insulin content,[18] findings consistent with our observations in adult mice with stretptozotocin-induced diabetes with severe hyperglycemia. Our results contravene the hypothesis that, compared with moderate hyperglycemia, severe hyperglycemia causes more intense overstimulation. The lack of beneficial effect of NN414 on recipient glycemia and graft insulin content may be due to its inhibitory effect, as demonstrated in treated recipients with low C-peptide concentration.[18]

In conclusion, diabetic recipients of a marginal islet mass exhibited no beneficial effects of posttransplantation treatment with a COX-2 inhibitor, rofecoxib, or a K-ATP channel opener, NN414.

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