Activated Protein C Preserves Functional Islet Mass After Intraportal Transplantation
A Novel Link Between Endothelial Cell Activation, Thrombosis, Inflammation, and Islet Cell Death

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Clinical studies indicate that significant loss of functional islet mass occurs in the peritransplant period. Islets are injured as a result of detrimental effects of brain death, pancreas preservation, islet isolation, hypoxia, hyperglycemia, and immune-mediated events. In addition, recent studies demonstrated that islets are injured as a result of their exposure to blood and of activation of intrahepatic endothelial and Kupffer cells, resulting in inflammation and thrombosis. Activated protein C (APC) is an anticoagulant enzyme that also exerts anti-inflammatory and antiapoptotic activities by acting directly on cells. Here, we report that exogenous administration of recombinant murine APC (mAPC) significantly reduced loss of functional islet mass after intraportal transplantation in diabetic mice. Animals given mAPC exhibited better glucose control, higher glucose disposal rates, and higher arginine-stimulated acute insulin release. These effects were associated with reduced plasma proinsulin, intrahepatic fibrin deposition, and islet apoptosis early after the transplant. In vitro and in vivo data demonstrated that mAPC treatment was associated with a significant reduction of proinflammatory cytokine release after exposure of hepatic endothelial cells to islets. mAPC treatment also prevented endothelial cell activation and dysfunction elicited by intrahepatic embolization of isolated islets inherent to pancreatic islet transplantation (PIT). This study demonstrates multiple remarkable beneficial effects of mAPC for PIT and suggests that APC therapy may enhance the therapeutic efficacy of PIT in diabetic patients. Diabetes 53:2804–2814, 2004

Pancreatic islet transplantation (PIT) has been validated as treatment for type 1 diabetes (1–3). However, wider implementation is hindered by the use of high numbers of islets, usually obtained from two organ donors, to achieve insulin independence. In addition, reduced functional islet mass (FIM) has been shown (4) in successfully transplanted recipients compared with healthy people. Multiple factors, such as injury during brain death, pancreas preservation, and islet isolation, exposure to proinflammatory cytokines (PICs), and both immunologic and nonimmunologic inflammatory responses, contribute to early islet graft loss (5,6). Although retransplantation is effective, it lacks cost-effectiveness and is constrained by the shortfall of donor pancreatic tissue. Therefore, strategies to preserve FIM are needed to enhance the therapeutic efficacy of PIT as a cure for diabetes.

Recent studies have demonstrated that pancreatic islets express tissue factor (7), a cell surface–bound glycoprotein that binds both the zymogen, factor VII, and the active serine protease, factor VIIa (8). Tissue factor/factor VII complexes activate the extrinsic coagulation pathways and are the major in vivo initiator of coagulation (9). During clinical PIT, when islets come into direct contact with blood in the portal vein, islet-produced tissue factor triggers a detrimental clotting reaction, referred to as instant blood-mediated inflammatory reaction (IBMIR), which is characterized by activation of the coagulation and complement systems, rapid binding and activation of platelets, and leukocyte infiltration into the islets (10–12). Together, these effects contribute to disruption of the islet morphology, islet dysfunction, and death. Furthermore, nonspecific activation and dysfunction of intrahepatic endothelial cells, characterized by upregulation of intracellular adhesion molecule (ICAM)-1, P-selectin, along with production of PICs, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, interferon-γ, and of nitric oxide (NO) have been demonstrated in vitro and in vivo after PIT. These effects contribute to the complex network of events that ultimately lead to early graft loss (13–19).

Activated protein C (APC) is an important physiological anticoagulant generated from protein C by the action of...
thrombin-thrombomodulin on the endothelial cells (20). APC inactivates factors Va and VIIIa, thereby limiting the generation of thrombin. APC also has profibrinolytic activity (21). In addition to its well-defined role in coagulation, APC acts directly on cells and promotes anti-inflammatory and antiapoptotic activities by binding to the endothelial protein C receptor and activating protease activated receptor 1 (22–26). APC appears to regulate the inflammatory process in part by blocking the activity of the transcription factor nuclear factor (NF)-κB, by inhibiting PIC production, and by limiting thrombin generation.

**FIG. 1.** mAPC promotes glucose control in syngeneic diabetic islet transplant recipients. At 24 h after islet isolation, streptozotocin-induced diabetic syngeneic recipients received an intraportal infusion of an optimal (500 IEQ) (A) or a suboptimal islet dose (either 250 [B] or 150 [C] IEQ). Recipients were treated with either vehicle or mAPC (0.2 mg/kg i.v.) 1 h before the transplant. Nonfasting glucose levels were assessed as described in **RESEARCH DESIGN AND METHODS**. Results in A–C are expressed as means ± SD (n = 8). D: Percentage of recipients that achieved nonfasting glucose <200 mg/dl. *P < 0.05.

**TABLE 1**

Glucose disposal rates obtained 10 days posttransplantation

<table>
<thead>
<tr>
<th>Islet dose (IEQ)</th>
<th>Vehicle</th>
<th>mAPC</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>2.3 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>0.032</td>
</tr>
<tr>
<td>250</td>
<td>1.3 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>0.022</td>
</tr>
<tr>
<td>150</td>
<td>0.76 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Data are means ±SE. Glucose disposal rates (% glucose · mg⁻¹ · dl⁻¹ · min⁻¹) were calculated after intraperitoneal glucose tolerance testing as described in **RESEARCH DESIGN AND METHODS**. Recipients (n = 6) received either vehicle or mAPC (0.2 mg/kg i.v.) 1 h before the transplant.
Moreover, recent studies (25) demonstrated that mAPC modulates several genes in the apoptosis pathway that resulted in important antiapoptotic effects. Because of its antithrombotic, profibrinolytic, anti-inflammatory, and antiapoptotic activities, we hypothesized that APC may decrease loss of FIM in the peritransplant period by decreasing IBMIR, apoptosis, and endothelial cell activation after intraportal PIT.

RESEARCH DESIGN AND METHODS
Male C57BL/6 mice (The Jackson Laboratories, Bar Harbor, ME) were used as islet donors and recipients. Surgical and nonsurgical procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Animals, under supervision of the Institutional Animal Care and Use Committee. Four days before islet transplantation, recipients were rendered diabetic by a single intraperitoneal injection of freshly reconstituted streptozotocin (Sigma, St. Louis, MO) at 200 mg/kg i.p. Blood glucose levels were obtained after tail snipping using a portable glucose meter (Roche AccuCheck III; Roche Diagnostics, Indianapolis, IN). Diabetes was defined as nonfasting glucose levels >250 mg/dl for ≥3 consecutive days.

Islet isolation and transplantation. Pancreatic islets were isolated and purified following standard techniques by intraductal injection of rodent Liberase (Roche Diagnostics) (30). The number of islets within each size class was converted to the standard number of islets of 150-μm diameter equal in volume to the sample (islet equivalents [IEQs]). Purity was assessed by separating, immediately frozen, and stored at −70°C until use. Total mRNA was prepared using the RNAeasy 96 Total RNA Isolation Kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. One attomol of TNF-α mRNA is ~430 fmg, and 1 amol of TNF-α mRNA is ~230 fmg.

Intrahepatic fibrin detection. Fibrin deposition was quantified in the whole liver by Western blotting with antibodies against fibrin B (NYBT2G1, dilution 1:500; Accurate Chemical & Scientific, Westbury, NY) as previously described (22,29). Briefly, equal amounts of protein (10 μg/lane), measured by Bradford assay (Bio-Rad Laboratories, Hercules, CA), were electrophoresed on 10% SDS-polyacrylamide gels, transferred onto nitrocellulose membranes, and probed with the described antibody. The relative abundance of each protein was determined by scanning densitometry using β-actin as an internal control.

FIG. 2. Arginine-stimulated insulin release after PIT. On day 10 posttransplantation, after an overnight fast, vehicle- or mAPC-treated recipients (500 [A], 250 [B], and 150 [C] IEQ) received arginine (0.3 g/kg body wt i.v.) in 30 s. Blood samples were collected in heparin tubes at −5, 0, 2, 5, and 7 min after stimulus injection. Mouse insulin was determined by ELISA, as described in RESEARCH DESIGN AND METHODS. Results are expressed as means ± SD (n = 6). ∗∗∗P < 0.001.
Fibrin abundance was expressed relative to intrahepatic fibrin concentration assessed in control diabetic mice, whose value was arbitrarily assigned as 1. Experiments demonstrated that 6 h was required for the peak of intrahepatic fibrin deposition after syngeneic PIT (data not shown); therefore, this time-point was selected for the experiments.

**Portal vein endothelial cell isolation and characterization.** Portal vein endothelial cells (PVECs) were isolated and characterized as described previously (19). Briefly, rings of the portal vein (C57BL/6 mice) were placed endothelial side down on Petri dishes and incubated overnight in a humidified incubator at 37°C in a 95% air/5% CO₂ atmosphere. Dulbecco’s modified Eagle’s medium (Life Technologies) supplemented with L-glutamine, 25 mmol/l HEPES, 10% fetal bovine serum, 100 units/ml of penicillin, and 100 mg/ml of streptomycin was used to keep the tissue moist. One day later, 1 ml of media was added per 10 cm² of tissue culture dish. Culture continued for 5–7 days until PVECs migrated onto the culture dish. Then, the rings were removed. Immunofluorescence staining of factor VIII antigen was used to identify PVECs (19). Following these procedures, it was possible to obtain PVECs with purity >85% after 6–10 days in culture (data not shown).

**PVEC and islet cocultures.** Hand-picked islets (C57BL/6 mice) were cocultured with PVECs at 1 IEQ/100 PVECs (19) in the presence of mAPC (50 mmol/l) or vehicle, as described (22). This ratio is comparable with the dose of islet cells normally infused into the portal vein during PIT. After 6 h in culture, a

**FIG. 3.** Serum proinsulin levels and islet apoptosis after PIT. **A:** Proinsulin levels were assessed in serum samples obtained from diabetic recipients given intraportal media without islets or an optimal islet dose (500 IEQ) in combination with either vehicle or mAPC (0.2 mg/kg i.v. at 1 h before the transplant, n = 6) by ELISA, as described in RESEARCH DESIGN AND METHODS. Results are expressed as means ± SD. +*-P < 0.001 vs. PIT + vehicle (+); *3P < 0.05. **B:** Islet apoptosis was evaluated 24 h after the transplant. Sections of the liver were stained for insulin, and intraislet apoptosis (arrows) was detected by the TUNEL assay as described in RESEARCH DESIGN AND METHODS. **C:** An apoptotic index (percentage of TUNEL-positive cells per islet) was determined after microscopic evaluation of a minimum of 15 intrahepatic islets per recipient (n = 5). Results are expressed as means ± SD, +P < 0.05, vehicle vs. mAPC.
islet function after transplantation. Nonfasting glucose levels were assessed as described above. In addition, recipients were subjected to metabolic testing 10 days after PIT. Glucose disposal rates (\(K_d\)) were calculated from intraperitoneal glucose tolerance testing (16-h fast; 1 g glucose/kg i.v. 1 h before the transplant, \(n = 6\)), the animals were killed, the whole liver excised, and fibrin deposition was assessed by Western blotting as described in RESEARCH DESIGN AND METHODS. Results are expressed as means ± SD. *P < 0.05, PIT + vehicle vs. PIT + mAPC.

FIG. 4. Intrahepatic fibrin deposition after islet transplantation. Six hours after intraportal infusion of media without islets or 500 IEQ/mouse in combination with either vehicle or mAPC (0.2 mg/kg i.v. 1 h before the transplant, \(n = 6\)), the animals were killed, the whole liver excised, and fibrin deposition was assessed by Western blotting as described in RESEARCH DESIGN AND METHODS. Results are expressed as means ± SD. *P < 0.05, PIT + vehicle vs. PIT + mAPC.

**RESULTS**

**Effect of mAPC on functional outcome of islet isografts.** A series of islet isografts were performed to evaluate the effects of mAPC on PIT. As demonstrated in Fig. 1A, diabetes was reverted within 3 days in 100% of the recipients that were transplanted with 500 IEQ (optimal islet dose) and treated with either vehicle or mAPC. However, lower nonfasting glucose levels were demonstrated early after the transplant in animals given mAPC compared with controls. These differences were more pronounced in recipients given a suboptimal islet dose (Fig. 1B–D). Only 25% of the recipients given vehicle and 250 IEQ presented nonfasting glucose <200 mg/dl after the transplant, compared with 75% of animals given mAPC in combination with the same islet dose (\(P < 0.05\)). Moreover, none of the recipients treated with vehicle, and 150 IEQ presented euglycemia compared with 37.5% of mAPC-treated recipients (\(P < 0.05\)). Interestingly, although all diabetic recipients transplanted with an optimal dose achieved excellent glucose control, higher glucose disposal rates were demonstrated 10 days after the transplant in animals given mAPC (3.2 ± 0.4% glucose · mg/l · min⁻¹) compared with vehicle-treated controls (2.3 ± 0.3% glucose · mg/l · min⁻¹, \(P < 0.05\)) (Table 1). To further evaluate the islet grafts, FIM was indirectly evaluated 10 days after the transplant by AIRarginine (Fig. 2). Significantly higher AIRarginine was demonstrated in all recipients given mAPC compared with vehicle after transplantation independently of the islet dose infused. Moreover, higher total insulin content assessed 30 days after the transplant was demonstrated in livers harvested from animals given mAPC (500 IEQ and insulin, 377 ± 88 ng) versus animals given vehicle (172 ± 32 ng, \(P < 0.05\)) (data not shown). Proinsulin levels, an established marker of islet injury after islet exposure to blood (12), were significantly reduced in animals given mAPC compared with vehicle-treated controls (Fig. 3A). In correlation with proinsulin levels, animals treated with mAPC presented improved glucose disposal.
lower islet apoptosis assessed 24 h posttransplantation compared with vehicle-treated controls (Fig. 3B and C). Taken together, these results demonstrate that mAPC significantly decreases loss of FIM after transplantation.

**Effect of mAPC on intrahepatic fibrin deposition after PIT.** Intrahepatic fibrin deposition was measured in recipients given 500 IEQ (Fig. 4). Minimal levels of fibrin deposition were demonstrated in control recipients given media without islets. A significant 20-fold increase in fibrin deposition was shown in vehicle-treated recipients that received 500 IEQ, and fibrin deposition was markedly reduced in animals given mAPC. Intrahepatic immunohistochemical analysis performed posttransplantation demonstrated that, in vehicle-treated recipients, a capsule of fibrin infiltrated by leukocytes and monocytes surrounding the islets, and these effects were abrogated by the administration of mAPC (Fig. 5). In accordance with the results obtained after the evaluation of total insulin content by biochemical methods, we found a higher islet insulin content in mAPC-treated animals as demonstrated by immunohistochemistry (Fig. 5E and F). These results demonstrate that activation of the coagulation cascade, leukocyte infiltration, and fibrin deposition occur after intraportal infusion of syngeneic islets and that these effects are abrogated by the administration of mAPC.

**Effect of mAPC on intrahepatic PIC activation after PIT.** Previous studies (14,16–18) demonstrated nonspecific activation of PIC after intraportal PIT, an effect that cannot be prevented using conventional immunosuppressive drugs. PICs have profound effects on islet dysfunction and death in diabetes and in PIT (33). Because mAPC inhibits PIC production in different clinical and experimental conditions (20,25,27–29,34), we evaluated the possibility that mAPC would prevent the loss of FIM by inhibiting PIC production. Preliminary experiments demonstrated that the peak of maximal liver mRNA TNF-α and
IL-1β expression after intraportal infusion of islets occurred at 6 h (data not shown). Therefore, we chose this time point for the analysis of the effect of mAPC on PIC expression (Fig. 6A and B). No difference in liver mRNA TNF-α and IL-1β expression was observed between normal animals compared with diabetic animals that received an intraportal infusion of media without islets. In contrast, a very large increase in PIC mRNA expression was demonstrated after PIT (500 IEQ/mouse), and this effect was significantly reduced by the administration of mAPC (P < 0.001). These results demonstrate potent anti-inflammatory effects of mAPC given shortly before the islet infusion.

**Effect of mAPC on endothelial cell activation after PIT.** Nonspecific activation and dysfunction of intrahepatic endothelial cells represents a key pathophysiological component in the early loss of islet grafts (14,15,19). Based on the importance of mAPC on endothelial cell physiology (25,35), we evaluated the possibility that mAPC might directly modulate intrahepatic endothelial function after intraportal PIT. Soluble forms of ICAM-1, E-selectin, and P-selectin arise in plasma from proteolytic cleavage from the cell membrane (36) in reactions following cell activation (37–39). When plasma sICAM-1, soluble E-selectin, and soluble P-selectin were evaluated serially after syngeneic intraportal infusion of an optimal islet dose, we found peak plasma levels at 6 h (data not shown). As demonstrated in Fig. 7A–C, no significant increases in plasma sICAM-1, soluble E-selectin, and soluble P-selectin were found between normal animals and control diabetic animals given media without islets. In contrast, four- to fivefold increases in sICAM-1, soluble E-selectin, and soluble P-selectin were shown after PIT in animals treated with vehicle. Strikingly, these increases were almost completely abolished by the administration of mAPC (P < 0.001). To evaluate the role of mAPC on endothelial cell dysfunction after intraportal PIT (14,15,40), we evaluated hyaluronic acid clearance in syngeneic islet recipients given 500 IEQ. Hyaluronic acid clearance, indicating intrahepatic endothelial dysfunction, was delayed in diabetic recipients treated with vehicle (30 min = 238.2 ± 39 mg/dl) compared with mAPC-treated recipients (140.4 ± 31 mg/dl, P < 0.05). Overall, these results demonstrate that mAPC decreased intrahepatic endothelial activation and dysfunction after intraportal PIT.

**Effects of mAPC on NF-κB nuclear translocation and TNF-α expression in PVECs activated by isolated islets.** We evaluated the effects of mAPC on activation of PVECs elicited by coculture with islets in vitro as reflected by the nuclear activity of NF-κB, a key transcription factor involved in endothelial cell activation (35). Within 3 h after PVEC incubation with syngeneic islets, we observed significant increase in PVEC NF-κB nuclear translocation (data not shown), with maximal activity at 6 h (Fig. 8A). mAPC caused a 67% reduction in nuclear activity of NF-κB in the cocultures of treated PVECs and islets compared with vehicle-treated controls (P < 0.001). mAPC also caused a significant reduction in TNF-α production in culture supernatants evaluated 24 h after PVEC exposure to islets (Fig. 8B). Overall, these results demonstrate that mAPC decreased nuclear translocation of NF-κB and, consequently, activation of PVECs elicited by pancreatic islets.

**DISCUSSION**

Thrombosis and inflammation are linked in many clinical conditions such as sepsis, atherosclerosis, and as recently described, PIT (7,10,20,34,41). Mechanisms for communication between coagulation and inflammation pathways have been uncovered, including reactions in which proinflammatory mediators may regulate coagulation activation and products of the clotting cascade may affect inflammation (34,42–44). Intrahepatic infusion of isolated islets is the preferred site for clinical PIT. However, instant coagulation/inflammation reaction (IBMIR) and activation of endothelial cells occur regularly during this procedure, even without clinical signs of intraportal thrombosis (7,14,15,19). The final outcome of these events is islet injury and, therefore, reduction in FIM. In the present
study, we demonstrated that mAPC is a potent inhibitor of IBMIR and intrahepatic activation/dysfunction of endothelial cells elicited by intraportal infusion of isolated islets in mice. Notably, diabetic recipients that received an optimal or suboptimal islet dose presented higher FIM when pretreated with mAPC, as demonstrated by better glucose control, higher capacity to dispose glucose, and reduced proinsulin levels assessed early after the transplant. In contrast to other antithrombotic agents (e.g., heparin or tissue plasminogen activator) that may predispose to bleeding, clinical and experimental animal studies report that APC generally does not cause bleeding, with the possible exception of severe sepsis patients (20,22,29,41,45). Consistent with the well-known anticoagulation effects of APC (20), we demonstrated a significant reduction in intrahepatic fibrin deposition after PIT in diabetic mice given mAPC. More specifically, we demonstrated significant reduction in fibrin and infiltration of leukocytes surrounding the intraportally infused islets.

Islets constitutively express tissue factor (7), which, in combination with factor VIIa, is the major activator of the extrinsic coagulation system, leading to thrombin formation and generation of fibrin clots (8,9). Thrombin also potentiates a prothrombic state when it activates the thrombin-activated fibrinolysis inhibitor. At high concentrations, thrombin may be a potent proinflammatory and proapoptotic mediator (46,47). Accordingly, thrombin inhibition abrogates IBMIR during islet exposure to blood (48). Consistent with these observations, mAPC administration downregulates the expression of tissue factor, decreases thrombin generation, and promotes fibrinolysis (20,48,49). In addition to islet-borne tissue factor, coagulation in PIT can occur indirectly through the release of cytokines that induce coagulation, such as TNF-α (50), which inherently arises from the islet transplant procedure (14,18,51).

It is becoming increasingly clear that anticoagulants can limit coagulation-induced increases in the inflammatory response by mechanisms that involve not only inhibition of coagulation proteases but also direct interactions with cells that generate inflammatory substances following cell activation (34,50). Herein, in addition to decreased fibrin deposition, we demonstrated a significant reduction in liver PIC expression after intraportal PIT in animals treated with mAPC. Novel mechanisms contributing to anti-inflammatory effects of mAPC involve both direct and indirect effects (20,34,50). Indirect effects may be based on downregulation of coagulation pathways and consequent
reduction in generation of thrombin and factor Xa, which can each, in turn, activate protease-activated receptors on cells to generate PICs and adhesion molecules. The direct effects may include downregulation of expression of various NF-κB–dependent PIC genes (20,25,27,34,50). Consistent with these concepts, we demonstrated significant downregulation of NF-κB nuclear translocation in mAPC-treated PVECs cocultured with islets and found decreased PVEC activation, demonstrated by reduction in TNF-α production in culture supernatants. These concepts were supported by in vivo studies showing that plasma levels of sICAM-1, soluble E-selectin, and soluble P-selectin, as well as intrahepatic endothelial cell function (evaluated by hyaluronic acid clearance) were improved by mAPC treatment. Overall, the results show that intrahepatic endothelial dysfunction elicited by embolism of isolated islets infused into the portal vein was significantly reduced in recipients treated with mAPC compared with controls. Therefore, mAPC treatment prevents activation and dysfunction of endothelial cells elicited by intraportal infusion of islets and represents a novel approach to preserve FIM after transplantation.

When islet grafts are implanted into the liver, cellular components other than endothelial cells serve as critical mediators of nonspecific inflammatory events, including resident macrophages (Kupffer cells). Previous studies (14,15) demonstrated that prevention of macrophage activation and the consequent release of inflammatory mediators preserve islet mass after transplantation, an effect also potentially beneficial from the immunological point of view because immunogenicity of the transplanted islets is not amplified by the presence of inflammatory cytokines or by efficient antigen presentation by macrophages. The tissue factor/factor VIIa complex induces proinflammatory effects in macrophages (52), whereas binding of APC to specific receptors on mononuclear phagocytes blocks responses of macrophages induced by lipopolysaccharide and PICs, including monocyte-dependent T-cell proliferation (28). Studies are underway in our laboratory to evaluate the hypothesis that APC protective effects also involve direct effects on Kupffer cells during PIT.

The beneficial effects of mAPC in our islet transplantation model could also be related to its antiapoptotic activity (22–26). In the present study, we demonstrated a significant decrease in intraislet apoptotic cells after the transplant in animals given mAPC compared with controls. Moreover, studies in our laboratory suggest that recombinant human APC induces significant protection against PIC-mediated human isolated islet cell death (53). Modulation of several genes in the islet apoptosis pathway, including the Bcl-2 homolog protein and the inhibitor of apoptosis protein, seems to be critical in this effect (J.L.C., C.E., C.A.S., G.B., M.V., S.E.R., C.Y., J.A.T., J.A.F., J.H.G., D.E.E., unpublished data).

In summary, we have demonstrated that mAPC can significantly reduce loss of FIM after intraportal islet transplantation in diabetic mice by downregulation of coagulation, inflammation, and probably apoptotic pathways. The protective effects of APC are associated with decreased activation of intrahepatic endothelial cells elicited by the intraportal embolism of isolated islets. These results suggest that APC therapy may enhance the therapeutic efficacy of islet transplantation in humans.

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