

Islet Transplantation and Regeneration for Treatment of Diabetes

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Islet transplantation has the potential to restore normoglycemia and prevent the development of diabetic complications such as retinopathy, nephropathy and neuropathy, and could therefore be a valuable treatment for diabetic patients. The scarcity of available islets is an obstacle for clinically successful islet transplantation. To resolve the problems, we have examined the two methods, islet transplantation with extracellular matrix¹ and *in vivo* expansion of islets with electrically-transfection of growth factors.

Key Words: Insulin, islets, fibronectin, HGF

Pretreatment of Islets with Fibronectin

It has proved difficult to achieve insulin independence following islet transplantation due to the high high rate of graft failure. One solution might be to increase the efficacy of the limited islets. Isolated islets are exposed to a variety of cellular stressors, and disruption of the cell-matrix connections damages islets. We examined the effect of fibronectin on islet viability, mass and function, and also examined whether fibronectin-treated islets improved the results of islet transplantation. Fibronectin is a major component of the extracellular matrix, inhibits apoptosis by upregulating the Bcl-2 adhering to the cell via $\alpha 5 \beta 1$ integrin in fibroblasts and by affecting Bcl-2 and Bax levels via $\alpha 4 \beta 1$ integrin in chronic B cell lymphocytic leukemia. fibronectin also induces secretion of growth factors and increases cell

survival via $\alpha 4 \beta 1$ integrin in eosinophils. Because integrins are also expressed in islets cells, fibronectin might also act as a survival factors in islets.

Pancreatic islets were isolated from male Wistar rats by collagenase digestion after anesthesia with pentobarbital and cultured with RPMI1640 medium with or without soluble 10 μ g/ml fibronectin. Islets cultured with fibronectin for 48 hours maintained higher cell viability (0.146 \pm 0.010 vs. 0.173 \pm 0.007 by MTT assay), and also had a greater insulin and DNA content (86.8 \pm 3.6 vs. 72.8 \pm 3.2 ng/islet and 35.2 \pm 1.4 vs. 30.0 \pm 1.5 ng/islet, respectively) than islets cultured without fibronectin. Absolute values of insulin secretion were higher in fibronectin-treated islets than in controls (Fig. 1); however, the ratio of stimulated insulin secretion to basal secretion was not significantly different (206.9 \pm 10.5 vs. 206.9 \pm 10.5).

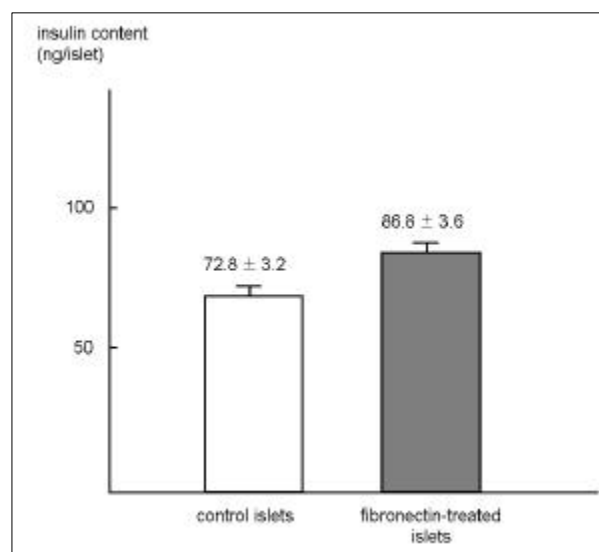


Fig. 1. Insulin content of the islets with or without fibronectin.

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23.3 vs. 191.7 \pm 20.2% when the insulin response to 16.7 mmol/l glucose was compared to that of 3.3 mmol/l glucose); the higher insulin secretion was thus mainly due to larger islet cell mass.

Syngeneic Wistar rats were made diabetic by a single injection of 50 mg/kg STZ through a tail vein. After cultivation, the adherent islets were removed from culture dishes by gentle pipetting, collected in a centrifuge tube, and washed twice with KRB buffer. The rats transplanted with fibronectin-treated islets had lower plasma glucose and higher plasma insulin levels within 2 weeks after transplantation, and had more favorable glucose tolerance 9 weeks after transplantation. These results indicate that cultivation with fibronectin might preserve islet cell viability, mass and insulin secretory function, which could improve glucose tolerance following islet transplantation.

HGF-Gene Transfer in STZ Mice

Hepatocyte growth factor (HGF) is originally identified and cloned as a mesenchyme-derived factor related to liver regeneration after hepatectomy or hepatic injury, and as a potent mitogen for hepatocytes. The active form of HGF is a disulfide-linked heterodimeric protein consisted of a 69-kDa α -chain and a 34-kDa β -chain, which contain four kringle domains and serine protease-like domain, respectively. HGF protein is produced as an inactive single chain precursor which is processed and activated by four proteases including blood coagulation factor XIIIa, urokinase, tissue-type plasminogen activator and a serum-derived serine protease named HGF-activator. Active HGF is now known to possess potent mitogenic, motogenic and morphogenic properties for a wide variety of cells and acts thorough a membrane-spanning tyrosine kinase receptor, the protein product of the proto-oncogene, c-met. To explore the possibility of HGF-gene therapy in diabetes mellitus, we investigated the effect of electric HGF-gene transfer in mice.

The pCAGGS-HGF plasmid, which drives HGF gene expression under the cytomegalovirus immediate-early enhancer-chicken β -actin hybrid promoter, was injected and transfected into the



Fig. 2. Electric transfer of HGF.

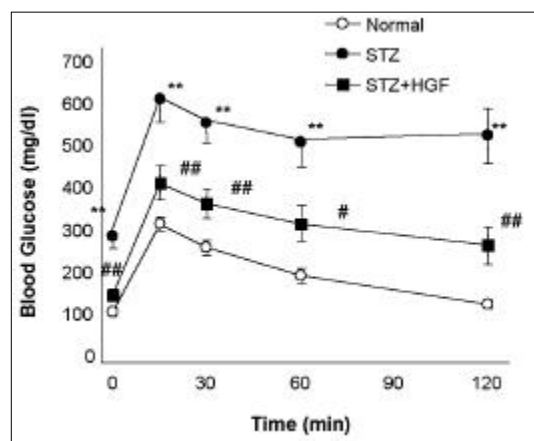


Fig. 3. Oral glucose tolerance test.

anterior tibial muscles by electroporation with a pair of electrode needles (Fig 2).

Following the HGF-gene transfer, plasma HGF levels was increased and detected at least for 7 days, whereas not detected in the untreated mice. In streptozotocin-induced diabetic mice, repetition of HGF-gene transfer at intervals of 7 days for 4 weeks suppressed elevation of blood glucose levels. In addition, both glucose tolerance (Fig. 3) and insulin secretion after oral glucose load were significantly improved, and pancreatic insulin contents and islet number were increased in HGF-gene transferred group.

These results suggested that HGF gene therapy may be potentially useful for the treatment of type 1 diabetes mellitus.

Conclusion

Treatment of islets with growth factors *in vivo* or *in vitro* will preserve or regenerate islet cells, which could improve glucose tolerance in diabetic animals.

REFERENCE

1. Hamamoto Y, Fujimoto S, Inada A, Takehiro M, Nabe K, Shimono D, et al. Beneficial effect of pre-treatment of islets with fibronectin on glucose tolerance after islet transplantation. *Horm Metab Res* 2003;35:460-5.