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Original article

A novel tissue for islet transplantation in diabetics

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A B S T R A C T

Background: Islet transplantation for diabetes therapy has remained a challenge. None of the currently used transplantation sites have provided satisfactory results as islets seem to require a specific tissue for survival and growth. Since the submandibular gland (SMG) shares physiological and anatomical similarities with the pancreas, we attempted to use this tissue as the transplantation site.
Methods: In Experiment 1, a group of 10 female Syrian Golden hamsters' (SGH) received isolated and purified homologous islets transplanted into their right SMG. In Experiment 2, 15 female SGH received islet transplant into their left SMG as above, except that the recipient hamsters were made diabetic by streptozotocin (STZ) before islet transplantation. In Experiment 3, isolated and purified human islets were transplanted into the SMG of 10 female hamsters.
Results: In 8 out of 10 hamsters in Experiment 1 the islets survived and showed the same morphological structure and endocrine cell content, as intrapancreatic islets and presented signs of rapid growth and distribution. Also, as in Experiment 1, well-established islets were present in Experiment 2. Ten of the 15 hamsters pretreated with STZ had blood glucose values between 96 and 125 mg/dl, whereas three hamsters remained hyperglycemic (glucose levels between 194 and 417 mg/dl). Remarkably, the islets in the pancreas of 10 STZ-treated hamsters with functioning SMG islets remained atrophic even after 12 weeks. In two hamsters transplanted islets showed degeneration and remained diabetic until their pancreatic islets regenerated .In Experiment 3, transplanted human islets were completely destroyed.
Conclusions: SMG appears to be the most suitable site for islet transplantation for the treatment of diabetes.

1. Introduction

Therapy for diabetes, especially of Type 1 diabetes has remained a challenge. The most logical approach for controlling diabetes, namely transplantation of islets either as isolated islets or as pancreas transplantation, did not deliver the expected results. One of the major problems in islet transplantation was the choice of host tissue. The currently favored transplant site, the liver, remains an imperfect treatment. Contrary to experimental results, different transplantation sites or the use of reprogrammed pluripotent stem cells showed similar disappointing results. Induction of islet cell neogenesis by glucagon-like peptide 1 agonists, gastrin, epidermal growth factor and islet neogenesis-associated protein (INGAP) are thought to stimulate neogenesis in rodents, but researchers have yet to find evidence of similar regeneration in humans. Also, other suggested possibilities, such as reprogrammed acinar cells to produce beta cells by injecting them with several transcription factors, or the use of mesenchymal stem cells, that are already being extensively studied for treatment of cardiovascular disease and other conditions, are presently illusive.

Despite numerous recommendations and approaches for successful replacement of nonfunctional islet cells, one fundamentally important physiological aspects of the issue has been largely ignored. Why are islets embedded within the pancreas and not as other endocrine tissues as a compact cell mass anywhere in the body? Also, why are these endocrine elements distributed within the pancreas with tight contact with the surrounding exocrine cells of the pancreas? We do not exactly know the answer, but we realize that their function requires pancreatic tissue where they find the optimal environment for their survival and function. As our numerous previous attempts to maintain islets transplanted into the liver, spleen and subcapsular region of the kidney failed (unpublished results), we were looking for tissues with a structure that resembles the pancreas. Therefore, we chose the submandibular

Abbreviations: SGH, Syrian golden hamster; SMG, submandibular glands; STZ, streptozotocin.

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gland and found that this tissue was a unique and ideal place for pancreatic islets to grow and function.

2. Material and methods

2.1. Animals

Eight-week-old out bred female Syrian Golden hamsters (SGH) of the Eppley colony were used. They were housed in the centralized Comparative Medicine Animal Facilities, an AAALAC International accredited animal facility, in plastic cages on corncob bedding (Bed-O-Cobs, The Anderson Cob Co., Maumee, OH) under standard laboratory conditions (temperature, 21 ± 2 °C; humidity, 40 ± 5%; light/dark cycle, 12 h/12 h; 10× air changes/hr). They were fed a commercial diet (Wayne Lab Blox, Allied Mills, Chicago, IL) and had free access to tap water. The maintenance and humane treatment of the animals followed the guidelines of the UNMC Animal Care and Use Committee.

2.2. Islet isolation and culture

Pancreatic islets of eight-week-old female SGH were isolated, purified and cultured as reported [1].

Human islets from a 38-year old male Caucasian healthy multiorgan donor who died of ruptured cerebral aneurysm was kindly provided by the Diabetes Research Institute, University of Miami School of Medicine, Miami, FL. The islets were prepared and purified as reported [2].

Plasma glucose was assayed by Glucose Oxidase method from blood samples taken after an overnight fast. The assays were performed before, 3 days and 4 weeks after streptozotocin treatment and at autopsy. Blood samples were taken from orbital vein of live hamsters and from the right heart at autopsy. Comparison of glucose levels was done by χ^2 test.

2.3. Experiment 1

2.3.1. Islet transplantation

400 freshly isolated and purified islets from 8-weeks-old female SGH were drawn into a 1-ml Hamilton Syringe modified with a pipette tip attachment. The settled islet pellet was slowly delivered into a 25-cm piece of PE-50 tubing with a 26-gauge needle attached as reported [3]. The tube tip was folded over and spun to form a packed pellet of islets. Under Phenobarbital anesthesia (50 mg/kg body weight), a 1-cm incision was made between the mandibular ridge and the clavicle. Islets were slowly injected into the fully exposed right submandibular gland (SMG) of 10 female SGH. The needle was inserted in the median lower pole of the SMG and pushed slowly and carefully into the upper left pole. While retracting the needle, islets were injected in the needle track. After injection, the SMG was returned to the normal position and the skin was closed by stainless steel surgical clips.

2.4. Experiment 2

In the subsequent experiment, a group of female hamsters received streptozotocin (Upjohn, Kalamazoo, MI, 50 mg/kg intraperitoneally) and the glucose levels were determined by retro orbital blood glucose readings. Fifteen hamsters with a reading greater than 200 mg/dl one day after streptozotocin treatment were selected. Three days after streptozotocin treatment, all hamsters received about 750 islets each in their right SMG as described above. They were then euthanized after 12 weeks and their SMG was subjected to a thorough microscopic examination. Glucose levels, using glucose oxidase methods, were assayed before transplantation and at the end of the study. The tissue sections were stained with H&E and processed for immunohistochemistry for the demonstration of the β, α, δ and PP cells, as well as TGF-α and Nestin by a multi-labeling technique developed in our

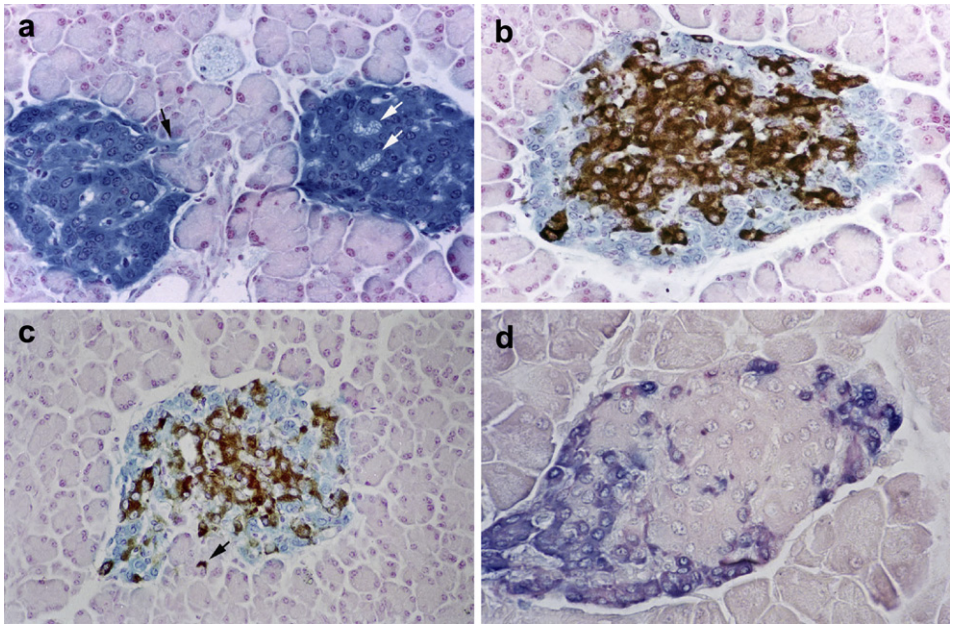

Fig. 1. Transplanted islets within the submandibular tissue of a SGH. a) Two well-preserved islets, immunoreactive with anti-insulin (*blue*) with the glandular tissue. Although the boundary of the islets is well delineated, a few cells appear to extend beyond the boundary into exocrine tissue (*black arrow*). Large and congested blood vessels were seen in many islets (white arrows). X65, anti-insulin antibody) and H&E. b) A single, well-demarcated islet in the SMG containing insulin (*brown*) and glucagon cells (*blue*). X65, double staining with anti-insulin and anti-glucagon) and H&E. c) An islet within the SMG tissue composed primarily of insulin (*brown*) and glucagon (*blue*). Note the presence of a single insulin cell in the exocrine tissue adjacent to the islet (*arrow*). X65, double staining with anti-insulin and anti-glucagon) and H&E. d) Expression of glucagon (*blue*) and TGF-α (*red*) in a large islet. The central portion of the islet contains β-cells. X75, double staining with anti-glucagon and anti-TGF-α. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)