Normalization of Blood Glucose in Totally Pancreatectomized Dogs by Use of Pancreatic Chambers

YOSHIMASA ARAKI, YASUNORI INOUE, KUNINORI YOSHIOKA, YOSHIHIDE NAKAMURA, TOSHIHIDE YOSHIDA AND MOTOHARU KONDO

First Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kyoto, 602, Japan

Abstract

Two ancreaticp chambers constructed of plastic tube and ultrafiltration membrane and containing the minced pancreases of five neonatal rats were implanted in the omentum of totally pancreatectomized diabetic dogs. Blood glucose levels in the diabetic dogs leveled off just after operation, in contrast with those in control animals, in which vacant chambers were implanted, and began to decrease about a week after the transplantation. Blood glucose levels fell to less than 150 mg/100 ml within three weeks. The neonatal rat pancreases in the pancreatic chamber produced a near normal plasma glucose and insulin response to an intravenous glucose tolerance test on the 20th day after transplantation. Removal of the pancreatic chamber from the experimental dogs after normalization of blood glucose levels, caused a rapid elevation of blood glucose levels, and lead to death within four days. No rejection reactions were observed in the course of the experiment. These findings suggest that pancreatic transplantation with the pancreatic chamber is potentially useful for the treatment of diabetes.

Although interest has focused on the transplantation of isolated pancreatic islets or minced pancreatic tissue for the treatment of diabetes mellitus (Matas et al., 1976; Mauer et al., 1974; Gray and Watkins, 1976), the biggest problem in the clinical use of allogeneic or xenogeneic transplantation is tissue rejection. The authors (Araki et al., 1979) reported in preliminary form that improvement of hyperglycemia and responsiveness of insulin to glucose and arginine loadings were observed in totally pancreatectomized dogs for about seven days following allotransplantation of dog islets via the portal vein. No adequate procedure to reduce the rejection has been found. Immunosuppressants have been shown to achieve minimal success in prolonging the survival of allogeneic islets of Langerhans in rats and man (Matas et al., 1976; Marquet and Heystek, 1975). One possible way to overcome the rejection problem is to isolate pancreatic tissue from immune destruction behind the physical barrier of a semipermeable membrane that is in permeable to molecules above a particular size, such as circulating antibodies and cellular elements, but allows nutrients to pass by diffusion from extra-membranous fluid. The pancreatic tissue thus remains capable of responding to the physiological needs of the diabetic host. In present study, blood glucose levels of dogs in which a pancreatic chamber containing minced pancreatic tissue xenograft was implanted were evaluated for a longer period of up to three weeks.

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Materials and Methods

Ten young mongrel dogs of both sexes, weighing 1.2 to 2.1 kg, were anesthetised with Ketamine hydrochloride (Ketalar®, Park, Davis and Sankyo LTD.) and Thyamylal sodium (Isozol®, Yoshitomi Pharm. Co.), and pancreatectomized under strictly aseptic conditions. Their blood glucose levels were then maintained from 200 to 300 mg/100 ml for about a week with NPH insulin until the day before transplantation.

Pancreases of ten five-day-old inbred Wister rats were suspended in ice-cold Hanks solution, minced with fine scissors, and incubated to prevent the introduction of fibroblastoid cells into the pancreatic chamber in Medium 199 supplemented with 10% fetal bovine serum (Microbiological associates), 7 mg/100 ml of aprotinin (Trasylol®, Bayer) and 0.1% of streptomycin in an atmosphere of 5% of CO₂.

The pancreatic chamber was constructed from the barrel of a 50-ml plastic syringe cut to a length of 0.5 cm, with a semipermeable membrane (XM 100A®, Amicon) fixed over each end with alphacyanoacrylate monomer (Aron alpha A®, Sankyo Co.) (Fig. 1). The chamber was sterilized before use.

Minced pancreatic tissue of ten neonatal rats was divided into two pancreatic chambers, which were fixed to the omentum of diabetic dogs. The experimental dogs were divided into two groups. Group I consisted of five diabetic dogs in which the device without pancreatic tissue was implanted, and Group II five diabetic dogs with the device containing pancreatic tissue.

After implantation, dogs were kept in metabolic cages and fed with dog food, meat and boiled rice. Fasting blood glucose, urinary glucose, urine volume and body weight were measured daily.

On the 20th day after transplantation, an intravenous glucose tolerance test (IVGTT) was performed on Group II by loading of 0.32 g/kg of 50% D-glucose in 2 min. Plasma glucose and immunoreactive insulin (IRI) levels were measured 5 min before and 0, 3, 10, 20, 30, 40, 50 and 60 min after injection of glucose. Blood glucose and urinary glucose levels were determined with a Beckman glucose analyzer, and plasma IRI values with a double antibody radioimmunoassay technique (Dinabot Radioisotope Lab.). The glucose disappearance rate (Kg) was calculated from the plasma glucose levels at from 10 to 30 min intervals.

Statistical differences were evaluated by Student's t-test.

After removal of the pancreatic chambers, the membranes were fixed with Bouin's solution and embedded in paraffin. Sections were stained with either hematoxylin and eosin, or aldehyde fuchsin.

Results

All dogs in Group I died between the 4th and 7th days after implantation of the pancreatic chamber without pancreatic tissue, with hyperglycemia, polyuria and glycosuria.

Implantation of the pancreatic chamber with minced pancreatic tissue produced a significant improvement in the diabetic condition. Blood glucose levels in Group II started to decrease within a week and reached 112±13 mg/100 ml on the 21st day after implantation. Glycosuria had disappeared by the 18th day after transplantation. There was no substantial change in daily urinary volume in Group II throughout the course of the experiment. When the pancreatic chambers were removed after 21 days, a sudden rise in blood glucose levels was observed. The urinary volume and glucose increased significantly from the day after removal of the device (Fig. 2). They continued to lose weight progressively up to 10 days following operation, then after about 4 days began gain weight, but it failed to exceed the prepancreatectomized weight throughout the course of the experiment.

Intravenous glucose tolerance tests in
Fig. 2. Effect of implantation of pancreatic chambers with minced neonatal rat pancreatic tissue in totally pancreatectomized dogs. Control animals received pancreatic chamber without pancreatic tissue. The effect on hyperglycemia is shown in the top series of curves. The effect on polyuria and glycosuria in the bottom.

Group II showed an increase in plasma glucose level from 110±5 to a maximum of 243±11mg/100 ml, followed by a gradual decrease with a glucose disappearance rate of 1.349±0.215%/min. The plasma IRI level increased significantly from 7.4±1.6 before the injection of glucose to a maximum of 36±7.6μU/ml (p<0.001) 3 minutes after the injection, with a gradual decline thereafter (Fig. 3).

After the removal of the pancreatic chambers, the filtration membranes were examined. Although microscopic examination revealed small groups of pale epithelial cells among the degenerated cells deposited on the internal surface of the membranes, no significant cellular elements were present (Fig. 4). On the external surface of the membranes, neovascularization and deposition of fibroblastoid cells were observed.
Discussion

Complete reversal of diabetes was observed in totally pancreatectomized dogs which received minced neonatal pancreases encased in plastic chambers. The device proved effective in normalizing plasma and urinary glucose levels and in restoring the substantial responses of plasma IRI in intraveous glucose tolerance tests essentially to normal. Furthermore, the recurrence of hyperglycemia after the removal of the pancreatic chamber suggests that implantation of the device containing neonatal rat pancreases ameliorated the diabetic state in totally pancreatectomized dogs.

Attempts have been made to overcome the rejection problem by using a millipore chamber holding isolated islets (Strautz, 1970, Gates et al., 1972) or bundles of artificial semipermeable capillaries with cultured beta cells (Sun et al., 1977; Whitmore et al., 1975). However, a major problem with the millipore chamber approach has been the overgrowth of fibroblasts adhering to the external surface of the chamber, leading to a reduction of membrane permeability. Recently, amelioration of the diabetic state has been achieved by Gates et al. (1977) using a chamber consisting of nucleopore membrane, and by Tze et al. (1979) using a semipermeable capillary unit for allo- and xenogeneic grafts. In this study, plasma glucose disappearance and insulin release patterns of the pancreatic chamber recipients following IVGTT on the day before removal of the device were much better than those in the untreated diabetic controls similarly tested in our preliminary study (Araki et al., 1979). No significant effect of fibroblast overgrowth on the external surface of the device was observed in the course of the experiment. Ten minced neonatal rat pancreases in two pancreatic chambers were found sufficient to ameliorate diabetes in young dogs, and histological examination and

Fig. 3. The plasma glucose and insulin levels of the IVGTT performed on 5 diabetic dogs implanted with two pancreatic chambers containing minced neonatal rat pancreases. The test was performed on the 20th day after implantation.
IVGTT showed that this device is capable of preventing rejection reaction for at least three weeks.

Further experiments over a longer period are now required to confirm the potential usefulness of this device.

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