Treatment of Diabetes by Xenogeneic Islets Without Immunosuppression

Use of a Vascularized Bioartificial Pancreas

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Tight glycemic control by intensive insulin therapy effectively delays the onset and slows the progression of diabetic complications but is associated with frequent dose adjustments and a high incidence of hypoglycemia. Successful pancreas transplantation corrects abnormal glucose metabolism but subjects patients to morbidity and mortality associated with chronic immunosuppression. A vascularized artificial pancreas device containing pancreatic islets is designed to provide glycemic control without immunosuppression. We report here that devices seeded with porcine islets implanted into pancreatectomized severely diabetic dogs maintained a marked improvement in glycemic control with reduced exogenous insulin requirements for up to 9 months with improved glucose tolerance and a reduction in glycosylated hemoglobin levels. No immunosuppression was used. Thus, use of a vascularized artificial pancreas containing xenogeneic porcine islets could be an alternative to intensive insulin therapy and pancreatic transplantation in treating diabetic patients before the development of severe diabetic complications. *Diabetes* 45:342–347, 1996

The Diabetes Control and Complications Trial (DCCT) has shown the importance of maintaining blood glucose levels close to the normal range in delaying the onset and slowing the progression of diabetic complications in patients with IDDM (1). The intensive insulin therapy in the DCCT, however, requires three or more insulin injections daily with frequent adjustments in insulin dosage to achieve target blood glucose levels and is associated with a high incidence of severe hypoglycemia. Moreover, primary-care physicians who treat the majority of diabetic patients may not be able to offer as rigorous and comprehensive treatment of diabetes as that provided by a team of well-trained diabetes professionals in the DCCT (2).

With the emergence of effective immunosuppressive drugs and improved surgical techniques, pancreas transplantation has become an increasingly used therapy for IDDM with much improved patient and graft survival. However, the necessity of chronic immunosuppression has been associated with significant morbidity and mortality due to opportunistic infection and spontaneous neoplasms as well as direct drug toxicity and metabolic complications. The shortage of human donor pancreases also limits application of this treatment modality.

The use of bioartificial pancreas devices capable of excluding immune lymphocytes and immunoglobulins has emerged as a potential alternative to insulin therapy and pancreas/islet transplantation for treatment of IDDM. The immune exclusion is achieved by separating islet grafts from the host by semipermeable membranes that allow only small molecules, such as glucose, insulin, nutrients, and metabolites, to pass through. Lymphocytes and immunoglobulins are excluded by the membrane and are unable to cause rejection of the islets. Three types of bioartificial pancreas devices, i.e., microcapsules, diffusion chambers, and perfusion devices, have been used to treat experimental diabetes (3).

Our group has been interested in the use of vascularized bioartificial pancreases (perfusion devices) for treatment of diabetes in experimental animals for the past 5 years. In a pancreatectomy-induced diabetic dog model, we previously reported that two devices containing allogeneic (canine) islets controlled severe diabetes with minimum use of exogenous insulin for up to 1 year (4–6). No immunosuppression was needed to maintain viability of islet allografts, confirming the immune exclusive nature of the devices. Because of the persistent shortage of human donor pancreases, we need to explore the use of xenogeneic islets rather than human allogeneic islets for the clinical application of bioartificial pancreas devices. The present study examines whether long-term control of diabetes in totally pancreatectomized dogs is attainable using xenogeneic porcine islets contained in a vascularized bioartificial pancreas without immunosuppression.

**RESEARCH DESIGN AND METHODS**

**Animals.** Adult mongrel female dogs weighing 18–22 kg were purchased from Buckshire (Perkasie, PA), evaluated by a veterinarian, and housed in accordance with U.S. Department of Agriculture Regulations.
TABLE 1

<table>
<thead>
<tr>
<th>Animal (device)</th>
<th>Preimplantation</th>
<th>Postimplantation</th>
<th>Duration of function (days postimplantation)</th>
<th>Plasma porcine C-peptide (ng/ml)</th>
<th>Body weight change (postimplantation)</th>
<th>Cause of termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP10 (AP0)</td>
<td>21.0 ± 2.3</td>
<td>10.4 ± 0.5</td>
<td>16</td>
<td>13–127</td>
<td>0.12 ± 0.02</td>
<td>144</td>
</tr>
<tr>
<td>GP18 (AP1)</td>
<td>26.6 ± 0.8</td>
<td>9.9 ± 0.3</td>
<td>12</td>
<td>7–270</td>
<td>0.21 ± 0.02</td>
<td>271</td>
</tr>
<tr>
<td>GP19 (AP1)</td>
<td>21.6 ± 1.8</td>
<td>11.3 ± 0.3</td>
<td>19</td>
<td>11–229</td>
<td>0.29 ± 0.01</td>
<td>230</td>
</tr>
<tr>
<td>GP14 (AP1)</td>
<td>16.4 ± 1.1</td>
<td>4.0 ± 0.2</td>
<td>6 ± 1</td>
<td>1–56</td>
<td>0.45 ± 0.08</td>
<td>57</td>
</tr>
<tr>
<td>GP23 (AP2)</td>
<td>22.9 ± 0.8</td>
<td>8.7 ± 0.5</td>
<td>16 ± 1</td>
<td>13–71</td>
<td>0.49 ± 0.04</td>
<td>90</td>
</tr>
<tr>
<td>GP24 (AP2)</td>
<td>22.2 ± 0.9</td>
<td>9.9 ± 0.6</td>
<td>8 ± 6</td>
<td>9–91</td>
<td>0.37 ± 0.06</td>
<td>157</td>
</tr>
<tr>
<td>GP25 (AP2)</td>
<td>21.0 ± 0.8</td>
<td>12.9 ± 0.6</td>
<td>14 ± 1</td>
<td>9–73</td>
<td>0.47 ± 0.05</td>
<td>84</td>
</tr>
<tr>
<td>GP27 (AP2)</td>
<td>26.8 ± 0.7</td>
<td>7.0 ± 0.5</td>
<td>13 ± 1</td>
<td>11–64</td>
<td>0.73 ± 0.07</td>
<td>77</td>
</tr>
<tr>
<td>GP28 (AP2)</td>
<td>19.1 ± 2.3</td>
<td>7.5 ± 0.4</td>
<td>4</td>
<td>1–78</td>
<td>0.55 ± 0.04</td>
<td>Ongoing (&gt;366)</td>
</tr>
</tbody>
</table>

Data are means ± SE. Three types of devices implanted into these animals are shown in parentheses. The prototype device (AP0) had a membrane length of 30 cm and a chamber volume of 7.1 ml. A new modified device (AP1) had the same membrane length and an increased chamber volume (to 15.1 ml). Another modified device (AP2) had increased membrane length (45 cm) and chamber volume (11.5 ml). The results of three AP0, four AP1, and one AP2 devices are not included in the table because of nonfunction of the device or early device failure (see text). Average preimplantation FBG and exogenous insulin requirement were measured 1 week before device implantation. Average FBG and exogenous insulin requirement are given for the period of function. The period of function is defined as a period during which the FBG levels were consistently maintained below 13.9 mmol/L. The first and last day of the period are shown. Average plasma porcine C-peptide values are given for the period of function. Body weight changes are given as body weight on the last day of function minus body weight 1 day before implantation. LF, loss of function; Th, thrombosis; RB, reduced bruit. For the ongoing study, Coumadin (0 mg) is given daily instead of aspirin after 80 days postimplantation.


**Islets.** The pancreas was removed from 6-month-old Yorkshire pigs (Tufts University School of Veterinary Medicine, Grafton, MA) weighing ~100 kg. Islets were isolated using a modification of a standard collagenase digestion and discontinuous Ficoll density gradient method described by Warnock and Rajotte (7) with the use of *Clostridium histolyticum* collagenase from Serva (Heidelberg, Germany). Approximately 2,600 islet equivalents (IEs) were isolated from a gram of pancreas tissue with 90% purity and viability assayed by diithizone staining and acridine orange and propidium iodide staining (8), respectively. An IE is a unit introduced to standardize the tissue mass consisting of varying sizes of islets by converting the numbers to those of islets of 150 μm in diameter (9). Islets were cultured in minimum essential medium with 10% heat-inactivated horse serum for 24 to 48 h, washed, suspended in 1% agar solution, and seeded into a device 2-3 h before implantation.

**Pancreatectomy.** Total pancreatectomy (>95%) was performed under general anesthesia as described previously (4), with antibiotic coverage and postoperative analgesia. After pancreatectomy, multivitamins and pancreatic enzymes were given daily mixed with food. All diabetic dogs were treated by daily exogenous insulin.

**Device implantation.** The device was implanted 2–7 weeks after pancreatectomy as described previously (4,6). Starting 2 days before implantation, 75 mg aspirin was administered daily. Upon laparotomy, the device was placed between the left common iliac artery and vein. The device was wrapped in the omentum and the anterior abdominal wall. No immunosuppression was used throughout the study period.

**Vascularized artificial pancreas.** The structure and the materials of the device were described previously (4,6). Briefly, the device consists of a single-coiled acrylic copolymer tubular membrane with a nominal molecular weight cutoff of 70 kDa contained within a disk-shaped acrylic housing. The space surrounding the membrane provided a compartment for the islets and was accessible via two seeding ports. Both ends of the tubular membrane were connected to vascular grafts with a matched inner diameter (IMPRA, Tempe, AZ), which were anastomosed to iliac vessels of the recipient animal, thus creating an arteriovenous shunt through the device. A single device was implanted into a totally pancreatectomized diabetic recipient. Three types of devices were used: the prototype device with a membrane length of 30 cm and a chamber volume of 7.1 ml (AP0) (4,5); a new modified device with the same membrane length but increased chamber volume (to 15.1 ml) (AP1); or a new modified device with increased membrane length (45 cm) and chamber volume (11.5 ml) (AP2). Modification of the devices was implemented to increase the insulin secretory capacity of the device and to avoid the use of two devices.

**Diabetes control.** Fasting blood glucose (FBG) levels were determined 2–3 times per week in the morning using chenstrip tapes and a glucose meter throughout the life of the device recipients. When the devices failed, they were removed under general anesthesia. After removal of the device, FBG monitoring was continued to document reversal to diabetes. Subcutaneous NPH insulin was administered once a day immediately before feeding. Insulin dose was adjusted to keep FBG levels <13.9 mmol/L rather than at normal glucose levels (5.1 ± 0.4 mmol/L; n = 16) to avoid the risk of hypoglycemia.

**Intravenous glucose tolerance tests (IVGTTs).** IVGTTs were performed by administering 50% dextrose (0.5 g/kg) intravenously and monitoring the blood glucose over a 2-h period. A rate of glucose disappearance from the circulation (K rate) for the IVGTT was calculated from the slope obtained from the results of the first 40 min of the test (10).

**Porcine C-peptide and glycosylated hemoglobin assays.** Porcine C-peptide was determined by a 2-day disequilibrium assay using a porcine C-peptide radioimmunoassay kit (LINCO Research, St. Louis, MO). The assay has the limit of sensitivity at 0.1 ng/ml and the limit of linearity >0.2 ng/ml. HbA1c was determined by a cation exchange chromatography technique using the Helena GLYCO Hb Quik Column Method (Helena Laboratories, Beaumont, TX). Blood samples for both assays were obtained once a week to once a month in the morning before insulin treatment and feeding. All assays were run in triplicate.

**Histology.** Tissues were fixed in 10% buffered formalin solution, processed, and paraffin embedded. Sections 3–5 mm thick were cut and stained for insulin at room temperature using a polyclonal rabbit anti-insulin antibody and peroxidase-antiperoxidase three-step procedure. Known positive tissues and negative controls were routinely run.

**Statistical analyses.** Statistical analyses were performed using unpaired Student's t test. A P value of <0.05 was considered significant.

**RESULTS**

**Glycemic control.** A total of 17 devices, including 5 AP0, 5 AP1, and 7 AP2 devices, were implanted into diabetic dogs and evaluated for their ability to control glucose metabolism. The number of porcine islets seeded into the devices was 114,000 = 18,000 IEs, 206,000 ± 28,000 IEs, and 341,000 ± 33,000 IEs for AP0, AP1, and AP2 devices, respectively. Out of 17 devices, 3 AP0 and 2 AP1 devices never improved FBG after implantation because of insufficient insulin secretion. One AP1 and one AP2 device were removed within the first month because of thrombosis, and another AP1 was removed because of recipient bowel obstruction. The remain-
ing nine dogs had a marked reduction in exogenous insulin requirement and improvement of FBG, as shown in Table 1. In seven of nine dogs, the FBG after implantation was maintained below 11.1 mmol/l with 4–16 U/day of exogenous insulin. Control of diabetes for >8 months was achieved in two dogs (GP18 and GP19). Overall reduction of exogenous insulin doses in comparison with the preimplantation doses was 54% for AP0 devices, 61% for AP1 devices, and 74% for AP2 devices. Porcine C-peptide (>0.2 ng/ml) was detected in animals carrying AP1 and AP2 devices but not in the dog with the prototype (AP0) device. This was also true when all 17 recipient animals were analyzed throughout the study periods for circulating porcine C-peptide (Fig. 1). None of the nine dogs lost body weight while the devices maintained glycemic control.

Figure 2 illustrates the representative profile of good glycemic control with reduced insulin requirements after device implantation in two dogs (GP18 and GP27). Dog GP18 underwent pancreatectomy 7 weeks before implantation of an AP1 device. One week before implantation, average weekly FBG was 26.6 ± 0.8 mmol/l, with an average weekly exogenous insulin requirement of 39 U/day. Immediately after implantation, FBG remained high (19.7 ± 3.4 mmol/l), with an exogenous insulin requirement of up to 38 U/day (or 22 ± 5 U/day). FBG dropped to 10.3 ± 0.9 mmol/l in the second week and stabilized after the third week at 10.3 ± 0.4 mmol/l, with exogenous insulin ranging from 10 to 16 U/day. After the 21st week, 8–12 U of insulin was required to maintain FBG at 9.3 ± 0.4 mmol/l. Low levels of circulating porcine C-peptide (0.22 ± 0.02 ng/ml; n = 33) were detected throughout the course. The IVGTT performed on day 247 was abnormal, with a K rate of 1.82 compared with the rate in normal dogs (K = 4.4 ± 0.4; n = 68), but it was much better than that of diabetic dogs (K = 0.65 ± 0.14; n = 4). There was a slight peak (~25% increase from baseline levels) in circulating porcine C-peptide during the IVGTT. The device was removed on day 271 because of a sudden increase in FBG associated with reduction of the bruit in the groin. After removal of the device, there was a rapid increase in FBG to 18.4 ± 3.3 mmol/l by the second week despite an increase in the exogenous insulin dose to 36 ± 1 U/day.

Dog GP27 received an AP2 device 4 weeks after pancreatectomy. Normoglycemia was achieved after day 12 postimplantation and was maintained thereafter for 7 weeks, with a mean FBG of 7.0 ± 0.5 mmol/l compared with a preimplant FBG of 26.8 ± 0.7 mmol/l. Despite much improved glycemic control, administration of low-dose exogenous insulin (13 ± 1 U/day) was continued. High levels of porcine C-peptide (0.73 ± 0.07 ng/ml; n = 9) were detected in the circulation during this period. Rapid deterioration of glycemic control on day 65 was followed by a loss of bruit and removal of the device on day 77 postimplantation.

**Glycosylated hemoglobin.** As a possible indicator of glycemic control by the bioartificial pancreas, we examined changes in HbA1c values over time after pancreatectomy and device implantation. As shown in Fig. 3, a significant rise in HbA1c values was observed within 30 days after pancreatectomy (P < 0.001 against prepancreatectomy HbA1c). HbA1c values at 31–80 or >200 days postpancreatectomy were significantly higher than those at 0–30 days postpancreatectomy (P = 0.034 and P = 0.001, respectively). No significant difference was observed between HbA1c values at 31–80 days and at >200 days, indicating that HbA1c appears to reach its peak level 30–80 days after total pancreatectomy. While a wide range of HbA1c values was seen during the 0–99 days after implantation, long-term control of FBG levels in two dogs (GP18 and GP19) was associated with a significant
FIG. 3. Changes in HbA1c after pancreatectomy and device implantation. Fasting blood was used for HbA1c analysis. HbA1c for normal healthy dogs before pancreatectomy was 6.5 ± 0.2% (n = 22).

Postpancreatectomy HbA1c values were collected from 19 dogs at various times after pancreatectomy. Mean ± SE HbA1c was 9.9 ± 0.2 (n = 23), 9.6 ± 0.4 (n = 13), and 10.1 ± 0.3% (n = 23) for 0–30, 31–80, and >200 days postpancreatectomy, respectively. Different symbols used for postimplantation HbA1c values represent individual animals. HbA1c for GP18 and GP19 after 100 days postimplantation was 8.3 ± 0.6% (n = 6) and 8.4 ± 0.2% (n = 6), respectively.

The results presented here provide clear evidence that vascularized bioartificial pancreas devices containing xenogeneic porcine islets provide a promising therapeutic modality for the treatment of IDDM. Considering the brittle nature of the total pancreatectomy–induced canine diabetes model used in the present study, marked improvement in glycemic control was achieved by the use of a single implanted device together with small doses of exogenous insulin. These results represent the longest survival of discordant xenogeneic islets thus far reported in a large animal species with or without immunosuppression. Maintenance of normal to near-normal FBG levels lasted for 2 to >8 months and was associated with an improved IVGTT and a significant reduction in HbA1c level. HbA1c levels rose rapidly after total pancreatectomy (8.8 ± 0.2 vs. 6.5 ± 0.2% for prepancreatectomy levels) and reached their peak 30–80 days after pancreatectomy. Considering that the pancreatectomized dogs maintained high HbA1c levels despite insulin administration, the significant reduction of HbA1c levels observed after 100 days postimplantation in two dogs (GP18 and GP19) illustrates the ability of the devices to achieve improved diabetes control. Although complete elimination of exogenous insulin therapy was not accomplished, we believe that the treatment of diabetes may be made less rigorous by the combined use of the devices and the daily administration of a small amount of insulin given in reduced numbers of injections.

In our previous study with allogeneic islets, two prototype devices were needed to control diabetes. The present study examined the feasibility of using a single device to treat a diabetic animal. As in the case with allogeneic islets, most of the single prototype devices (AP0) seeded with porcine islets failed to provide proper glycemic control. When larger devices (AP1 and AP2) were used, glycemic control was markedly improved, with a much higher level of circulating porcine C-peptide. It appeared that the devices with both increased membrane and increased islet chamber volume (AP2) had more insulin secretory capacity than the devices with increased chamber volume alone (AP1), as evidenced by a greater reduction of exogenous insulin usage and higher circulating porcine C-peptide levels. Although AP2 devices generally contained more islets than AP1 and AP0 devices, higher numbers of seeded islets were not always associated with better glycemic control. Thus, further studies are needed to analyze the intricate roles played by the blood-membrane contact surface area (membrane length) and total numbers of islets seeded as well as the density of the islets within the islet chamber (islet numbers/chamber volume).

The devices used in this study were associated with late vascular thrombosis. The clotting was at either the anastomosis sites or the junction of a polytetrafluoroethylene graft and tubular acrylic copolymer membrane. This clotting problem may be uniquely associated with the use of dogs in our studies; the difficulty in maintaining the patency of vascular grafts in dogs has been well recognized because of their blood hypercoagulability (11). To prevent late thrombosis, we have recently initiated a series of experiments in which low-dose (4–6 mg) warfarin (Coumadin) was used instead of aspirin after 60 days postimplantation to maintain prothrombin time (PT) at 14–20 s (in a normal dog, 5.9–9.1 s). Three of four devices are currently maintaining vascular patency at...
160–366 days postimplantation, while one device was thrombosed when the PT fell to a normal range. Because low-dose warfarin treatment is well tolerated by many patients, the use of warfarin as well as further modification of the device may reduce the incidence of vascular thrombosis in patients.

An important feature of the vascularized bioartificial pancreas is that immunosuppression is not required for maintenance of islet xenograft function. Histological evaluation of a device removed after 271 days (GP18) showed positive insulin staining in the islets with no evidence of lymphocyte or inflammatory cell infiltration. In addition, porcine islets continued to maintain glycemic control despite the fact that serum obtained from normal dogs and dogs implanted with devices strongly lysed porcine lymphocytes in vitro in the presence of complement (data not shown), thus indicating the presence of natural antibody. At present, many believe that only patients with severe diabetic complications, such as renal failure, who require kidney transplantation can be justifiably transplanted with the whole pancreas or pancreatic islets under immunosuppression. Nonuremic diabetic patients who are treated by pancreas transplantation alone have a greater propensity to lose grafts from rejection in spite of high-dose immunosuppression than patients with chronic renal failure who receive simultaneous pancreas and kidney transplantation (12). Because immunosuppression is not required, the vascularized bioartificial pancreas may be used as an alternative to pancreas transplantation for treatment of IDDM at an early stage, before the development of debilitating diabetic complications.

The use of xenogeneic islets in the devices provides a solution to the limited availability of human donor organs. Pigs are the species of choice as a potential xenogeneic organ/tissue donor because of the many physiological similarities between swine and humans (13,14). While isolated porcine islets remain functionally viable for >14 months in diabetic nude mice (15), we do not know how long porcine islets can function in the devices. Should isolated porcine islets function for only a limited period, replacement of islets in the device may become necessary. Studies are currently in progress to develop a retrievable islet suspension matrix for reseeding of the devices.

ACKNOWLEDGMENTS
We thank M. Wyche, K. Schwarz, P. Mazzoni, and T. Nishioka (Deaconess Hospital, Boston, MA); C. Tosone, D. Nicholson, J. Stegemann, C. Doyle, A. Foley, M. Curley, and J. Underwood (W.R. Grace & Co.-Conn); K. Lightbown and P. Kintzer (Tufts Veterinary School, Grafton, MA); and M. Lage, A.E.
Warner, and H.B. Warren (Animal Resource Center) for their assistance.

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