Rapid Publications

Treatment of Severely Diabetic Pancreatectomized Dogs Using a Diffusion-Based Hybrid Pancreas

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Long-term survival of dog islet allografts implanted in diabetic pancreatectomized dogs was achieved by islet encapsulation inside cylindrical chambers fabricated from permselective acrylic membranes (nominal M_r exclusion of 50,000–80,000). Dog islets were isolated from the pancreases of outbred mongrel dogs by collagenase digestion. Chambers containing mean ± SE 316 ± 63K islet equivalents (mean islet volume, 558 ± 111 mm^3, purity 90–95%) were peritoneally implanted into six totally pancreatectomized dogs. The dogs were monitored for glycemic control by fasting and postprandial blood glucose determinations, and responses to both intravenous glucose (intravenous glucose tolerance test 0.5 g/kg) and oral glucose (oral glucose tolerance test 1 g/kg). All of the dogs required appreciably lower dosages of exogenous insulin therapy for control of fasting blood glucose levels, with the mean daily insulin dose dropping from 38 ± 7 to 5 ± 1 U/day during the 1st wk. Three recipients required no insulin for >82, >68, and 51 days. Intravenous glucose tolerance test K values (decline in glucose levels, %/min) at 1 and 2 mo postimplantation were 2.7 ± 0.4 and 2.0 ± 0.5, respectively compared with 3.5 ± 0.5 before pancreatectomy. The glucose values during oral glucose tolerance tests at 2 wk, although returning to <125 mg/dl (<7.0 mM) by 2 h, exceeded the normal range, with peak values of 174 to 202 mg/dl (9.7 to 11.3 mM). These preliminary results are encouraging, and represent an important step in determining the feasibility of using this type of diffusion-based hybrid artificial pancreas as treatment for diabetes mellitus in humans. *Diabetes* 41: 886–89, 1992

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Recent advances in the area of islet immunoisolation place the hybrid artificial pancreas on the verge of clinical trials (1,2). Sullivan et al. (3), with a perfused intravascular device, recently reported the first extensive, long-term in vivo implantations of immunoisolated allografts in diabetic pancreatectomized dogs without immunosuppression. These devices require vascular surgery for implantation. Consequently, effort has focused on developing an extravascular device that could be inserted under the skin or placed in the peritoneal cavity. One approach is to encapsulate the donor islet tissue in hollow fibers. In 1980, Archer et al. (4) first examined such a system in rodents. They seeded islet cells from neonatal mice and hamsters inside Amicon XM-50 membranes (nominal M_r exclusion of 50,000), which were sealed at the ends and implanted into streptozocin-induced diabetic hamsters. Normoglycemia was restored and glycosuria was suppressed for as long as several months. Histological analysis of recovered implants, however, showed a fibrous tissue layer surrounding the membrane. Altman et al. (5–7) obtained similar results with the same type of fibers seeded with allogeneic and xenogeneic rodent islets and human insulinoma cells. Although minimal fibrosis was observed in the NOD mouse (7), the fibers elicited an inflammatory pericapsular response, both in the rat (6,7) and the pig (8). These experiments were conducted with fibers having a fenestrated outer surface. Our laboratory found that these fibers elicited a fibrotic reaction in rats when implanted intraperitoneally. In contrast, wider bore fibers with a smooth outer surface revealed little or no evidence of an inflammatory response. Pork, beef, and dog islets encapsulated with these fibers restored normoglycemia in streptozocin-induced diabetic rats for >10 wk (9). The external membrane surfaces were generally free of fibrotic overgrowth and exhibited only occasional host cell adher-

TABLE 1
Mean insulin requirement (U/day) each week after membrane implantation

Animal days	Preimplantation	Wk 1	2	3	4	5	6	7	8	9	10	11	12	Function w/membrane (days)
SI75	28	3	0	0	0	0	0	0	3	7	9	4	5	140*
SI81	42	6	13	10	13	9	17	8	15	20	35	R		62
SI84	38	3	0	0	0	0	0	0	0	0	0	0	0	82*
SI86	38	0	0	0	0	0	0	0	0	0	0			68*
SI88	38	3	13	23	28	25	28	36	37	R				15
SI89	42	13	34	37	R									5

Values are means.
R = membranes removed.
*Implants are ongoing.

ence. These results led to experiments designed to investigate the effectiveness of islets transplanted within these tubular membrane diffusion chambers in treating diabetic pancreatectomized dogs.

RESEARCH DESIGN AND METHODS

Islets were prepared from outbred adult male and female dogs by modifiying the method of Warnock and Rajotte (10), as previously reported from our laboratory (3,9). Pancreatic tissue was dissociated on a discontinuous Ficoll density gradient. Isolated islets were cultured for 1 day at 37°C in M199/Earle's medium, supplemented with 10% (vol/vol) fetal bovine serum, 20mM HEPES, 5.6 mM (100 mg/dl) glucose, and 400 IU/ml penicillin in a humidified atmosphere of 5% CO_2/95% air. The islets were then seeded into XM-50 tubular membranes with a nominal M_r cutoff of 50,000–80,000 (W.R. Grace & Co.-Conn, Lexington, MA). Before implantation, the membranes were cultured overnight.

Donor and recipient dogs were obtained from Biomedical Associates (Friedensbury, PA). Outbred adult female dogs weighing 15–20 kg were used as transplant recipients, and were housed in compliance with USDA Regulations Part III (Animal Welfare Act) at the Animal Resources Center of the Harvard Medical School. Surgeries were performed, as previously described (11). Pancreatic enzymes were replaced by administering Viokase powder (A.H. Robbins, Richmond, VA) mixed with multivitamins in the food. Fasting blood samples were taken daily, and glucose levels were determined by means of an AccuCheck II blood glucose monitor (Boehringer Mannheim, Indianapolis, IN). A daily injection of pork lente insulin (NovoLabs, U-100) was administered with the dose adjusted for maintaining circulating fasting glucose levels <14.0 mM (<250 mg/dl).

The encapsulated islets were implanted 2–3 wk after pancreatectomy. Laparotomy was performed through a short midline abdominal incision. One-hundred fifty-five to 248 chambers (containing 2–4 × 10^5 islet equivalents from multiple donors) were distributed randomly into the peritoneal cavity. One animal (SI81) received 112 additional membranes (containing 1.7 × 10^5 islets) on postoperative day 41. After implantation, the abdominal wall muscles and the skin wound were closed with continuous 2–0 and 3–0 Ethilon, respectively.

Intravenous glucose tolerance tests were performed before pancreatectomy and monthly after membrane implantation. Fifty percent (wt/vol) glucose (0.5 g/kg body wt) was infused intravenously, and blood glucose concentrations were measured before the injection and at 5, 10, 20, 30, 40, 50, and 60 min after the glucose injection. K values (decline in glucose levels, %/min) were calculated according to standard methods (12). Oral glucose tolerance tests were performed before pancreatectomy and at 2 wk postimplantation by administering 2 ml 50% dextrose/kg body wt. Blood samples were drawn at 0, 15, 30, 45, 60, 75, 90, and 120 min after dextrose administration.

Statistical analysis. Data are means ± SE and compared with the use of the unpaired Student's *t* test or one-way analysis of variance. Differences were consided significant at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 lists the postoperative insulin requirements of the implant recipients. The average exogenous insulin requirement (U/day) after implantation is listed for the first 12 wk. All of the dogs had varying degrees of reduced insulin requirements for 21 to >140 days. Three dogs showed only partial function for 3–10 wk, failure being attributed to membrane breakage in dog SI88, and bacterial infection of the implants in dogs SI81 and SI89. Examination of membranes removed from these three dogs revealed only a scattering of viable islets; the external surfaces were generally free of fibrosis and host cell adherence. Implantation of the chambers completely supplanted exogenous insulin therapy in the remaining three dogs for 51 to >82 days (each of these implants is ongoing, although as of day 52, SI75 required small dosages of insulin). This includes two animals (SI84 and SI86) in which the preimplantation insulin requirement was ≥38 U/day. The mean ± SE fasting glucose values averaged 4.0 ± 0.4 mM (71 ± 7 mg/dl) for these two animals (Fig. 1). This was lower than the fasting glucose levels before pancreatectomy, which averaged 5.0 ± 0.2 mM (91 ± 0.3 mg/dl). The reason for these lower levels is unclear.

Intravenous glucose tolerance test K values at 1 and 2 mo postimplantation were 2.7 ± 0.4 and 2.0 ± 0.5 compared with 3.5 ± 0.5 before pancreatectomy. The rate of glucose decline was slowed significantly ($P < 0.05$, ex-

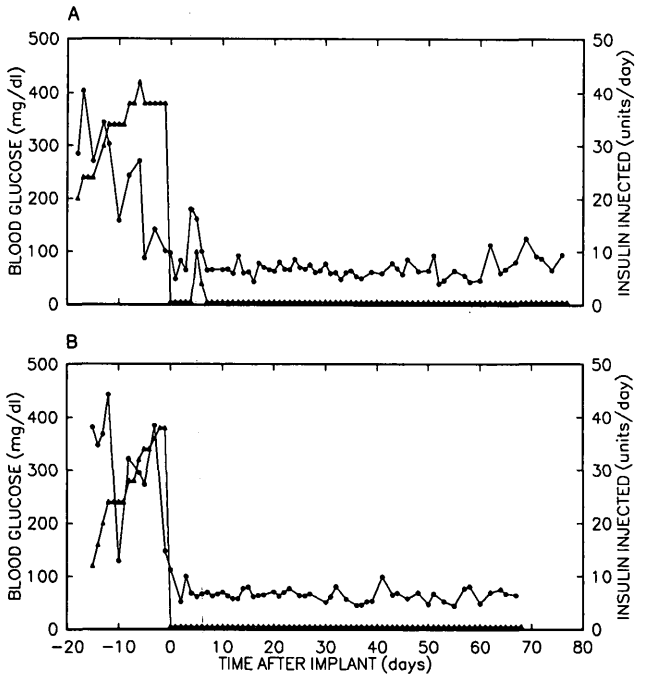

FIG. 1. Exogenous insulin requirements (▲) and fasting blood glucose concentrations (○) before and after membrane implantation. These data were obtained for animals SI84 (*A*) and SI86 (*B*), which had preimplant insulin requirements of 38–42 U/day.

cept the 60-min samples 1 mo postimplant, which were not significantly different from normal) but remained well above the level considered diabetic (K < 1.0). Figure 2 illustrates the responses during intravenous glucose tolerance tests and oral glucose tolerance tests in dogs SI84 and SI86; neither dog required insulin therapy during the first 2 mo. During oral glucose tolerance tests at 2 wk, the blood glucose concentrations were not appreciably different from normal at 15, 30, 45, and 120 min after administering oral dextrose. The maximal glucose levels ranged from 9.7 to 11.3 mM (174 to 202 mg/dl) and returned to <7.0 mM (<125 mg/dl) by 2 h.

These data are consistent with the rapid in vitro kinetic performance of diffusion chambers with the same membrane wall thickness. When encapsulated and nonencapsulated dog islets were perifused with either 5.6 or 16.8 mM glucose for 60 min, an approximately fourfold average increase from basal insulin secretion was observed in both groups (9). The secretory response of the encapsulated islets was sustained for 60 min of glucose stimulation (300 mg/dl) 16.8 mM and returned to basal levels after perifusion with low-glucose solution. The increase in insulin release from the encapsulated islets was delayed by only 7 min compared with nonencapsulated islets.

These results clearly indicate that the diffusion-based hybrid artificial pancreas is capable of responding to glycemic stress, and can function in a large diabetic animal to control blood glucose concentrations. Importantly, it proved possible to achieve exogenous insulin independence for prolonged periods of time without the need for immunosuppressive or anti-inflammatory agent therapy. These results represent an important step toward bringing the hybrid organ approach to clinical reality.

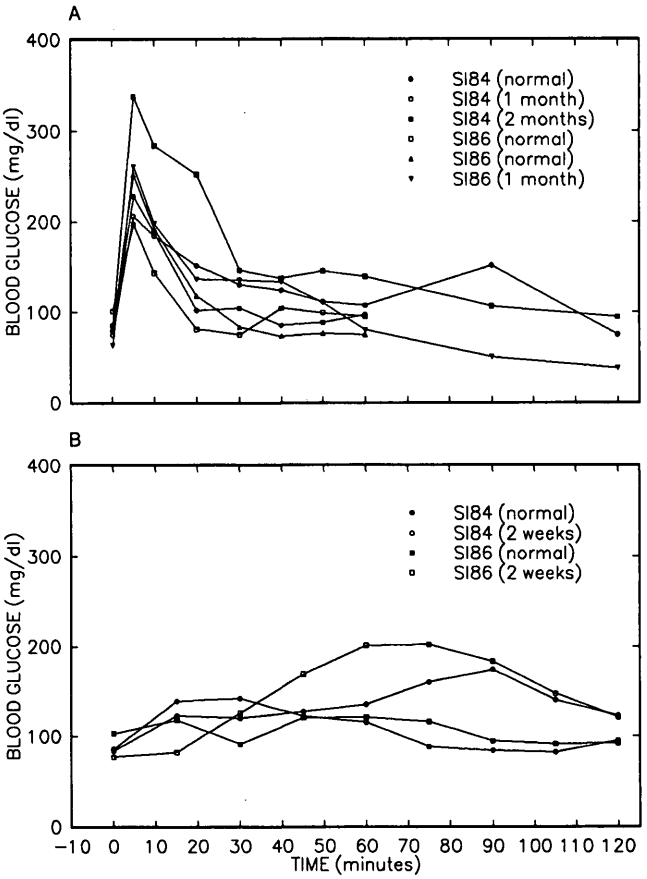

FIG. 2. Intravenous (*A*) and oral (*B*) glucose tolerance tests in dogs SI84 and SI86. The intravenous glucose tolerance test K values at 1 mo postimplantation were 1.8 and 2.6, respectively, compared with 3.0, 4.4, and 3.9 before pancreatectomy. The K value for SI84 at 2 mo was 3.1.

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