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Studies with Implantable Artificial Capillary Units Containing Rat Islets on Diabetic Dogs

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Summary. An implantable artificial endocrine pancreas consisting of a coiled single acrylic copolymer capillary surrounded by rat islets (1000/kg body weight) was implanted in 10 streptozotocin-alloxan diabetic dogs. About 5 h following implantation plasma glucose decreased from an initial mean value of 350 mg/dl to 150 mg/dl, and then to 100 mg/dl at 12 h. Plasma insulin increased to a mean of 39 mU/l (range 23–83 mU/l) at 5 h in the recipient animals. In addition a much improved plasma glucose disappearance rate ($K = 1.9 \pm 0.3$) with slightly delayed insulin responses was seen after intravenous glucose tolerance tests performed in 4 dogs at 7, 8, 10 and 18 h following implantation. These findings suggest that xenogeneic rat islets implanted as an artificial endocrine pancreas can improve glucose metabolism in the diabetic dog.

Key words: Artificial endocrine pancreas, capillary unit, xenogeneic, pancreatic islets, transplantation, diabetes.

Experimental studies have shown that transplanted isogeneic pancreatic islets can restore normoglycaemia in diabetic animals, and prevent the secondary renal changes associated with diabetes mellitus [1–3]. The use of islet transplantation for the treatment of diabetes has been hindered by the two major problems of immune rejection and the shortage of donor tissue [4]. The application of a semipermeable membrane as a mechanical barrier has been suggested as a possible means to protect the transplanted cell from immunological destruction [5]. This would have the potential to overcome the shortage of donor islet tissue through utilization of allogeneic or xenogeneic islet cells for transplantation.

Recently a lack of immunorejection of allogeneic and xenogeneic cells by a previously sensitized rat recipient has been demonstrated when the cells are contained in an implantable artificial capillary unit [6]. These implanted islet cells have been able to achieve normalization of the hyperglycaemic state in the diabetic recipient rat [7, 8].

We report here modifications of the early prototype of the implantable artificial endocrine pancreas (IAEP) to improve the function and the effectiveness of the unit. The important modifications include improvements in the size and configuration of the unit, the fibre arrangement, the internal diameter and thickness of the capillary wall, the surface area of the capillary and the extra-capillary volume. Using xenogeneic rat islets we have investigated the metabolic effects of implanting this unit into the diabetic dog.

Materials and Methods

Preparation of Animals

Mongrel dogs of 10–12 kg (Animal Service, University of British Columbia) were used. For induction of diabetes a combination of streptozotocin (30 mg/kg) and alloxan (50 mg/kg) was administered intravenously. Diabetes was confirmed by the presence of a fasting plasma glucose of over 300 mg/dl. Two weeks following induction of diabetes the animals were divided into two groups. (i) The control group underwent sham operation with the implantation of an artificial capillary unit (ACU) alone. (ii) The experimental group was implanted with an ACU containing xenogeneic rat islets.

Construction of the Implantable Artificial Capillary Unit

The islet chamber was constructed with 2 pieces of plexiglass. The chamber piece (14 × 44 × 44 mm) and the cover (38 mm diameter, 3.2 mm thick) were as illustrated in Figure 1. Two 17G bore

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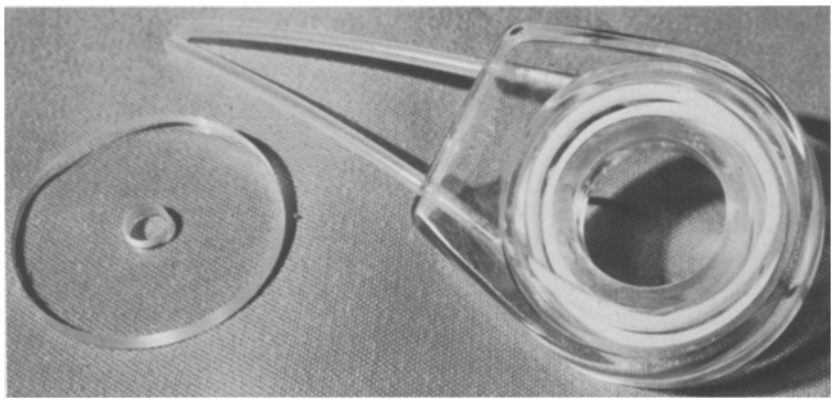

Fig. 1. Implantable artificial capillary unit consisting of a single 60 cm long XM50 artificial capillary coiled 5 times in a plexiglass islet chamber. The ends of the capillary are connected to 2 bevelled vessel tips

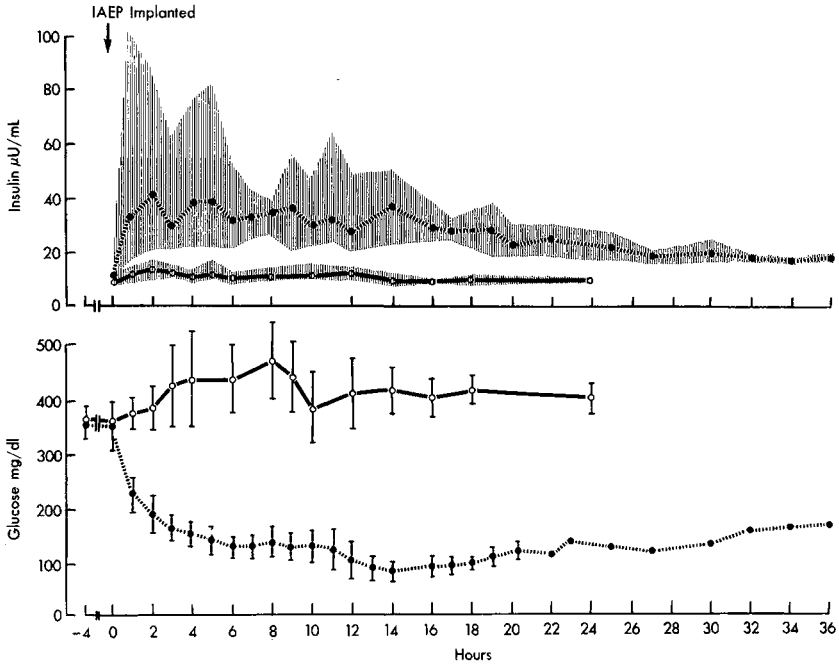

Fig. 2. Plasma insulin and glucose levels in diabetic dogs following implantation of artificial capillary unit containing xenogeneic rat islets (●·····●) or in 4 sham operated diabetic dogs (○————○). Glucose levels between 0–12 h (n = 10), 12–20 h (n = 5), and 12–36 h (n = 2) are shown as mean ± SEM. The shaded area shown in the upper panel is the range of insulin levels

hypodermic needles were press fitted into the plexiglass to serve as injection portals. The chamber is doughnut shaped (inner diameter 25 mm, outer diameter 30 mm, height 6.4 mm) and 2 holes were drilled horizontally through the plexiglass for entry and exit of the XM50 artificial capillary (Amicon Corp.). A total of 600 mm of capillary (internal diameter 1.10 mm) was coiled 5 times within the chamber. Polyethylene tubes were fitted between the capillary and the unit at the entry and exit holes to prevent kinking. The ends of the capillary were glued end to end to internally bevelled vessel tips. After the capillary has been placed into the unit, the plexiglass cover was fixed onto the chamber piece with silicon glue.

The extracapillary chamber was sterilized with 50% ethanol (v/v) for 24 h, soaked in two changes of sterile Hanks' balanced salt solution (HBSS) for 1 h each, then rinsed ten times with sterile HBSS. Finally it was filled with Medium 199 supplemented with 10% fetal calf serum, 100 mU/l penicillin and 0.1 g/l streptomycin (complete culture medium) prior to the introduction of the pancreatic islets. Immediately before implantation, the capillary was filled with 0.154 mol/l saline containing 100 U/ml heparin.

Preparation of Islets

Pancreatic islets were isolated from male Wistar rats (300–400 g) by the modified digestion-filtration method of Scharp et al. [9]. The isolated islets were purified from the exocrine debris by a single layer Hypaque-Ficoll density centrifugation technique [10]. Approximately 300–400 islets free from exocrine tissues were obtained from each rat pancreas. The isolated islets were stored in complete culture medium in a humidified 5% CO_2 incubator at 37° C until enough islets were collected for transplantation of 1,000 islets/kg body weight of the recipient diabetic animal. They were introduced into the IAEP unit through one of the portals prior to connection of the unit to a preformed arterio-venous (A–V) shunt.

Surgical procedures and systemic anticoagulation: General anaesthesia of the dog was achieved with thiopentone and halothane. An A–V shunt between the right carotid artery and jugular vein was formed with a silastic arteriovenous cannula (S-300 series Saf-T-Shunt, Extracorporeal, Pa, USA) 5–7 days

before the implantation of the IAEP. Two weeks after induction of diabetes the IAEP was inserted into a skin pocket created by blunt dissection in the neck, and the vessel tips were connected to the A-V shunt. All the animals received oral aspirin (65 mg/kg body weight) and dipyridamole (25 mg/kg) pre-operatively. The left deep circumflex iliac vein of the dog was catheterised with silastic tubing to facilitate systemic heparinisation. Before the release of the clamps on the A-V shunt 1500 U of heparin was given intravenously as a priming dose. This was followed by continuous heparinisation at 500–700 U/h from a portable infusion pump. The patency of the unit was monitored at hourly intervals by means of a Medsonics Ultrasound Stethoscope (BF5a). Four dogs were sham operated and 10 dogs implanted with the IAEP.

Blood samples were collected prior to and at 30 to 60 min intervals following IAEP implantation for the duration of the experiment. Intravenous glucose tolerance tests (IVGTT) was performed during a control period, a diabetic period after streptozotocin-alloxan injection, and after implantation of the IAEP. For the IVGTT during the control period, dogs were fasted overnight, then glucose (500 mg/kg body weight) administered through the cephalic vein catheter. Blood samples were withdrawn at 0, 1, 3, 5, 10, 15, 30, 45, 60, 75, 90 and 120 min for insulin and glucose determination. Plasma glucose levels were measured with a Beckman glucose analyzer. Plasma insulin was determined by the double antibody immunoassay technique of Hales and Randle [11] with human insulin as standard.

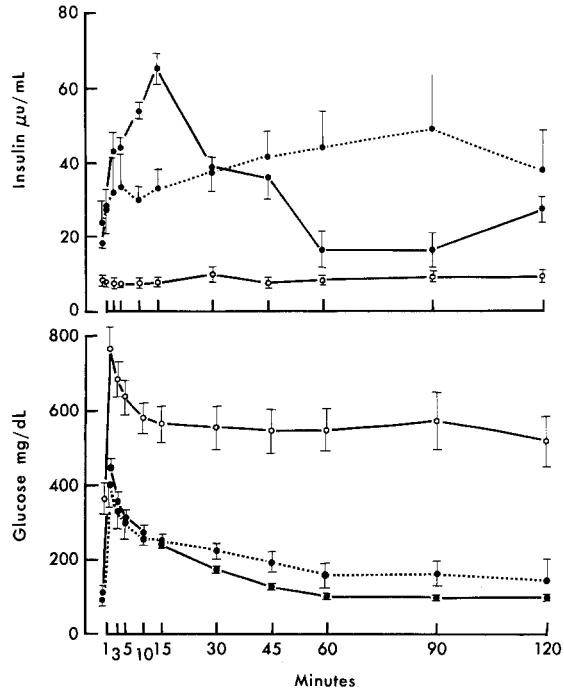

Fig. 3. Plasma insulin and glucose levels during an intravenous glucose tolerance test in dogs, normal controls (●———●) (n = 8), sham operated diabetics (○———○) (n = 4), and with IAEP implant (●·····●) (n = 4). Values shown are mean ± SEM

Results

Figure 2 demonstrates the mean plasma glucose and insulin levels in diabetic dogs which received the IAEP implants. Plasma glucose levels declined to 150 mg/dl by 5 h, and were maintained at less than 150 mg/dl for about 12 h in all 10 dogs. Among these 10 dogs, the duration of the study in 5 dogs extended to 20 h and 2 to 36 h. The mean plasma insulin levels of 5 diabetic dog recipients of IAEP increased from the pre-implantation value of 10 ± 6 mU/l to over 40 mU/l from 1 to 17 h, and were maintained above the basal level for the duration of the experiment in each animal. There was, however a fairly wide range of insulin levels between animals. The 4 sham operated diabetic dogs remained hyperglycemic with a relatively low insulin level for the duration of the study. All the experiments were terminated prematurely, mainly because of thrombus formation within the artifical capillary unit.

Figure 3 shows the plasma glucose and insulin levels following IVGTT in normal control dogs (n = 8), sham operated diabetic dogs (n = 4) and those with the IAEP implant (n = 4). IVGTT was performed in the 4 dogs at 7, 8, 10 and 18 h following IAEP implantation. Even though plasma glucose disappearance (K = 1.9 ± 0.33) and insulin release patterns of the IAEP recipients following IVGTT were slower than in normal animals (K = 4.07 ± 0.33) ($p < 0.01$), they were much improved over the untreated diabetic controls (K = 0.34 ± 0.55) ($p < 0.05$).

Figure 4 shows the insulin and glucose levels in dog #1, one of the two dogs which were studied for 36 h. After the implantation blood glucose level decreased from the initial value of 300 mg/dl to 100 mg/dl in 2 h and stabilised within the normal range. This paralleled the rise in plasma insulin from the pre-implantation value of 12 mU/l to a peak of 32 mU/l. At 21 h, the unit was removed from the animal. Plasma insulin then fell to pre-implantation levels within an hour. The glucose level rose progressively reaching 186 mg/dl at 28 h at which time an IVGTT yielded a diabetic curve. Reimplantation of the unit at 30.5 h was followed by a rise in plasma insulin level and a fall in glucose levels. At 36 h the unit became occluded, glucose rose and plasma insulin level fell again.

Table 1 shows the plasma glucose disappearance rates in 2 IAEP recipients following IVGTT in the following 4 periods of the experiment: (i) normal state before induction of diabetes, (ii) during diabetic state, (iii) after achieving normoglycaemia by IAEP implant, (iv) following the subsequent removal of the IAEP. Much improved glucose disappearance rates were achieved when the IAEP were present in the diabetic dogs and they reverted to diabetic values upon removal of the unit.

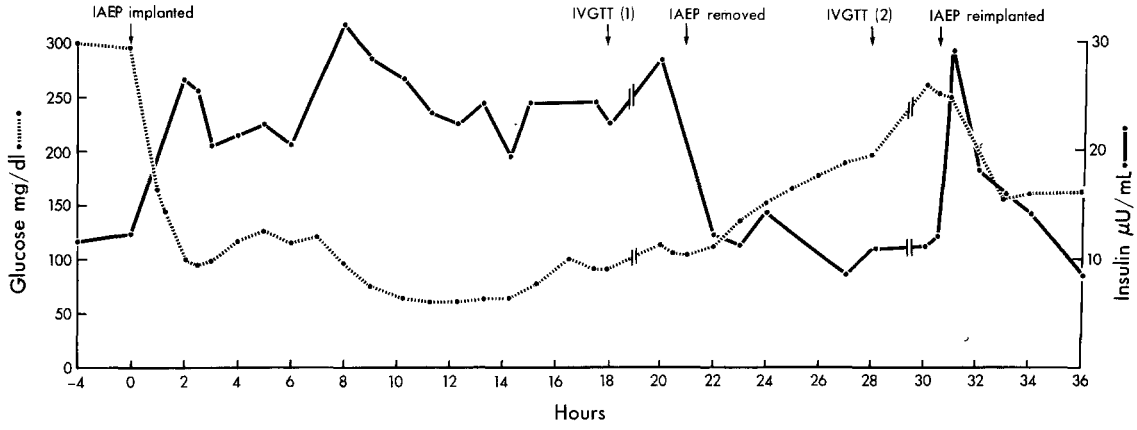

Fig. 4. The plasma glucose (●·····●) and insulin (●——●) levels in one dog following implantation of an artificial capillary unit containing xenogeneic rat islets (1000 islets/kg body wt). The time of implantation, removal, re-implantation and IVGTT's are indicated

Table 1. Glucose disappearance rates (K) during IV glucose tolerance tests in two diabetic dogs with repeated implantation of an artificial capillary unit (IAEP) containing rat islets

K rate / Dog	Normal state	Diabetic	Diabetic with IAEP	Diabetic subsequent to IAEP removal
1	3.3	0.3	1.8	0.3
2	3.7	0.5	2.5	0.4

Discussion

Data obtained from this study demonstrate the ability of the IAEP containing rat islets to release insulin and restore normoglycemia in experimental diabetic dog recipients. In addition, the IAEP was effective in releasing insulin following an intravenous glucose tolerance test, with an improvement in the glucose disappearance pattern. The quantity of pancreatic islets used for transplantation in the dog model was found to be critical [12]. In the present study, a quantity calculated at 1000 islets/kg body weight was found sufficient to improve glucose homeostasis in the streptozotocin-alloxan diabetic dog. The metabolic response of the diabetic dogs to the IAEP was variable, as demonstrated by the wide range of plasma glucose and insulin values. Since xenogeneic rat islets were used, individual sensitivity of the recipient dogs to rat insulin may be one contributing factor to this variation. Another factor could be variation in the quality of the islets used in the study. Since islets are collected for implantation over a period of 2 days, it is possible that some islet preparations were better than others. The delay in insulin response during IVGTT in IAEP recipients is likely to be due to the transit time across the artificial capillary membranes. Only further improvement of the membrane characteristics could allow more rapid responses to blood glucose concentrations.

The design in the prototype used in the present study furnishes some of the basic requirements for an IAEP. The artificial capillary made from acrylic copolymer (XM50) is non-toxic, sterilisable, has a nominal molecular weight cut off and a very smooth inner surface. Despite the smooth inner surface the material is still thrombogenic. To improve the biocompatibility of the unit with respect to blood coagulation the artificial capillary had a significantly greater internal diameter was used than previously. Since the thickness of the fibre wall has not been changed, the efficiency of transport of smaller molecules such as insulin and glucose through the capillary membrane should not be affected. This is evident from the data which showed that the lag time in the rise of insulin level following IAEP implantation was similar to those of rat experiments reported previously [7]. A single capillary in the form of a coil is of importance since it improves the contact surface area between the implanted islets and the host circulation. This design also eliminates the manifold system which is a major site of thrombus formation in multifibre units. The enlarged extra-capillary space of 1.5 ml in this unit accommodates the required number of implanted islets adequately and also provides for rapid exchange of insulin and glucose between the implanted islets and the host circulation. Two portals in the unit allow for the introduction of donor islets and nutrient medium in vitro as well as for the introduction of additional islets in vivo following implantation. The capillary fibre when connected

end to end to the internally bevelled connector tips should minimise turbulence where blood enters and leaves the unit.

In the present investigation a long term study of the dogs would have been desirable, but the duration has been minimised by either excessive bleeding from an intensive anticoagulation regime or thrombus formation within the artificial capillary unit. Long term study of this system must await improvement of the blood compatibility of artificial capillary fibres, perhaps through regional anticoagulation by grafting heparin onto the capillary fibre to reduce the thrombogenicity, or growing an endothelial cell lining on all blood contact surfaces of the IAEP.

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