

C-Peptide responses after meal challenge in mice transplanted with microencapsulated rat islets

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Abstract

Aims/hypothesis. This study aimed to assess a response of microencapsulated rat islets to a meal challenge after being transplanted intraperitoneally into diabetic mice.

Methods. Microencapsulated rat islets or control naked syngeneic mouse islets were transplanted intraperitoneally into mice with streptozotocin-induced diabetes. Meal challenges were done 3, 6 and 9 weeks after transplantation. Glucose-induced insulin secretion from microencapsulated islets before and after transplantation was assessed in vitro.

Results. Within the first week, all animals transplanted with either microencapsulated rat islets or with syngeneic murine islets became normoglycaemic (< 11 mmol/l). At 4 and 6 weeks, body weight was less than normal in the non-diabetic control mice. Mice with the encapsulated rat islets had lower fasting glucose concentrations and more rapid glucose clearance after a meal challenge than the control mice. The group of mice with transplanted syngeneic

islets had similar glucose profiles to control mice, except for slightly accelerated glucose clearance. The C peptide responses of mice with either microencapsulated or naked islets were clearly lower than the controls. An increase of C peptide appeared as early as 20 min in the plasma of the group with encapsulated islets, but this was considerably slower than in the other two groups. Microencapsulated rat islets retrieved 9 weeks after transplantation did not lose their ability to respond to glucose, but their output was less than half of the pretransplant control islets.

Conclusion/interpretation. The delivery of C peptide and presumably the accompanying insulin are delayed by restrictions of the capsules and the peritoneal location. However, this delay in reaching peripheral target organs does not prevent microencapsulated grafts from efficiently clearing glucose after a meal. [Diabetologia (2001) 44: 646–653]

Keywords Microencapsulation, transplantation, pancreatic islet, C peptide, meal challenge, diabetic mice.

For transplantation of pancreatic islets into patients with Type 1 (insulin-dependent) diabetes mellitus, microencapsulation could provide protection against immune destruction. Such protection has been dem-

onstrated in several animal models, in which random glucose concentrations have been maintained in the normal range for extended periods of time: in rodents [1–8], dogs [9,10], or cynomolgus monkeys [11]. At present, it has only been proved practical to place microcapsules into the peritoneal cavity but it is not clear if this site allows adequate exchange of nutrients, metabolites and insulin between the encapsulated islets and the systemic circulation. To our knowledge, there are few studies of glucose regulation by transplanted microencapsulated islets and these mostly describe only glucose concentrations without providing information about corresponding insulin

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Abbreviations: STZ, Streptozotocin; KRH, Krebs-Ringer Hepes buffer, GLP-1, glucagon-like peptide 1.

secretion. A previous study from our group, which evaluated rats with microencapsulated islet allografts with an intravenous glucose tolerance test and a meal challenge, showed a lack of insulin responses and slower clearance of blood glucose compared to normal animals [12]. Improvement of biocompatibility of microcapsules did not improve glucose clearance [13]. Insulin infusion into the peritoneal cavity, which was intended to mimic insulin release from microcapsules, was followed by a sluggish rise of insulin in the peripheral circulation [14]. These results suggest that insulin had been secreted into the peritoneal cavity but probably in a manner that adsorption by local tissues prevented insulin from reaching the peripheral concentrations in detectable amounts. In contrast to our previous results, plasma insulin responses have been found in rats transplanted with microencapsulated islets, but this response was impaired when compared to normal animals and the authors did not exclude regeneration of the host pancreas [15].

We assessed how beta cells in microencapsulated rat islets respond to a meal challenge after intraperitoneal transplantation into diabetic mice. The challenges were done at 3, 6 and 9 weeks after transplantation and instead of measuring insulin, we assessed C peptide, which is released in equimolar concentrations with insulin and does not undergo hepatic extraction [16]. Glucose-induced insulin secretion was carried out *in vitro* to compare the response of microencapsulated islets before transplantation and after retrieval at the end of the study.

Methods

Animals. Male B6AF₁ mice (Jackson, Bar Harbor, Me., USA) aged 6 to 7 weeks were made diabetic with *i.p.* injections of streptozotocin (STZ) (180 mg/kg body weight, Sigma, St. Louis, Mo., USA) freshly dissolved in citrate buffer pH 4.5. Animals with blood glucose concentrations above 19.4 mmol/l were used as transplant recipients. Male Sprague Dawley rats (Taconic, Germantown, N.Y., USA) or male B6AF₁ mice were used as donors. Non-fasting blood glucose concentrations of all animals and body weights were measured on the day of transplantation and then weekly for next 9 weeks. Blood glucose concentrations were measured with a One Touch portable glucometer (Lifescan, Milpitas, Calif., USA). Of note, blood glucose values obtained with this method are about 40% lower than plasma concentrations found with standard glucose oxidase techniques [17]. Graft failure was recognized when blood glucose surpassed 11 mmol/l on two consecutive measurements. All animal procedures were approved by the Animal Care Committee of the Joslin Diabetes Center.

Islet isolation. Rat and mouse islets were isolated with a technique described elsewhere [18]. Briefly, 1 to 2 mg/ml of a collagenase P (Boehringer Mannheim, Indianapolis, Ind., USA) solution was injected into the pancreatic duct, and the pancreas was digested for 19 min at 37°C; islets were then separated from the exocrine tissue using discontinuous density gradient centrifugation with Histopaque-1077 (Sigma Chemical, St.

Louis, Mo., USA). Islets with diameters of 50 to 250 µm were hand-picked, counted, and cultured for 1 to 2 days in RPMI-1640 containing 10 mmol/l glucose and supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 mg/ml streptomycin (all from Mediatech, Herndon, Va., USA).

Microencapsulation. Before encapsulation, alginate (40% guluronic acid and 60% mannuronic acid, Keltone LV, Kelco International, London, UK) was purified according to a method described elsewhere [13]. Microcapsules were produced using a 3% alginate solution [13], this being a concentration that provides the viscosity required to provide mechanically strong and uniformly spherical capsules; it also minimizes the effect of swelling and shrinking on the formation of inadequate capsules [19]. Before encapsulation, islets were washed with culture medium and then with Ca⁺⁺-free Krebs-Ringer HEPES Buffer (KRH). The pellet of islets was suspended in sterile filtered alginate and transferred to an air-driven droplet generator. The alginate solution containing 2000 islets/ml was extruded through a blunted 23-gauge needle with an airstream pressure of 9 to 10 cm H₂O to form droplets, which were instantly gelled in 100 mmol/l CaCl₂. The alginate beads were then coated with 0.1% Poly-L-lysine (Sigma, mol. wt. 15 000–30 000) for 10 min followed by 5 min incubation in a 10 times diluted alginate solution and finally triple washed with KRH. The diameter of microcapsules was in the range of 600 µm to 800 µm. It has been shown previously, that capsules prepared with our method were not permeable to substances larger than 100 000 M_r [20].

Transplantation and retrieval of microcapsules. Recipient mice were anaesthetized with Metofane (Schering-Plough Animal Health, Union, N.J., USA). The microcapsules (volume of 0.4–0.5 ml) were suspended in 2 ml of KRH and injected into the peritoneal cavity with a 16-gauge cannula via a small incision (3 mm) in the linea alba. The muscle layer of the abdomen was closed with a suture and the skin layer was closed with surgical clips.

Nine weeks after transplantation, peritoneal lavage was performed by infusing 5 ml of KRH through a 2 cm incision into peritoneal cavity with subsequent flushing of the abdomen above a 50 ml centrifuge tube. The abdominal cavity was then flushed two to three times with KRH.

Samples of microcapsules were fixed in 4% paraformaldehyde, embedded in paraffin and stained with haematoxylin.

Experimental groups. Two experimental groups and one control group were followed for 9 weeks: (1) STZ-diabetic mice transplanted intraperitoneally with 1000 xenogeneic microencapsulated rat islets; (2) STZ-diabetic mice transplanted intraperitoneally with 1000 syngeneic murine non-encapsulated islets; (3) Age-matched normal non-diabetic mice.

Meal Challenge. Three, 6 and 9 weeks after transplantation animals were fasted overnight (15 hr) and then given food pellets for 10 min (Mouse Chow 9F containing 20% protein, 9% fat, 3% fiber, 6.5% ash, and 2.5% minerals; Purina Mills, Richmond, Ind., USA). To avoid competition for food, each mouse was subjected to a meal challenge in a separate cage. To obtain plasma, blood samples from snipped tails were collected into heparinized capillary tubes before food intake, and 20, 60 and 120 min thereafter. For C peptide measurement, plasma samples were stored at –80°C prior to radioimmunoassay (Linco, St. Charles, Mo., USA). Radioimmunoassay kits were modified for small blood samples by reducing the manufacturer's recommended sample and diluent volumes by two; from 100 µl to 50 µl. The cross-reactivity of antibodies used in assay

was 100% for rat and mouse C peptides. Rat C peptide was used for standard concentrations.

Insulin secretion in vitro. Glucose-induced insulin secretion was studied in two groups of microencapsulated islets: those cultured for 4 days after encapsulation and those retrieved from animals by peritoneal lavage 9 weeks after transplantation. Samples of handpicked non-overgrown microcapsules containing a total of 10 islets with 100 to 150 μm diameter were collected into 12 mm cell culture inserts with 3.0 μm pore membrane (Millicell-PC, Millipore Corporation, Bedford, Ma., USA). Before glucose stimulation, microcapsules containing islets were washed twice with RPMI containing no glucose by transferring them in cell culture inserts to wells containing fresh medium. After 30 min preincubation in RPMI at a glucose concentration of 5.6 mmol/l, capsules were moved to wells containing RPMI with either 5.6 mmol/l glucose, 16.7 mmol/l glucose or 16.7 mmol/l glucose supplemented with 10 mmol/l theophylline. After a 60 min incubation, inserts with microcapsules were removed and media were collected and frozen at -20°C pending insulin measurement with radioimmunoassay using rat insulin standards (Linco). The same procedure was applied to control non-encapsulated rat islets obtained from the same isolation cultured under the same conditions for 4 days.

Statistical analysis. Results are presented as means \pm SEM. For statistical comparison, the unpaired, paired Student's *t*-test or analysis of variance (ANOVA) with PLSD Fisher test were used. Differences were considered to be statistically significant if the *p* value was less than 0.05.

Results

Transplantation and retrieval of microcapsules. All animals transplanted with either microencapsulated rat islets or with syngeneic murine islets became normoglycaemic (< 11 mmol/l) within the first week after transplantation. Blood glucose concentrations remained stable for the rest of the study in 7 of 11 mice transplanted with microencapsulated rat islets. Two grafts failed, probably because of substandard quality of the islets, which fits with the poor performance of islets from these batches maintained in tissue culture. The other two grafts were probably unsuccessful because of severe cellular overgrowth that could have been caused by infection introduced during transplantation. The rejection time for SD rat islets transplanted into B6AF₁ mice in our previous study was 14 ± 1 days [21]. For mice transplanted with syngeneic islets, 5 out of 6 remained normoglycaemic (one mouse became hyperglycaemic 2 weeks after transplantation). The blood glucose profiles of mice with stable grafts are shown in Figure 1A. During the first 2 weeks after transplantation, mice with transplanted microencapsulated rat islets had glucose concentrations of 4.1 ± 0.2 mmol/l and 4.6 ± 0.6 mmol/l at 1 and 2 week, respectively, which were lower than concentrations of normal control mice (6.8 ± 0.2 mmol/l and 6.8 ± 0.3 , respectively) ($p < 0.01$). Mice with naked syngeneic islets were in-

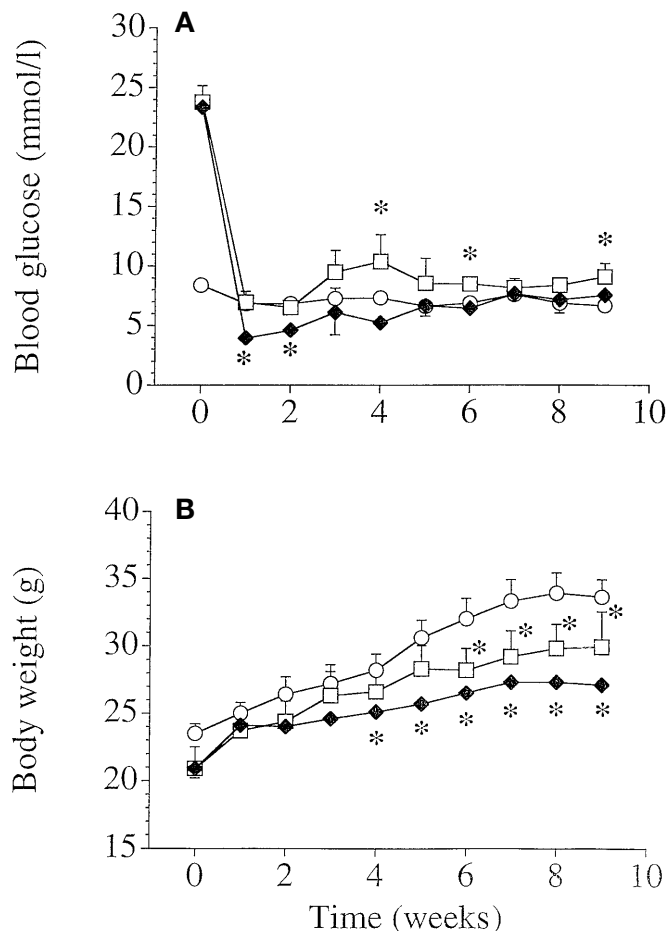


Fig. 1(A, B). Non-fasting blood glucose concentrations (A) and body weights (B) of STZ diabetic B6AF₁ mice transplanted intraperitoneally with either microencapsulated xenogeneic rat islets (◆) ($n = 7$) or syngeneic non-encapsulated mouse islets (□) ($n = 5$). As controls, normal non-diabetic B6AF₁ mice were used (○) ($n = 6$). Data are presented as means \pm SEM; Standard error bars for the group of animals transplanted with microcapsules were very small and therefore overlapped with the plot symbols. * $p < 0.05$ vs. normal mice

clined to have higher glucose concentrations than normal controls 3 weeks after transplantation. This tendency persisted for the rest of the study reaching a statistical difference at several time points (4, 6, and 9 weeks, $p < 0.02$).

At the beginning of the experiments, diabetic mice were slightly lighter than normal controls (Fig. 1B). After early weight gain, the mice with either intraperitoneal transplanted islets or encapsulated islets had statistically significant lower body weight than the normal control mice.

As measured by volume, most of the capsules could be retrieved by peritoneal lavage (60–100% of the initial volume). Microscopic evaluation showed that the fraction of fully overgrown capsules varied from 9 to 48% of the total. Histology has shown that the cellular overgrowth did not necessarily accompany a lack of surviving beta cells inside the capsules

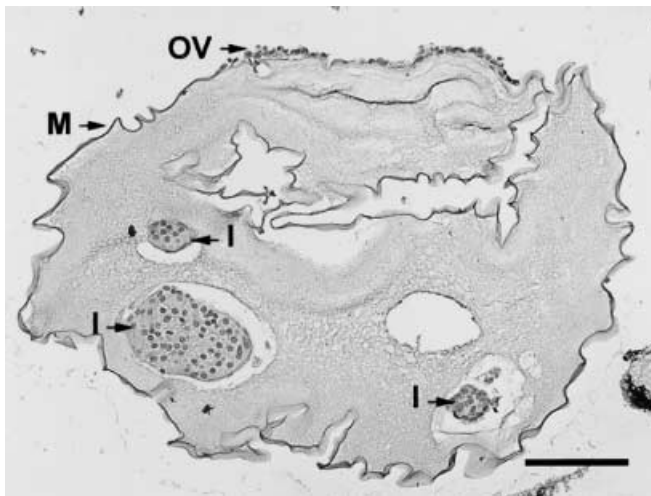


Fig. 2. Alginate-polylysine microcapsule containing rat islets retrieved 9 weeks after transplantation into STZ-diabetic mouse (5 μ m paraffin-embedded histological section, haematoxylin stain). M = membrane; I = islet; OV = layer of cells overgrowing the capsule; magnification bar = 100 μ m

(Fig. 2). In fact, in some non-overgrown microcapsules, only remnants of islets could be seen.

Meal challenge. Animals tolerated the overnight fast very well and, after receiving food pellets, ate without hesitation. Plasma glucose values and areas under the curve (AUC) after food intake for all experimental groups at three different time points after transplantation are shown in Table 1. Glucose profiles at 9 weeks (Fig. 3) provide an example of the patterns, which are similar to 3 and 6 weeks. The results for the group with the encapsulated rat islets are strikingly different from those of the other two groups. The fasting glucose concentrations of the mice with encapsulated rat islets were clearly lower at all time points. This phenomenon might have been caused by differences in fasted and fed blood glucose concentrations in mice and rats. Normal B6AF₁ mice have

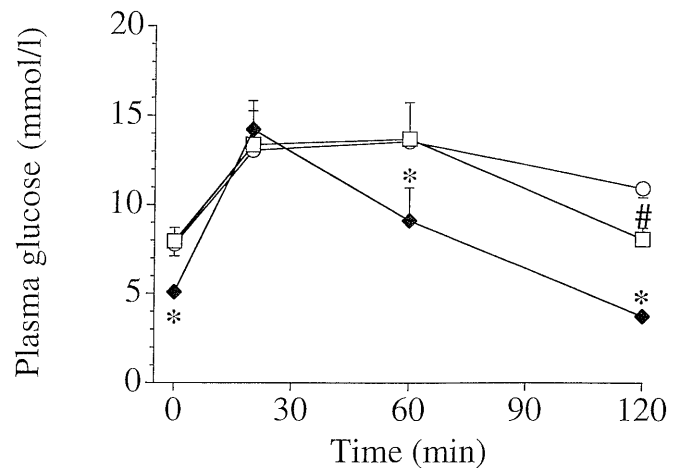


Fig. 3. Plasma glucose concentrations during meal challenges of STZ diabetic B6AF₁ mice. The test was performed 9 weeks after i.p. transplantation of either microencapsulated xenogeneic rat islets (◆) ($n = 7$) or syngeneic non-encapsulated mouse islets (□) ($n = 5$). As controls, age-matched normal non-diabetic B6AF₁ mice were used (○) ($n = 9$). Data are presented as means \pm SEM; * $p < 0.02$ microcapsules vs normal and syngeneic, # $p < 0.001$ syngeneic vs normal

fasted blood glucose concentrations of 5.1 ± 1.1 mmol/l whereas SD rats have 3.3 ± 0.1 mmol/l and fed blood glucose concentrations of 6.9 ± 0.2 mmol/l and 4.4 ± 0.1 mmol/l, respectively. We have reported previously similar results in Swiss Webster nude mice transplanted subcutaneously with macroencapsulated SD rat islets [22]. Despite the lower starting values, the peak glucose concentrations at 20 min were comparable to those of the other groups. Moreover, the microencapsulated group had a much faster rate of glucose clearance, with glucose concentrations decreasing to considerably lower values at 120 min. In fact, for the 6 and 9 week groups, glucose concentrations were even lower than the fasting values, being about 3.6 mmol/l at 120 min compared with fasting values of about 5.0 mmol/l.

Table 1. Plasma glucose after meal challenge in STZ diabetic B6AF₁ mice transplanted with microencapsulated xenogeneic islets

| Groups | Weeks after Tx | 0 min | 20 min | 60 min | 120 min | Area under the curve | Number of animals |
|----------------------|----------------|---------------|----------------|----------------|----------------|-----------------------------|-------------------|
| | | mmol/l | | | | mmol · min /l | |
| Microencapsules-xeno | 3 | 4.3 \pm 0.2 | 11.7 \pm 1.1 | 6.5 \pm 1.0 | 5.7 \pm 1.8 | 890 \pm 126 | 6 |
| | 6 | 4.8 \pm 0.4 | 13.3 \pm 1.8 | 7.5 \pm 1.3 | 3.5 \pm 0.4 | 926 \pm 126 ^a | 7 |
| | 9 | 5.1 \pm 0.3 | 14.2 \pm 1.6 | 9.1 \pm 1.0 | 3.7 \pm 0.2 | 1045 \pm 141 ^b | 7 |
| Syngeneic | 3 | 6.7 \pm 0.6 | 12.5 \pm 1.2 | 9.4 \pm 1.3 | 6.7 \pm 0.6 | 1154 \pm 115 | 5 |
| | 6 | 6.2 \pm 0.6 | 12.8 \pm 1.1 | 11.0 \pm 1.0 | 8.7 \pm 1.0 | 1189 \pm 67 | 5 |
| | 9 | 8.2 \pm 0.7 | 14.0 \pm 1.6 | 14.2 \pm 1.7 | 8.1 \pm 0.5 | 1456 \pm 118 | 5 |
| Normal | 1 | 5.6 \pm 0.4 | 11.9 \pm 0.4 | 9.4 \pm 2.1 | 8.5 \pm 1.4 | 1207 \pm 179 ^c | 9 |
| | 6 | 7.9 \pm 0.7 | 11.3 \pm 0.3 | 11.7 \pm 0.5 | 9.6 \pm 0.4 | 1292 \pm 29 ^c | 9 |
| | 9 | 7.8 \pm 0.7 | 13.1 \pm 0.4 | 13.6 \pm 0.4 | 11.0 \pm 0.5 | 1468 \pm 42 | 9 |

As controls, syngeneic non-encapsulated islets transplanted i.p. and normal non-diabetic B6AF₁ mice were used. Data are presented as means \pm SEM; ^a $p < 0.01$ vs normal at 6 weeks, ^b $p < 0.02$ vs syngeneic and normal at 9 weeks, ^c $p < 0.05$ vs normal at 9 weeks

Table 2. Plasma C peptide after meal challenge in STZ diabetic B6AF₁ mice transplanted with microencapsulated xenogeneic islets

| Groups | Weeks after Tx | 0 min | 20 min | 60 min | 120 min | Area under the curve | Number of animals |
|----------------------|----------------|----------|-----------------------|-----------|----------|---------------------------|-------------------|
| | | pmmol/l | | | | pmol · min/l (thousands) | |
| Microencapsules-xeno | 3 | 58 ± 19 | 117 ± 18 ^a | 239 ± 43 | 174 ± 43 | 21.2 ± 3.6 | 6 |
| | 6 | 86 ± 15 | 127 ± 31 | 258 ± 38 | 238 ± 26 | 24.7 ± 2.8 | 7 |
| | 9 | 91 ± 22 | 132 ± 20 | 259 ± 38 | 236 ± 47 | 24.9 ± 3.5 | 7 |
| Syngeneic | 3 | 114 ± 49 | 235 ± 60 | 135 ± 40 | 85 ± 32 | 15.9 ± 5.7 | 5 |
| | 6 | 155 ± 50 | 217 ± 62 | 164 ± 26 | 163 ± 64 | 22.0 ± 8.0 | 5 |
| | 9 | 141 ± 30 | 296 ± 52 | 236 ± 42 | 216 ± 29 | 28.6 ± 3.9 | 5 |
| Normal | 1 | 217 ± 33 | 426 ± 143 | 241 ± 111 | 199 ± 40 | 33.2 ± 6.3 | 9 |
| | 6 | 297 ± 36 | 582 ± 39 | 407 ± 28 | 378 ± 48 | 52.1 ± 3.1 ^b | 9 |
| | 9 | 229 ± 38 | 571 ± 68 | 534 ± 74 | 562 ± 98 | 66.0 ± 9.0 ^{c,d} | 9 |

As controls, syngeneic non-encapsulated islets transplanted i. p. and normal non-diabetic B6AF₁ mice were used. Data are presented as means ± SEM; ^a $p < 0.02$ vs microcapsules at 0 min, ^b $p < 0.01$ vs microcapsules and syngeneic at 6 weeks, ^c $p < 0.01$ vs microcapsules and syngeneic at 9 weeks, ^d $p < 0.02$ vs normal at 1 week

The group of mice with syngeneic islets in the peritoneal cavity had similar glucose profiles to control mice, except for faster glucose clearance as evidenced by lower glucose values at 120 min (at 9 weeks, 8.1 ± 0.6 versus 10.9 ± 0.5 mmol/l; $p < 0.001$).

The area under the plasma glucose curve was significantly lower for mice with microencapsulated rat islets at 6 and 9 weeks compared to normal controls ($p < 0.02$) and at 9 weeks compared with the group transplanted with syngeneic islets. This difference is largely accounted by the decreased glucose concentrations found at 0, 60 and 120 min. In all three groups there was a tendency for the glucose AUC to increase from 3 to 9 weeks.

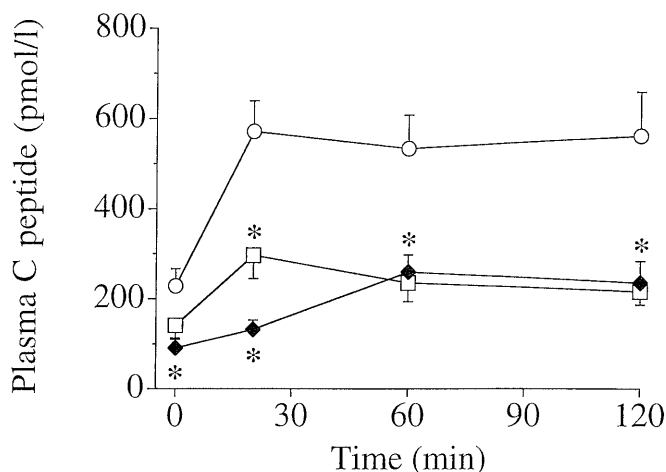


Fig. 4. Plasma C peptide concentrations during a meal challenge of STZ diabetic B6AF₁ mice. The test was done 9 weeks after i. p. transplantation of either microencapsulated xenogeneic rat islets (◆) ($n = 7$) or syngeneic non-encapsulated islets (□) ($n = 5$). As controls, age-matched normal non-diabetic B6AF₁ mice were used (○) ($n = 9$). Data are presented as means ± SEM; * $p < 0.01$ microcapsules and syngeneic vs normal

C peptide responses in vivo. Plasma C peptide values and AUCs from the meal challenges are shown in Table 2. Values for the 9 week point are depicted in Figure 4. For the group with encapsulated rat islets at 3, 6 and 9 weeks, C peptide values at 20 min were higher than fasting values but significance was only found at 3 weeks (58 ± 19 rising to 117 ± 18 pmol/l, $p < 0.02$). Unambiguous increases in this group were found at 60 and 120 min at all three time points after transplantation. Notably, the responses of the group with encapsulated islets were considerably slower than in the other two groups, with this being most obvious at the 20 min time point. It should be noted that the C peptide responses of the mice with naked syngeneic islets in the peritoneal cavity were clearly lower than those of control mice. This was surprising because the glucose clearance was faster in spite of these lower C peptide values, while the glucose values at 120 min were significantly lower at the 6 and 9 week time points (Table 1). When the AUCs of the C peptide responses were analysed, the values for the microencapsulated rat islet group and naked syngeneic islet group were similar. However, the AUCs for the C peptide responses of the normal mice were about twice as high as those of the two transplanted groups.

Insulin secretion in vitro. A glucose concentration of 5.6 mmol/l was used as a basal concentration prior to high glucose stimulation, since plasma concentrations found after overnight fasting in rodents were approximately in this order of magnitude. Microencapsulated rat islets cultured for 4 days responded to the high glucose stimulus; insulin release rose from 41 ± 8 pg · islet⁻¹ · h⁻¹ in the basal state to 150 ± 40 pg · islet⁻¹ · h⁻¹ with 16.7 mmol/l glucose, and to 531 ± 70 pg · islet⁻¹ · h⁻¹ with 16.7 mmol/l glucose and 10 mmol/l theophylline (Fig. 5). The amount of se-

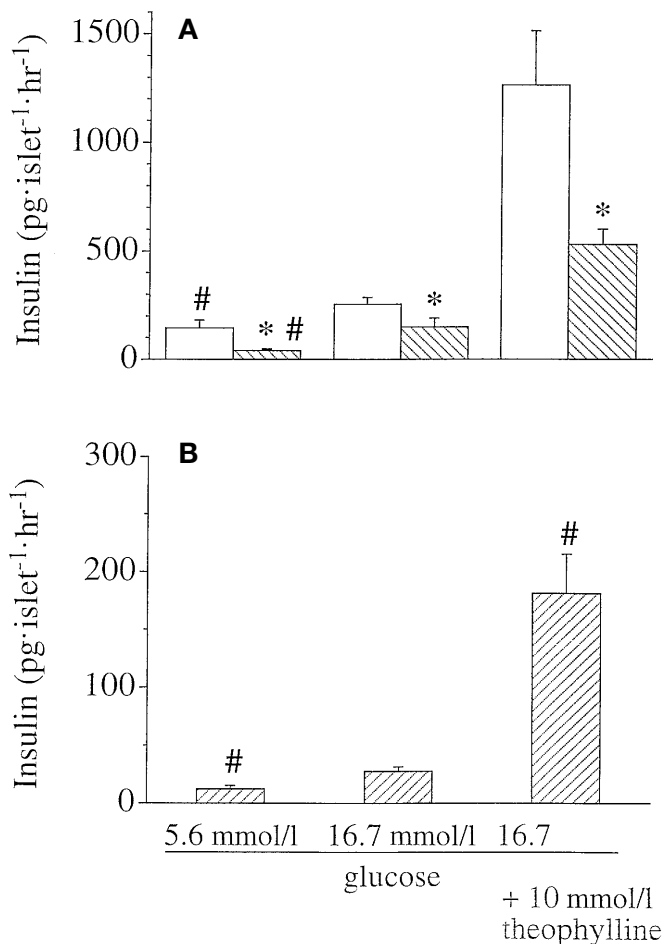


Fig. 5 (A, B). Glucose-induced insulin release by microencapsulated (▨) and non-encapsulated (□) rat islets cultured for 5 days (A), and by microencapsulated rat islets retrieved (▨) from STZ diabetic B6AF₁ after being transplanted for 9 weeks (B) (Please note the difference in scale). Data are presented as means from 2–4 separate experiments done in triplicates ± SEM; **p* < 0.01 microencapsulated vs non-encapsulated, #*p* < 0.05 vs. secretion at 16.7 mmol/l glucose from the same group

creted insulin, however, was substantially lower than that secreted by control non-encapsulated rat islets, which increased from a basal concentrations of 144 ± 36 to 254 ± 32 $\text{pg} \cdot \text{islet}^{-1} \cdot \text{h}^{-1}$ in the presence of high glucose. A further increase was found in the combined presence of glucose and theophylline. Microencapsulated rat islets retrieved 9 weeks after transplantation did not lose their ability to respond to either glucose or theophylline, but their output was less than half of encapsulated islets cultured for 4 days.

Discussion

Little information is available about the in vivo dynamics and quantity of insulin release from encapsulated islets. Our study provides information about

the delivery of C peptide from microencapsulated rat islets into the peripheral circulation of mice. We conclude that the delivery of C peptide and presumably the delivery of insulin, which accompanies C peptide with equimolar quantities, are delayed by restrictions of the capsules and the peritoneal location. That delay in reaching peripheral target organs does not prevent these grafts from being efficient enough to maintain normal glucose concentrations.

We found at 3 weeks after transplantation, a significant increase of C peptide released from microcapsules at the 20 min time point, although the increases at 6 and 9 weeks did not reach statistical significance. Clear statistical significance was found 40 min later in all groups. These data suggest that some release occurs before 20 min, which might have more important effects upon liver than muscle. Much more release occurs after 20 min and it seems likely that samples taken at the 30 min point would have shown statistically significant increases. The question arises as to how much insulin effect might be exerted during the 10 to 20 min period, compared with later periods. Perhaps the rat islets used in this study are uniquely sensitive to gut hormones released from mice during a meal. Unfortunately, it was not feasible to carry out intravenous challenges with glucose or other secretagogues such as arginine or glucagon-like peptide 1 (GLP-1) because of the small size of the mice.

When C peptide and insulin released in the peritoneal cavity enter the portal circulation, a large portion of the insulin is taken up by the liver, while C peptide is hardly extracted at all. Therefore, in our previous study, insulin could have been released and exerted important effects on the liver but have been difficult to measure in peripheral plasma. This explanation might be supported by the fact that intraperitoneal insulin infusions mimicking insulin release from capsules were associated with only modest insulin increases in systemic circulation, and delayed glucose clearance [14].

Similarly, in diabetic mice transplanted with islets encapsulated in hollow fibers, glucose concentrations were normalized, but peripheral insulin concentrations were low and did not increase after glucose challenges [23]. We have also observed rapid glucose clearance in rats with alginate macrobeads (about 3 mm in diameter) containing syngeneic islets, but found no measurable increases in circulating insulin concentrations after challenges with intravenous glucose, arginine and GLP-1 [24]. In contrast, modest and delayed increases of insulin concentrations were found after meal challenges. We concluded that much of the restriction was due to the long time required for diffusion out of such large capsules.

Studies on encapsulation within smaller microcapsules (diameter 250–350 μm) or by conformal coating (50 μm layer) resulted in fast glucose clearance during glucose tolerance tests in mice transplanted with

porcine islets [25, 26]. Another study, however, showed completely normal oral glucose tolerance test in rats with pig islets in conformal microcapsules [27]. Neither insulin nor C peptide data were reported in these papers.

Studies in larger animals do not provide much additional information. A previous study reported that in monkeys transplanted with microencapsulated porcine islets, intravenous glucose challenges were accompanied by about a threefold increase in insulin concentrations but some of the insulin might have been from residual beta cells in the monkeys' pancreata. These responses were not compared with normal control monkeys [11]. In a study of diabetic dogs transplanted with canine islets in relative large microcapsules, C peptide results were limited to one dog, but the C peptide response gradually increased for the 60 min after the intravenous glucose challenge [10]. In other dog experiments, a flat shaped dose-response curve for C peptide was found after the administration of intravenous glucose [9].

In attempts to understand the dynamics of insulin release from encapsulated islets, investigators have often looked at the rate of release with *in vitro* experiments. Unfortunately, these kinds of studies are not likely to provide useful insights into what is happening *in vivo*. Rapid release of insulin has been found when either microencapsulated or macroencapsulated islets were perfused [5, 28] but the movement of fluid on the surface of the capsules during perfusion would be expected to markedly accelerate the movement of insulin from inside the capsules. The size of capsules should be associated with the rate of insulin release as can be predicted by the laws of diffusion [29, 30].

Many questions can be posed about the peritoneal site as a location for either naked or encapsulated islets. In the present experiments, the C peptide concentrations in plasma were considerably decreased in the groups with either naked or encapsulated islets in the peritoneal cavity than in the control mice. Surprisingly, glucose clearance was about the same for these transplanted mice although C peptide concentrations were half of those of the controls.

One possible explanation is that the peritoneal cavity is known to be less accommodating to transplanted islets. It has been found that naked syngeneic islets transplanted under the kidney capsule or into the portal vein of diabetic rats were much more efficient in normalizing glucose concentrations than when transplanted into the peritoneal cavity [31–33]. Additionally, islets in microcapsules are possibly exposed to relative hypoxia because of lack of vascularization in peritoneal space. Of note, mice in this study received 1000 syngeneic islets whereas only 400 are usually required to normalize glucose concentrations when placed under the kidney capsule [34]. We have shown previously that about 40% of encapsulated islets are lost after transplantation [35].

The differences in body weight among the groups are potentially important. In spite of a rapid increase in weight in the two weeks after transplantation, the mice with encapsulated rat islets remained substantially lighter than the normal control rats. Even the mice with syngeneic islets in the peritoneal cavity became lighter than the control mice. Similar limitation in weight gain compared with controls was observed in rats transplanted with microencapsulated or naked islets [12, 33]. It is also possible that the presence of substantial volume of capsules in peritoneal cavity led to a loss in appetite. During these studies, food uptake was not measured but the experimental animals looked and behaved normally. Inflammation caused by the encapsulated islets could cause appetite problems but the majority of retrieved microcapsules were free of overgrowth and macroscopic evaluation did not show any signs of inflammation in the peritoneal membranes. An alternative explanation might be that the difference in body weight is caused by the substantially lower insulin amount in the systemic circulation of transplanted animals when compared to normal controls [35]. The decreased insulin concentration in the transplanted animals is adequate to provide optimal glucose clearance but it could be too low to completely abolish lipolysis between meals. By transplanting more islets, in order to account for the loss of functional islets associated with the isolation and transplantation procedures [35], we could increase the systemic insulin concentrations and thereby correct the differences in body weight.

Results obtained in free-feeding caged rodents might not allow us to fully understand the requirements for humans, who must be able to change insulin delivery to accommodate intermittent meals, overnight fasts and the challenges of exercise. It is known from experience with pump delivery of insulin in patients with Type I diabetes, that a constant rate of delivery of insulin will not provide adequate control of blood glucose concentrations. Satisfactory control can only be obtained if insulin is provided for meals and adjustments in delivery are made during an overnight fast. It seems likely based upon work with mechanical artificial pancreases that delays of insulin release of 10 to 15 min could be tolerable [36]. In conclusion, we suggest that the dynamics of insulin release from microencapsulated islets in the peritoneal cavity could be rapid enough to meet the demands of a normal lifestyle in humans.

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