Gradual Absorption of Intraperitoneal Insulin Results in Impaired Glucose Tolerance in Recipients of Microencapsulated Islet Grafts

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513

GRADUAL ABSORPTION OF INTRAPERITONEAL INSULIN RESULTS IN IMPAIRED GLUCOSE TOLERANCE IN RECIPIENTS OF MICROENCAPSULATED ISLET GRAFTS

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As a consequence of its volume, a microencapsulated islet graft can only be implanted into the peritoneal cavity. We studied the function of intraperitoneally implanted islet allografts microencapsulated in alginate-polylysine capsules. Recipients were subjected to intravenous (IVGTT) and oral glucose tolerance tests (OGTT) 4 to 6 weeks after implantation. All became normoglycemic within 5 days after implantation but were found to be glucose intolerant during IVGTT and OGTT, with maximal bloodglucoses of 11.7 ± 0.4 mM and 8.8 ± 0.4 mM, respectively, and there was no increase of plasma insulin levels. This impaired glucose tolerance may very well be the consequence of the fact that microencapsulated islets remain freely floating in the peritoneal cavity without vascular access which implies that insulin is not directly absorbed into the circulation, like with vascularized islets, but has to be transferred from the peritoneal fluid to the bloodstream. To investigate whether the transplantation site as such contributes to the interference with optimal transport kinetics between the islets and blood we infused insulin into the peritoneal cavity of conscious and freely moving rats to mimic the gradual release of insulin from an encapsulated islet graft. We observed virtually no rise of insulin levels and it took 30 minutes until glucose levels had dropped significantly, with infusion of 20 pM/min insulin during 15 min (the amount of insulin produced by a native rat pancreas in response to OGTT). With higher insulin doses, there was a dose dependent rise of insulin and decrease of glucose levels. When compared to intraportal infusions with the same insulin dosages, however, they were strongly delayed and reduced as well as prolonged. This strongly delayed effect of peritoneally infused insulin on both plasma insulin levels and glucose levels explains, the lack of elevation of insulin levels and the disturbed glucose tolerance in recipients of an encapsulated islet graft. With view on the clinical efficacy of the bioartificial pancreas, our findings indicate that we should focus on finding or creating a transplantation site which, more than the unmodified peritoneal cavity, permits for close contact between the bloodstream and the encapsulated islet tissue.

514

NORMOGLYCEMIA RESTORES BETA CELL REPLICATIVE CAPACITY IN TRANSPLANTED ISLETS EXPOSED TO CHRONIC HYPERGLYCEMIA.

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To study the effects of chronic hyperglycemia on beta cell replication in transplanted (Tx) islets, four groups of streptozocin diabetic C57BL/6 mice were Tx with 100 (Tx-100) syngeneic islets, an insuficient beta cell mass to restore normoglycemia. One month later (day 30), groups 3 and 4 received a second Tx of 250 islets (Tx-250), sufficient to restore normoglycemia, which was harvested 30 days later (day 60). Tx-100 was harvested 14 (group 1), 60 (groups 2 and 3) and 74 (group 4) days after Tx. Beta cell replication was determined by bromodeoxiuridine incorporation and expressed as percentage of positive beta cells. Groups 1 and 2 remained hyperglycemic through the study; groups 3 and 4 were hyperglycemic from day 0 to day 30 and normoglycemic from day 30 to day 60. Group 4 showed mild hyperglycemia between day 60 and 74 (harvesting of Tx-250 and Tx-100 respectively). Hyperglycemia increased beta cell replication after 14 (1.02±0.18%, $p<0.05$), but not after 60 days (0.60±0.23%), compared to islets exposed to normoglycemia (group 3: 0.42±0.07%). In group 4 beta cell replication was increased (1.20±0.29%, $p<0.05$) in response to increased metabolic demand after Tx-250 extraction. In summary, when Tx beta cells are chronically exposed to hyperglycemia beta cell replication became limited. Normoglycemia restored the beta cell replicative response to glucose even after one month of exposure to severe hyperglycemia, suggesting that prolonged hyperglycemia did not cause permanent damage.

515

CORRECTION OF GLUCOSE TOLERANCE TEST IN DIABETIC MICE BY PORCINE ISLETS ENCAPSULATED IN AN69 MICROTUBES.

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Purified porcine islets were encapsulated within a 80 cm long, 400 μm (i.d.), 600 μm (o.d.) microtube made of a hydrogel of polyacrylonitrile-sodium methallyl sulphonate (AN69, HOSPAL R&D). Two microtubes in the form of a spire were implanted in the peritoneal cavity of mice made diabetic by 5 consecutive injections of subdiabetogenic doses of streptozotocin. The aim of this work was to study correction of diabetes and the effect of a glucose load. Islets of Langerhans were isolated and purified from pig pancreas and maintained in culture for 3 days before transplantation. AN69 hollow fibers were seeded with islets suspended in 600 μl culture medium, corresponding to approx. 1/100 of the islets recovered from one porcine pancreas. 17 mice were transplanted. 1) In 11 mice plasma glucose decreased from 354 ± 16 to 197 ± 14 mg/dl within 3 days, and subsequently to 162 ± 17 mg/dl at day 17. Plasma glucose from the other 6 mice was higher at the time of implantation (444 ± 41 mg/dl, $p<0,05$), decreased to 322 ± 48 mg/dl at day 3, but then gradually increased to reach 410 ± 34 mg/dl at day 17. Implantation of empty tubes had no effect on glycaemia of diabetic mice. 2) Glucose tolerance tests (1 g/kg body weight, IP) were performed in 7 corrected mice 14 days after implantation. Initial plasma glucose concentrations were 368 ± 21, 181 ± 5, 165 ± 17 mg/dl in non treated diabetic mice, normal control mice, and in corrected-transplanted mice, respectively. Peak values, observed at 20 min, were 635 ± 85, 346 ± 11, and 308 ± 19 mg/dl, respectively, decreasing at 2 hours to 654 ± 118, 199 ± 16, and 192 ± 6 mg/dl. Fibers were explanted from 2 normalized mice. Thirteen days later, blood glucose concentration was in the diabetic range (345 and 407 mg/dl). In conclusion, diabetes was corrected in two thirds of the animals, and a complete correction of glucose tolerance test can be achieved in diabetic mice by xenografting porcine islets in AN69 microtubes.

516

CHARACTERISATION OF XENOGRAFT REJECTION OF MICRO-ENCAPSULATED ISLETS BY FACS-ANALYSIS

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Xenotransplantation of islets of Langerhans would help to overcome the limitations of human organ donor shortage. In this study, we present a method for the characterisation of xenograft rejection by Facs analysis of peritoneal cells. Material and Methods: Porcine islets were microencapsulated in barium alginate microbeads (mc-Tx). Non-encapsulated islets and empty microcapsules (empty mc) served as controls. 12,000 - 15,000 islet equivalents were transplanted intraperitoneally into non-diabetic Lewis rats (n=3 in each group). After 7 days cells were harvested by peritoneal lavage and characterised by Facs analysis. The following antibodies were used: 1F4 (CD3 T-lymphocytes), OX33 (B-lymphocytes); OX6 (MHC class II); OX42 (CD11b, iC3b); ED1 and ED2 (subgroups of monocytes and macrophages). Results: Total cell number was highest after mc-Tx (149,4 ± 30,1 x 10^6 cells) compared to empty mc (41,4 ± 19,7 x 10^6) and non-encapsulated Tx (18,1 ± 3,3 x 10^6). After mc-Tx the percentage of CD3 positive T-lymphocytes rose to 44,5 ± 11,5% compared with 4,9 ± 2,4% for empty controls. In case of non-encapsulated islets CD3 expression was 19,2 ± 8,2%. Moreover OX6 positivity was dramatically increased in the mc-Tx group (60,2 ± 8,9% vs. 15,2 ± 7,0% free islets vs. 4,9 ± 1,2% empty mc). Only a small amount of OX33 positive cells were detected with a slightly increased number after free Tx. ED1 expression was markedly higher after mc-Tx (37,6 ± 18,9%), but did not differ between the two other groups (1,0 ± 0,6% vs 1,0 ± 0,3%). OX42 as a constitutive marker of mononuclear cells was strongly positive in all three groups. Conclusion: Facs analysis of peritoneal cells after xenotransplantation shows a considerable T-cell activation and a hyperexpression of MHC class II molecules on mononuclear cells. Further studies have to evaluate the time course of the reaction, the metabolic aspect and the relevance of cut-off modifications.