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SUCCESSFUL REVERSAL OF HYPERGLYCAEMIA IN DOGS WITHOUT IMMUNOSUPPRESSION

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SUMMARY

A natural macro-capsule consisted of a full-term pregnant bitch's amniotic membrane and preserved porcine pancreatic islets of Langerhans was implanted intra-abdominally either in the omental pouch (group 1, n=6) or freely in the peritoneum (group 2, n=4) of alloxan induced diabetic mongrel dogs. This macro-capsule constituted a biocompatible and permi-selective immune barrier containing purified porcine islets. It was able to reverse hyperglycemia in the diabetic dogs and did not lead to the hazards of artificial devices such as interstitial acute and chronic inflammatory reactions, development of granulation tissue and intestinal adhesions. Moreover, revascularization of this natural membrane prolonged the survival and viability of islets.

INTRODUCTION

The immunologic problems related to islets transplantation may be overcome by the immunoisolation of the graft with micro- or macrocapsules. It is mandatory for the capsules to be biocompatible, impermeable to the immunocompetent cells, and freely permeable to nutrients and hormones (Soldani et al., 1989). Several studies examined the use of micro-capsulation techniques, using agarose gel capsules modified with oil (Iwata et al., 1989), polyornithine alginate (Calafiore et al., 1989).

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and polylysine alginate (Wu et al., 1988) or macro-capsules with cuprophan hollow fibers (Zekron et al., 1989) and polyurethane - silicone (Soldani et al., 1989). All of these trials have only been partially successful; because of allograft rejection was prevented, but graft function deteriorated due to massive fibrosis around the foreign material (Van Dervliet et al., 1991). To solve this problem, the authors have attempted the use of the amniotic membrane as a biocompatible natural membrane to form a macro-capsule (Mahgoub et al., 1992).

The aim of the present study was to assess the efficiency of Moghazy capsule as bioartificial pancreas in xenotransplantation.

MATERIALS AND METHODS

Pancreatic tissue was harvested from the pig after being anaesthetized and slaughtered. Warm ischemia was 10 to 15 minutes and cold ischemia was about 30 to 60 minutes. It was transferred to the lab under aseptic conditions. Preliminary preparation of the pancreatic tissue included removal of the fat and blood vessels. Digestion was done by collagenase type Xl (C), (sp. Act. 1.890U/mg-ó) in concentration of 1.8 mg C/1 ml pancreatic tissue (George et al., 1989). Purification of the digested tissue was done by Ficoll purification (Lymphoflot, density gradient solution 1.077 gm/ml, Biotest AG, Frankfurt) solution (Olack et al, 1991). Collection of islets from the interface was done and the viability was assessed microscopically using Trypan blue stain. The islets were then cultured on RPMI 1640 media (Biochrom KG, Berlin) for 24 hours at 37°C. The secretory function was assessed by glucose challenge test.

The ammniotic membrane was obtained from full-term pregnant bitch after laparo-hysterectomy. The membrane was washed with cold saline and crystalline penicillin (50.000 IU/100 ml saline) was added. The membrane was molded to form the macro-capsule into which the pig's pancreatic islets were introduced.

Diabetes was induced in ten dogs by injection of alloxan (100mg/kg b.w.). Fifteen days later their mean fasting blood glucose became 319.29±193 mg % and their mean weight 9.86±0.85kg. The prepared macrocapsule containing (12.000 purified porcine islets/kg.b.w. of diabetic dog) was then implanted into the peritoneum after preparing an omental pouch in six diabetic dogs (group l), while the same number of islets were implanted freely in the peritoneum in each of the other four dogs as a control group (group 2). Both groups did not receive any forms of immunosuppression. One- year post transplantation two animals with the functioning graft (group 1) were sacrificed. The capsule was harvested from the omentum and fixed in 10% buffered formaldehyde, subsequently paraffin embedded, sectioned, and stained with (H & E). Statistical analysis of data was performed according to the method of Snedecor and Cochran (1967) using T-test.

RESULTS

Three to six days post transplantation the insulin dose given to diabetic dogs of group (1) before transplantation was tapered and then stopped when the blood glucose level decreased to 136.14 ±37.47mg %. Dogs were considered insulin independent when this blood glucose was maintained without exogenous insulin for at least one week. While in group (2) the insulin dose was decreased for two days post-implantation then increased due to rising of blood glucose level to 384.7 ±42.76 mg %.

Six days post transplantation insulin assay by RIA was found to be increased from 8.29±1.98 I.U/ml to 12.16 ± 0.99 I.U/ml in group (1), while in group (2), it decreased to 7.47±1.8 I.U/ml.

One- month post transplantation there was a significant weight gain in group (1) from 9.88±0.8 kg to 13.38±1.8 kg .while in group (2) no significant change was recorded.

One- year post transplantation the histopathological examination revealed healthy viable islets with no infiltration with mononuclear inflammatory cells or islet necrosis as well as neovascularization of its wall (Fig. 1).

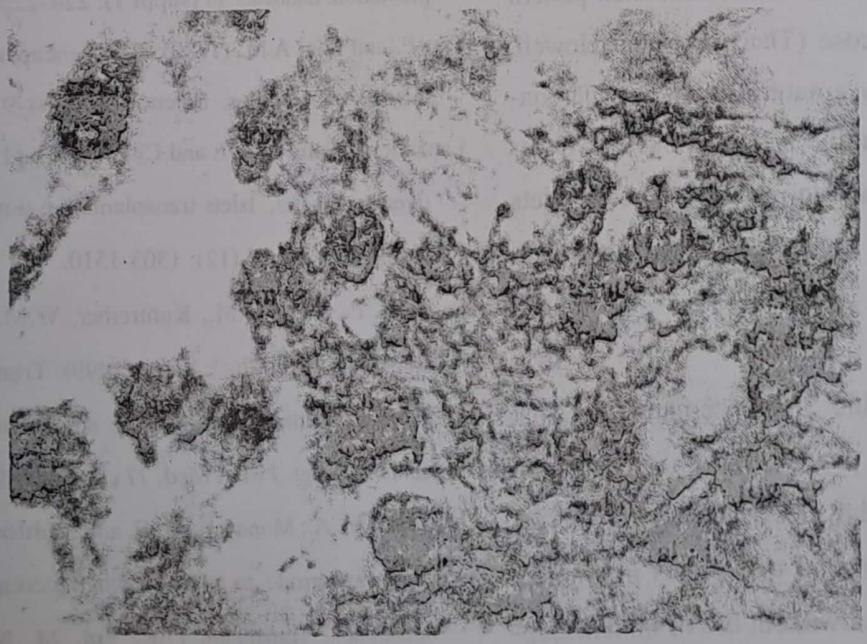

Fig. (1): Microscopic findings of pig pancreatic islets inside the capsule (H & E magnification x 400).

DISCUSSION

Immuno-isolation is mandatory to prevent islet graft directed immune destruction upon a graft in non immune suppressed diabetic high mammalian (O'Shea and Sun, 1986). One of the obstacles preventing successful clinical pancreatic islet transplantation is rejection, which was overcome by microencapsulation as agarose gel capsules modified with oil (Iwata et al., 1989), polyornithine alginate (Calafiore et al., 1989) and polylysine alginate (Wu et al., 1988). But long term failure, secondary to foreign body reaction, has not been overcome. In the present study a trial to solve this problem by using the amniotic membrane, because of its characteristics of histo-compatibility which does not express any foreign body reaction (Akle et al., 1981) as well as its diffusion pattern of insulin and glucose (Theodorou and Howell, 1979). In this study a natural macrocapsule constitutes a biocompatible and permi-selective immune barrier containing purified porcine islets was able to reverse hyperglycemia in alloxan induced diabetic dogs.

On conclusion: This study demonstrates that macroencapsulated porcine islets in a natural membrane exhibit the functional capacity to secrete insulin and respond to changes in ambient glucose concentration without use of immunosuppression.

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