O7-4 **A novel approach for local and temporary release of anti-inflammatory agent for prolongation of survival of islet xenografts**

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INTRODUCTION AND OBJECTIVES

During recent years, islet transplantation has been shown to be an efficacious method for the treatment of Diabetes Mellitus. Unfortunately, the technology of islet transplantation is only applied on a minor scale due to the necessity to apply lifelong immunosuppression. This obstacle can be overcome by microencapsulation of the islets.

Important advances have been made in encapsulation research during recent years. In spite of these advances survival of the graft is still limited to periods up to 6 months which is too short to merit clinical application. This limited graft survival is due to loss of up to 60% of the endocrine islet volume (De Vos et al. 1997) in the immediate period after transplantation. Recently it has been shown that this loss is caused by an up to now unrecognized inflammatory response in the immediate period after implantation.

Due to the mandatory surgery, after implantation the number of macrophages is increased at the transplantation site. In response to islet-derived cytokines (MCP-1) these macrophages secrete high quantities of deleterious cytokines (IL-1 and TNF) which stimulate the islets to produce even more islet-derived cytokines with a harmful circle of activation as a consequence. Deoxyspergualin (DSG) reduces the response and loss of encapsulated islets to a large extent (Hsu et al Cell Transplantation, 1999).

For the present study we developed an new encapsulation method where small poly-L-Lysine capsules with islets or liposomes are encapsulated in an second bead consisting of high-G alginate. Prior to the experiments we have studied *in vitro* the release of encapsulated DSG prepared with different lipid formulas. We studied the efficacy of the system by comparing survival rates of rat-islet xenograft implanted in the peritoneal cavity of mice.

MATERIALS AND METHODS

Concept

To reduce the immuunresponse directly after transplantation we developed an local and temporally diffusion system by encapsulating liposomes. We determent the optimal diffusion by testing the release profiles of three different liposomes composition encapsulated in alginate. The optimal composition was determined by comparing release profiles of carboxyfluorescein (CF, Sigma, St. Louis MO, USA) containing liposomes instead of DSG, since it has the same chemical properties and is less laboriously measurable.

The release profile of DSG was determined with the optimal liposomal formulation and the beneficial effect on transplantation survival was tested in an diabetic xenogenic transplantation model.

Liposome preparation

Inflammatory responses occur predominantly in the first two weeks afer implantation . In order to achieve local controlled release of the antiinfammatory drug DSG we tested three types of liposomes with varying lipid compositions. The release was always studied after encapsulation of the liposomes in alginate-capsules.
We tested the following types of liposomes: Eggphosphatidylcholine (EPC, from Avanti Polar Lipids, Inc Alabaster, AL, USA), EPC/Cholesterol (Sigma, St. Louis MO, USA) (9:1), and EPC/1-palmitoyl,2-oleoyl-sn-glycero-3-phosphoserine (Avanti Polar Lipids, Inc Alabaster, AL, USA) (POPS) /Cholesterol (5:1:4).

For studying the release profiles from the different types of liposomes we incubated 20 alginate-capsules containing calcein-liposomes. Evry day we replaced the KrH buffer and determine the calcein concentration in these samples by measuring the fluorescence on aFL600 microplate fluorescence reader with excitation and emission wavelengths of 485 nm and 530 nm, respectively. For the maximal release we applied 1% Triton X-100 incubation which results in desintegration of the liposomes. As a negative control we applied 25 mM KRH buffer-containing 5 mM calcium dihydrate. The release was expressed as a percentage of the total amount of carboxyfluorescein in the liposomes. Only selected experiments were performed with DSG.

Transplantation model

Male inbred AO-rats (AO/G, Harlan) weighing 300-350 gram served as donors. Islets were isolated as previously described, with a collagenase digestion technique. Male BL-6 mice weighing 25 to 31 gram were used as recipients of encapsulated islet grafts. Diabetes was induced by injection of 190 mg/kg of streptozotocin (Sigma, The Netherlands) via peritoneal injection. Glucose concentration in blood was determined with glucose test bandelettes (Accu-Chek, Roche). Only animals with severe weight loss, polyuria, polydipsia and blood glucose levels exceeding 20 mM over a period of at least four weeks were used as recipients. Under halothane anaesthesia, the microencapsulated AO islets were injected into the peritoneal cavity recipient diabetic BL6 mice. Recipients

with a blood glucose level less than 8.4 mM were considered normoglycemic

Microcapsule fabrication

After culture, islets were washed three times with RPMI containing 10% FCS and were subsequently washed with Ca^{2+}-free KRH containing 135 mM NaCl. After washing the islets were mixed with 2,7 % Intermediate-G sodium alginate (~ 40% guluronic acid, Alginates UK Ltd in the ratio of 1000 islets in 100 µl Alginate. The alginate solution was converted into droplets using an electrostatic bead generator.

Incorporation of the liposomes and encapsulated islets into alginate beads was done by suspending 100 µL liposomes into 1 ml 1.9 % and high-G sodium alginate (> 50% guluronic acid, ISP Alginates UK Ltd)) solution for a second envelopment in beads.

RESULTS AND DISCUSSION

Release profiles liposomes

The release profiles of the three encapsulated liposomes formulations is measured for 12 days (figure 1).

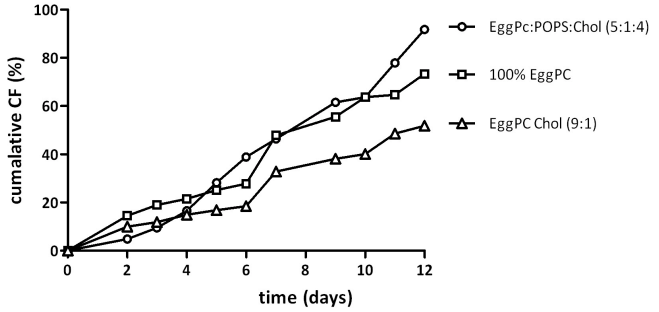

Figure 1. Release of calceine entrapped in liposomes with different lipid compositions which are encapsulated in intermediate-G alginate bead.

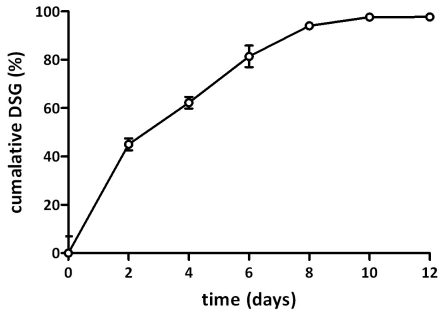

Figure 2. Release of DSG entrapped in liposomes with lipid composition EPC/Chol (9:1) are encapsulated in intermediate-G alginate bead.

All three compositions showed an gradual release in time out of the encapsulated liposomes. The lipid composition EPC/POPS/Chol (5:1:4) released finally about 92% of the carboxyfluorescein whereas approximately 74% of carboxyfluorescein was released from encapsulated liposome with lipid composition 100% EPC. Moreover, 52% was released from encapsulated liposome with lipid composition EPC/Chol (9:1).

The liposome formulation EPC/Chol (9:1) was chosen for the subsequent study with DSG because it shows the slowest gradual release in time. As seen in figure 2 were we obtained a nice sustained release and the liposomal DSG content was secreted completely after 12 days.

Transplantation

All transplanted mice became normoglycemic. After eleven days the blood glucose levels reached the lowest level 4,9 mM for the single and 5.3 mM for the liposomal double encapsulated islets encapsulated islets.

The transplanted mice with single encapsulated islets had a lower mean basal blood glucose level with more than 1 mM this lower level had no prolonged effect on the graft survival. The maximal survival of the single encapsulated islets is 60 days in contrast to 140 days of the liposomal encapsulated (figure 3).

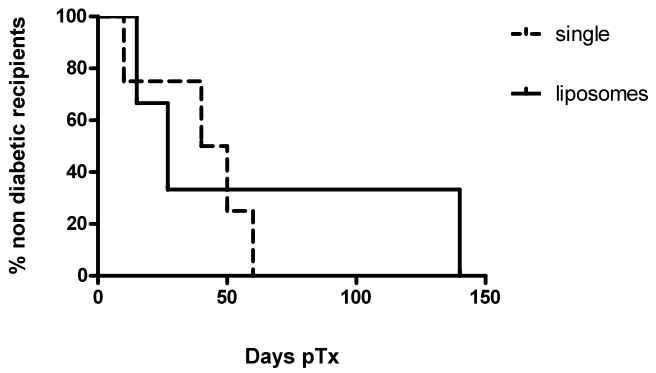

Figure 3. Graft survival of DSG entrapped in liposome encapsulated islets compared with encapsulated in single alginate capsules.

CONCLUSIONS

We developed an liposomal encapsulation system to obtain local and temporarily release of DSG. With this new concept DSG is released out encapsulated liposome for 12 days. The efficacy of the system was demonstrated in islet xenografts in mice that showed longer survival times when compared to traditional capsules.

REFERENCES

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