Feasibility of localized immunosuppression: 1. Exploratory studies with glucocorticoids in a biohybrid device designed for cell transplantation

P. BUCHWALD1,2, N. BOCCA3, S. MARZORATI1, G. HOCHHAUS2, N. BODOR3, C. STABLER1,4,7, N. S. KENYON1,4,7, L. INVERARO1,5,6, R. D. MOLANO4, C. RICORDI1,4,7, A. PILEGGI1,7

Received December 6, 2009, accepted February 2, 2010

Emerging biotechnologies, such as the use of biohybrid devices for cellular therapies, are showing increasing therapeutic promise for the treatment of various diseases, including type 1 diabetes mellitus. The functionality of such devices could be greatly enhanced if successful localized immunosuppression regimens could be established, since they would eliminate the many otherwise unavoidable side effects of currently used systemic immunosuppressive therapies. The existence of local immune privilege at some specialized tissues, such as the eye, CNS, or pregnant uterus, supports the feasibility of localized immunomodulation, and such an approach is particularly well-suited for cell transplant therapies where all transplanted tissue is localized within a device. Following the success of syngeneic transplantation in a subcutaneous prevascularized device as a bioartificial pancreas in a rodent model, we now report the first results of exploratory in vivo islet allograft studies in rats using locally delivered glucocorticoids (dexamethasone phosphate and the soft steroid loteprednol etabonate). Following in vitro assessments, in silico drug distribution models were used to establish tentative therapeutic dose ranges. Sustained local delivery was achieved via implantable osmotic mini-pumps through a central sprinkler, as well as with a sustained-delivery formulation for loteprednol etabonate using poly(D,L-lactic) acid (PLA) microspheres. Doses delivered locally were approximately hundred-fold smaller than those typically used in systemic treatments. While several solubility, stability, and implantation problems still remain to be addressed, both compounds showed promise in their ability to prolong graft survival after tapering of systemic immunosuppression, compared to control groups.

1. Introduction

1.1. Islet transplantation and biohybrid devices

In type 1 diabetes mellitus (T1DM; juvenile onset or insulin dependent), the insulin producing β-cells of the pancreatic islets are destroyed by an autoimmune process and glycemic metabolism can only be controlled by administration of exogenous insulin. T1DM is characterized by infiltration of the pancreatic islets by immune cells, which after some time destroy the β-cells by T-cell-mediated mechanisms (Faustman and Davis 2009; Green and Flavell 1999). Unfortunately, even with a careful insulin treatment, chronic and degenerative complications, such as retinopathy, nephropathy, neuropathy, atherosclerosis, and lipid disorders, still occur in a considerable fraction of patients with T1DM due to the metabolic abnormalities associated with diabetes. Precise metabolic control, in a manner that cannot be achieved with exogenous insulin, can be attained by the transplantation of pancreatic islets (Mineo et al. 2009). With recent clinical advancements, insulin independence can be consistently attained following the transplantation of an adequate allogenic islet numbers resulting in clear benefits for patients with brittle diabetes (Mineo et al. 2009). Nevertheless, there are still important limitations that have to be solved including: the need for large islet numbers (generally requiring more than one donor per recipient); the side effects of the systemic immunosuppression (which is required to avoid graft-rejection); the progressive loss of graft function; and the development of allo-sensitization (Fig. 1) (Pileggi et al. 2006b; Ricordi et al. 2005) – all of which limit the applicability of islet transplantation to only the most severe cases of brittle T1DM in adults (Mineo et al. 2009; Pileggi et al. 2006b; Ricordi et al. 2005). It is also becoming increasingly clear that the liver, the currently favored site of clinical islet transplantation, does not represent an optimal site for islet cell transplants, and there is an ongoing search for alternative sites (Pileggi et al. 2006b). Hence, our ongoing focus is on developing improved immunosuppressive regimens (Bocca et al. 2008; Pinto et al. 2010; Margolles-Clark et al. 2009; Marzorati et al. 2009a,b), improving devices and immunoisolation techniques (Fort et al. 2008; Pileggi et al. 2006c), as well as finding superior alternative sites (Berman et al. 2009) for islet transplantation. During the last decades, various extravascular approaches for a bioartificial pancreas have been explored for islet grafts using either immunoisolated (i.e., encapsulated) or non-encapsulated...
allow long-term maintenance of function, while also avoiding
that LIS may provide sufficient protection against rejection and
localized entirely within the device. Hence, our hypothesis is
the graft and in its immediate surroundings. Our report herein
important immunological events leading to rejection occur in
With this strategy, elevated drug concentration levels are lim-
to implement a localized immunosuppression (LIS) regimen.
and, ultimately, immune tolerance (Ricordi and Strom 2004;
Obviously, the true potential of such a device for cell trans-
planted relatively easy implantation, biopsy, and retrieval (Galletti et al.
Kizil et al. 2005; Narang and Mahato 2006; Pileggi et al.
Within the general framework of beta-cell replacement therapies (Pileggi et al. 2006a; Ricordi and Strom 2004), our cur-
current focus is on the evaluation of subcutaneous, neo-vascularized,
biohybrid devices (BHD) as possible therapeutic options toward
islets. They all aimed at providing adequate mechanical pro-
tection and sustained graft function while also making possible
imperative organ recovery/preservation. (2) Post-transplant insults:
(1) islet mass (continuous blue line) is lost due to both pre- and
post-transplant events: (1) imperfect organ recovery/preservation, (2, 3)
suboptimal islet isolation and culture, (4) immediate post-transplant islet loss,
and (7) progressive loss due to the diabetogenic and antiproliferative effects
of the immunosuppressive agents used as well as to chronic rejection and
recurrence of autoimmunity. Immune response (dashed red line) needs to be
controlled with immunosuppressive therapies to prevent graft rejection. The
efficacy of immunosuppression needs to take into account (i) graft
immunogenicity, (ii) immune activation, (iii) expansion, while, ideally, not
preventing (iv) immunosuppression and (v) regulatory mechanisms
and minimizing memory

![Fig. 1: Schematic illustration of the problems hindering islet transplantation (Ricordi et al. 2005).](image)

**ORIGINAL ARTICLES**

**1.2. Local immune privilege**

One support for the feasibility of LIS is provided by the existence of local immune privilege at some specialized tissues within the
body. It has been long known that donor allografts can elicit
adoptive immunity of variable potency depending on the site of
the transplantation. For example, some specialized tissues
such as the eyes, the central nervous system (CNS), the preg-
nant uterus, and the testes, have been found to possess intrinsic
immune privilege (Acke et al. 2008; Niederkorn 2006). The
‘immune privilege’ term was originally introduced by Medawar
in 1948. For a long time, it was assumed to be mainly due to
anatomic segregation; however, it is becoming increasingly clear
that it is maintained by a combination of anatomical, physi-
ological, and immunoregulatory processes (Acke et al. 2008;
Niederkorn 2006). It also requires some localized active sup-
pressive processes and applies to more than the few originally
assumed sites. Furthermore, similar processes are also likely
exploited by cancerous tumors and chronic infections in order
to acquire their unique immune privileges (Mellor and Munn
2008). Hence, the mechanisms involved in this protection are of
particular relevance for our goal of achieving long-term func-
tion of tissues transplanted into the well confined boundaries of
a BHD. The main potential regulatory checkpoints involved in
the creation and maintenance of immune privilege in local tissue
microenvironments have been summarized as follows (Mellor and Munn 2008): (1) Local inflammatory responses to tissue
insults or generation of ‘danger’ (Matzinger 1994) as tissue
insults generally tend to induce some degree of local inflam-
mation consisting of the influx and activation of immune cells,
increased cytokine production, altered cell differentiation and
metabolic stress responses, and perhaps unmasking of normally
cryptic antigens; (2) Dendritic cell maturation and migration
to local draining lymph nodes to deliver antigens in immune-
stimulatory or suppressive manner following their maturation,
which is often mediated by ‘danger’ signals working through
the pathways of innate receptors and chronic inflammation in T-cell
receptors and inflammatory cytokine receptors; (3) Antigen
presentation and T-cell activation in lymph nodes (a good tar-
get to maximize the impact of an intervention as T-cell priming

---

Pharmazie 65 (2010)
occurs here and involves small numbers of dendritic-, T-, and, in some settings, T<sub>reg</sub>-cells); and (4) Regulation of effector cells (e.g., by local delivery of corticosteroids) to the potential toxic and diabetogenic effects of some of these agents. Impaired glucose metabolism (Fernandez et al. 1999) and post-transplant diabetes mellitus (First 2003) are common in patients receiving systemically chronic therapy with calcineurin inhibitors (i.e., CsA and TACR). The deleterious effects of systemic administration of calcineurin inhibitors on islet graft function have long been recognized (Alejandro et al. 1989; Alejandro et al. 1988; Ricordi et al. 1992). Systemic immunosuppression and cultivation of islet cells in vitro with SIR have been associated with impaired islet function and islet cell toxicity (Bell et al. 2003; Marcelli-Tourvieille et al. 2007; Zahr et al. 2007; Zhang et al. 2006). Mycophenolate mofetil and some of the newer, emerging compounds (e.g., fingolimod or leflunomide) seem to have less effect on glucose metabolism at therapeutic doses (Egidii 2005). Administration of systemic glucocorticoids in the peri-transplant period has been associated with the development of islet graft dysfunction (Rilo et al. 1994), and corticosteroids (including DEX) show some inhibitory insulin release by islet β-cells (Lambillotte et al. 1997; Fierabracci et al. 1986; Wazalich et al. 2006). Steroids are known to produce whole-body insulin resistance when administered systemically for longer periods and to exacerbate diabetes (Qi and Rodrigues 2007); however, if insulin resistance is mainly due to suppression of glucose transport, this problem might be circumvented via local drug delivery, as much lower levels are present systemically (Pagano et al. 1983; Sakoda et al. 2006). Essentially all currently used systemic immunosuppression regimens have serious negative effects on
the engraftment, function, and survival of transplanted islets, thereby hindering the success of islet implantation (Marzorati et al. 2009a). Hence, a LIS approach can become successful only if (i) locally active agents can be identified, (ii) they are active at concentration levels that are immunosuppressive, but non-β-cell toxic, and (iii) the nontrivial problems of delivering and maintaining them locally can be solved (at least until immune tolerance inducing protocols are available).

1.5. Local corticosteroids
Corticosteroids, potent antiinflammatory and immunosuppressive agents, should be a strong first choice as possible LIS agents, since they are commonly utilized in a large variety of clinical diseases. Due to their broad spectrum of activity, they also are the most widely used class of immunosuppressive agents. Here, we report the first exploratory results of in vivo islet allograft studies in rat BHDs using two locally delivered glucocorticoids: dexamethasone phosphate (DEXP), which was selected on the basis of solubility considerations, and loteprednol etabonate (LE), which was selected on the basis of its safety and localized activity. LE is a soft steroid specifically designed to produce targeted local activity with no systemic side effects due to its prompt metabolic (preferably extrahepatic, e.g., hydrolytic) inactivation (Bodor and Buchwald 2006; Buchwald and Bodor 2004; Druzgala et al. 1991). Soft drugs are new, active therapeutic agents (often isosteric-isoelectronic analogues of a lead compound) with a chemical structure specifically designed to allow for predictable metabolism into inactive metabolites after exerting the desired therapeutic effect(s) (Bodor and Buchwald 2008). Both LE and DEX bind to the glucocorticoid receptor with a dissociation constant (KD) that is in the 5–10 nM range (Buchwald 2008). On the basis of this and the result of previous in vitro investigations (Bocca et al. 2008), concentration levels of 5–500 nM (2–250 ng/mL) can serve as a first estimate of a target therapeutic range that could be immunosuppressive, but not significantly β-cell toxic.

2. Investigations, results and discussion
2.1. COMSOL Multiphysics computational drug delivery models
Achieving sustained local drug delivery at a tissue engineered site is a considerable challenge; several possibilities have been considered with only limited success (Saltzman and Olbricht 2002). Using the 5–500 nM range as a tentative therapeutic target for active corticosteroid molecules, we performed a series of geometrically accurate fully 3D finite element method (FEM)–based COMSOL Multiphysics computational simulations (Bocca et al. 2008, 2007). These were built by combining the diffusion/convection and the incompressible Navier-Stokes fluid mechanics application modes of the software, and were used to obtain first estimate of local doses for various possible local delivery methods that could be implemented with our currently used rodent BHD model (Fig. 2). As Fig. 3 shows, the model-predicted flow profiles seem in strong agreement with those measured experimentally for an essentially aqueous fluid with a multi-hole cylindrical sprinkler. Accordingly, models for various delivery possibilities were built (Bocca et al. 2008, 2007); the two corresponding most closely to those used here in the in vivo transplantation models are for a cylindrical BHD with a continuous pump-driven infusion through a central ‘sprinkler’ system and with multiple, randomly distributed, sustained-release spherical beads (Fig. 4). These calculations suggested that, as long as no significant local metabolic degradation takes place, a drug delivery rate of approximately 1 nmol/day (corresponding to approximately 0.5 μg/day) can provide adequate coverage inside the cylindrical chamber for steroid-sized molecules. The multiple spherical bead approach might provide the most uniform coverage, but only if the beads can be uniformly distributed and maintained (Fig. 4). Calcu-
sustained release of both hydrophilic and lipophilic drugs (Li and Jast 2006). PLA microspheres are of particular interest for targeted drug delivery since they are biocompatible, biodegradable, and can decrease unwanted side effects while maintaining therapeutic effects (Okada and Toguchi 1995). Various microsphere formulations have been prepared (Pinto et al. 2010; Pinto 2008); those used here were prepared by solvent evaporation and had a drug loading of 3.9 ± 0.2%, an estimated mean particle diameter of 50 μm, and provided an in vitro drug release duration of approximately three months.

2.3. Exploratory in vivo studies

A first set of exploratory in vivo rodent experiments investigating the functionality of allogeneic implants in BHDs with maintenance LIS therapy were performed. Chemically diabetic rats were transplanted with allogeneic islets into prevascularized BHDs, maintained on systemic immunosuppression (ALS induction followed by maintenance on mycophenolic acid) for at least two weeks, and then withdrawn from the systemic immunosuppression and maintained only on the LIS treatment regimen. To assess the function of the islets transplanted into the prevascularized BHD, glucose levels were measured daily. For local delivery, implantable osmotic mini-pumps (Alzet®, 0.25 mg/L) were used for both DEXP and LE by connecting it to the central sprinkler of the BHD (Fig. 2). Both the prevascularized BHD and the pump were implanted subcutaneously in the dorsal region of the rodents (Pileggi et al. 2006c), and they were connected via polyethylene tubing. For LE, the PLA microsphere-based sustained-delivery formulation was also explored. Most of these early exploratory experiments were hampered by a number of problems mainly related to solubility and stability limitations, as well as to the implantation of the mini-pump. Nevertheless, both glucocorticoids tested (DEXP and LE) showed some promise. Results of these early in vivo tests, obtained while the animal models were still being developed, were somewhat inconsistent because of the mentioned problems; nonetheless, local delivery showed significant prolongation of graft function when compared to control animals that reject the transplant within 6–12 days after tapering of the systemic immunosuppression (p<0.05).
3. Experimental

3.1. In silico drug distribution models

Computational models with a finite element method (FEM) (COMSOL Multiphysics 3.4, COMSOL AB, Stockholm, Sweden) were performed as described by Bocca et al. (2007; Bachwald 2009). Briefly, diffusion was assumed to be governed by the generic diffusion equation in its nonconservative formulation (incompressible fluid):

$$\nabla \cdot (\nabla c - D\nabla c) = 0
$$

(1)

Notation: $c$ concentration of the species of interest (mol m$^{-3}$), $D$ diffusion coefficient (m$^2$ s$^{-1}$), $R$ reaction rate (mol m$^{-3}$ s$^{-1}$), $\eta$ viscosity (kg m$^{-1}$ s$^{-1}$), $\rho$ density (kg m$^{-3}$), $F$ volume force (kg m$^{-1}$ s$^{-2}$), $p$ pressure (Pa).

For models with convection (sprinkler infusion), the diffusion model was replaced with the generic momentum balance equation (for incompressible fluids):

$$\nabla \cdot (\rho u + p - \nabla V) + \nabla \cdot (\rho u \cdot \nabla u) = 0
$$

(2)

Notation: $\rho$ density (kg m$^{-3}$), $u$ velocity field (m s$^{-1}$), $p$ pressure (Pa).

For models with convection (sprinkler infusion), the diffusion model was replaced with the generic momentum balance equation (for incompressible fluids):

$$\nabla \cdot (\rho u + p - \nabla V) + \nabla \cdot (\rho u \cdot \nabla u) = 0
$$

(2)

Notation: $\rho$ density (kg m$^{-3}$), $u$ velocity field (m s$^{-1}$), $p$ pressure (Pa).

For models with convection (sprinkler infusion), the diffusion model was replaced with the generic momentum balance equation (for incompressible fluids):

$$\nabla \cdot (\rho u + p - \nabla V) + \nabla \cdot (\rho u \cdot \nabla u) = 0
$$

(2)

Notation: $\rho$ density (kg m$^{-3}$), $u$ velocity field (m s$^{-1}$), $p$ pressure (Pa).

For models with convection (sprinkler infusion), the diffusion model was replaced with the generic momentum balance equation (for incompressible fluids):

$$\nabla \cdot (\rho u + p - \nabla V) + \nabla \cdot (\rho u \cdot \nabla u) = 0
$$

(2)

Notation: $\rho$ density (kg m$^{-3}$), $u$ velocity field (m s$^{-1}$), $p$ pressure (Pa).

For models with convection (sprinkler infusion), the diffusion model was replaced with the generic momentum balance equation (for incompressible fluids):

$$\nabla \cdot (\rho u + p - \nabla V) + \nabla \cdot (\rho u \cdot \nabla u) = 0
$$

(2)

Notation: $\rho$ density (kg m$^{-3}$), $u$ velocity field (m s$^{-1}$), $p$ pressure (Pa).

For models with convection (sprinkler infusion), the diffusion model was replaced with the generic momentum balance equation (for incompressible fluids):

$$\nabla \cdot (\rho u + p - \nabla V) + \nabla \cdot (\rho u \cdot \nabla u) = 0
$$

(2)

Notation: $\rho$ density (kg m$^{-3}$), $u$ velocity field (m s$^{-1}$), $p$ pressure (Pa).

For models with convection (sprinkler infusion), the diffusion model was replaced with the generic momentum balance equation (for incompressible fluids):

$$\nabla \cdot (\rho u + p - \nabla V) + \nabla \cdot (\rho u \cdot \nabla u) = 0
$$

(2)

Notation: $\rho$ density (kg m$^{-3}$), $u$ velocity field (m s$^{-1}$), $p$ pressure (Pa).

For models with convection (sprinkler infusion), the diffusion model was replaced with the generic momentum balance equation (for incompressible fluids):

$$\nabla \cdot (\rho u + p - \nabla V) + \nabla \cdot (\rho u \cdot \nabla u) = 0
$$

(2)

Notation: $\rho$ density (kg m$^{-3}$), $u$ velocity field (m s$^{-1}$), $p$ pressure (Pa).

For models with convection (sprinkler infusion), the diffusion model was replaced with the generic momentum balance equation (for incompressible fluids):

$$\nabla \cdot (\rho u + p - \nabla V) + \nabla \cdot (\rho u \cdot \nabla u) = 0
$$

(2)

Notation: $\rho$ density (kg m$^{-3}$), $u$ velocity field (m s$^{-1}$), $p$ pressure (Pa).

For models with convection (sprinkler infusion), the diffusion model was replaced with the generic momentum balance equation (for incompressible fluids):

$$\nabla \cdot (\rho u + p - \nabla V) + \nabla \cdot (\rho u \cdot \nabla u) = 0
$$

(2)

Notation: $\rho$ density (kg m$^{-3}$), $u$ velocity field (m s$^{-1}$), $p$ pressure (Pa).

For models with convection (sprinkler infusion), the diffusion model was replaced with the generic momentum balance equation (for incompressible fluids):

$$\nabla \cdot (\rho u + p - \nabla V) + \nabla \cdot (\rho u \cdot \nabla u) = 0
$$

(2)

Notation: $\rho$ density (kg m$^{-3}$), $u$ velocity field (m s$^{-1}$), $p$ pressure (Pa).
ORIGINAL ARTICLES


