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Intraportal delivery of immunosuppression to intrahepatic islet allograft recipients

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Abstract Local delivery of immunosuppressive agents may dampen local alloreactive events with avoidance of systemic toxicity. We investigated the innovative strategy of intraportal (IPO) delivery of three immunosuppressive agents in streptozotocin diabetic rat recipients of islet allografts (Lewis to Wistar-Furth) transplanted intrahepatically. IPO budesonide (BUD, 240 or 360 μg/kg per day), a potent steroid, and cyclosporin (CyA, 2 or 4 mg/kg per day) did not prolong graft mean survival time [MST ± standard deviation (SD)] as compared to nonimmunosuppressed recipients. Fourteen days of IPO FK 506 (0.16 mg/kg per day) significantly increased MST as compared with untreated controls (49 ± 29 vs 7 ± 1 days, $P < 0.01$) and was more effective than intravenous (IV) FK 506 (17 ± 7 days, $P < 0.01$). When FK 506 was given for 28 days, the benefit of IPO over IV delivery was reaffirmed (MST 81 ± 32 vs 34 ± 4 days, $P < 0.01$). The potential for toxicity was lessened by lower mean systemic levels in the IPO group as compared to the IV group (1.3 ± 0.6 vs 3.5 ± 0.9 ng/mg, $P < 0.02$). The strategy of continuous IPO FK 506 was effective in the prevention of rejection of intrahepatic islet allografts.

Key words Immunosuppression, local, islet allografts · Islet allografts, FK 506 · FK 506, local, islet allografts · Budesonide, islet allografts

Introduction

The major drawback to clinical transplantation is long-term systemic immunosuppression with increased susceptibility to infection and drug-related toxicities. An alluring strategy is the delivery of immunosuppressive agents directly to the allograft without systemic effect. The current schema of alloreactivity supports the concept that effector cells differentiate and mature in the allograft and that this process is dependent upon locally produced lymphokines [3, 10]. Based on this recent understanding, local immunosuppression merits re-evaluation.

Intra-arterial delivery of immunosuppressive agents to vascularized allografts has been shown to prolong allograft survival in several experimental models [8, 16, 17, 19]. The dual blood supply to the liver – via the hepatic artery and portal vein – presents a unique opportunity for local delivery of immunosuppressive agents intraportally (IPO). This approach has not been investigated. In a review of local immunosuppression, Gruber characterizes the ideal immunosuppressive agent as cleared rapidly outside and/or highly metabolized by the target organ to produce a regional advantage (increased efficacy) and/or systemic advantage (decreased toxicity) [7]. In theory, local delivery of the ideal agent could establish high local levels in the allograft and low levels systemically. We selected agents – cyclosporin (CyA), budesonide (BUD), and FK 506 – that are metabolized or avidly bound by the liver to evaluate the effect of local immunosuppression by IPO delivery on intrahepatic is-

let allograft survival versus systemic intravenous (IV) delivery.

Materials and methods

Recipients were male Wistar-Furth ($RT1^U$) rats, weighing 200–250 g, that were rendered diabetic [blood glucose (BG) > 300 mg/dl] with a single IV injection of streptozotocin (80 mg/kg; Zanosar, Upjohn, Kalamazoo, Mich.). BG determinations were done by tail bleeding, using a Glucometer (Ames Co, Miles Laboratories, Elkhart, Ind.). While awaiting transplantation, diabetic rats received long-acting insulin protamine zinc insulin (Eli Lilly, Indianapolis, Ind.) in doses of 2–4 U/day, depending on BG determinations to prevent weight loss and general debility. Rats had free access to standard rat chow and water. Surgery was performed and animal care administered in accordance with the policies of the Institutional Animal Care and Use Committee at Stanford University Medical Center.

Islets were isolated from four to six Lewis ($RT1^l$) rats using standard techniques of collagenase digestion (Type V, Sigma, St. Louis, Mo.), passage through a nylon filter, and density gradient separation using Ficoll [22]. Between 1000 and 1500 islets were slowly injected IPO in a 1-cc suspension of Hank's solution. The agent (CyA, BUD, or FK 506) was placed in an implantable osmotic minipump (Alzet, Palo Alto, Calif.) that continuously delivered the agents for either 14 or 28 days. The proximal splenic vein was cannulated for IPO delivery with a ligature placed on the splenic side, and the jugular vein was cannulated for systemic delivery using a polyethylene catheter (PE 50, Clay Adams, Parsippany, N.J.) from the minipump placed subcutaneously on the back. The minipumps were checked by laparotomy for proper position and flow on the 7th day after implantation. Fourteen or 28 days after implantation, depending on the experimental protocol, the minipump was removed to assure that the reservoir was empty.

Based on experiments by Ruers et al., the dose of BUD (generously provided by Astra Pharmaceutical Production, Lund, Sweden) was 240 or 360 μg/kg per day [11]. The Ruers et al. study found BUD to be stable and biocompatible with the minipump. CyA (Sandimmun, Sandoz, Basel, Switzerland) in the IV formulation was administered in a dose of 2 or 4 mg/kg per day via minipump, based on prior work in the rat cardiac allograft model [19]. CyA levels were assayed on whole blood by high pressure liquid chromatography in the Toxiciology Laboratory of the Department of Pathology at the University of Pennsylvania [18]. FK 506 (generously provided by Fujisawa Pharmaceutical, Osaka, Japan) was dissolved in propylene glycol. The dose employed (0.16 mg/kg per day) was based on reports of experimental and clinical use [11, 15]. FK 506 levels were determined on serum collected 14 days after transplantation using a two-step competitive binding immunoassay developed by Tamura et al. [20].

Recurrent diabetes after islet transplantation was defined as blood glucose above 250 mg/dl on two consecutive daily determinations. Three recipients that became hyperglycemic due to islet allograft rejection were retransplanted with Lewis islets injected IPO without immunosuppression to study the phenomenon of sensitization following IPO immunosuppression. Histological study of the livers of islet allograft recipients with recurrent diabetes was done in three recipients and two normoglycemic recipients. After adequate fixation in Bouin's solution, sections were stained with hematoxylin and eosin. Aldehyde fuchsin staining was used to demonstrate insulin granules.

Statistical analysis was carried out using the Statworks software program (Cricket Software, Philadelphia, Pa.). To assess statistical differences in nonparametric MST data between experimental groups, the Wilcoxon signed rank test was used. Categorical differences were analyzed by the chi-square test. A P value below 0.05 was considered significant.

Results

At the time of sacrifice, there was no evidence of splenic infarction (an unwanted consequence that could theoretically confound the experiment), presumably because of venous decompression via the short gastric veins. Minipump reservoirs were empty.

Results are summarized in Table 1. Neither CyA nor BUD delivered IPO significantly increased the MST of islet allografts over untreated controls. In six recipients of CyA (4 mg/kg per day), levels were determined on portal and peripheral venous blood 7 days after transplantation. Levels were not significantly different in portal venous blood (508 ± 374 ng/ml) and in peripheral blood (771 ± 467 ng/ml); that is, there was no CyA gradient across the liver.

Immunosuppression with FK 506, either by the IPO (groups 6 and 8) or IV (groups 7 and 9) route, significantly extended islet allograft survival as compared to

Table 1 Graft survival after intrahepatic transplantation of islet allografts (*MST* mean survival time)

Group	Agent	Daily dose	N	Route	Duration	MST ± SD
1	None	–	7	–	0 days	7 ± 1 (5,6,6,7,8,8,9 days)
2	CyA	2 mg/kg	8	IPO	14	17 ± 29 (5,5,5,5,6,9,9,88)
3	CyA	4 mg/kg	5	IPO	14	13 ± 18 (3,4,6,8,45)
4	BUD	240 μg/kg	4	IPO	14	4 ± 1 (3,4,4,5)
5	BUD	360 μg/kg	4	IPO	14	7 ± 5 (4,5,6,14)
6	FK 506	0.16 mg/kg	7	IPO	14	49 ± 29 (9,23,49,50,52,59, > 100)
7	FK 506	0.16 mg/kg	7	IV	14	17 ± 7 (8,10,11,19,24,24,25)
8	FK 506	0.16 mg/kg	7	IPO	28	81 ± 32 (58,62,68,68,72,92, > 100)
9	FK 506	0.16 mg/kg	7	IV	28	34 ± 4 (28,32,33,34,36,36,42)

* $P < 0.01$ for group 1 vs groups 6–9, for group 6 vs 7, for group 8 vs 9, and for groups 8 and 9 vs group 2 (Wilcoxon signed rank test)
Groups 3, 4 and 5 were of insufficient size to generate valid statistical comparisons

non-immunosuppressed recipients (group 1) or recipients immunosuppressed with BUD or CyA administered IPO (groups 2–5). IPO delivery of FK 506 for 14 and 28 days of treatment was superior to IV delivery in prolonging islet allograft MST: 49 ± 29 vs 17 ± 7 days and 81 ± 32 vs 34 ± 4 days, respectively. Only 1 of 14 (7 %) recipients rejected the islet allograft while maintained on IPO FK 506 as compared to 4 of 11 (29 %) on IV FK 506, but this difference did not achieve statistical significance. In the IV FK 506 group, rejection uniformly occurred either during infusion or within 14 days of discontinuation. The mean FK 506 levels on recipient serum from the 14th day after transplantation in the IPO FK 506 group (group 8) was 1.3 ± 0.6 ng/ml. This was significantly lower than the mean level of 3.5 ± 0.9 ng/ml ($P < 0.02$) in the IV group.

When the minipump reservoir was depleted after 14 or 28 days in the IPO FK 506-treated animals (groups 6 and 8), 14 normoglycemic recipients remained so for a mean of 42 ± 25 days (range 9–100 +) and 46 ± 15 days (range 30–100 +), respectively.

Histological study of the liver from normoglycemic recipients demonstrated healthy, well-granulated islets in the sinusoidal space. Histological examination showed that three recipients with recurrent hyperglycemia had acute rejection of the islet allografts, manifested as an infiltrate of mononuclear cells into the portal triad where degranulated islets were also seen.

Three recipients in group 8 underwent retransplantation without immunosuppression using Lewis islets. Normoglycemia was established in each before recurrent hyperglycemia occurred 4, 14, and 19 days after transplantation (MST 12.3 ± 8 days). In comparison, two recipients of islet regrafts that were not immunosuppressed at the time of either islet allograft developed recurrent hyperglycemia after 3 and 4 days (MST 3.5 days). There was no mortality in the experimental groups and no overt drug toxicity.

Discussion

This study of local immunosuppression differed from others by its use of the IPO route of delivery. We found that IPO delivery of FK 506 markedly prolonged survival of islet allografts transplanted intrahepatically into diabetic rats as compared to IV delivery. Furthermore, this survival advantage was realized with significantly lower systemic FK 506 levels in the IPO delivery group.

For many years, the prevailing schema of alloreactivity made local immunosuppression illogical. The understanding was that a vascularized allograft was recognized by host immune cells circulating through the graft. Subsequent proliferation of responder cells occurred in the spleen or lymph nodes; the, sensitized lymphoid cells returned to the graft to destroy cells bearing foreign histocompatibility antigens. Given this schema, the value of a high concentration of an immunosuppressive agent in the graft was questionable.

The importance of the local environment has been recognized. The sponge matrix allograft model and others have advanced the understanding of intragraft events [3, 10]. This has resulted in a conceptual change in the schema of alloreactivity. Currently, it is thought that changes in local vascular permeability and the elaboration of chemotactic factors within the allograft result in infiltration by both nonspecific and specifically alloreactive immune cells. T-helper cells produce cytokines locally that support maturation and expansion of specific cytotoxic T cells in the graft. Based on these concepts, there has been a resurgence of interest in local immunosuppression.

A recent review summarized the various models studied and the theory behind local immunosuppression [7]. Most investigations have employed intra-arterial delivery of immunosuppressives to vascularized cardiac or renal allografts. The intra-arterial introduction of immunosuppressive agents carries the risk of arterial thrombosis with graft loss. This potential risk is shared by the IPO route, but cannulation of a venous branch and the high flow portal system may provide protection against thrombosis. Our model employed IPO delivery to the nonvascularized "free" islet allograft. Aebischer and coworkers demonstrated effective local immunosuppression using drug-impregnated polymer rods in an islet xenograft (rat-to-mouse) model [1]. Other than this intriguing report, studies of local immunosuppression have used vascularized, solid organ allografts. In our studies, a constant IPO infusion delivered high-dose immunosuppressive agents to the microenvironment of transplanted islets lodged in the distal hepatic venules and sinusoids. According to Andersson and coworkers, 14 days after islet transplantation an arterial blood supply develops [2]. Therefore, after 14 days, the hepatic parenchyma surrounding the islets received the IPO levels, but the islets per se were exposed to arterial systemic levels of the immunosuppressive agent. We found an additional 14 days of FK 506 IPO delivery was protective against rejection as compared to IV delivery.

The ideal immunosuppressive agent for local delivery must have certain pharmacological characteristics to provide a regional advantage. The ideal agent should have high first-pass metabolism by the target organ or rapid clearance outside the target organ. This will result in high allograft levels of immunosuppression and low systemic levels. Tissue-binding characteristics are also important since selective allograft tissue-binding with early saturation kinetics may have the undesirable effect of equalizing the efficacy of local and systemic delivery.

The first agent we studied was BUD, a potent steroid, that is biotransformed in the liver into inactive metabolites [6]. Exploiting this characteristic, Ruers and colleagues infused BUD into the coronary arteries of heterotopic cardiac allografts drained into the portal vein [17] with the expectation that high levels would be established in graft tissue and that systemic levels would be low due to hepatic degradation. In fact, graft survival was comparable to systemically treated recipients because of selective binding of BUD to heart muscle. This phenomenon did not confound our studies because selective binding of BUD to hepatic tissue does not occur. Nevertheless, despite the attractive pharmacological features of BUD for IPO delivery, our pilot studies found IPO BUD did not prolong intrahepatic islet allograft survival. The dose employed was two to three times the dose that Ruers and colleagues used successfully in the heterotopic heart allograft model, so failure for immunological reasons due to an inadequate dose is improbable. It is possible that high-dose BUD prevented engraftment or was damaging to engrafted islets.

CyA was the second agent studied via the IPO route. This was based on known hepatic clearance from blood with further hepatic metabolism during the enterohepatic circulation [13]. In addition, the liver is a major depot for CyA. Stepkowski et al. found that a finite period of low-dose CyA (2 mg/kg per day) into the coronary arteries of heterotopic cardiac allografts resulted in high CyA levels in the graft tissue and markedly prolonged graft survival as compared to systemic administration [19]. Using the same dose and a double-dose regimen (4 mg/kg per day) we were not able to demonstrate an advantage in islet allograft survival over untreated controls. CyA islet toxicity might have been implicated [9] except that islets engrafted, restored normoglycemia, and then were rejected in a typical time course (3–9 days). Comparable portal vein and systemic vein CyA blood levels suggested that first-pass hepatic extraction was too low to result in a regional advantage. In addition, hepatic metabolites of CyA have been shown to be biologically active [13].

FK 506 was thought to be well suited for IPO delivery and the establishment of high intrahepatic levels because of its lipophilic nature [12]. Although the precise biodegradable pathways of FK 506 have not been elucidated, there are data to support hepatic and intestinal metabolism [1, 15]. According to other studies, the selected dose of FK 506 (0.16 mg/kg IPO or IV) was too low to cause islet toxicity and did not prolong islet allograft survival when given subcutaneously [21]. This dose approximated the parenteral dose used clinically and resulted in blood levels in the low to mid-therapeutic range [15]. Systemic levels were significantly lower in recipients of FK 506 delivered IPO than IV. Most importantly, islet allograft survival was markedly better in the IPO delivery groups. In fact, only 1 of 14 recipients experienced rejection to allografted islets during the course of IPO FK 506. The protective effect of IPO FK 506 was more pronounced in recipients that were treated with 4 weeks as compared to 2 weeks. When the IPO FK 506 was depleted in the minipump, normoglycemia was maintained for over 40 days rather than the abrupt hyperglycemia experienced in the IV delivery groups within 14 days of minipump depletion. The delay in rejection may reflect a gradual dissipation of high FK 506 levels in tissue depots or a residual immunosuppressive effect secondary to high local levels.

Interestingly, retransplanted donor-specific islets in recipients of IPO FK 506 with recurrent hyperglycemia enjoyed freedom from second-set rejection even in the absence of immunosuppression. This result was reminiscent of reported "local unresponsiveness" following intrahepatic islet transplantation and an induction course of CyA [14]. In this report, successful islet allografts were destroyed with streptozotocin and diabetic hosts retransplanted using donor-specific islets without immunosuppression. Unresponsiveness to the allograft was site-dependent. Islets embolized into the liver were accepted, whereas islets transplanted under the renal subcapsular site were rejected. Several studies have found that Kupffer cells process antigen in a tolerogenic manner [4]. The strategy of IPO delivery of certain immunosuppressive agents in association with donor antigen may use this characteristic to an advantage.

IPO delivery of immunosuppressives could find application in the clinical arena. It is entirely feasible to implant a pump and deliver immunosuppressive agents IPO to islet or liver transplant recipients. Alternatively, refractory acute rejection episodes could be treated using IPO delivery instituted by transhepatic access to the portal vein (obtained by percutaneous puncture or through transjugular cannulation of the hepatic veins) to provide high local concentrations of immunosuppressive agents without systemic toxicity.

In summary, these studies showed that IPO FK 506 improved islet allograft survival with low systemic levels, delayed rejection after discontinuation of FK 506, and prevented second-set rejection upon retransplantation of donor-specific islets. IPO delivery of FK 506 and other agents is an approach worthy of continued investigation.

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