



Article scientifique

Article

2011

Published version

Open Access

This is the published version of the publication, made available in accordance with the publisher's policy.

AEB071 (sotrastaurin) does not exhibit toxic effects on human islets in vitro, nor after transplantation into immunodeficient mice

Merani, Shaheed; McCall, Michael; Pawlick, Rena L; Edgar, Ryan L; Davis, Joy; Toso, Christian; Emamaullee, Juliet A; Kin, Tatsuya; Shapiro, A M James

How to cite

MERANI, Shaheed et al. AEB071 (sotrastaurin) does not exhibit toxic effects on human islets in vitro, nor after transplantation into immunodeficient mice. In: Islets, vol. 3, n° 6, p. 338–343. doi: 10.4161/isl.3.6.17766

This publication URL: <https://archive-ouverte.unige.ch/unige:25032>

Publication DOI: [10.4161/isl.3.6.17766](https://doi.org/10.4161/isl.3.6.17766)

AEB071 (sotrastaurin) does not exhibit toxic effects on human islets in vitro nor after transplantation into immunodeficient mice

Shaheed Merani,¹ Michael McCall,¹ Rena L. Pawlick,¹ Ryan L. Edgar,¹ Joy Davis,¹ Christian Toso,³ Juliet A. Emamaullee,¹ Tatsuya Kin² and A.M. James Shapiro^{1,2,*}

¹Alberta Diabetes Institute; ²Clinical Islet Transplant Program; University of Alberta; Edmonton, AB, Canada; ³Abdominal and Transplantation Surgery; Department of Surgery; University Hospital; Geneva, Switzerland

Keywords: AEB071, islet transplant, sotrastaurin, toxicity

Abbreviations: AEB, AEB071 or sotrastaurin; PKC, protein kinase C; IE, islet equivalents; DAPI, 4',6-diamidino-2-phenylindole; TUNEL, terminal deoxynucleotidyl transferase; dUTP, nick end labeling

AEB071 (AEB, sotrastaurin), a specific inhibitor of protein kinase C, reduces T lymphocyte activation and cytokine release. AEB delays islet allograft rejection in rats and prevents rejection when combined with cyclosporine. Since many immunosuppressive agents have toxic effects on the function of transplanted islets, we investigated whether this was also the case with AEB. Human islets were transplanted into Rag-knockout mice randomly assigned to vehicle control, AEB or sirolimus treatment groups. Non-fasting blood glucose levels, body weight and glucose tolerance was measured in recipients. In a separate experiment, human islets were cultured in the presence of AEB and assayed for glucose dependent insulin secretion and level of β -cell apoptosis. Eighty-six percent of the AEB-treated recipients achieved normoglycemia following transplant (compared with none in sirolimus-treated group, $p < 0.05$). AEB-treated recipients exhibited similar glucose homeostasis as vehicle-treated controls, which was better than in sirolimus-treated recipients. Human islets cultured with AEB showed similar rates of β -cell apoptosis ($p = 0.98$ by one-way ANOVA) and glucose stimulated insulin secretion ($p = 0.15$) as those cultured with vehicle. These results suggest that AEB is not associated with toxic effects on islet engraftment or function. AEB appears to be an appropriate immunosuppressive candidate for clinical trials in islet transplantation.

Introduction

Islet transplantation is a growing therapeutic option for patients with labile type 1 diabetes mellitus. As observed in the early Edmonton series¹ and later in a multi-center trial,² there is concern that initial transplant success has followed by partial graft failure over time.³ The attrition of β -cell mass and function may be due to the combined effects of allo-rejection, autoimmune diabetes recurrence and toxicity of immunosuppressive agents on the islet cell; but the relative contribution of these factors remains incompletely defined. The optimal immunosuppressive protocol for islets has yet to be defined that can provide both complete protection from allo- and autoimmune injury while avoiding specific β -cell toxicity.

The use of sirolimus, tacrolimus in islet transplantation have been associated with insulin resistance⁴ and reduced insulin secretion.⁵ The selective protein kinase C (PKC) inhibitor, AEB071 (AEB, international nonproprietary name: sotrastaurin), which targets the conventional (PKC α , PKC β I, PKC β II, PKC γ) and novel (PKC δ , PKC ϵ , PKC η , PKC θ), but not atypical

(PKC ι , PKC ζ , PK-N1, PK-N2) isoforms of PKCs,⁶⁻⁹ may potentially avoid risk of islet toxicity. Unlike other kinase inhibitors, AEB is specific for PKC rather than other tyrosine or serine/threonine kinases.^{6,7,9} AEB has been investigated for its ability to delay whole organ^{10,11} and cell transplant rejection¹² among other indications.¹³

PKC θ is involved in the signaling cascade leading to activation of T-cells and works downstream to both signal 1 (through T-cell receptor) and signal 2 (CD28 and other co-stimulation).¹⁴ PKC and calcineurin (the target of calcineurin inhibitor tacrolimus and cyclosporine) work synergistically to increase NFAT activation, while PKC also acts to increase NF κ B mediated transcription. PKC and calcineurin show synergistic activation of NFAT and IL-2 production in vitro and in vivo,¹⁵⁻¹⁷ and we have shown complementary immunosuppressive function between their inhibitors AEB and CsA in rodents after islet transplantation.¹² Furthermore, AEB can delay the onset of autoimmune diabetes in the NOD mouse.¹⁸

We have previously demonstrated that AEB is not toxic to β -cell function or glucose homeostasis in naïve rodents.¹² Here,

*Correspondence to: A.M. James Shapiro; Email: amjs@islet.ca
Submitted: 06/15/11; Revised: 08/13/11; Accepted: 08/15/11
DOI: 10.4161/isl.3.6.17766

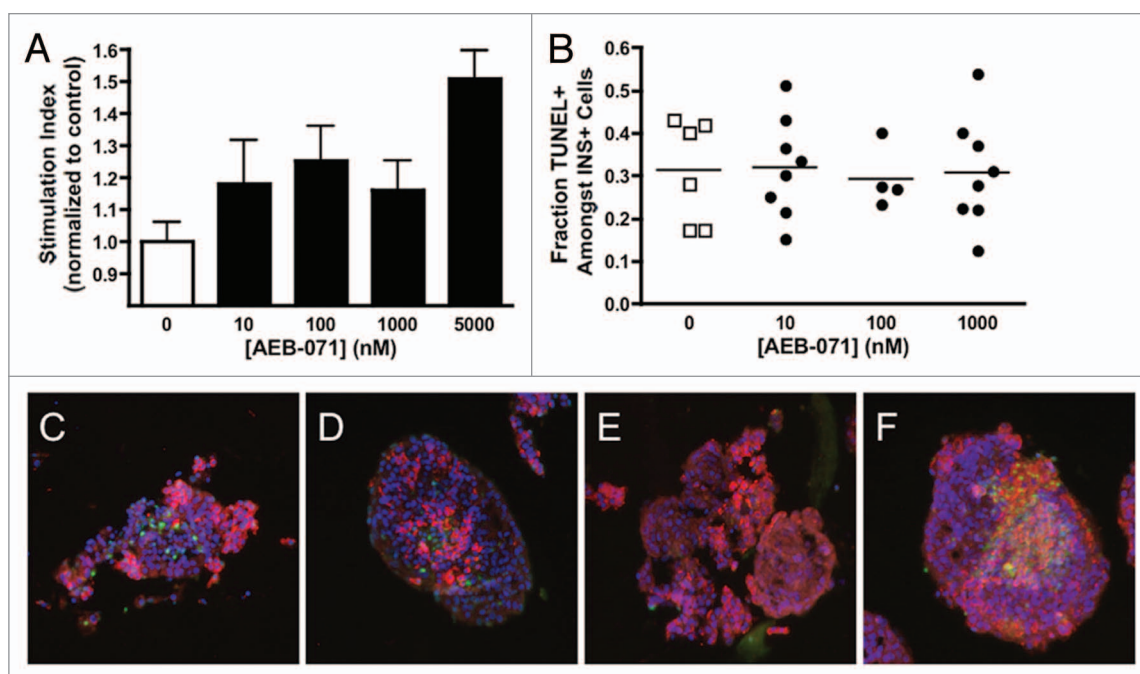


Figure 1. Human islets cultured for 48 h in the presence of AEB show no perturbation in insulin secretion or β -cell apoptosis. Stimulation indexes of cultured human islets in the presence of AEB are similar to that of islets cultured in vehicle control (A, data from three separate islet isolation experiments). Rates of spontaneous apoptosis among β -cells when cultured in AEB were similar with AEB and vehicle control (B). Representative photos of immunohistochemistry staining of islets cultured in presence of AEB, colors represent insulin (red), apoptosis (by TUNEL method, green) and nuclei (blue) (C, 0 nM; D, 10 nM; E, 100 nM; and F, 1,000 nM).

we explored potential AEB toxicity on human islets, particularly those undergoing the delicate processes of tissue culture, transplantation and engraftment. The present study used freshly isolated human islets and investigated the effect of AEB in vitro on β -cell glucose stimulated insulin secretion and spontaneous β -cell apoptosis known to occur in culture. In the second part, diabetic immunodeficient mice transplanted with a well-defined marginal mass of human islets were used to determine if oral AEB altered engraftment and glucose homeostasis offered by the human islet graft.

Results

AEB does not alter stimulated insulin secretion nor apoptosis of human islets in vitro. Human islets cultured in the presence of AEB (10, 100, 1,000 and 5,000 nM) or vehicle for 48 h demonstrated similar glucose stimulated insulin secretion (Fig. 1A and $p = 0.15$ by Kruskal-Wallis test). Furthermore, the rate of β -cell apoptosis after 48 h culture was similar between treatment groups and vehicle control (Fig. 1B and C, $p = 0.98$ by Kruskal-Wallis test), as quantified by the proportion of apoptotic nuclei (TUNEL⁺) amongst insulin⁺ (β -cells).

AEB, unlike sirolimus, does not alter time to normoglycemia, glucose homeostasis or body weight in human islet transplant recipients. To determine the influence of therapeutic immunosuppressive exposure to AEB on transplanted human islets, a previously established model using immunodeficient mice incapable of human islet xenograft rejection transplanted

with human islets was to investigate the engraftment and function in a rejection-free environment.¹⁹ Recipients were randomly assigned to either of an AEB, sirolimus or vehicle control treatment group. A sirolimus treatment group was chosen as a positive control of drug induced toxicity on β -cell engraftment based on previous reports of this phenomenon.^{20,21}

The first day following transplant on which recipients began to maintain non-fasting blood glucose less than 12.0 mM, defined as normoglycemia, was no different between AEB and vehicle treated controls (Fig. 2A and $p = 0.40$). With a median time of 19 d following transplant, 85.7% (5 of 7) AEB treated recipients achieved normoglycemia; and 57.1% (4 of 7) of controls achieved normoglycemia with a median time of 30 d. None of the seven sirolimus treated recipients achieved normoglycemia ($p = 0.0055$ vs. AEB treated animals). Correspondingly, blood glucose levels among sirolimus treated animals were higher than those of both vehicle and AEB treated recipients (Fig. 2B and $p < 0.0001$ for treatment effect by two-way ANOVA).

Body weight of transplants recipients increased to $102.9 \pm 3.2\%$ of pre-transplant weight in AEB treated and $101.0 \pm 3.2\%$ in vehicle treated control animals ($p = 0.63$) by 3 weeks following transplant. Conversely, a decline in body weight to $86.3 \pm 3.5\%$ was observed in sirolimus treated transplant recipients ($p = 0.009$ vs. vehicle).

To further investigate the level of β -cell reserve among recipients who achieved normoglycemia, these animals were subject to a 3 g/kg dextrose bolus challenge (Fig. 3A and B). The area under the curve was used as a cumulative measure of glucose

homeostasis during this tolerance test, and found to be significantly higher in sirolimus treated recipients vs. AEB treated and vehicle control recipients ($p < 0.05$ in both comparisons, Fig. 3B).

Islet grafts were assayed by histology for insulin at the time of experimental end point (Fig. 4). All recipients showed insulin-positive staining regardless of treatment group.

AEB and sirolimus blood levels.

One hour peak AEB levels among treated recipients was 423.2 ± 60.1 ng/ml and AEE-800 was 666.6 ± 184 ng/ml, which correspond to the therapeutic range of AEB previously reported in pancreatic islet transplantation in rodent islet allotransplant.¹² A sirolimus dose of 0.2 mg/kg ip daily was initially chosen based on our previous experience in Balb/c mice where this dose corresponded to a trough levels of 5.93 ± 3.1 ng/ml.²² Therapeutic maintenance trough level in human pancreatic islet transplant is considered to be 7–12 ng/ml.^{1,2} In these B6 background mice, 24 h through sirolimus levels was observed to be 1.7 ± 0.25 ng/ml, which is below the therapeutic window.

Discussion

AEB, a protein kinase C inhibitory immunosuppressant, has shown early promise in clinical and experimental renal transplantation.^{23,24} When human islets were challenged in vitro and in vivo after transplantation in immunodeficient mice, we could find no evidence for β -cell toxicity or impairment of function when exposed to AEB at therapeutic dosing. In a marginal islet transplant mass model using 1,500 IE human islets, neither the fraction of transplant recipients achieving normoglycemia, the time taken for recipients to achieve normoglycemia, nor the maintenance of glucose homeostatic function out to 6 weeks was compromised by treatment of recipients with AEB. Conversely, even a small dose of sirolimus, which is below the therapeutic level target, was found to reduce glucose homeostatic outcomes offered by transplantation of human islets.

We have previously reported that AEB does not have a diabetogenic effect on naïve rodents treated with a therapeutic level dose for 6 weeks.¹² Here, we expand these findings by investigating the impact of AEB on human islets. The nature of these investigations is particularly important since the target of AEB, protein kinase c, is known to be a widely used signaling molecule in endocrine cells including β -cells.²⁵ Others have suggested that inhibition of PKC function may reduce diabetic complications by reducing oxidative stress and endothelial cell destruction in diabetes.²⁶ Either way, there is suspicion of deleterious effects of PKC inhibition by AEB on β -cell engraftment and function. We have shown in our models that no detectable benefit or toxicity

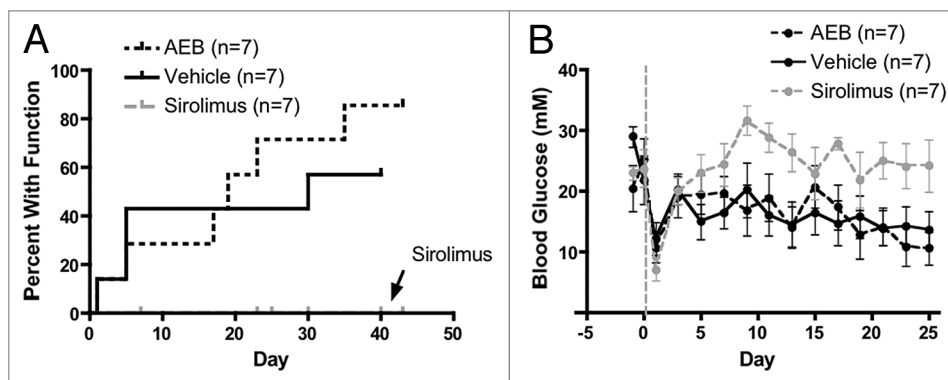


Figure 2. AEB-treated human islet transplant recipients show better engraftment and glucose homeostasis than sirolimus treated recipients. STZ-diabetic immunodeficient mice transplanted with a marginal mass human pancreatic islet transplant show improved reversal of hyperglycemia (A) and glucose homeostasis (B) in AEB and vehicle control treated groups vs. sirolimus. Reversal of hyperglycemia is statistically significant between vehicle vs. sirolimus and AEB vs. sirolimus (both $p < 0.001$, ANOVA).

detectable in engraftment and function of human pancreatic islets undergoing transplantation. The implications of the findings are important to both pancreatic islet transplantation, as well as all emerging therapeutic indications for AEB such as whole organ transplantation (renal and liver), psoriasis, ulcerative colitis and uveitis. In these settings, one can extrapolate that β -cell function should not be altered by AEB dependent mechanisms, and that incidence of post-treatment diabetes will not increase.

Others have shown AEB to be a suitable immunosuppressive agent in both pre-clinical and clinical trials.^{6,7,9-11,13} Here, we show that it also does not have toxic effects on glucose homeostasis. In clinical pancreatic islet transplant, sirolimus is a commonly used immunosuppressive agent and has been shown to have negative effects on β -cell function and be diabetogenic in some,^{4,5,20} but not all, animal models of β -cell function.^{27,28} Our findings support continued investigation into AEB as an alternate immunosuppressive agent since it provides clear benefit over current immunosuppressive agents insofar as it has no detectable toxicity on β -cells nor glucose homeostasis.

Overall, this study demonstrates that human islets both in culture and undergoing transplantation in a marginal mass setting, which are exposed to a therapeutic range of AEB, do not experience reduced engraftment. Rather, AEB treated animals show better engraftment and function when compared with those treated with the current clinical immunosuppressive agent sirolimus. This study admittedly does not study the engraftment of islets in the setting of a human recipient environment, which may reveal the interaction of PKC inhibition in the host as well as on transplanted tissue. Furthermore, although our data here complements our previous investigations in allograft rejection¹² and autoimmune dysfunction;¹⁸ it does not employ an experimental model which combines these mechanism. Perhaps the clinical allograft islet transplant model in patients with autoimmune diabetes would be most definitive toward this understanding.

Ongoing Phase I and II clinical trials of AEB are currently underway, and will undoubtedly better define the toxicity profile of AEB in humans. An ideal agent in clinical islet transplant

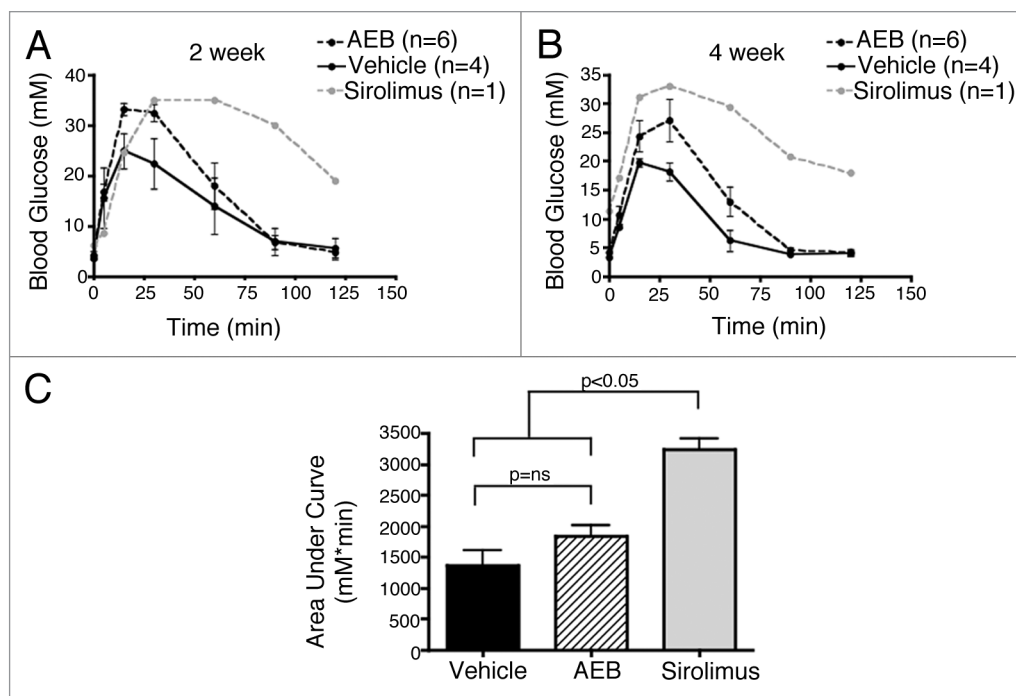


Figure 3. AEB-treated human islet transplant recipients better glucose homeostasis at 2- and 4-weeks post-transplantation compared with sirolimus treated recipients. Glucose tolerance test of human islet recipients shown at 2 weeks (A) and 4 weeks (B). Area under the glucose curve of cumulative data (C) illustrates similar glycemic control among marginal mass transplant recipients in vehicle and AEB treatment groups, vs. poor glycemic homeostasis in sirolimus treated recipients.

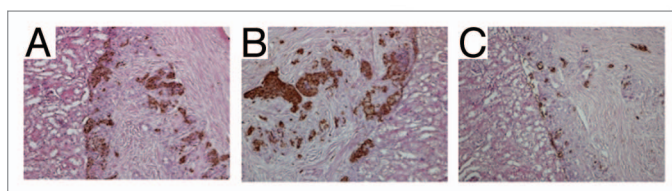


Figure 4. All transplant recipients showed insulin-positive staining of their islet grafts on histological investigation. Transplant recipients had their islet-graft bearing kidney assayed for insulin using histology. Representative samples of (A) vehicle control, (B) AEB and (C) sirolimus treated recipients show that all animals showed insulin staining grafts. Insulin stains brown, hematoxylin and eosin were used as counter-stains.

would preserve the transplanted β -cells through mechanisms of allograft rejection, prevent autoimmune disease recurrence, and have minimal to no toxic effects on glucose homeostasis. Our findings demonstrate that, in animal models at least, AEB has all three properties, and therefore, support continued exploration of AEB as an immunosuppressive agent in islet transplantation.

Materials and Methods

Animals and reagents. Female C57Bl6-Rag^{-/-} mice were purchased at 6 weeks of age from Jackson Laboratory. All animals were housed using conventional methods following Canadian Council on Animal Care guidelines, and experiments were conducted under approval of the University of Alberta Health

Sciences Animal Care and Use Committee. All chemicals were purchased from Sigma-Aldrich unless otherwise noted. AEB (MW = 438.49) was provided by Novartis and was dissolved in polyethylene glycol 400 and distilled water for in vivo experiments. Sirolimus (Rapamune) was purchased from the University of Alberta Hospital Pharmacy.

Human islets. Research ethics approval for the use of human islets was obtained through the University of Alberta Human Research Ethics Board. Human islets from the Clinical Islet Laboratory at the University of Alberta designated for research purposes were enumerated by microscopy and cultured at a density of 1,000 islet equivalents (IE)/ml in CMRL-1066 supplemented with 10% FBS. For in vitro studies, islets were cultured in non-treated tissue culture flasks for 48 h in media containing 0, 10, 100, 1,000 or 5,000 nM of AEB (corresponding to 0, 4.38, 43.8, 438 and 2,190 ng/ml respectively) at 37°C and 5% CO₂. AEB was dissolved in 0.1% DMSO for culture and 0.1% DMSO was used as vehicle control. Three separate islet isolates were used in the in vitro experiments and two additional isolates for the in vivo experiments.

Glucose stimulated insulin secretion static incubation assay. Triplicate aliquots of five hundred IE per treatment group, in each experiment, were cultured in 5 ml RPMI-1640 supplemented with low concentration glucose (2.8 mM) in a 6-well plate within a 40-micron cell strainer at 37°C and 5% CO₂. Following 30 min incubation, the same islet aliquot was transferred to a well containing 5 ml RPMI-1640 supplemented with high concentration glucose (25 mM) for an additional 30 min.

Insulin content was quantified in supernatants from each incubation phase, in triplicate, by the Special Investigations Laboratory at the University of Alberta Hospital using ELISA (Roche Diagnostics). Insulin stimulation indices (SI) were calculated as insulin content in the high glucose media after culture divided by that in low glucose media.

Apoptosis assay. Human islets in culture were prepared for histology by fixing in formalin, followed by embedding within agar and subsequent preparation in paraffin-embedded blocks. Using the method described by Emamaullee et al. islets were stained for insulin and apoptotic cells concurrently to quantify the ratio of apoptotic to non-apoptotic β -cell.²⁹ Guinea pig anti-insulin antibody (Dako) at a dilution of 1:500 was used for insulin staining. Secondary TRITC-conjugated goat anti-guinea pig antibody (Jackson ImmunoResearch) was used for detection of the primary antibody. 4',6-Diamidino-2-phenylindole (DAPI) or Hematoxylin/Eosin were used as counter-stains for immunofluorescence and light microscopy respectively. DeadEnd™ fluorometric TUNEL kit (Promega) was used as per manufacturer's instructions to detect apoptotic cells. A minimum of 100 insulin-staining cells per slide was counted for each treatment group in each islet isolation experiment.

Diabetes induction, islet transplantation and post-operative monitoring. Streptozotocin (190 mg/kg i.p.) was administered to induce diabetes in mice at least 3 d prior to transplant. Transplantation was performed when non-fasting blood glucose level was ≥ 20.0 mM for two or more consecutive days. Under isoflurane anesthesia, the left kidney of the recipients was exposed. The renal capsule was incised with the tip of a 27 gauge needle and kept moist with saline. A glass rod was inserted under the capsule to allow transplantation of 1,500 IE of human islets per diabetic mouse through a PE90 catheter.

Recipients were treated starting on the day of transplant and continuously thereafter with either AEB at 30 mg/kg po bid, sirolimus 0.2 mg/kg ip daily or vehicle (saline 100 μ l po bid). Non-fasting blood glucose of recipients was monitored daily following islet transplantation for two weeks, and on alternate days thereafter using a One Touch Ultra (LifeScan Inc.,) glucometer. Primary graft function (a return to normoglycemia after transplantation) was defined as a minimum of 3 d of blood glucose levels less than 12.0 mM. Animals were investigated for six post-operative weeks, at which time their islet graft was retrieved by nephrectomy and their return to hyperglycemia used to confirm graft dependent normoglycemic status vs. spontaneous remission. No animals showed spontaneous remission from diabetes.

Oral glucose tolerance test. At post-operative week two and four, islet transplant recipients who exhibited a non-fasting blood glucose less than 20 mM were challenged with a 3 g/kg body weight oral dextrose bolus following an 18 h fast. Blood glucose was monitored before glucose bolus and after 5, 15, 30, 60 and

120 min. Absolute area under the glucose curve was calculated using the trapezoidal method.

Blood concentration of immunosuppressive drugs. At the experimental endpoint of 6 weeks, terminal blood collections were conducted by cardiac puncture at either 1 h (peak level for AEB) or 24 h (trough level for sirolimus) after drug administration. Whole blood samples were assayed by Novartis Bioanalytics for AEB and its chief metabolite (AEE-800) or by the University of Alberta Hospital Clinical Trials Laboratory for sirolimus.

Histology. At experimental end point, transplant recipients had their islet-bearing kidney harvested, fixed in formalin and prepared in paraffin-embedded blocks for sectioning. Guinea pig anti-insulin antibody (Dako) at a dilution of 1:500 was used for insulin staining. Secondary biotinylated goat anti-guinea pig antibody visualized with avidin-binding complex with diaminobenzidine as a chromagen (Jackson ImmunoResearch) was used for detection of the primary antibody. Hematoxylin/Eosin were used as counter-stains.

Statistical analysis. Data was analyzed using GraphPad Prism (GraphPad Software, version 4.0a). Kaplan-Meier survival function curves were compared using the log-rank statistical method. Non-parametric one-way or two-way ANOVA and appropriate post hoc tests were used for analysis of data as described in the results section. Statistical significance was considered to be a p-value less than 0.05. All graphical representation of data are as mean \pm standard error of the mean (SEM) indicated by error bars.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Funding for this project was provided by charitable donations from the Roberts family, the Diabetes Research Institute Foundation of Canada, and the Victor family. We would like to thank Drs. Jurgen Wagner, Walter Schuler and Colin Anderson for their contribution to experimental design and thoughtful contribution to interpretation of data. A.M.J.S. is a Senior Clinical Scholar supported through the Alberta Heritage Foundation for Medical Research (AHFMR). S.M. is a recipient of the AHFMR M.D./Ph.D., Studentship, the CIHR Walter and Jessie Boyd and Charles Scriver M.D./Ph.D., Studentship and Lionel E. McLeod Award. C.T. is supported by the Swiss National Science Foundation, a F.S. Chia award and the AHFMR. M.M. is the recipient of an Alberta Diabetes Foundation/Wirtanen Scholarship. S.M., C.T., J.A.E. and A.M.J.S. participated in research design. S.M., M.M., C.T., J.A.E. and A.M.J.S. participated in writing of the paper. S.M., M.M., R.L.P., R.L.E., J.D. and T.K. participated in performance of the research. S.M., R.L.E. and M.M. participated in data analysis.

References

- Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; 343:230-8; PMID:10911004; DOI:10.1056/NEJM200007273430401.
- Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, et al. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med* 2006; 355:1318-30; PMID:17005949; DOI:10.1056/NEJMoa061267.
- Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, et al. Five-year follow-up after clinical islet transplantation. *Diabetes* 2005; 54:2060-9; PMID:15983207; DOI:10.2337/diabetes.54.7.2060.
- Larsen JL, Bennett RG, Burkman T, Ramirez AL, Yamamoto S, Gulizia J, et al. Tacrolimus and sirolimus cause insulin resistance in normal sprague dawley rats. *Transplantation* 2006; 82:466-70; PMID:16926589; DOI:10.1097/01.tp.0000229384.22217.15.
- Paty BW, Harmon JS, Marsh CL, Robertson RP. Inhibitory effects of immunosuppressive drugs on insulin secretion from HIT-T15 cells and Wistar rat islets. *Transplantation* 2002; 73:353-7; PMID:11884930; DOI:10.1097/00007890-200202150-00007.
- Evenou JP, Brinkmann V, Towbin H, Welzenbach K, Cottens S, Wagner J, et al. Enzymatic cellular characterization of NVP-AEB071 (AEB), a novel selective protein kinase c (PKC) inhibitor that blocks early T cell activation, and its use to define the role of PKC in T cells. *Am J Transplant* 2006; 6:4-1145.
- Evenou JP, Thuille N, Cottens S, Kovarik J, Wagner K, Zenke G, et al. NVP-AEB071 (AEB), a novel protein kinase c inhibitor, abrogates early mouse T cell activation without affecting activation induced cell death. *Am J Transplant* 2006; 6:4-1145.
- Yabu JM, Vincenti F. Novel immunosuppression: small molecules and biologics. *Semin Nephrol* 2007; 27:479-86; PMID:17616278; DOI:10.1016/j.seminephrol.2007.03.009.
- Evenou JP, Wagner J, Zenke G, Brinkmann V, Wagner K, Kovarik J, et al. The potent protein kinase C selective inhibitor AEB071 (Sotrastaurin) represents a new class of immunosuppressive agents affecting early T cell activation. *J Pharmacol Exp Ther* 2009; 330:792-801; PMID:19491325; DOI:10.1124/jpet.109.153205.
- Bigaud M, Wiecek G, Riesen S, Menninger K, Barbet I, Jean C, et al. NVP-AEB071 (AEB), a novel oral inhibitor of early T cell activation, prolongs the survival of non-human primate kidney allografts when used as monotherapy or at non-effective doses combined with a non-effective dose of cyclosporine. *Am J Transplant* 2006; 6:4-1145.
- Bruns C, Pally C, Beerli C, Wiecek G, Weckbecker G, Wagner J, et al. NVP-AEB071 (AEB), a novel oral inhibitor of early t-cell activation, prolongs rat cardiac allograft survival when used alone and in combination with cyclosporine, everolimus or FTY720. *Am J Transplant* 2006; 6:4-1145.
- Merani S, Pawlick RL, Edgar RL, Toso C, Emamaullee J, Anderson CC, et al. Protein kinase C inhibitor, AEB-071, acts complementarily with cyclosporine to prevent islet rejection in rats. *Transplantation* 2009; 87:59-65; PMID:19136892; DOI:10.1097/TP.0b013e31819198b4.
- Skvara H, Dawid M, Kleyn E, Wolff B, Meingassner JG, Knight H, et al. The PKC inhibitor AEB071 may be a therapeutic option for psoriasis. *J Clin Invest* 2008; 118:3151-9; PMID:18688284; DOI:10.1172/JCI35636.
- Hayashi K, Altman A. Protein kinase C θ (PKC θ): a key player in T cell life and death. *Pharmacol Res* 2007; 55:537-44; PMID:17544292; DOI:10.1016/j.phrs.2007.04.009.
- Sun Z, Arendt CW, Ellmeier W, Schaeffer EM, Sunshine MJ, Gandhi L, et al. PKC θ is required for TCR-induced NF κ B activation in mature but not immature T lymphocytes. *Nature* 2000; 404:402-7; PMID:10746729; DOI:10.1038/35006090.
- Werlen G, Jacinto E, Xia Y, Karin M. Calcineurin preferentially synergizes with PKC θ to activate JNK and IL-2 promoter in T lymphocytes. *EMBO J* 1998; 17:3101-11; PMID:9606192; DOI:10.1093/emboj/17.11.3101.
- Ghaffari-Tabrizi N, Bauer B, Villunger A, Baier-Bitterlich G, Altman A, Utermann G, et al. Protein kinase C θ , a selective upstream regulator of JNK/SAPK and IL-2 promoter activation in Jurkat T cells. *Eur J Immunol* 1999; 29:132-42; PMID:9933094; DOI:10.1002/(SICI)1521-4141(199901)29:01<132::AID-IMMU132>3.0.CO;2-7.
- Merani S, Edgar RL, Toso C, Emamaullee J, Thiesen A, Shapiro AM. AEB-071 has minimal impact on onset of autoimmune diabetes in NOD mice. *Autoimmunity* 2009; 42:242-8; PMID:19301207; DOI:10.1080/08916930802587950.
- Emamaullee JA, Davis J, Pawlick R, Toso C, Merani S, Cai SX, et al. The caspase selective inhibitor EP1013 augments human islet graft function and longevity in marginal mass islet transplantation in mice. *Diabetes* 2008; 57:1556-66; PMID:18356409; DOI:10.2337/db07-1452.
- Laugharne M, Cross S, Richards S, Dawson C, Ilchysyn L, Saleem M, et al. Sirolimus toxicity and vascular endothelial growth factor release from islet and renal cell lines. *Transplantation* 2007; 83:1635-8; PMID:17589348; DOI:10.1097/01.tp.0000266555.06635.bf.
- Nanji SA, Shapiro AM. Islet transplantation in patients with diabetes mellitus: choice of immunosuppression. *BioDrugs* 2004; 18:315-28; PMID:15377174; DOI:10.2165/00063030-200418050-00004.
- Merani S, Truong W, Emamaullee JA, Toso C, Knudsen LB, Shapiro AM. Liraglutide, a long-acting human glucagon-like peptide 1 analog, improves glucose homeostasis in marginal mass islet transplantation in mice. *Endocrinology* 2008; 149:4322-8; PMID:18511515; DOI:10.1210/en.2008-0501.
- Budde K, Sommerer C, Becker T, Asderakis A, Pietruck F, Grinyo JM, et al. AEB071, a novel protein kinase c-inhibitor: first clinical results of an AEB071 plus tacrolimus regimen in renal transplant recipients. *Am J Transplant* 2010; 10:571-81.
- Friman S, Arns W, Banas B, Chan L, Mulgaonkar S, Nashan B, et al. AEB071, a novel protein kinase C-inhibitor: evaluation of an AEB071 plus mycophenolate regimen in renal transplant recipients. *Am J Transplant* 2011; 11:1444-55.
- Liu XJ, He AB, Chang YS, Fang FD. Atypical protein kinase C in glucose metabolism. *Cell Signal* 2006; 18:2071-6; PMID:16787739; DOI:10.1016/j.cellsig.2006.04.007.
- Potenza MA, Gagliardi S, Nacci C, Carratu MR, Montagnani M. Endothelial dysfunction in diabetes: from mechanisms to therapeutic targets. *Curr Med Chem* 2009; 16:94-112; PMID:19149564; DOI:10.2174/092986709787002853.
- Melzi R, Maffi P, Nano R, Sordi V, Mercalli A, Scavini M, et al. Rapamycin does not adversely affect intrahepatic islet engraftment in mice and improves early islet engraftment in humans. *Islets* 2009; 1:42-9; PMID:21084848; DOI:10.4161/isl.1.1.8881.
- Kneteman NM, Lakey JR, Wagner T, Finegood D. The metabolic impact of rapamycin (sirolimus) in chronic canine islet graft recipients. *Transplantation* 1996; 61:1206-10; PMID:8610419; DOI:10.1097/00007890-199604270-00015.
- Emamaullee JA, Rajotte RV, Liston P, Korneluk RG, Lakey JR, Shapiro AM, et al. XIAP overexpression in human islets prevents early posttransplant apoptosis and reduces the islet mass needed to treat diabetes. *Diabetes* 2005; 54:2541-8; PMID:16123341; DOI:10.2337/diabetes.54.9.2541.