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IMPROVED ISLET FUNCTIONAL RECOVERY, SURVIVAL, AND PRESERVATION OF ENGRAFTMENT CAPACITIES POST-THAWING USING A CGMP VITRIFICATION SYSTEM FOR SUBSEQUENT ISLET TRANSPLANTATION

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Advanced Cell-Based and Regenerative Therapies in Mexico.

Introduction: Islet transplantation is now an elective therapy for diabetes mellitus. However, there is a global shortage of pancreas donors and once islets are isolated cannot be maintained for long-time in culture. Cryopreservation is an attractive method to maintain isolated islets for a subsequent use if their viability and function are not compromised post-thawing. In the present study, we investigated the utility of islet vitrification to preserve viable and functional islets that kept their engraftment capacities post-thawing for islet transplantation.

Material and Methods: Islets were isolated form Balb/c mice and briefly culture in RPMI 1640 medium for 16 hours, then were divided into the three following experimental groups: G1; Islets cultured (IC), which served as positive control, G2; Islets vitrified (IV) G3; Islets cryopreserved with 10% DMSO and 10% human serum albumin (HSA) (IDH), which served as negative control. To perform in vitro analyses firstly, either IV or IDH were thawed and then culture for 16 hour to allow recovery post-thawing before starting the experiments. All three groups were comparatively assessed for viability, apoptosis, mitochondrial function, glucose-stimulated insulin secretion (GSIS), and pro-inflammatory cytokines expression. To assess the islet capacities of engraftment and in vivo function islets from all groups were transplanted into streptozotocin-induced diabetic mice beneath the kidney capsule and follow their blood glucose levels.

Results and Discussion: The viability assay showed similar results between G1 and G2, in contrast G3 had a significant reduction to >40%. The apoptosis was detected about 8% for G1, and 10% for both G2 and G3. The mitochondrial-staining functional assay revealed recovery for G2 equal to those islets, which were just maintained in culture G1. Instead, G3 mitochondrial function could not be restored post-thawing. The secretory function evaluated by GSIS was 5.17 for G1, 3.92 for G2, and 1.71 respectively. Interestingly, the panel of pro-inflammatory cytokine expression showed low levels for G1 and G2 and significantly higher levels for G3, which correlates with the viability. The experiments in vivo has showed a well-preserved early capacity of engraftment within a week for G1 equal to G2, meanwhile G3 has a graft function unable to control blood glucose levels. However, longer follow up and potency test are ongoing to fully support the usefulness as functional grafts.

Conclusions: The results of a cGMP vitrification system has promising results by preservation of viable islets post-thawing and functional recovery similar to culture islets. In addition, the secretory function was also preserved while maintaining a low inflammatory profile, which could be suitable for islet transplantation. Therefore, the use of vitrification may be a useful tool to preserve islet mass long-time until need for clinical islet transplantation.

R&R Research and Development Solutions to have been funded the present study. To Mexican Society of Transplantation who always motivate young researchers.

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DURABLE EFFECT OF BETA CELL REPLACEMENT THERAPY ON IMPROVEMENT IN BLOOD GLUCOSE CONTROL AND PREVENTION OF PROGRESSION OF SECONDARY DIABETIC COMPLICATIONS IN NONUREMIC PATIENTS WITH TYPE 1 DIABETES MELLITUS AND PROBLEMATIC HYPOGLYCEMIA

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Introduction: Beta cell replacement therapy in form of pancreas or islet transplantation is the only effective treatment for patients suffering from hypoglycemic unawareness despite intensive insulin treatment. Transplant provide endogenous insulin allowing for physiologic optimal blood glucose control.

Materials and Methods: 13 consecutive nonuremic patients with "brittle" type 1 diabetes (T1DM) received 28 islet transplants (up to 3 islet infusions) and 4 of them subsequently pancreas transplantation to extend benefit of insulin independence. Thymoglobulin was used during first islet transplant and basiliximab prior to subsequent islet and pancreas transplants for induction, whereas tacrolimus and mycophenolate for maintenance immunosuppression. Patient received Reparixin, etarnecept or no anti-inflammatory therapy in peritransplant period.

Results: Three patients developed donor specific antibodies (DSAs) with antibody mediated rejection and 1 severe cytokine release syndrome and 1 bleeding, which compromised islet graft function. Overall 1, 2, 3 and 5 year insulin independence rate after first islet transplantation was 11/13 (85%), 11/13 (85%) and 8/13 (61%), 6/13 (46%), respectively. Four patients received pancreas tx after median 4.5 years (3.5 - 6.5) increasing 5 and 6 year insulin independence rate to 61.5% (8/13) and 60% (6/10), respectively. Currently, 61.5% (8/13) are still off insulin with median follow up 6 years (5.3 - 7.6) and 2 more with partial islet function with A1c of 5.5% and 6.6% leading to 10/13 (77%) patients without severe hypoglycemic episodes and hypoglycemia unawareness. Remaining 3 patients dropped the study due to social reasons right after their first or second islet transplant, or one due to leukemia. Secondary diabetic complications such as the diabetic neuropathy remained stable but retinopathy improved in 4/13 patients (30%). None of the patients experienced any of the cardiovascular events. One patient with creatinine 1.55 mg/ml (GFR = 59) received kidney together with pancreas transplantation.

Conclusion: Beta cell replacement therapy in form of islet and subsequent pancreas transplantation has proven its long-term efficacy in restoring normoglycemia and alleviating the immediate burden of hypoglycemic unawareness as well as preventing progression of secondary complications.