Harmine Nanoemulsion-Mediated Immune Preconditioning Elicits Durable Protection from Rejection of Allogeneic Islet Grafts in the Anterior Chamber of the Eye

Maxwell Cusnier[1], Brandon Hugh Watts[2], Oscar Alcazar[2], PhD, Midhat Abdulreda[2], PhD, Peter Buchwald,[2] PhD and Christopher A Fraker[2], PhD

1. Lincoln Memorial University, DeBusk College of Osteopathic Medicine

2. University of Miami, Leonard M Miller School of Medicine, Diabetes Research Institute

Abstract

Background: Islet or stem cell derived β-cell (SC-β) transplantation offers a promising therapy for patients with type 1 diabetes, but its success is severely limited by early inflammatory events and immune-mediated loss of transplanted tissue. These challenges create a critical need for non-invasive strategies that can modulate inflammation prior to transplant or in lower doses at the transplant site, thereby reducing early graft loss, and potentially eliminating or reducing reliance on chronic systemic immunosuppression

Objectives:. This study aimed to formulate and test nanoemulsions made of Harmine, a naturally occurring alkaloid that's demonstrated potential in β-cell proliferation and anti-inflammatory properties in models' neural inflammation. By improving bioavailability and implementing a brief preconditioning strategy, we hypothesized that our nanoemulsified formulations could impart durable protection against early inflammatory responses from antigen presenting cells that lead to cytotoxic immune cell recruitment and graft destruction.

Method: Harmine nanoemulsions were produced by high-pressure homogenization, then sterile-filtered and quantified by UV spectrophotometry. The optimized harmine nanoemulsion dose (0.2µg per mouse per day; ~10µM for in vitro dosing) was used to precondition isolated mouse islets for two hours before transplantation into the anterior chamber of allogeneic recipients.

Results: Of the five animals in the harmine nanoemulsion group, 80% had durable engraftment past 90 days, with some currently POD 120. In a smaller subset, animals were rechallenged with a second allogeneic transplant in the contralateral anterior chamber with freshly isolated islets.

Conclusions: These findings indicate that brief harmine-nanoemulsion preconditioning, followed by 14 days of low-dose local delivery, is sufficient to provide durable protection against allogeneic graft rejection. We propose that this effect stems from dampened activation of local antigen-presenting innate immune cells, reducing pro-inflammatory surveillance signals and shifting them toward a more tolerogenic, quiescent state.

Introduction

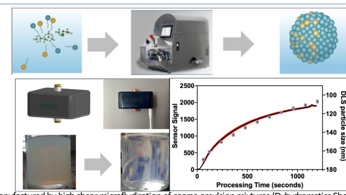
Islet or stem cell derived β-cell (SC-β) transplantation offers a promising therapy for patients with type 1 diabetes, but its success is severely limited by early inflammatory events and immune-mediated loss of transplanted tissue. Strategies that reduce antigen shedding and early inflammatory signaling are critical to prolonging graft survival. Despite advances in immunosuppression, most systemic agents utilized carry significant toxicity, particularly problematic because patients with diabetes already exhibit immune dysfunction, making lifelong systemic suppression both risky and detrimental to graft health. These challenges create a critical need for non-invasive strategies that can modulate inflammation prior to transplant or in lower doses at the transplant site, thereby reducing early graft loss, and potentially eliminating or reducing reliance on chronic systemic immunosuppression.

This study aimed to formulate and test nanoemulsions made of Harmine, a naturally occurring alkaloid that's demonstrated potential in β-cell proliferation and anti-inflammatory properties in models' neural inflammation. By improving bioavailability and implementing a brief preconditioning strategy, we hypothesized that our nanoemulsified formulations could impart durable protection against early inflammatory responses from antigen presenting cells that lead to cytotoxic immune cell recruitment and graft destruction.

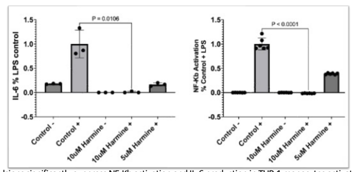
Methods

Harmine nanoemulsions were produced by high-pressure homogenization, then sterile-filtered and quantified by UV spectrophotometry. Harmine dosing was subsequently titrated and optimized in genetically engineered THP-1 cells by colorimetric measurement of secreted alkaline phosphatase triggered by activation of the NF-κB pathway with lipopolysaccharide. The optimized harmine nanoemulsion dose (0.2µg per mouse per day; ~10µM for in vitro dosing) was used to precondition isolated mouse islets for two hours before transplantation into the anterior chamber of allogeneic recipients. Post-transplant, recipients received harmine nanoemulsion eyedrops for 14 days. Islet grafts were imaged one to two times weekly to monitor immune responses and detect rejection using dual-photon confocal microscopy.

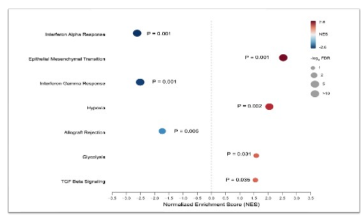
Results



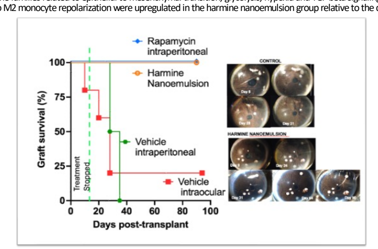
Nanoemulsions are manufactured by high shear microfludization of coarse emulsion mixtures (Dyhydromatics ShearJet HL60). The process is monitored using custom designed optical sensors to insure uniform particle size and low polydispersity. This generates transparent nanoemulsions from the historically cloudy mixtures.



10uM harmine nanoemulsions significantly suppress NF-Kb activation and IL-6 production in THP-1 monocytes activated with 50ng/mL LPS. Notably, the nanoemulsion lacking LPS does not produce any inflammatory signalling, indicating the safety and biocompatibility of the GRAS materials used for manufacture.



RNAseq analysis of THP-1 monocytes stimulated with LPS (50ng/mL) +/- 10uM Harmine nanoemulsion showed differential gene expression in the harmine nanoemulsion group relative to the control group. Inflammatory signaling and notably, allograft rejection gene familes were significantly down regulated. Gene families related to epithelial to mesenchymal transition, glycolysis, hypoxia and TGF beta signaling, all cytoprotective families and signals of M1 to M2 monocyte repolarization were upregulated in the harmine nanoemulsion group relative to the control.



In MHC-mismatched islet cell allografts transplanted into the anterior chamber of the eye in recipient mice, preconditioning with 10uM harmine nanoemulsion and brief 14 day eye drop treatment resulted in durable and complete prevention of rejection. The only other group with similar results was systemic IP administration of rapamycin at molar equivalent doses 250X that of the harmine nanoemulsion. This demonstrates the potential for the harmine nanoemulsions to provide local and long-term graft protection without systemic immunosuppression and without off target effects. Additionally, using the harmine nanoemulsion, all treatments were stopped POD 14 and the protection persisted.

Discussion

Discussion: These findings indicate that brief harmine-nanoemulsion preconditioning, followed by 14 days of low-dose local delivery, is sufficient to provide durable protection against allogeneic graft rejection. Although treatment lasted only 14 days, most treated animals retained their grafts for over 90 days, suggesting that the early immune response is a key driver of rejection. We propose that this effect stems from dampened activation of local antigen-presenting innate immune cells, reducing pro-inflammatory surveillance signals and shifting them toward a more tolerogenic, quiescent state. The results suggest that localized early immune modulation may reduce the need for long-term immunosuppression.

Limitations: Limitations of this study include data collected from a small number of animals, which hinders the ability to generalize the findings to larger populations. Additionally, the study was performed in a transplant model that doesn't fully mimic Type 1 Diabetes, in which the immune system is already attacking beta cells. Furthermore, longer experimentation is needed to fully confirm the permanence of the graft protection.

Future Studies: Future work will evaluate graft protection in chemical and autoimmune models of hyperglycemia to assess performance under disease-relevant stress. Larger studies will be used to ensure more reliable results and to check for long-term safety. Future work could also explore whether harmine protects the graft and supports β-cell growth simultaneously.

References

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