DEVELOPMENT OF A SMALL-VESSEL ISCHEMIA-REPERFUSION MODEL TO ASSESS ENDOTHELIAL-TARGETED DRUG DELIVERY

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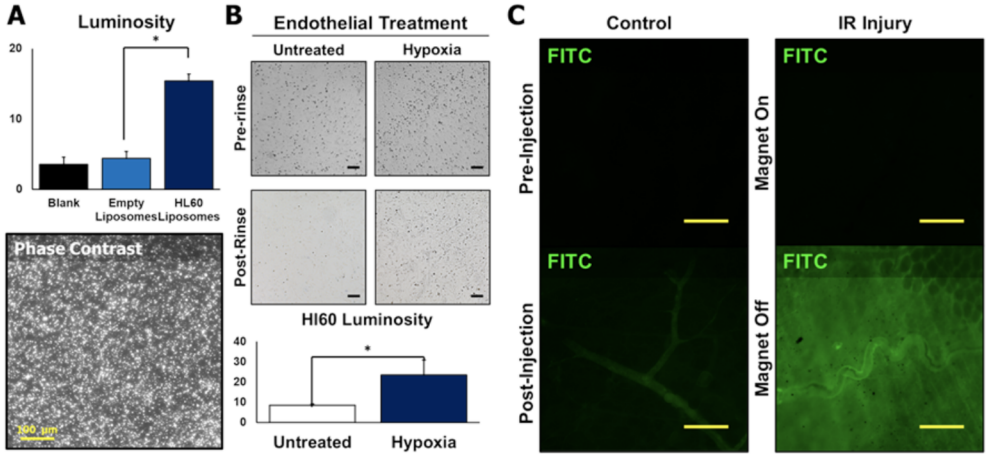
Introduction

- Ischemia-reperfusion (IR) is a common mediator of injury in the initiation and propagation of chronic wounds.
- IR results in endothelial dysfunction with resultant local and systemic propagation of inflammatory signals. This results in expression of endothelial surface markers which bind and localize leukocytes to sites of injury.
- We propose a system to transfer leukocyte-derived surface markers to liposomes to enhance drug delivery to dysfunctional endothelium.
- Here we describe our model of small-vessel IR in the skin as well as our system for liposomal transfer and validation of adherence.

Material and Methods

Liposomal Loading: Loading of liposomes with leukocyte proteins was demonstrated via luciferase activity/luminosity assay in vitro between loaded and unloaded liposomes.
In Vitro Attachment Assay: To confirm endothelial expression of PMN-binding proteins with ischemia; we examined PMN-differentiated HL60-luc2 cells attachment to endothelial cells cultured under hypoxic conditions following a washout assay.
Magnet Induced IR Mouse Model: To generate replicable soft-tissue IR injury we utilized a murine model of magnet-induced skin compression with ring-shaped magnets. Degree of compression is controlled by the number of stacked magnets and the extent of injury is controlled by the duration and number of cycles of IR. IV administration of high molecular weight FITC-dextran was utilized to assess blood flow and post-injury extravasation.

Results



A. Liposomal Loading: Average luminosity with luciferase activity in HL60-luc2 loaded vs. unloaded liposome *(top)*; representative sample of liposome under phase contrast post-extrusion *(bottom).*

B. Adherence Assay: Adherence of Hl60-luc2 PMNs to endothelial cells pre- and post-hypoxia *(top)*; and average post-wash luminosity of adherent PMNs *(bottom)*.

C. Magnet Induced Cutaneous Ischemia-Reperfusion Injury: Absence of endogenous FITC signal prior to intravenous injection of FITC-dextran (500 kd) *(top left)*; presence of localized intravascular signal in native skin post-FITC administration *(bottom left)*; absence of intravascular FITC signal with I/R magnet clamp in place *(top right)*; extensive extravasation of FITC into surrounding tissues post-magnet release *(bottom right).*

Conclusions

- On liposomal loading we noted successful transfer of luciferase activity to our loaded vs. unloaded liposomes.
- Utilizing our in vitro adherence assay, we demonstrated persistent adherence of Hl60-luc2 PMNs to endothelial cells pre- and post-hypoxia with sustained luminosity.
- On liposomal loading we noted successful transfer of luciferase activity to our loaded vs. unloaded liposomes.
- In our murine model, we noted absence of endogenous FITC signal prior to intravenous injection and presence of localized intravascular signal in native skin post-FITC administration.

Future Directions

We are currently using paired magnets to induce IR injury with varying duration and cycles of ischemia and reperfusion in order to characterize levels of endothelial dysfunction in the tissue.

References

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