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PROPRANOLOL AS A NOVEL THERAPY FOR LYMPHATIC MALFORMATIONS

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Purpose:
Lymphatic malformations (LM) are associated with significant morbidities for which there are currently no consistently effective treatments. Development of novel therapies has been limited by lack of biologic understanding of LMs. We characterized a novel human LM progenitor cell (LMPC) population that recapitulates LMs in a mouse model. Propranolol has been proven to be an effective therapy for infantile hemangiomas, another vascular anomaly with a progenitor cell origin. We hypothesized that propranolol would alter development of experimental LMs.

Methods:
De-identified patient samples (IRB-AAAA7338) of LMs and uninvolved tissues (N=8) were immunostained for beta1/2/3-adrenergic receptors and podoplanin, a lymphatic endothelial marker. Propranolol effects on LM-derived LMPCs and LM endothelial cells (LMECs) on *in vitro* proliferation were assessed (WST-8 cell-counting kit). Cytotoxicity was determined by fluorescence-based digital image microscopy. Next, NCR nude mice were randomized to treatment/control arms (N=6 each) and pretreated with propranolol (40mg/kg/day) or vehicle for 5 days prior to implantation of 1.8 x106 LMPCs in each cohort. Propranolol administration was continued throughout the experiment. Implants were removed at 5 weeks for analysis.

Results:
Immunohistochemistry demonstrated an increase in all three beta -adrenergic receptors in LM tissues. A significant decrease in LMEC proliferation was observed at 25uM propranolol, with LMPC proliferation significantly decreased at 50uM. Propranolol-induced cytotoxicity was found at 10-3M ($p < 0.05$) for both LMECs and LMPCs. *In vivo*, implants from propranolol -treated mice exhibited 3.2-fold decrease in lymphatic channel area and a 2-fold increase in adipose area as compared to controls.

Conclusion:
Human LMs contain a progenitor cells that can recapitulate LMs in an *in vivo* model. Propranolol dose -dependently represses proliferation and induces cytotoxicity in LMPCs *in vitro*, and decreases lymphatic channel area *in vivo*. Our data suggests that beta -adrenergic blockade may prove useful in therapy