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John Macbeth  
*Pepperdine University*

Donna Nofziger-Plank  
*Pepperdine University*

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Lyrosphosphatidic Acid Stimulates Lymphangiogenesis in Human Lymphatic Endothelial Cells

John Macbeth, Dr. Donna Noziger Plank

Department of Biology, Natural Science Division, Pepperdine University, Malibu, CA

Abstract
Lympangiogenesis is the process by which new lymphatic vessels sprout and grow from existing vessels whether under developmental, immunological, or cancerous conditions. Proper lymphatic vessel formation is important in working alongside normal angiogenesis in order to help regulate the body's tissue fluid as well as aid in immunosurveillance. Various factors regulate lymphangiogenesis such as members of the vascular endothelial growth factor family (VEGF). Another factor that has recently been identified to play a role in lymphangiogenesis is the bio-active phospholipid lysophosphatidic acid (LPA) however the molecular mechanism by which LPA regulates lymphangiogenesis has not been well characterized. In this study, human lymphatic endothelial cells (HLECs) were treated with LPA in the presence or absence of VEGF and the late stage effects of lymphangiogenesis were examined. Preliminary evidence suggests that VEGF and LPA induces proliferation in HLECs, however there is no increase in this stimulation when both factors are added together. A Matrigel tube formation assay revealed that LPA induces an increase in cellular extensions as well as in tube length as compared to the control. Another factor that has recently been implicated in regulating lymphangiogenesis is the VEGF. VEGF is a type of growth factor family (VEGF).

Introducro
Lymphatic vessel formation, lymphangiogenesis, is known to occur during normal development and during tissue stress such as inflammation and wound healing. In an inflammatory response, there is an excess collection of fluid near the wound. Lymphatic vessels grow into the damaged area to relieve the fluid and to allow more leukocytes or white blood cells to enter the affected area [1]. Thus, lymph vessel formation is vital for regulating the body’s tissue fluid and recruiting leukocytes as part of the body’s immune response. One important mediator of the inflammatory response is the bio-active phospholipid lysophosphatidic acid (LPA). LPA belongs to a family of lipid growth factors that is present in low concentrations in serum and biological fluids but is found in higher concentrations at sites of inflammation and tumor growth [2]. LPA has been shown to modulate inflammatory responses through the stimulation of chemokines, cytokines, and cytokine receptors expression as well as regulate cytoskeletal rearrangement and migration of target cells. LPA evokes its biological effects through binding to G-protein coupled receptors. There are five known receptors for LPA, LPA[1-5]. It has previously been found that binding to LPA[1-4] induces the production of pro-angiogenic factors such as VEGF, Interleukin 6 (IL-6) and Interleukin 8 (IL-8) [4].

In this study, we will look at the effect of LPA on lymphangiogenesis using human lymphatic endothelial cells (HLECs). Using an in vitro assay, I measured the ability of HLECs to proliferate in the presence of LPA in the presence or absence of vascular endothelial growth factor (VEGF). Additionally, a BD Matrigel tube formation assay was used to measure the effect of LPA on the ability of HLECs to extend and form rudimentary tubes.

Proliferation Assay

Figure 2. Proliferation assay results following 24 hour exposure to factors. Following serum starvation, 3,000 HLECs were plated in each fibronectin-coated well of a 96-well plate in EGM-2-MV media under the following conditions: control (EGM-2-MV media alone), LPA (10uM), LPA (1uM), VEGF-A, or both LPA (10uM) and VEGF-A. The cells were then incubated at 37°C for 24 hours and a cell proliferation assay was performed using a Cell Counting Kit-8 (D DJindo). Briefly, a water soluble tetrazolium salt, WST-8, was added to each well and allowed to incubate for 4 hours. WST-8 is reduced by dehydrogenases in the cells producing an orange colored formazan dye which can be detected by measuring the absorbance at 450 nm using a microplate reader. The amount of dye generated is directly proportional to the number of living cells that well. Multiple assays were performed and the ratio of absorbance relative to the control was determined. The average of this ratio for the multiple assays is shown here. There is no significant difference between the number of viable cells between the different treatment groups.

LPA Structure

Figure 1. Diagram of the structure of lysophosphatidic acid

Literature Cited
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Conclusions
• Preliminary evidence suggests LPA stimulates proliferation in HLECs
• No significant co-stimulatory response detected with both LPA and VEGF-A.
• Results from Matrigel lymphangiogenic tube formation assay suggest
  • At two hours, compared to control, 1 µM LPA exposure resulted in an increase in both cell extension number and length while 10 µM LPA induced more extensions but no difference between extension length was found.
  • At 6 hours, there was a significant difference in tube length between 1 µM LPA and the control with a p value of 0.0003.