



Abstract

Background: The lymphatic system is important for bodily processes, such as absorbing fats, immune response, and balancing fluids. High saturated fatty acid (SFA) consumption disrupts lymphatic function within the mesentery. Impaired mesentery lymphatic function leads to increased permeability and abnormal lymph leakage causing visceral obesity and insulin resistance.

Objective: To test anti-inflammatory compounds, methylsulfonylmethane (MSM) and epigallocatechin gallate (EGCG), against dysfunction induced by exposure to palmitic acid, a high SFA, or inflammatory stimuli, TNF-a, and VEGF-C.

Method: Cell culture models of lymphatic endothelial cells will be used to evaluate the effect of MSM and EGCG during exposure to palmitic acid or TNF-a, and VEGF-C. Cell viability, cytotoxicity, and permeability of Evans Blue dye will be tested at various concentrations of treatments to identify proper dosages. Once treatment doses are verified, primary outcomes will include the transwell permeability assay, protein concentration and mRNA transcript expression of growth and inflammation markers by western blot and real-time polymerase chain reaction, respectively.

Expected Outcomes: We hypothesize MSM and EGCG will improve cell barrier function and decrease markers of inflammation in lymphatic endothelial cells exposed to high SFA or inflammatory stimuli.

Introduction

Overview: The lymphatic system is part of the immune system. It is a network of tissues, vessels, and organs that work to move a colorless, watery fluid, known as lymph, back into the bloodstream. It protects the body from invaders that may cause illness, balances body fluid levels, absorbs digestive tract fats, and removes waste products. Dysfunction, such as blockages or infections, can affect the lymphatic system leading to disease. Some causes of lymphatic dysfunction are high exposure to saturated fats and inflammation.³

Current treatment: Treatment may include compression bandages, massage, compression stockings, sequential pneumatic pumping, careful skin care and, rarely, surgery to remove routes.⁸ swollen tissue drainage to create new

Potential Treatment: To emphasize the preservation of lymphatic endothelial function and to reduce high saturated fat exposure in patients through proper education and dietary supplementation.

Osteopathic Aims: To apply models of osteopathic medicine to investigate techniques to improve the functional relationship between lymphatics, cardiovascular system, digestion and metabolism, and the use of osteopathic manipulative treatment in lymph-related dysfunction.

Research Aims: To test anti-inflammatory compounds against lymphatic dysfunction induced by exposure to high saturated fat and/or inflammatory stimuli.



Anti-Inflammatory Interventions for Lymphatic Endothelial Cell Dysfunction

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Cell Cultures: Lymphatic endothelial cells (LECs) obtained from Cell Biologics Inc. will be utilized. The LECs will be exposed to Palmitic acid, VEGFC, or TNF- α to mimic a high saturated fat diet or inflammatory stimuli. The protective effects of anti-inflammatory compounds methylsulfonylmethane (MSM) and epigallocatechin gallate (EGCG) following exposure to a diet high in saturated fat (HSF) or inflammatory stimuli will be investigated. Each model of dysfunction (palmitic acid, VEGF-C, TNF- α) will be tested alone and in combination with each anti-inflammatory supplement (EGCG and MSM).

Primary Outcomes: Endothelial Transwell Permeability Assay will be performed as direct indicator of cell barrier function (figure 2). Western Blot and Real-Time Quantitative PCR will be used to analyze protein and mRNA transcript expression of markers related to VEGF signaling, inflammatory markers, and lymphangiogenesis. The targets that will be identified are VEGFC, VEGFR3, inflammatory markers IL-6, IL-1 β , TNF- α , TGF- β , and the lymphangiogenesis marker Prox1.

Preliminary Studies: At this stage, we are working to establish optimum treatment concentrations. Once the concentrations are verified, we can begin co-treatment of the cells with the antiinflammatory and inflammatory compounds. For this purpose, the Evans Blue Dye assay will be used and has been recently verified in initial preliminary experiments. The basic assay protocol is visualized in Figure 2. The cells are cultured on an insert that is placed into an individual culture well. Evans blue dye is added to the top compartment. If the cell layer is not intact, dye will diffuse into the lower chamber. Therefore, the concentration of dye in the lower chamber reflects cell layer permeability. Media samples are removed from the lower chamber and dye concentration is quantified using spectrophotometry. Data presented in Figure 3 depicts dye concentration at various concentrations of cells in the culture insert.



Figure 3. Evans Blue Permeability Assay on different concentrations of cells

Lymph (interstitial fluid)

Methods

Expected Outcomes: In our initial experiments, we expect the cell cultures that are treated with MSM or EGCG will maintain LEC barrier function after exposure to palmitic acid, VEGFC, or TNF- α . Primary outcomes will include direct measurement of cell barrier function via the transwell permeability assay, and assessment of markers related to inflammation by western blot of qPCR.

Significance/Application: Studies have shown that a diet high in saturated fat results in an impaired barrier function of lymphatic endothelial cells within the mesentery, leading to leakage of lymph.¹ The leakage of lymph into the surrounding tissue leads to an increased accumulation of adipose and a decreased sensitivity to insulin the area, leading to visceral obesity and metabolic dysfunction. ^{1,3–5} Evidence has shown that the abnormal lymphatic function following exposure to high saturated fatty acids is due to inflammatory signaling pathways that result in an upregulation of VEGFC and its receptor, VEGFR3. ^{1,2,6} We hypothesize that administering MSM and EGCG will act as protectants against the LEC permeability after a high saturated fat or inflammatory exposure by disrupting VEGFC-VEGFR3 cell signaling. These studies are a first step in investigating prevention and treatment strategies for impaired lymphatic function.

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Discussion

References

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