Haeme-containing proteins suppress Lymphatic Pumping

Abstract:

Red blood cells (RBCs) and lysate products (erythrolysate) are consistently observed in lymph draining inflammatory reactions and from tissues subjected to trauma or surgical procedures. We determined previously that erythrolysate modulates lymphatic pumping by altering the pressures over which the lymph pump is active. The purpose of this study was to test the hypothesis that oxyhemoglobin was the active material within erythrolysate. To quantitate lymphatic pumping, bovine lymphatics were suspended in an organ bath preparation with the vessels cannulated at both inflow and outflow ends. By raising the heights of the Krebs reservoir and the outflow catheters appropriately, a transmural pressure could be applied to the vessels. This procedure stimulated pumping activity. Erythrolysate was prepared from sheep RBCs by lysis in Tris buffer and a portion of this was purified by column chromatography using DEAE-Sephadex A-50. Both the purified hemoglobin (10(-5) M) and crude erythrolysate (the latter diluted appropriately in Krebs solution to contain 10(-5) M hemoglobin) reduced lymphatic fluid pumping approximately 70% over a period of 2 h. To determine whether this activity was due to the heme or the protein portion of the molecule, we compared the activity of purified oxyhemoglobin with that of its oxidized methemoglobin derivative. This was achieved by conversion with potassium ferricyanide. Methemoglobin was inactive, suggesting that the heme portion was important for the lymphatic effect. Further confirmation of this observation was provided by experiments with myoglobin which was purified from sheep heart. Oxymyoglobin, which shares an identical heme but has a different protein component, inhibited lymphatic pumping, when tested on the bovine lymphatics.