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Mobilization of human $CD34^+CD133^+$ and $CD34^+CD133^-$ stem cells in vivo by consumption of an extract from *Aphanizomenon flos-aquae*—related to modulation of CXCR4 expression by an L-selectin ligand?☆

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Abstract

Objective: The goal of this study was to evaluate effects on human stem cells in vitro and in vivo of an extract from the edible cyanobacterium *Aphanizomenon flos-aquae* (AFA) enriched for a novel ligand for human CD62L (L-selectin).

Experimental approach: Ligands for CD62L provide a mechanism for stem cell mobilization in conjunction with down-regulation of the CXCR4 chemokine receptor for stromal derived factor 1. Affinity immunoprecipitation was used to identify a novel ligand for CD62L from a water extract from AFA. The effects of AFA water extract on CD62L binding and CXCR4 expression was tested in vitro using human bone marrow $CD34^+$ cells and the two progenitor cell lines, KG1a and K562. A double-blind randomized crossover study involving 12 healthy subjects evaluated the effects of consumption on stem cell mobilization in vivo.

Results: An AFA extract rich in the CD62L ligand reduced the fucoidan-mediated externalization of the CXCR4 chemokine receptor on bone marrow $CD34^+$ cells by 30% and the $CD62L^+$ $CD34^+$ cell line KG1A by 50% but did not alter the CXCR4 expression levels on the $CD34^-$ cell line K562. A transient, 18% increase in numbers of circulating $CD34^+$ stem cells maximized 1 hour after consumption ($P<.0003$). When 3 noncompliant volunteers were removed from analysis, the increase in $CD34^+$ cells was 25% ($P<.0001$).

Conclusion: AFA water extract contains a novel ligand for CD62L. It modulates CXCR4 expression on $CD34^+$ bone marrow cells in vitro and triggers the mobilization of $CD34^+$ $CD133^+$ and $CD34^+$ $CD133^-$ cells in vivo.

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Abbreviations: AFA, *Aphanizomenon flos-aquae*; PBMC, Peripheral blood mononuclear cells; PMN, Polymorph-nucleated cells.

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1. Introduction

Much recent research has focused on the role of selectins and their ligands in mobilization of bone marrow stem cells. L-selectin belongs to the selectin family of cell adhesion molecules involved in cellular migration during normal immunosurveillance and inflammatory conditions. L-selectin is best known as a homing molecule for recirculating lymphocytes to recognize high endothelial venules during the process of extravasation [1–3] and for leukocytes to recognize and home to inflamed tissues [4–8]. However, L-selectin plays significant roles in other physiological cell adhesion processes as well, including the retention vs. release of bone marrow stem cells into the blood circulation [9–11].

Of special importance are findings that engagement of L-selectin by some ligands will modulate the expression of the CXCR4 chemokine receptor [12]. The CXCR4 receptor specifically recognizes the chemokine stromal derived factor 1 (SDF-1), which acts as a potent chemoattractant for stem cells and assists in retaining stem cells within the bone marrow environment [13–16]. The chemoattractant properties of SDF-1 on stem cells were shown in vitro [17] as well as in vivo to be directly associated with recruitment of stem cells into kidney [18] and liver [19]. The mobilization of recruitment of stem cells is associated with repair of the central nervous system [20,21], heart [22,23], and other tissues [24]. Stem cell mobilization and homing involve a series of G-protein-coupled receptors that can interact with each other as well as with adhesion molecules [25,26]. It is proposed that loss of responsiveness towards CXCR4 may be one of several contributing mechanisms that allow some bone marrow stem cells to detach and leave the bone marrow as part of the mobilization process [27,28].

The use of selectin ligands has been proposed as a mechanism for stem cell mobilization [29]. Some L-selectin ligands (LSLs), including fucoidan and sulfatide, have a proven effect on stem cell mobilization [29,30]. The mobilization appears to happen in selectin-dependent and -independent mechanisms in tandem. As an example, the sulfated polysaccharide fucoidan can act as an LSL and up-regulate the chemokine receptor CXCR4, a receptor for SDF-1. However, fucoidan also assists stem cell detachment within the marrow by binding to another adhesion receptor, CD11b, during stem cell mobilization [30].

The objective of this study was to evaluate the effects on human stem cells in vitro and in vivo of an extract from *Aphanizomenon flos-aquae* (AFA), enriched for a novel ligand for human L-selectin. We report here that a novel compound from the blue-green algae AFA binds to the ligand-binding area of human L-selectin. The effect of this compound was tested in various in vitro assays as well as on stem cell mobilization in humans.

2. Materials and methods

2.1. Buffers and media

For cell cultures, freshly isolated human marrow cells, as well as the KG1a and K562 cell lines, were resuspended and cultured in RPMI-1640 with 10% fetal calf serum (Gibco, Grand Island, NY, USA), 1% penicillin and streptomycin, and L-glutamine. For immunostaining, cells were washed, resuspended, and stained in phosphate-buffered saline (PBS) containing 0.02% azide and 1% fetal calf serum or bovine serum albumin.

2.2. Cyanobacterial extracts

Dried powder of the freshwater blue-green algae AFA was obtained from Desert Lake Technologies, Keno, OR, USA. Dried powder of *Spirulina platensis* was obtained from Healthforce Nutritionals, Escondido, CA, USA. One gram of dried algal material was resuspended in 10 ml PBS and incubated for one hour at 4°C and protected from light. The resulting slush was mixed by repeated inversion of the vial and centrifuged at 400 g for 10 min. The bright blue supernatant was decanted and sterile-filtered using a 0.22-mm filter. This filtrate of AFA water extract, AFA-W, was stored cold and dark and used within the same day of preparation.

2.3. Monoclonal antibodies

The CD62L monoclonal antibody TQ1 (specific for the ligand-binding area of the L-selectin molecule) linked to phycoerythrin (PE) was purchased from Coulter (Hialeah, FL, USA). CD45-PerCP, CD11b-PE, CD14-PE, and isotype control antibodies were obtained from Becton–Dickinson (San Jose, CA, USA). Monoclonal antibodies for CXCR4 (clone 12G5) and CCR9 were obtained from R&D Systems (Minneapolis, MN, USA).

2.4. Capturing of ligand using Dynabeads and chimera proteins

In order to identify the molecular weight of the L-selectin binding compound, we used a cell-free method in which Dynabeads (Dynal Biotech, Lake Success, NY, USA) coated with protein G were incubated with an L-selectin chimera protein (R&D Systems). The chimera protein is a fusion of the extracellular domain of human L-selectin with the F_c portion of human immunoglobulin G (IgG), thereby facilitating binding to protein G. The chimera protein was captured and subsequently covalently linked to the protein G-coated Dynabeads using the protocol recommended by the manufacturer. Beads were incubated for 1 h in a freshly made 5.4-mg/ml solution of dimethyl pimelimidate × 2HCl (Sigma Aldrich, St Louis, MO, USA) in 0.2 M triethanolamine buffer (pH 8.0)