A novel cyanobacterial ligand for human L-selectin extracted from *Aphanizomenon flos aquae* – potential role for stem cell biology in vitro and in vivo?

Introduction

The objective of this study was to evaluate the in vitro and in vivo effects of StemEnhance®, an extract from *Aphanizomenon flos-aquae* (AFA) enriched for a novel ligand for human L-selectin, on stem cell physiology. L-selectin is a cell adhesion molecules involved in cellular migration, cellular adhesion, and the retention versus release of bone marrow stem cells into the blood circulation. Stimulation of L-selectin leads to the externalization of pre-formed CXCR4 chemokine receptors, which are specific for the chemokine Stromal Derived Factor-1 (SDF-1) (Figure 1). Binding to SDF-1 to CXCR4 leads to the externalization of adhesion molecules that anchor the stem cell in the bone marrow. SDF-1 acts as a potent attractant for stem cells and therefore assists in retaining stem cells within the bone marrow environment.

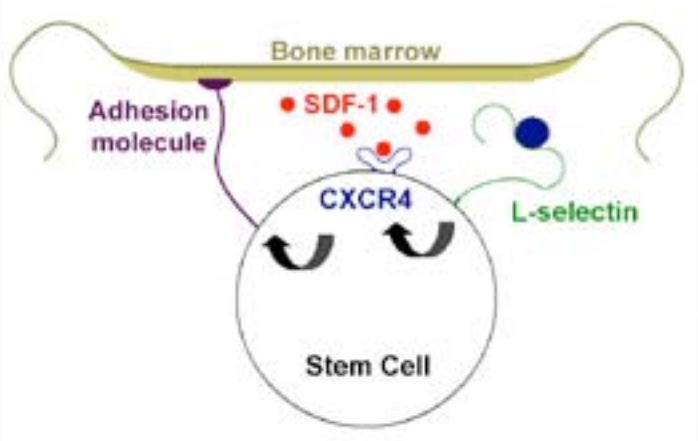

Figure 1

It was demonstrated that any interference with the CXCR4/SDF-1 axis is one of several contributing mechanisms involved in the release of stem cells from the bone marrow. Therefore, any compound that interferes with CXCR4 or SDF-1 has the potential of acting as a stem cell mobilizer.

There are many ways to support stem cell mobilization. For example, Granulocyte Colony-Stimulating Factor (G-CSF), the natural compound in the body stimulating stem cell mobilization works at least in part by raising the level of specific proteolytic enzymes that degrade SDF-1, thereby disrupting the CXCR4/SDF-1 axis. Other compounds such as AMD-3100 promote stem cell mobilization by blocking CXCR4, once again disrupting the CXCR4/SDF-1 axis. Finally, L-selectin blockers reduce the density of CXCR4 on the surface of the stem cells' membrane, thereby down-regulating the CXCR4/SDF-1 axis. Due to the physiological processes involved in each of these mechanisms of action, the mobilizations triggered by each of these mechanisms show different magnitude, time of onset, and duration. Mobilization triggered by G-CSF and AMD-3100 begins within a few days, last for a few days and can lead to an increase in the number of circulating stem cells by up to 100-fold. Conversely, mobilization triggered by L-selectin blockers is more transient and of a much lesser magnitude. The mobilization observed after consumption of StemEnhance was rapid, transient and mild, therefore we hypothesized that AFA contained an L-selectin blocker.

Methods & Results

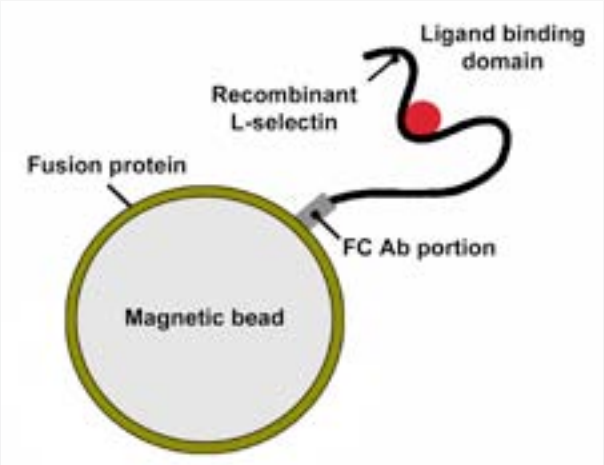

Figure 2

AFA contains a ligand for human L-selectin

In order to determine whether AFA contained an L-selectin ligand (binding molecule), paramagnetic Dynabeads coated with human L-selectin were incubated with a water extract of AFA (AFA-W) (Figure 2). After incubation, Dynabeads were washed and any bound material from the AFA extract was detached from the L-selectin molecules and run on gel-electrophoresis.

This process revealed that AFA contains an L-selectin ligand that appears to be a dimer made of two proteins having apparent molecular weights of 57 and 54 kDa respectively (Figure 3).

Using the same protocol on Spirulina, it was determined that Spirulina does not contain an L-selectin ligand.

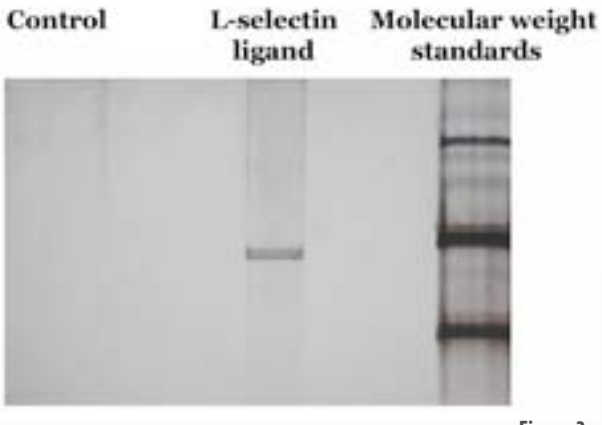

Figure 3

AFA-W specifically reduces TQ1 immunostaining of L-selectin on human PMN cells

L-selectin possesses one specific binding site whose activation leads to the externalization of CXCR4. In order to determine whether the L-selectin ligand present in AFA was binding to the active binding site of L-selectin, we tested the effect of AFA-W on the binding properties of TQ1 anti-human L-selectin monoclonal antibody. TQ1 is an antibody that specifically binds to the physiological active binding site of L-selectin. Incubation of lymphocytes with AFA-W reduced the binding of TQ1 by approximately 50-fold, indicating that the AFA L-selectin ligand does bind to the active binding site of L-selectin.

AFA-W inhibits the fucoidan-induced CXCR4 expression on CD34+ cells from bone marrow

It was important to determine whether the L-selectin ligand found in AFA was a stimulant or an inhibitor of L-selectin. We know that stimulation of L-selectin leads to an increase in the externalization of CXCR4, which can be quantified by measuring the density of CXCR4 receptors on the surface of stem cells. Incubation of bone marrow stem cells with AFA-W did not have any effect on CXCR4 density, indicating that the AFA L-selectin ligand was not a stimulant of L-selectin (Figure 4; green line).

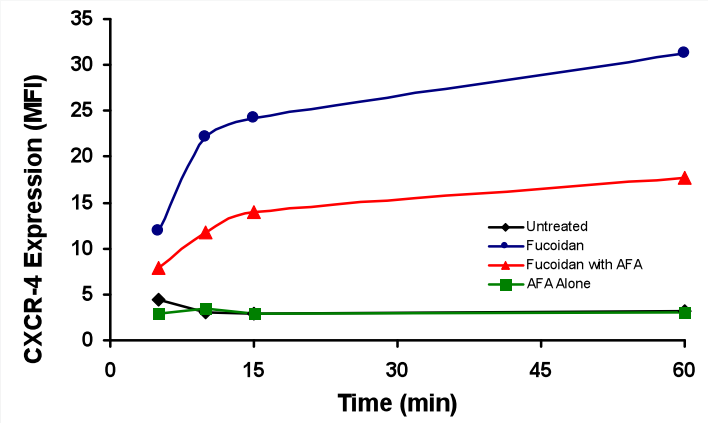

Figure 4

To investigate whether the ligand was a blocker of L-selectin we tested the effect of AFA-W on fucoidan-induced increase in CXCR4 density (Figure 4). Fucoidan is a sulfated polysaccharide known to stimulate L-selectin. Fucoidan triggered an 8-fold increase in CXCR4 density (blue line) which was inhibited (≈50%) by incubation with AFA-W (red line). Therefore, AFA contains a blocker of L-selectin.

Consumption of StemEnhance® resulted in a transient increase of circulating CD34+ cells.

As previously described in the scientific literature, L-selectin blockers have the potential of being effective stem cell mobilizers by modulating the CXCR4/SDF-1 axis. Therefore, we tested the mobilizing ability of the AFA L-selectin ligand in humans. Using a double-blind cross-over paradigm, the level of circulating CD34+ stem cells was compared in 15 individuals before and after ingestion of 1 gram of StemEnhance® or placebo. StemEnhance® (Stemtech HealthSciences, Inc., CA) is a proprietary blend of the cytoplasmic and cell wall-rich fractions of the whole plant biomass, enriched approximately 5-fold in content of the L-selectin ligand compared to the raw AFA biomass.

Consumption of StemEnhance® resulted in an average increase of 3-4 million circulating stem cells at 60 minutes ($p < 0.0001$) (Figure 5). The number of circulating CD34+ stem cells returned to baseline level around 3-4 hours after consumption. This was in contrast to placebo, which resulted in only minor fluctuations of the levels of CD34+ cells in the blood circulation over 2 hours.

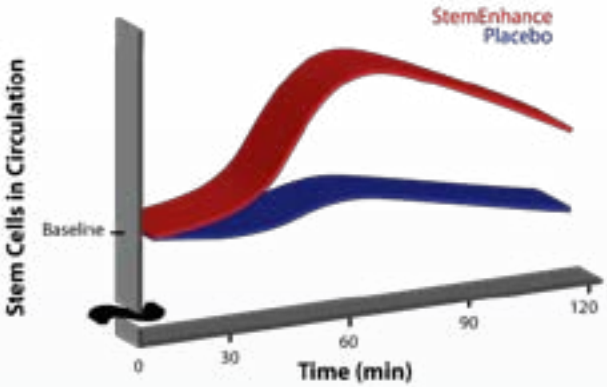

Figure 5

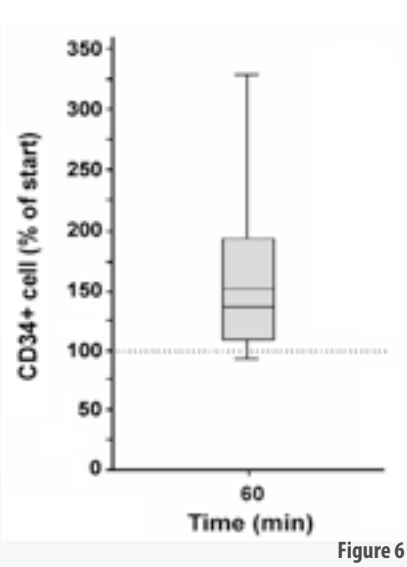

Figure 6

In order to test the repeatability of the effect of consumption of StemEnhance® on the levels of CD34+ cells in the peripheral blood, 16 separate experiments were performed on one volunteer. The average increase in the number of circulating stem cells was 53 ± 16%, with a median of 36% and a highest and lowest increase of 233% and -4%, respectively (Figure 6).

Discussion

Dietary strategies for supporting stem cell biology represent an emerging field of nutritional and medical research. The cyanobacterium AFA has been studied for its anti-oxidant properties and immuno-modulatory effects both in human and in vitro. AFA contains a number of compounds that have been subject to much research, including the potent antioxidant phycocyanin, a complex polysaccharide with potent immuno-modulatory properties, and the neuromodulator phenylethylamine responsible for the experience of mental energy reported by consumers.

It is reported here that AFA also contains a novel compound that specifically binds to the ligand-binding area of human L-selectin. It is composed of two subunits with apparent molecular weight around 54-57 kDa. This ligand for human L-selectin, obtained from AFA water extract, was able to modulate the functional response on human lymphocytes in vitro. The expression of the chemokine receptor CXCR4, which is induced by the known L-selectin ligand fucoidan, was down-regulated when fucoidan and AFA water extract were added simultaneously, indicating that the L-selectin ligand from AFA was competing with fucoidan for binding to L-selectin.

A double-blinded placebo-controlled cross-over study showed that consumption of StemEnhance® resulted in a small but significant increase in the number of circulating CD34+ stem cells, peaking at 1 hour after consumption. The effect was statistically significant ($p<0.0001$). There is however a significant fluctuation from one day to another in the effect of StemEnhance® or in the ability to quantify the effect accurately. Therefore, in order to test the nature of this fluctuation, we tested one individual on 16 different experimental days. The increase in the number of circulating stem cells after consumption of StemEnhance® averaged 52 ± 16% and varied greatly from 96% to 333% of baseline value. Interestingly, the average response in the one individual tested repeatedly and the average response to StemEnhance® in the double-blind randomized study involving 12 people were similar, indicating the relative consistency of the response and that the double-blind trial may in fact have understated the effect of StemEnhance®.

Recent studies have put in evidence the potential role of stem cell mobilizers in the maintenance of optimal health. Recently, a number of studies concluded that the level of circulating CD34+ stem cells was a good indicator of health.