Studying the Changes of High Frequency, High Dose Granulocyte Colony Stimulating Factor (GCSF) in the Immune System of Patients with Amyotrophic Lateral Sclerosis (ALS)

Samuel Dang[1], Tuan Vu[2], Phillip Pham[1], Xiaoyang Lin[1, 3], Chuanhai Cao[1, 3]

1 Dept. of Pharmaceutical Sciences, College of Pharmacy; 2 Dept. of Neurology, Morsani College of Medicine; 3 USF Health Byrd Alzheimer's Center

Abstract

Background: Granulocyte colony stimulating factor (GCSF) is a known glycoprotein, acting as both a cytokine and hormone, stimulating bone marrow production of both granulocytes and stem cells. Some studies have even shown levels of neuroprotection. In this study, we use a high frequency, high dose regimen of Filgrastim on 3 ALS patients to study the various immunomodulatory effects over 3-4 months.

Results: T cell and CD34+ cells were detected by using flow cytometry assay on blood samples. Blood levels saw increases in CD34+ cells in the blood after treatment with dose dependent response. The correlation of GCSF versus neuro growth factor was analyzed. GCSF was also correlated to increases in levels of BDNF. Some immune cytokines and factors, such as TNFα, were all modulated after treatment with GCSF.

Conclusion: Our results indicate that GCSF can modulate immune function by increasing CD34+ cell numbers and increase TNFα level.

Introduction

GCSF can mobilize a decent population of CD34+ hematopoietic cells into the peripheral blood. Recently, neural stem cell transplantation has been used to treat neurodegenerative diseases[1]. Apoptosis also appeared to be inhibited by GCSF treatment, and it also promoted locomotor recovery and neuroprotection in acute spinal injuries by regulation of nucleophosmin-1 receptors[2].

The GCSF cytokine may show neuroprotection when it comes to motor neuron cell lines in ALS[3]. In recent studies, examining the microstructural neural damage in ALS, it appeared as if GCSF treatment could modulate the spread and progression of this damage[4]. In terms of apoptotic cell death in neurodegenerative disease, GCSF shows protective effects on motor neurons through direct action[5], and may also play a role in neurogenesis[6]. From current research, it seems that GCSF can attenuate the autoimmune causes of ALS, while also providing neuroprotection for the motor neurons that may be under apoptotic influence due to the increasing misfolded proteins.

In this current study, we are continuing our pilot study of frequent, high doses of GCSF in ALS patients. From our previous results, we have seen that this type of GCSF treatment can effectively up-regulate bone marrow peripheral cells (BMPCs), and we are presenting the additional data gained from our continuing pilot study.

Materials and Methods

Blood sample collection and preparation: Blood was drawn (30 ml) biweekly, and delivered to the lab within 24 hours. Plasma was isolated by centrifugation and stored at -80C and the blood cells were diluted with RPMI to the original volume, then submitted for PBMCs isolation using the Ficol assay. Isolated PBMCs were then separated and used for T-cell population assay by flow cytometry, and patient plasma was used to study cytokine and chemokine levels by Luminex assay.

Flow Cytometry Assay: PBMCs isolated at different times or stimulated PBMCs removed from the cultured plate were washed with 1XPBS twice. Vials were then stained with the following antibody cocktails: 1) I: CD3, CD4, CD8, CD19, CD69, CD71, ICOS, Aqua; 2) II: CD3, CD4, CD8, CCR7, CD45RA, CD57, CD28, CD27, Aqua; 3) III: CD3, CD4, CD8, CD19, CD45, Annexin, Aqua. Cells were incubated with panel I, II, or III antibody mixtures for 30 minutes at 4C, then washed with 1XPBS containing 0.5% heat inactivated Human serum albumin 2x, and re-suspended with reading buffer. Then data analysis was done by Flowjo (Tree Star).

Luminex Multiplex Assay (Millipore): Cytokine expression profiles were detected using Luminex assay according to the manufacturer's protocol.

Statistical Analysis: Data are expressed as the mean ± SD and analyzed using a one-way analysis of variance (ANOVA), followed by the Tukey post hoc test using Prism 6.0 (GraphPad Software Inc.) The level of statistical significance is $p < 0.05$. For single ALS vs. control comparisons, a 2 group T-test was used. Significant levels starting at α=0.05.

Results

1. Injections of Filgrastim were significantly correlated to increases i CD34+ cells and GCSF levels in the total cell and lymphocyte-only populations.

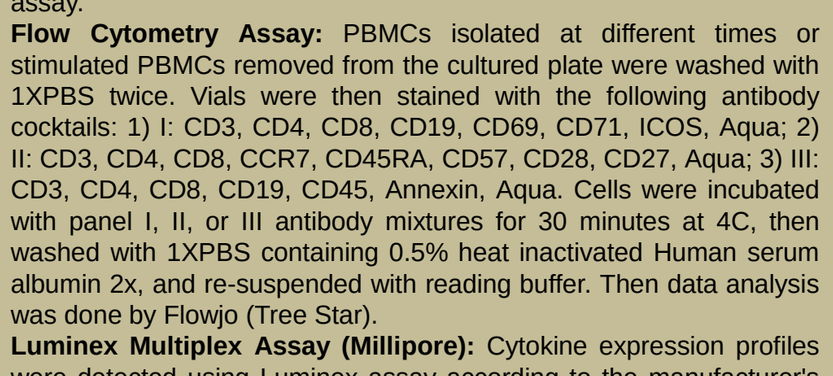

Figure 1: A selection of 15 time points was taken from all three patients. Injection time points are T5, T10, and T14; high-GCSF levels are T1, T6, T11, and T15. Patient 2 (Figure 1B) was not given the GCSF injection till T15, and high GCSF level was seen at T16 (not shown).

Results (cont.)

***Table 1*. Spearman correlation results for GCSF and cell count**

	GCSF v CD34- (Lymph+Mono)	GCSF v CD34+ (Lymph+Mono)	GCSF v CD34- (Lymph Only)	GCSF v CD34+ (Lymph Only)
Pt 1	-0.118 (p=0.291)	0.591 (p=9.1e-5)	-0.359 (p=0.385)	0.512 (p=3.5e-4)
Pt 2	-0.156 (p=0.255)	0.527 (p=0.008)	-0.188 (p=0.214)	0.417 (p=0.034)
Pt 3	0.140 (p=0.162)	0.369 (p=0.011)	-0.379 (p=0.069)	0.221 (p=0.035)

The values depicted in each row are the correlation coefficients after running a 1-tailed Spearman correlation, followed by the equivalent p values for each. Values highlighted in red are deemed to be significant correlations (α=0.05).

2. Increases in Brain-Derived Neurotrophic Factor (BDNF) were seen following increased GCSF.

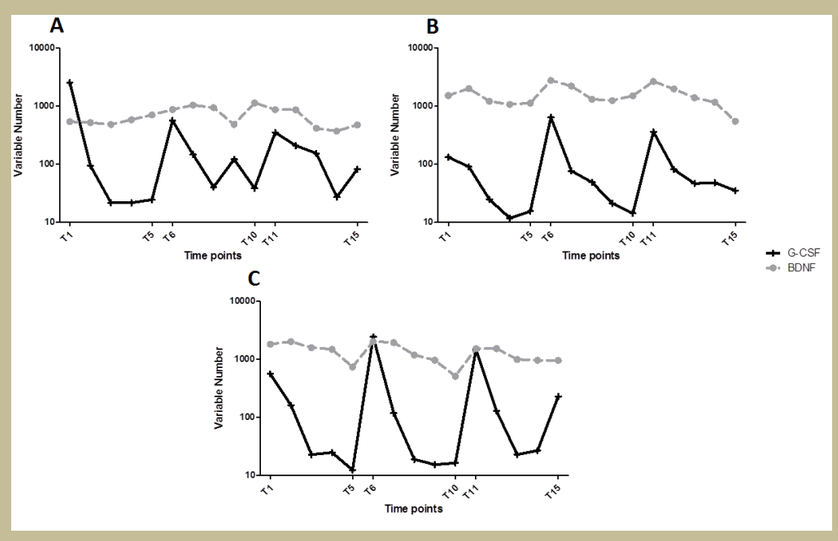

Figure 2: Timeline showing patient levels of BDNF measured in comparison to levels of GCSF. **I**njections of Filgrastim were significantly correlated to increases in BDNF over the complete course of treatment in patients and also in matched time points between patients. A selection of 15 time points was taken from all three patients. injection time points are T5, T10, and T14; high-GCSF levels are T1, T6, T11, and T15. Patient 2 (Figure 2B) was not given the GCSF injection till T15, and high GCSF level was seen at T16 (not shown).

***Table 2*. Spearman correlation results for GCSF and BDNF**

	GCSF v BDNF (Matched)	GCSF v BDNF (Complete)
Patient 1	0.135 (p=0.264)	0.187 (p=0.0925)
Patient 2	0.779 (p=2.597e-5)	0.779 (p=2.597e-5)
Patient 3	0.569 (p=0.002)	0.491 (p=0.003)

The '*Matched*' column dates were selected from each of the patient treatments to account for 4 GCSF treatments over similar spans of time. '*Complete*' shows the full correlations of each patient. The values depicted in each row are the correlation coefficients after running a 1-tailed Spearman correlation, followed by the equivalent p values for each. Values highlighted in red are deemed to be significant correlations (α=0.05).

3. Lymphocyte populations after GCSF injection correspond to modulation of TNF-α

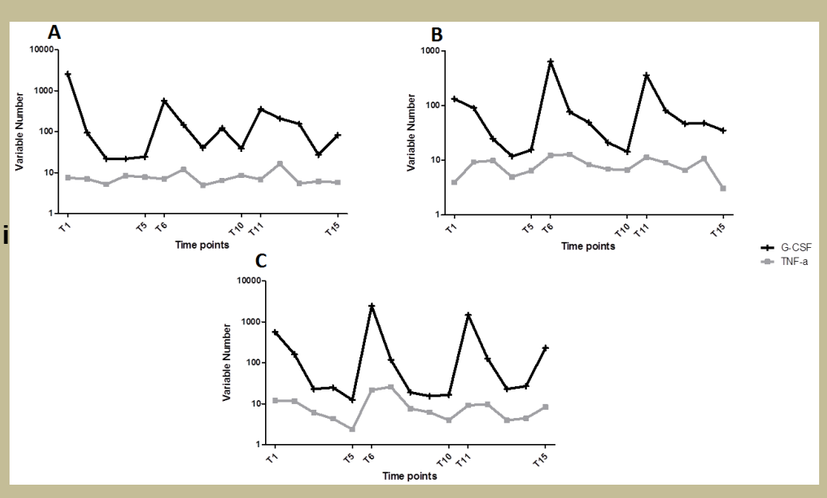

Figure 3. Timeline displaying the patient levels of TNFα measured in comparison to levels of GCSF. A selection of 15 time points was taken from all three patients to be more closely matched in terms of the dates of injection and blood draws. In Figure 3, injection time points are T5, T10, and T14; high-GCSF levels are T1, T6, T11, and T15. Patient 2 (Figure 3B) was not given the GCSF injection till T15, and high GCSF level was seen at T16 (not shown).

Results (cont.)

***Table 3*. Correlations run between cell numbers and various cytokine and inflammatory markers**

	TNF α	IFN γ	MCP-1	RANTES	IL-12(p70)	IP-10
Pt 1	0.441 (p=0.0005)	-0.122 (p=0.237)	-0.436 (p=0.052)	0.099 (p=0.243)	0.418 (p=0.005)	-0.245 (p=0.190)
Pt 2	0.639 (p=0.001)	N/A	-0.182 (p=0.258)	-0.241 (p=0.153)	0.866 (p=0.167)	-0.282 (p=0.154)
Pt 3	0.758 (p=0.001)	0.058 (p=0.382)	0.415 (p=0.013)	0.656 (p=0.005)	0.011 (p=0.483)	0.392 (p=0.018)

The values depicted in each row are the p values for each after running a 1-tailed Spearman correlation. Values highlighted in red are deemed to be significant correlations (α=0.05). Results that were N/A were unable to be read in ELISA due to low levels.

Discussion

GCSF and its analogs have been tested extensively for CNS disorders due to its degree of neuroprotection[6]. Receptors for GCSF are found in T cells and dendritic cells throughout the adaptive immune system, but they are also located on neurons throughout the brain, leading to believe this factor has a unique, neuroimmune modulatory function.

GCSF elicits neuroprotection through activation of anti-apoptotic pathways, mobilization of hematopoietic stem cells, such as CD34+, to sites of brain injury, increase trophic factors such as BDNF, drive neuronal differentiation, and enhance angiogenesis within the CNS. Activation of anti-apoptotic pathways in the CNS occur through GCSF up regulation of Stat3, pStat3, and Bcl-233. Mobilization of CD34+ cells after GCSF injection in mouse models of ischemic attack showed improvement of neurological function, and animals treated with GCSF showed reduced infarct volume and a lower mortality rate when compared to the control[7]. A theory is that CD34+ cells move to the site of damage in the nervous system, clear the site of damaged cells and help rebuild the neural circuitry. Another is that CD34+ cells interact with parenchymal cells to release trophic factors at the site of injury. GCSF also increases levels of brain-derived neurotrophic factor (BDNF) in the CNS, in addition to the mobilization of CD34+ cells. BDNF is believed to play a role in the level of recovery after neurological dysfunction.

In patients with amyotrophic lateral sclerosis (ALS), recent studies have suggested that GCSF may play a role in treatment. Mouse models of ALS have shown improved motor performance and neuron survival while also reducing the levels of denervation[8].

Conclusion

As ALS has a limited number of treatments and therapeutic options currently available, our previous publication implies that increase GCSF level can benefit neurological disease like AD. Here, we show that GCSF can increase CD34+ cells in the blood, and stem cells have been used to treat all kinds of neurological disease. We believe that GCSF may be worth further study to delay the advancement of symptoms in ALS. Our results indicate that Filgrastim treatment can modulate immune function and can possibly mobilize neurogenesis.

References

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