Stem cell mobilization utilizing granulocyte colony stimulating factor in advanced chronic heart failure: lessons from a pilot study

Jacob Joseph1,2*, Paulette Mehta3,6, Asem Rimawi3,6, Michele Cottler-Fox4,6, Anjan Sinha3,6, Balkrishna Mansingh3,6, Eugene S. Smith III3,6, and Jawahar L. Mehta3,5,6

1Department of Medicine, Cardiology Section (111), VA Boston Healthcare System, 1400 VFW Parkway, West Roxbury, Boston, MA 02132, USA
2Department of Medicine, Boston University School of Medicine, Boston, MA, USA
3Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA
4Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR, USA
5Department of Physiology and Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR, USA
6Central Arkansas Veterans Healthcare System, Little Rock, AR, USA

Mobilizing haematopoietic progenitor cells (HPC) to repair the failing heart is a promising intervention to halt the progression of this deadly disease. We postulated that low doses of granulocyte colony stimulating factor (GCSF) could successfully mobilize HPC in advanced systolic heart failure and lead to beneficial effects. In a pilot study involving patients with advanced systolic heart failure, we established that a low dose (5 μg/kg/day for 5 days) of GCSF was sufficient to mobilize HPC into the peripheral blood in adequate numbers (>10 cells/μL) in spite of advanced heart failure and subject age. A striking observation was the significant elevation of plasma interleukin-10 levels in response to GCSF, without any change in tumour necrosis factor-α or interferon-γ levels. Left ventricular function improved significantly in subjects with ischaemic cardiomyopathy at 9 months after a single 5-day cycle of GCSF. Our results suggest the potential for improving left ventricular function in advanced systolic heart failure utilizing GCSF.

Despite major therapeutic advances in the management of chronic heart failure over the last two decades, morbidity and mortality from this common malady remain high. Abnormal tissue remodelling involving loss of myocytes, structural and functional alterations of surviving myocytes, and fibrosis drive the process of progressive cardiac dysfunction and failure. Invoking the endogenous repair mechanisms of the body by mobilizing stem cells is an attractive approach to the treatment of heart failure. Results of various preclinical and small clinical trials suggest a beneficial effect of stem cell mobilization and infusion, mainly in the post-infarct state, although the results have not been conclusive.1–4 Since chronic systolic heart failure is a systemic illness with generalized effects on various organs including the bone marrow, it is important to undertake careful clinical studies in advanced chronic heart failure to understand the dosing, safety, and efficacy of haematopoietic growth factors as a prelude to larger clinical trials. In this review, we offer insights gained from a pilot clinical study,5 which aimed to establish a safe dose of granulocyte colony stimulating factor (GCSF) that would effectively mobilize haematopoietic progenitor cells (HPC) in advanced systolic heart failure.

KEYWORDS
Stem cells; Heart failure; Ventricle; Cytokines

* Corresponding author. Tel: +1 857 203 6841; fax: +1 857 203 5550, E-mail address: jacob.joseph@med.va.gov
This pilot study was designed as an escalating dose study\textsuperscript{5} to establish the lowest dose capable of safely mobilizing HPC in patients with advanced systolic heart failure. We were able to establish a safe and effective dose after enrolling a total of six patients (all male) with markedly impaired cardiac contractile function. We observed that GCSF in the dose of 5 $\mu$g/kg/day, in two divided doses, for 5 days was sufficient to elevate peripheral HPC number above a predetermined efficacy level of 10 cell/$\mu$L in all patients including a patient who received only 2 days of therapy. The dose is lower than used in recent trials of post-infarct stem cell mobilization\textsuperscript{11,4} and similar to doses used in small pilot trials in heart failure and advanced coronary disease.\textsuperscript{6,7} White blood cell (WBC) counts increased in all six patients in response to GCSF. Interestingly, the baseline or peak WBC counts did not correlate with the peripheral HPC counts suggesting that the WBC count cannot be reliably used to predict HPC response or to adjust the dose of GCSF (as was done in a recent study, reference\textsuperscript{6}) other than for safety (our study was designed to stop therapy if WBC count rose $>$75,000/$\mu$L). Our study did not have any ‘non-responders’ unlike trials using GCSF for mobilization in cancer patients, which may be due to the small number of subjects or to a real difference in the patient populations. Our study suggests that lack of adequate HPC mobilization may not be a limiting problem in heart failure, even in the presence of advanced symptomatology or advanced age (one of our subjects was an octogenarian).

Safety was a major endpoint analysed in the study. All patients who entered the study were required to have implantable cardioverter defibrillators in situ, since we wanted to eliminate the risk of arrhythmic death which we postulated could occur if there was significant stem cell implantation and transdifferentiation into myocytes with ensuing electrical heterogeneity. It is of note that the study conducted by Huttman et al.\textsuperscript{6} described earlier utilized four cycles of GCSF over 10 days each; they encountered one death among the 14 patients studied due to ventricular fibrillation. Bone pain was the commonest side effect in our study, and angina did not occur in any patient despite the fact that four out of the total of six patients had ischaemic cardiomyopathy (ICM). Based on pre-specified safety criteria, GCSF was discontinued in one patient after a total of 10 $\mu$g/kg GCSF, since his alkaline phosphatase value rose above three times the baseline value, even though the patient was asymptomatic except for bone pain. Interestingly, the patient’s serum creatinine started to rise after discontinuing GCSF, and he developed signs of worsening heart failure/volume overload, which rapidly responded to diuretics with symptomatic relief and reduction in serum creatinine. Since this patient had a history of frequent admissions for heart failure, it is not known whether this event was related to GCSF administration. However, this underscores the need for careful clinical monitoring during GCSF therapy in this patient population. There were no episodes of ventricular arrhythmias or defibrillator shocks during the 9 months of follow-up after GCSF administration. Overall our study suggests that a low dose of GCSF is effective and safe if proper precautions of monitoring clinical and laboratory parameters are instituted.

We measured left ventricular function by echocardiography at various time points to 9 months. No adverse effect on left ventricular function was observed during or immediately following drug administration. At 9 months, a trend towards improved left ventricular ejection fraction was seen compared with baseline when all patients were included in the analysis. When analysis was restricted to four patients with ICM, a significant increase in left ventricular ejection fraction was observed (absolute mean increase in ejection fraction of $\sim$9%). Even though this was not our primary endpoint, and although there is no Control placebo group or sophisticated quantitative measurements of regional ventricular function, our study implies the potential for improving myocardial function utilizing GCSF. The difference between the ischaemic and non-ischaemic dilated cardiomyopathy (DCM) groups could be the result of the small sample size; however it is also possible that ICM patients may respond better to stem cell mobilization. A recent report indicates that peripheral stem cell numbers in ICM patients are considerably lower than in DCM patients, while ICM hearts demonstrate a marked upregulation of homing factors in the myocardium compared with DCM hearts.\textsuperscript{5} In keeping with this hypothesis, it may be suggested that the optimal therapy in DCM patients should be upregulation of homing factors in the myocardium, while the strategy in ICM patients should focus on mobilizing stem cells safely.

An intriguing finding in our study was the effect of GCSF on plasma cytokine levels. Various preclinical studies have implicated pro-inflammatory cytokines in the pathogenesis of heart failure. The balance between pro-inflammatory cytokines such as tumour necrosis factor-$\alpha$ (TNF-$\alpha$) and the anti-inflammatory cytokine interleukin-10 (IL-10) is disturbed in heart failure. For example, Stumpf et al.\textsuperscript{9} showed that IL-10 levels are significantly reduced in heart failure patients compared with Control and that the ratio of TNF-$\alpha$ to IL-10 increased with worsening heart failure. Kaur et al.\textsuperscript{10,11} have demonstrated that IL-10 reduces TNF-$\alpha$-induced oxidant stress in cultured adult cardiac myocytes, and that IL-10 levels, and the ratio of IL-10 to TNF-$\alpha$ decreased concomitant with a progressive decline in cardiac function in a rat model of post-myocardial infarction heart failure. In a clinical study utilizing the drug levosimendan, it was observed that concomitant with an improvement in right ventricular function, IL-10 levels were increased significantly, with reduction in the ratio of the level of the pro-inflammatory cytokine IL-6 to the level of IL-10.\textsuperscript{12} Hence it is possible that IL-10 may be a major beneficial cytokine in heart failure. Among the cytokines analysed in our study, IL-10 levels increased significantly with GCSF treatment, while TNF-$\alpha$ levels did not change significantly. Mielcarek et al.\textsuperscript{13} have shown that GCSF mobilized peripheral blood mononuclear cells, specifically monocytes, significantly increase IL-10 production. It is conceivable that the increase in IL-10 is a major mechanism of the beneficial
effect of GCSF in heart failure. Since the alteration in cytokine levels occurred during or shortly following drug administration and well before improved myocardial function, the increase in IL-10 was most likely a cause of, and not a result of improved myocardial function.

Our experience demonstrates that in spite of advanced symptoms or increasing age, heart failure patients can safely mobilize HPCs in response to a relatively small dose of GCSF. These results raise the intriguing possibility that ICM patients are ‘primed’ to respond to HPC mobilization with GCSF, as suggested by Theiss et al. More importantly, our study also raises the possibility that HPC mobilization utilizing haematopoietic factors may have effects beyond engraftment and transdifferentiation, specifically by altering cytokine profile. Regardless, our study underscores the importance of further preclinical or mechanistic clinical investigations to understand the sequence of events that occur after mobilization of HPCs, their homing to and fate within the myocardium, direct effects of haematopoietic growth factors on the myocardium, as well as endocrine and paracrine effects of HPC mobilization independent of engraftment into the myocardium.

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**References**


