CLINICAL—LIVER

Granulocyte Colony–Stimulating Factor Mobilizes CD34+ Cells and Improves Survival of Patients With Acute-on-Chronic Liver Failure

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Podcast interview: www.gastro.org/gastropodcast. Also available on iTunes; see Covering the Cover synopsis on page 416.

BACKGROUND & AIMS: Acute-on-chronic liver failure (ACLF) develops in patients with chronic liver disease and has high mortality. Mobilization of bone marrow–derived stem cells with granulocyte colony–stimulating factor (G-CSF) could promote hepatic regeneration. **METHODS:** Consecutive patients with ACLF were randomly assigned to groups given 5 μg/kg G-CSF subcutaneously (12 doses; group A, n = 23) or placebo (group B, n = 24) plus standard medical therapy. We assessed survival until day 60; Child–Turcotte–Pugh (CTP), Model for End-Stage Liver Disease (MELD), and Sequential Organ Failure Assessment (SOFA) scores; and the development of other related complications. **RESULTS:** After 1 week of treatment, group A had higher median leukocyte and neutrophil counts than group B ($P < .001$). Sixteen patients in group A (69.6%) and 7 in group B (29%) survived; the actuarial probability of survival at day 60 was 66% versus 26%, respectively ($P = .001$). Treatment with G-CSF also reduced CTP scores in group A by a median of 33.3% compared with an increase of 7.1% in group B ($P = .001$), along with MELD (median reduction of 15.3% compared with an increase of 11.7% in group B; $P = .008$) and SOFA scores (median reduction of 50% compared with an increase of 50% in group B; $P = .001$). The percentages of patients who developed hepatorenal syndrome, hepatic encephalopathy, or sepsis were lower in group A than in group B (19% vs 71% [$P = .0002$], 19% vs 66% [$P = .001$], and 14% vs 41% [$P = .04$], respectively). After 1 month of treatment, G-CSF increased the number of $CD34^+$ cells in the liver (by 45% compared with 27.5% in group B; $P = .01$). **CONCLUSIONS: G-CSF therapy more than doubles the percentage of patients with ACLF who survive for 2 months; it also significantly reduces CTP, MELD, and SOFA scores and prevents the development of sepsis, hepatorenal syndrome, and hepatic encephalopathy.**

Keywords: Liver Regeneration; Liver Stem Cells; Growth Factor; Cirrhosis.

Acute-on-chronic liver failure (ACLF) is a serious acute insult of the liver on an underlying compensated chronic liver disease. ACLF has been defined as an acute hepatic insult manifesting as jaundice (serum bilirubin level ≥5 mg/dL) and coagulopathy (international normalized ratio ≥1.5), complicated within 4 weeks by ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease (Asian Pacific Association for the Study of the Liver criteria).[1,2] ACLF is characterized by a high mortality rate caused by multiorgan failure.[2] Kjaergard et al reported a mortality rate of 51% in their systemic meta-analysis.[3] The short-term mortality may be as high as 65% at 3 months.[4]

Available therapeutic options are limited. The Molecular Adsorbent Recirculating System is an option for patients with ACLF to give them additional time for recovery or to serve as a "bridge" to transplant. However, in a recent meta-analysis,[5] treatment with the Molecular Adsorbent Recirculating System did not appear to reduce mortality significantly compared with standard medical treatment.

Liver transplant remains the only definitive therapy for patients with ACLF; however, the limited availability of donor organs, prohibitive costs, limited expertise, and lack of widespread availability limit its usefulness in the management of patients with ACLF. Furthermore, in patients with acute alcoholic hepatitis and ACLF, liver transplant cannot be considered because of a necessary requirement of abstinence from alcohol use for 6 months. These patients are often quite sick and require repeated hospitalization with poor outcomes. Thus, there is an urgent need to evaluate new treatment options for patients with ACLF.

The intriguing capability of blood-derived stem cells that differentiate into multiple cell lineages raises the exciting opportunity of using these cells for tissue repair when the pool of tissue-intrinsic stem cells is overwhelmed.[6]

In the liver regeneration process, together with hepatocytes and intrahepatic stem cells, bone marrow–derived

Abbreviations used in this paper: ACLF, acute-on-chronic liver failure; CTP, Child–Turcotte–Pugh; G-CSF, granulocyte colony–stimulating factor; HVPG, hepatic venous pressure gradient; MELD, Model for End-Stage Liver Disease; SOFA, Sequential Organ Failure Assessment.

0016-5085/$36.00
doi:10.1053/j.gastro.2011.11.027

stem cells may represent a third proliferative compartment.[7] Animal and human studies have suggested that such cells might contribute to the regeneration after different kinds of liver injuries.[8]

A potential approach to improve bone marrow–derived stem cell engraftment to the damaged liver could be their mobilization by using cytokine administration.[9] Granulocyte colony–stimulating factor (G-CSF) therapy has been widely studied in bone marrow transplant recipients and in the oncologic population.

In an experimental rat model of fulminant hepatic failure and encephalopathy, rG-CSF not only ameliorated the histologically evident liver injury in a statistically significant manner but also enhanced the proliferative capacity of the hepatocytes.[10] In a recent study, mobilization of bone marrow–derived stem cells induced by G-CSF was observed in patients with severe cirrhosis.[11] In another study, it has been shown that G-CSF mobilizes $CD34^+$ cells, increases hepatocyte growth factor, and induces hepatic progenitor cells to proliferate within 7 days of administration in patients with alcoholic steatohepatitis.[12] Mookerjee et al have shown that neutrophil dysfunction in patients with alcoholic hepatitis superimposed on cirrhosis leads to the development of sepsis and further to the development of hepatorenal syndrome and hepatic encephalopathy in patients with alcoholic hepatitis superimposed on cirrhosis.[13] G-CSF therapy, on the other hand, has been shown to improve neutrophil function in patients with glycogen storage disease type Ib.[14] There are no data whether G-CSF therapy prevents sepsis and improves survival in patients with ACLF.

We undertook this study to evaluate the safety and efficacy of G-CSF therapy in reducing morbidity and mortality in patients with ACLF. We also investigated whether G-CSF therapy could improve the indices of severity of liver disease, such as Child–Turcotte–Pugh (CTP), Model for End-Stage Liver Disease (MELD), and Sequential Organ Failure Assessment (SOFA) scores. Sepsis, which sets in rather early in patients with ACLF, is partly related to neutrophil dysfunction.[13,15] The effect of G-CSF therapy on short-term (day 60) survival in patients with ACLF and prevention of new-onset hepatic encephalopathy and hepatorenal syndrome was also studied. Whether $CD34^+$ cells are mobilized to the damaged liver after G-CSF administration in vivo by counting these cells in the peripheral venous blood and hepatic parenchyma was analyzed.

Patients and Methods

Patients

Between December 2008 and August 2010, every consecutive patient with ACLF (as defined by an acute hepatic insult manifesting as jaundice [serum bilirubin level ≥5 mg/dL] and coagulopathy [international normalized ratio ≥1.5), complicated within 4 weeks by ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease [Asian Pacific Association for the Study of the Liver criteria])[1] was enrolled and included in the study. The exclusion criteria included age younger than 12 and older than 75 years, hepatocellular carcinoma or portal vein thrombosis, refusal to participate in the study, any concurrent evidence of sepsis, any significant comorbidities, multi-organ failure, grade 3 or 4 hepatic encephalopathy, HIV seropositivity, pregnancy, and any previous known hypersensitivity to G-CSF.

Methods

The patients were evaluated in terms of their clinical presentation and were investigated for the etiology of the liver disease. The diagnosis of the underlying chronic liver disease was based on clinical, radiologic, and endoscopic criteria. The presence of any of the following was taken as evidence of underlying chronic liver disease: high serum-ascites albumin gradient, grade ≥2 esophageal varices, hepatic venous pressure gradient (HVPG) ≥10 mm Hg, stage ≥2 fibrosis on histologic analysis, or portal vein ≥13 mm on ultrasonography. Wherever needed, HVPG measurement and a transjugular liver biopsy were performed to confirm the presence of underlying chronic liver disease.

CD34 cell quantification. CD34 cells were assessed at baseline in the peripheral venous blood and the liver tissue. Briefly, peripheral blood mononuclear cells were isolated on the baseline and follow-up samples from peripheral blood by Ficoll–Hypaque density gradient centrifugation of fresh blood samples and were stored in freezing media containing 10% dimethyl sulfoxide and 90% fetal calf serum at −80°C for 24 hours and then were shifted to liquid nitrogen until used. The expression of CD34 on different cell types was assessed by using 3-color flow cytometry. The fluorochrome-labeled antibodies were used and single-color compensation was performed before acquisition of samples. Lymphocytes were incubated with the antibodies for 30 minutes at 4°C and washed twice with fluorescence-activated cell sorter buffer containing 1× phosphate-buffered saline and 10% fetal calf serum. Cells were fixed with 4% paraformaldehyde. The surface expression of CD34 was measured on a BD Calibur Flow cytometer (BD Biosciences, San Diego, CA) and analyzed using CellQuest Pro software (BD Biosciences). Immunohistochemistry using antibody to CD34 (Spring Biosciences RTU) was performed on 4-μm-thick sections of paraffin-embedded liver tissues using standard protocol. Antigen retrieval was performed in citrate buffer at pH 6 in a microwave for 2 minutes at half power and 2 minutes at full power. After the application of primary antibody, slides were incubated overnight in a humid chamber and subsequently washed in 3 changes of Tris buffer for 30 minutes. Biotinylated secondary antibodies were applied for 1 hour in the same chamber at room temperature followed by washing with Tris buffer. Next, streptavidin peroxidase reagent was applied and incubated in the same chamber for 1 hour at room temperature. Finally, reaction product was visualized by developing color using diaminobenzidine tetrahydrochloride. Sections were counterstained with hematoxylin. CD34 scores were calculated by counting CD34-positive cells in sinusoids (arteries were not counted).

Within 48 hours of admission, the patients were enrolled and randomized into 2 groups. A randomization code was generated. Randomization was performed with sequentially numbered envelopes to either standard medical therapy with or without G-CSF, and the investigators as well as the patients were blinded to the treatment allotted. Patients in group A received G-CSF at a dose of 5 μg/kg subcutaneously, 12 doses over a period of 1 month (daily doses for the first 5 days and then every third day), along with the standard medical therapy, and patients in group B received placebo (1 mL saline subcutaneously each time) along with the standard medical therapy.