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Feasibility and safety of G-CSF administration to induce bone marrow-derived cells mobilization in patients with end stage liver disease☆

Silvia Gaia[1,*], Antonina Smedile[1], Paola Omedè[2], Antonella Olivero[1], Fiorella Sanavio[2], Federico Balzola[1], Antonio Ottobrelli[1], Maria Lorena Abate[1], Alfredo Marzano[1], Mario Rizzetto[1], Corrado Tarella[2]

[1]*Gastroenterology, San Giovanni Battista Hospital, 10126 Turin, Italy*
[2]*Haematology, San Giovanni Battista Hospital, 10126 Turin, Italy*

***Background/Aims*: To evaluate feasibility, safety and pattern of bone marrow-derived cells (BMC) mobilization in patients with end stage liver cirrhosis following granulocyte-colony stimulating factor (G-CSF) administration.**

***Methods*: Eight patients with severe liver cirrhosis (Child–Pugh score B-C, spleen diameter less than 170 mm) were included. They were treated with G-CSF (5 μg/kg b.i.d for three consecutive days) to mobilize BMC, evaluated as circulating CD34+ve cells (flow cytometry) and myeloid CFU-GM progenitors (in vitro colony growth assay). Co-expression in CD34+ve cells markers of differentiation (Thy1, CD133, CXCR4, c1qRp) were investigated on CD34+ve cells by double direct immunofluorescence. Data from 40 healthy haematopoietic stem cell donors were used as controls.**

***Results*: Mobilization of CD34+ve cells occurred in all patients. It was paralleled by expansion of circulating CFU-GM progenitors. Circulating CD34+ve cells co-expressed epithelial and stem cell markers in both cirrhotics and volunteer stem cell donors. G-CSF was well tolerated, no adverse event occurred, a significant reversible increase of splenic longitudinal diameter was observed.**

***Conclusions*: (i) G-CSF mobilization of BMC co-expressing epithelial and stem markers occurred in all cirrhotic patients; (ii) splenomegaly up to 170 mm does not prevent safe BMC mobilization following G-CSF in patients with end stage liver disease; (iii) mobilized BMC may represent an easy immature cell source potentially useful for novel approaches for liver regeneration.**

***Keywords*: Bone marrow-derived stem cell; Liver disease; G-CSF**

1. Introduction

Bone marrow-derived cells (BMC) can trans-differentiate into a variety of adult cell types including hepatocytes [1–5]. In response to acute or chronic liver damage, BMC can spontaneously populate liver and differentiate into hepatic cells [6,7]. Human BMC express multiple epithelial and liver markers when transplanted into rats with damaged liver [8] and, after peripheral blood stem cell transplantation, BMC repopulate liver and differentiate into epithelial and hepatic cells [7]. In

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* Corresponding author.
E-mail address: silvia.gaia@fastwebnet.it (S. Gaia).

Abbreviations: BMC, bone marrow-derived cells; G-CSF, granulocyte-colony stimulating factor; PB, peripheral blood; US, ultrasound; WBC, white blood cell.

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particular, BMC can significantly contribute to hepatic regeneration after acute or subacute liver necrosis, when mature hepatocytes are inhibited in cellular duplication [9,10].

However some studies describe a fusion of BMC into liver cells as mechanism underlying liver tissue regeneration after injury, and the factual capacity of BMC to trans-differentiate or to fuse into liver cells is still a great controversial issue [5,11,12].

These observations have stimulated emerging interest in the potential use of BMC to repair severely damaged liver. Unfortunately, at basal conditions, a very low rate of BMC is involved into liver regeneration [13] while a massive BMC mobilization in peripheral blood (PB) circulation might lodge in the severely damaged liver.

A non-invasive and potentially effective approach to obtain large quantities of BMC is their mobilization into PB by cytokines [14,15]. Granulocyte-colony stimulating factor (G-CSF), routinely used in haematological malignancies for BMC transplantation purposes, has potent mobilization capacity in donors and chronic haematological patients [16,17]. Moreover some studies suggest that cytokine-induced mobilization could also promote tissue repair in chronic or acute liver disease. G-CSF treatment significantly improved survival and liver histology in chemically injured mice, predominantly by promoting endogenous repair mechanisms [18].

Conversely splenomegaly, associated with liver cirrhosis and portal hypertension, might result in a potential restriction to BMC mobilization. Cell sequestration by the enlarged spleen can lessen or even abolish PB circulation of mobilized BMC [19] and liver failure might somehow affect the distinctive capacity of immature marrow cells to be released in PB in response to cytokine stimulation [20]. Moreover, splenic rupture is an uncommon though well-known complication, occasionally reported in a few patients and healthy donors undergoing BMC mobilization with G-CSF [21,22]. Thus, splenomegaly in patients with cirrhosis can considerably magnify the risk of splenic rupture following cytokine stimulation.

To address these issues, a pilot study has been performed evaluating G-CSF-induced mobilization in selected patients with end stage liver cirrhosis. The aims of this study were to define the feasibility and safety of BMC mobilization and to characterize the phenotype of mobilized cells following G-CSF administration.

2. Patients and methods

2.1. Patients

Between February 2004 and March 2005, eight patients with advanced liver cirrhosis entered this phase I/II study.

Table 1
Demographic and basal clinical features

Patients	
Male/female	5/3
Median age (range)	59 years old (49–80)
Alcoholic cirrhosis	5
Cryptogenic cirrhosis	3
Child–Pugh score $\geqslant 9$	8
Median WBC[a] (range)	2780/μL (1250–4490)
Median spleen LD[b] (range)	150 mm (108–170)

[a] WBC, white blood cells.
[b] LD, longitudinal diameter.

Eligibility criteria included age between 18 and 80 years and advanced liver cirrhosis of any origin with a Child–Pugh score $\geqslant 9$ and a Meld score >10. Criteria of exclusion were the following: enlisted for liver transplantation, spleen diameter larger than 170 mm, diagnosis of hepatocellular carcinoma or other cancers, digestive haemorrhage due to portal hypertension in the last 7 days, recent diagnosis of portal vein thrombosis, severe renal or cardiac dysfunction, acute infection or disseminate intravascular coagulation.

Demographic and clinical features are summarized in Table 1. Five patients had alcoholic and three cryptogenic cirrhosis. Chronic alcohol assumption had been interrupted at least 12 months before enrollment. All patients had one or more episodes of liver decompensation in the previous 3 months (porto-systemic encephalopathy, abdominal ascites or variceal bleeding).

Data from 40 adult healthy volunteers undergoing G-CSF mobilization at analogous dosage for allogenic haematopoietic stem cell transplantation were taken as controls.

The protocol was approved by the Regional Ethical Committee. The study was carried out according to the Declaration of Helsinki. All patients gave formal written informed consent before entering the study protocol.

2.2. Mobilization protocol

BMC mobilization was induced by G-CSF (Myelostim®), given subcutaneously at a dose of 5 μg/kg every 12 h for three consecutive days. All patients were hospitalised in our Liver Unit 1 day before starting on G-CSF and discharged 3 days after G-CSF discontinuation.

Patients were monitored daily by physical examination and laboratory tests. In addition, ultrasound (US) scan and a US-Doppler were performed daily from day 0 to day 6, in order to evaluate changes of spleen diameter and of portal flow (vein thrombosis). Discharged patients were monitored monthly by clinical and laboratory investigations and US scan.

2.3. Assessment of BMC mobilization

BMC mobilization was monitored by daily evaluation of circulating CD34+ve cells and in vitro clonogenic cells (CFU-GM assay) during cytokine administration and for 3 days thereafter. In addition, circulating CD34+ve cells were further characterized for the expression of various differentiation markers such as CD90, CD93, CD133, CD184 [23–32], see below.

2.3.1. Evaluation of circulating CD34+ve cells

Mobilized BMC were assessed as CD34+ve cells by flow cytometry. The assay was performed on freshly isolated cells. Briefly, 100 μL of whole blood was incubated with 20 μL of anti-CD34 PE monoclonal antibody (BD Biosciences, San Jose, CA) for 20 min at room temperature in the dark. Three microlitres of ammonium chloride lysing solution was added and incubated for 15 min for red blood cell lyses. Cells were then centrifuged at 1500 rpm for 5 min, washed in phosphate-buffered saline (PBS), and re-suspended in PBS for acquisition. The acquisition was performed by a FACSCalibur flow cytometer