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PROTOCOL

RANDOMIZED CONTROLLED PILOT STUDY ON THE CHARACTERIZATION AND IDENTIFICATION OF CD34+ STEM CELLS IN THE SPLANCHNIC DISTRICT AFTER G-CSF STIMULATION IN CIRRHOTIC PATIENTS

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(English version, brief version)

RESEARCH CENTER

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SYNOPSIS

One of the main clinical issues of transplantation medicine consists in the discrepancy between the increasing number of patients affected by chronic liver diseases and the lack of organs. This explains the high clinical relevance of researching and developing new therapies against hepatic failure. Regenerative medicine and recent outcomes achieved in the field of stem cell biology could actually provide some answers to this emergent problem.

Adult stem cells include, among others, hematopoietic stem cells (HSC) and mesenchymal cells (MSC). Scientific evidence supports the action of hematopoietic stem cells (CD34+ and CD133+) in the liver regeneration process after liver resection or transplantation, although the mechanism of such intervention is still unclear. When cultured, CD133+ cells express hepatocyte markers such as albumin and alpha-fetoprotein. In light of the data available in literature, it is presumable that hepatic damage favors the fate of bone marrow stem cells probably mobilized by a gradient of chemokines and chemoreceptors.

The information available on the therapeutic use of stem cells on humans for the treatment of chronic liver diseases is reassuring, but little is known about the fate of the stem cells used and the mechanisms they adopt to foster liver regeneration. In fact, according to studies carried out on rodents, bone marrow stem cells, which have a significant degree of plasticity, appear to affect liver regeneration, fibrogenesis, and fibrotic degradation in different ways. However, there is also other data showing that the medullary contribution favors the cells of the fibrous matrix (macrophages and myofibroblasts), instead.

Recent studies have shown that the infusion of bone marrow stem cells in cirrhotic patients is beneficial in terms of residual liver function improvement. However, despite encouraging preliminary results, many issues remain to be solved. In fact, organ stem cell repopulation is a multistep process that can be affected negatively by the poor ability of hematopoietic stem cells (CSE) to migrate and reside in proper niches (homing), or by the low “engrafting” and self-renewal efficiency.

G-CSF (granulokine) is the growth factor capable of mobilizing bone marrow hematopoietic progenitor cells by interrupting the connection between the CXCR4 (receptor expressed on the HSC) and the SDF-1 (present on bone marrow stromal cells). Today’s use of G-CSF is widely validated by European oncohematology centers, and applied in 70% of allogenic transplants. In addition to stimulating the bone marrow, this growth factor also stimulates extramedullary hematopoiesis regions such as the red pulp of the spleen, which in this case becomes an area of massive proliferation of granulocyte precursor cells. Therefore, in light of current knowledge, our goal is to understand if cirrhotic patients with portal hypertension (and splenomegaly) produce stem cells prevalently in the splanchnic system when stimulated with hematopoietic growth factors, and if these cells are capable of liver homing and hence foster the liver regeneration process.

Studies on GCSF use in patients with liver disease

Author	GCSF dose and days	Patients diagnosis	Clinical/Histological effects	Side Effects
Gaia et al 2006	10 µg/kg/ 3 days	8 liver cirrhosis*	Meld/N.A.	splenomegaly
Di Campli et al 2007	5 µg/kg/6 days vs 15/ µg/kg/5 days	acute on HCV/HBV/ alcohol cirrhosis	Not/N.A	bone pain
Spahr et al 2008	10 µg/kg/5 days	13 alcoholic steatohepatitis	Not/yes	1 patient (digestive bleeding)
Gordon et al 2008	520 µg per 5 days	9 alcohol cirrhosis	bilirubin/N. A.	not
Lorenzini et al 2008	2 vs 4 vs 6.6 vs 10 vs 15/ µg/Kg for 7 days	HCV/HBV/ alcohol cirrhosis	Not/N.A	bone pain and fever up to 6.6 µg/kg

N.A.: Not assessed; * Alcoholic and Cryptogenetic; CLD: chronic liver disease.

Type of study

Randomized pilot study

Protocol title

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Main objectives:

The main objectives of our study where to establish: 1) If the production of CD34+ hematopoietic stem cells after G-CSF stimulation in cirrhotic patients with portal hypertension occurs prevalently in the splanchnic region; 2) if CD34+ cells are capable of liver homing.

Secondary objectives:

1) Correlation between CD34+ cells and Hepatic Venous Pressure Gradient (HVPG), the dimensions of esophageal varices, splenic volume, and functional classification of the disease. 2) Phenotypic characterization and functional assessment of CD34+ stem cells.

Target patients for inclusion/indications:

Adult cirrhotic patients with indication for Transjugular Intrahepatic Portosystemic Shunt (TIPS).

Estimated enrolment: 15 patients, 5 of which controls. Enrolled patient will be covered by insurance.

Description of study: In order to achieve the goals of the study, we will enrol adult patients with indications to elective TIPS positioning. In addition to the procedure, patients enrolled in the study will undergo blood sampling to assess, by cytofluorimetry, the amount of CD34+ cells in basal conditions. Ten patients will be randomized for 5 days in a treatment with subcutaneous injection of GCSF at a dosage of 15µg/kg/da subdivided in 2 daily administration. On day 5 the second blood sample will be drawn to evaluate, by

cytofluometry, the amount of CD34+ cells present per microliter, after stimulation. On the same day, the TIPS will be positioned. During the catheterization, four 3-cc blood samplings will be drawn from each of the following districts:

1. hepatic vein;
2. portal vein;
3. splenic vein;
4. mesenteric vein.

The different blood samplings (pre- and post-hepatic) will help verify whether a distribution gradient of CD34+ stem cells exists after stimulation with GCSF, which will be called porto-sistemic. Five patients in the control group with indication to TIPS positioning will undergo 2 blood samplings during catheterization:

1. from hepatic vein
2. from splenic vein.

Blood sampling and tube analysis will be blind.

Patients randomized for treatment will undergo splenic ultrasound on day 0, after 5 days of stimulation with GCSF and after one month to monitor possible increase of the spleen volume. In addition, fibroscan will be performed on day 0, day 6, and after 3 months.

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