ENUMERATION, ISOLATION AND ELECTRON MICROSCOPIC STUDY OF HEMATOPOIETIC STEM CELLS FROM PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC LIVER AFFECTION

Thesis
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ABSTRACT

Background: The contribution of HSCs to liver regeneration in different forms of liver injury remains debatable. Accordingly, many studies were carried out to verify whether various liver lesions (as liver resection, transplantation and acute and chronic forms of liver failure) can activate bone marrow by mobilizing peripheral blood hematopoietic stem cells (HSCs) (CD34/ CD133+ cells) putatively able to induce liver repopulation. Many conflicting data were reported.

Aim of the work*:* It is intended in this current study to determine the degree of mobilization of BM-derived HSCs into the peripheral blood of patients with chronic hepatic affection and correlating it with various grades of liver damage.

Subjects and Methods: this study was conducted on 30 patients with Child A, B and C grades of chronic liver disease (10 patients for each stage). 10 healthy subjects were enrolled as controls. The percent of circulating HSCs was determined by flowcytometric phenotypic analysis and compared among the different groups of patients. Also, isolation of such cells was done by magnetic cell sorting technique using the Mini-MACS separator for further ultrastructural assessment by Transmission Electron Microscopy (TEM).

Results: The chronic liver disease group, compared to the healthy control group, exhibited no significant difference in the percentage of circulating $CD133^{+}$cells (0.419 % ± 0.194, 0.419% ± 0.252 and 0.277% ± 0.160 in Child A, B and C respectively versus 0.456 % ± 0.119 in control). Regarding the level of CD34+ cells, a statistically significant increase was found between child A chronic liver disease group and control group (mean ± SD; 1.183% ± 0.785 in child A versus 0.519% ± 0.201 in control group) ($p<0.05$). However no statistical significant difference could be found between Child B and C chronic liver disease patients and control group or between each other (0.532% ± 0.370 and 0.768% ± 0.332 in Child B and C respectively vs. 0.519% ± 0.201 in healthy controls). It was even found that in Child C, circulating HSC levels were decreased, though not significantly, as compared to the healthy subjects as well as to the milder Child A and B liver patients**.** Ultrastructural characteristics of isolated cells were compared in healthy and hepatic patients. The cells described in both groups were generally similar in appearance with no evidence of structural changes.TEM analysis revealed typical features of an immature phenotype.

Conclusion: Our data showed that chronic lesions of any degree of severity did not evoke bone marrow (BM) to mobilize HSCs into the circulation This could be due to: i) Defective hemopoiesis usually encountered in chronic liver diseases or ii) The toxic mileu of the chronic-damage liver interfering with extrahepatic stem cell-mediated liver repair.The recovery process seems to be mainly dependent on proliferating endogenous liver progenitors. Hence defining the underlying mechanisms interfering with BM activation and HSC mobilization is warranted.

Key words: HSCs, CD133/34, Chronic liver diseases

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List of abbreviations

5-FU	5- fluorouracil
AAF	Acetyl aminofluorene
AFP	Alpha feto protein
ALDH	Aldehyde dehydrogenase
AS	Adult stem cell
BM	Bone marrow
BMSCs	Bone marrow derived stem cells
BMT	Bone marrow transplantation
C/EBPβ	Transcription factor
CBC	Complete blood count
CCl_4	Carbon tetra chloride
CXCR4	Chemokine receptor
CDE diet	Choline deficient/ethionine-containing diets
CK19	Cytokeratin 19
DDPIV	Dipeptidyldipeptidase-IV
D-galN	D-galactosamine
EG cells	Embryonic Germ Cells
EGF	Epidermal growth factor
ES cells	Embryonic stem cells
FACS	Fluorescence activated cell sorting
FAH	Fumarylactoactate hydrolase (FAH) (–/–)
G-CSF	Granulocyte-colony stimulating factor
GFP	Green fluorescent protein

HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HGF	Hepatocyte growth factor
Ho-33342	Hoechst 33342 dye
HSCs	Hematopoietic stem cells
ICM	Inner cell mass
IL-6	Interleukin 6
INR	International normalized ratio
MMP-9	Matrix metalloproteinase-9
MSCs	Mesenchymal stem cells
NK cells	Natural killer cells
NOD/SCID	Non obese diabetic severe combined immune deficiency
OLT	Orthotopic liver transplantation
PG	Primordial germ
PH	Partial hepatectomy
PT	Prothrombin time
RBCs	Red blood cells
Rho-123	Rhodamine- 123
SDF-1	Stromal derived factor-1
TGF-α	Transforming growth factor-alpha
TGF-β1	Transforming growth factor-beta 1
TNF- α	Tumor necrosis factor-alpha
UCB	Umbilical cord blood

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INTRODUCTION AND AIM OF THE WORK

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Stem cells are defined functionally as regeneration units responsible for the development and regeneration of tissues and organs, being capable of both self-renewal and multilineages differentiation (Smith, 2001; Weissman *et al*., 2001).

Adult stem cells are present in a variety of organs including bone marrow. Their role is to replenish multiple mature differentiated cell types and thereby achieve long-term tissue reconstitution (Robey, 2000).

Hematopoietic stem cells (HSCs), as an example of adult stem cells, are approved to be able to differentiate into cells in other lineages that constitute various tissues, a property known as plasticity (Anderson *et al.*, 2001).

As regards the liver, a close relation between liver consisting cells and hematopoietic cells has been reported (Lagasse *et al.,* 2000; Quintana *et al.*, 2006). In this respect, recent data have shown that oval cells (the hepatic progenitor cells within the terminal branches of the intralobular biliary tree known as the canals of Hering) may be derived from a population of HSCs originating in the bone marrow (Roskams *et al.*, 2004). This is evidenced by expression of hematopoietic markers (CD34, C-kit) on oval cells (Grisham and Thorgeirsson, 1997) and the shared effect of hepatocyte growth factor on hematopoiesis & hepatopoiesis (Nishino *et al.,* 1996).

Furthermore, evidence from human & animal model bone marrow transplantation studies has suggested hepatocyte differentiation of transplanted bone marrow derived stem cells (BMSCs) after acute or chronic liver injury (Petersen *et al.,* 1999; Theise *et al.,* 2000).

It has thus been hypothesized that hepatic injury may constitute a trigger to mobilization of HSCs putatively able to differentiate into hepatocytes, thus starting the recovery process of the liver (De Silvestro *et al.,* 2004).

However, inspite of the documented improved regeneration of hepatocytes and fibrosis resolution in bone marrow transplantation studies, current evidence has denied hepatocyte regeneration from transplanted BMSCs and has not demonstrated a stable or long-term engraftment (Duffield *et al.,* 2005; Oyagi *et al.,* 2006).

Some reporters have explained such conflicting results by the fact that BMSCs may support liver repair only through delivery of growth factors that promote liver regeneration, fibrosis resolution or new blood vessel formation (Ueno *et al*., 2006; Xia *et al.*, 2006). However, the toxic milieu of the diseased liver may hamper the direct differentiation of stem cells into hepatocyte population (Kallis *et al.*, 2007).

Therefore, before this phenomenon of transdifferentiation of BMSCs to hepatocytes can be used clinically, several issues have to be resolved. Primarily it is necessary to establish the relationship between the degree of bone marrow (BM) activation to mobilize HSCs, destined to migrate to the injured hepatic tissue, and the severity of liver disease which is the triggering signal for mobilization of BMSCs.

Aim of the work:

It is intended in this current study to determine the degree of mobilization of BM-derived HSCs into the peripheral blood of patients with chronic hepatic affection and correlating it with various grades of liver damage. This aims at the following:

- Demonstrating that mobilized HSC count could serve as a prognostic marker to evaluate the extent of liver damage.

- Providing a baseline to preview the current status of stem cells mobilization in patients with liver disorders before clinical trials of administration of BMSCs or granulocyte-colony stimulating factor (G-CSF)**.**