Pathol. Oncol. Res. (2008) 14:381–385
DOI 10.1007/s12253-008-9091-3

ORIGINAL PAPER

Reduced Mobilisation of Hematopoietic Stem Cells After Hepatic Resection for Malignant Liver Disease

Marinella Menegazzo · Paola Bagatella · Piero Marson · Carla Donadel · Giustina De Silvestro · Augusto Corsini

Received: 31 January 2008 / Accepted: 28 July 2008 / Published online: 28 August 2008

Abstract Recent studies have demonstrated that hematopoietic stem cells (HSCs) can mobilize following liver resection, thus contributing to the repair of hepatic damage. Aim of this study has been to determine whether the nature of the hepatic lesion (benign vs. malignant disease) can give rise to a different degree of mobilisation of HSCs. Two groups of patients were selected: the first included seven patients undergoing hepatic resection (five major and two minor) for a benign liver disease (focal nodular hyperplasia, hemangioma cavernosa, angioma, biliary adenofibroma) and the second included seven patients undergoing hepatic resection (five major and two minor) for a malignant (either primary or secondary) liver disease. White blood cell count and CD34+ (percentage and total number) at time T_0 (basal value before surgery) and at time T_1 (value on the sixth–eighth day after surgery) have been evaluated by standard methods. In the group undergoing hepatic resection for a benign liver disease, a significant increase of CD34+ cells, both in percentage (0.082±0.043 vs. 0.048±0,026, p=0.041) and in absolute number (8.14±5.95 vs. 3.26±2.63, p=0.018) have been documented, as opposed to the group of patients affected with a malignant liver disease, where no significant variation has been observed (CD34+ %: 0.044±0.033 vs. 0.041±0.031, p: n.s.; CD34+ total number: 3.52±2.56 vs. 2.27±2.01, p=n.s.) These results show a different bone marrow response to the surgical liver resection depending on the nature of the lesion, thus emphasizing a reduced mobilisation of HSCs in the malignant diseases. Since it has been documented that the type of the hepatic lesion can induce a different regenerative response, it has to be explained how the neoplastic lesions can negatively influence the mobilization of HSCs. It can be hypothesized that a variety of humoral factors, including stromal cell-derived factor, matrix metalloproteinases, hepatocyte growth factor and interleukin-8 can influence the process of mobilization of HSCs after liver resection surgery. These substances are also involved in the mechanisms of development and metastasising of many tumours. It is probably in this context that a reason may be found for the different mobilisation of hematopoietic stem cells, depending on the nature of the hepatic lesion treated, that was encountered in this study.

Keywords Hematopoietic stem cell · Liver resection · Cancer · CD34+ mobilisation

Abbreviations

HSC	hematopoietic stem cells
WBC	white blood cell
SDF-1	stromal cell-derived factor
MMPs	matrix metalloproteinases
HGF	hepatocyte growth factor
IL-8	interleukin-8

M. Menegazzo · A. Corsini
Division of Surgery,
Azienda Ospedale Università,
Padova, Italy

P. Bagatella · P. Marson (✉) · C. Donadel · G. De Silvestro
Apheresis Unit, Blood Transfusion Service,
Azienda Ospedale Università di Padova,
via Giustiniani 2,
35128 Padova, Italy
e-mail: piero.marson@sanita.padova.it

M. Menegazzo · P. Bagatella
Istituto Oncologico Veneto,
Padova, Italy

Introduction

Stem cell is a cellular element that exhibits two distinctive properties: first, it is endowed with a long survival due to its capacity of self-regeneration in time; secondly it is able of differentiating into cellular elements with different specific functions. Moreover, stem cells are few in number and can replace damaged or pathological cells in various tissues [1, 2].

The hematopoietic stem cell (HSC) is the cellular element from which all peripheral blood cells derive. A pool of quiescent stem cells is present at the hematopoietic bone marrow compartment. These cells can become active under certain stimuli and differentiate or change into mature cells of different tissues. Several studies have demonstrated the ability of these cells to change into hepatocytes, oval cells, cholangiocytes, skeletal muscular cells, neurons, epithelial lung cells, cells of the gastrointestinal tract and of the skin [3–6].

Stem cells with a differentiating or potentially changing ability towards cellular elements of tissues different from that of their origin are also present in the various tissues of the organism. This interchanging capacity, common to bone marrow stem cells and to those of other tissues, defines the concept of "plasticity", that is typical of these cellular elements [7–10].

Following hepatic damage, it has been noted that the proliferative response occurs at three levels: in the first place, through proliferation of the mature hepatocytes, then through the activation of the progenitor cells of the terminal bile ductules i.e., oval cells, and, finally, through the action of circulating stem cells originating from the bone marrow [11, 12].

The extent of the contribution by the HSCs seems to be related to the severity of the hepatic damage and would appear to be made mainly through two mechanisms, namely the transdifferentiation of the HSCs into hepatocytes and, on the other hand, the cellular fusion of the HSCs with inadequate or insubstantial hepatocytes. There are several investigations on this issue, but the matter is still controversial. In addition to the debate, it has been hypothesized that the role of the HSCs in hepatic regeneration is mainly associated with the production of humoral factors required for an adequate recovery of hepatic function [13, 14].

Another very important aspect is the study of the mechanisms that, after hepatic damage, bring about the mobilisation of HSCs from the bone marrow to the peripheral blood and their homing in at the hepatic compartment [15].

Recent clinical studies have shown that surgery for hepatic resection induces a mobilisation of HSCs significantly greater than that induced by a general surgical trauma [16]. Aim of this clinical study has been to assess whether the nature of hepatic lesion (benign vs. malignant primary or secondary disease) brings about a different degree of mobilisation of HSCs from the bone marrow compartment to the peripheral blood. Results of these clinical observations could contribute to directing the study of the humoral mechanisms that determine the mobilisation and homing of the HSCs after hepatic damage.

Patients and Methods

Two groups of patients undergoing surgery for hepatic resection at the General Surgery Unit of Padova Hospital between January 2002 and June 2006, have been selected.

The first group included seven patients (five women and two men—mean age 59 years) who had undergone to hepatic resection for benign liver disease; on these patients two minor (metastasectomy or single segmentectomy) and five major resections (resection of two or more segments) had been carried out (Table 1). Patient VI-A underwent major liver resection since intramucous gallbladder carcinoma had been occasionally found on cholecystectomy because of gallstone disease. Liver histology resulted to be negative for

Table 1 Group 1: patients undergoing liver resection for benign hepatic disease

Patient	Sex	Age (years)	Disease	Surgery
I-A	M	79	Biliary adenofibroma	Left hepatectomy enlarged to segment V
II-A	F	35	Focal nodular hyperplasia	Left hepatectomy
III-A	F	52	Focal nodular hyperplasia	Resection of segment V
IV-A	F	69	Hepatic angioma	Right hepatectomy
V-A	F	39	Hemangioma cavernosa + focal nodular hyperplasia	Resection of segments V, VI and VII
VI-A	F	66	Gallbladder cancer	Resection of segment IV—histology: negative for neoplasia
VII-A	M	70	Hepatic nodular lesion in patient resected for colon cancer	Wedge resection of segment VI—histology: hemangioma

Table 2 Group 2: patients undergoing liver resection for malignant hepatic disease

Patient	Sex	Age (years)	Disease	Surgery
I-B	M	37	Cholangiocarcinoma	Left hepatectomy + wedge resection of segment V
II-B	M	64	Hepatocarcinoma	Left hepatectomy
III-B	F	33	Hepatic metastases of breast cancer	Resection of segment VI
IV-B	F	76	Hepatic metastases of rectal cancer	Right hepatectomy
V-B	F	67	Hepatic metastases of colon cancer	Resection of segments V, VI and VII
VI-B	F	64	Hepatic metastases of colon cancer	Complete resection of segment IV
VII-B	M	54	Hepatic metastases of colon cancer	Wedge resection between VI and VII segments

neoplasia. Moreover, patient VII-A underwent hepatic surgery owing to a nodular lesion which has been detected 2 years after an intervention for colon carcinoma. Liver histology did not show any evidence of malignancy and the nodular lesion was classified as hemangioma. None of them was previously treated with chemo- and/or radiotherapy.

The second group included seven patients (four women and three men—mean age 56 years) who had undergone to hepatic resection (two minor and five major resections) for liver cancer, both primary and secondary (Table 2). In all patients surrounding liver was not cirrhotic and concurrent major diseases were absent. As indicated in Tables 1 and 2, the surgical option, in terms of amount of liver resection, was substantially similar in both groups.

The following laboratory parameters have been evaluated: white blood cell (WBC) count, CD34+ % and CD34+ per microliter total number at time T_0 (basal value prior to surgery) and at time T_1 (value on the sixth–eighth day after surgery). The timing of the second sampling fixed for the sixth–eighth day after the operation has been chosen as a result of a preliminary study designed to define the peak of circulating HSCs in the post-operative period [16].

The determination of the peripheral CD34+ cell count has been carried out at the Transfusion Blood Unit of Padova Hospital by flow cytometry (Cytoron Absolute, Ortho Diagnostic System Inc. USA, and Beckton Dickinson FACSCalibur System, BD Biosciences, USA).

Given the non-normality of the distribution due to the limited number of tested samples, Wilcoxon non-parametric test has been employed for statistical analysis. P values <0.05 have been considered to be significant.

Results

All data are reported in the Table 3. In the group of patients undergoing liver resection for a benign disease, when comparing the values of T_1 and T_0, a significant increase has been observed in the CD34+ cells both in percentage (0.082±0.04 vs 0.048±0.03, p=0.041) and in absolute number (8.1±5.9 vs 3.3±2.6, p=0.018).

In the group of patients undergoing liver resection for cancer, when comparing the values of T_1 and T_0, there was a significant increase in WBC count (8.40±1.65 vs 5.86±1.93, p=0.030), while the variations of CD34+ cells, both in percentage and in absolute number, did not reach significant values.

Discussion

Our results, although obtained by evaluating a small series of patients, allow some considerations to be made. First, the increase of circulating HSCs has been shown to be significantly greater in patients undergoing to liver resection for a benign disease. This finding is even more significant, since in the group of patients undergoing liver resection for cancer a significant increase of the WBC

Table 3 Variations of laboratory parameters in patients undergoing liver resection for benign or malignant hepatic disease

	Group 1		Group 2	
	T_0	T_1	T_0	T_1
WBC count ($\times 10^6$/μL)	7.21±2.89	9.52±2.81	5.86±1.93	8.40±1.65***
CD34+ (total number/μL)	3.26±2.63	8.14±5.95*	2.27±2.01	3.53±2.56
CD34+ (%)	0.048±0.026	0.082±0.043**	0.041±0.031	0.044±0.033

Values are expressed as mean ± standard deviation

GROUP 1 Patients undergoing liver resection for benign hepatic disease, *GROUP 2* patients undergoing liver resection for malignant hepatic disease.

*p=0.018, **p=0.041, ***p=0.030

count has been documented, likely linked to the basic neoplastic disease. It is known that leucocytes, through the release of cytokines, can stimulate the bone marrow compartment. Nevertheless, the increase in the number of mobilised HSCs has been significantly lower in comparison with patients with a benign liver disease.

Furthermore, attention is drawn to the fact that the mean age, at the time of surgery, in the two groups tested has been quite similar, ranging from 56 (malignant disease) to 59 years (benign disease). This issue has allowed an additional "standardisation" or uniformity of the two groups of patients we considered. Indeed, it is known that the myelopoietic activity tends to decrease with age, causing a reduction of the number of circulating CD34+ cells [17].

The fact that the nature of the hepatic lesion can bring about a different regenerative response in comparison with a common or usual stimulus of the hepatic resection poses the problem of how the neoplastic disease can influence the liver regenerative process.

This process involves, on the one hand, cells permanently located in the liver and cells of the hematopoietic compartment and, on the other hand, a variety of humoral factors, including particularly stromal cell-derived factor (SDF-1), matrix metalloproteinases (MMPs), hepatocyte growth factor (HGF) and the interleukin-8 (IL-8) [18].

SDF-1 is a powerful chemoattractor for the HSCs and is produced by cells of various tissues, among which those of the liver. This factor acts through a receptor, CXCR4, that is released by the HSCs, the CD34+ and other cells of stromal origin. It is hypothesized that the release of HSCs from the bone marrow into the peripheral blood takes place owing to a concentration gradient of SDF-1. An increased production of SDF-1 by cells of different tissues, causing a concentration gradient, would hence favour the introduction into the bloodstream of HSCs from the bone marrow. As far as the liver is concerned, it is considered that in a liver damaged by a viral or autoimmune disease, the release of SDF-1 is increased [19, 20].

The MMPs are proteolythic enzymes capable of degrading the protein of the cellular matrix; they are produced by a variety of cells, including neutrophils, hepatocytes, and tumoral cells. Among these, MMP-9 appears to play a role in the reshaping of the liver parenchyma in the course of inflammatory processes and of cirrhosis and in regeneration after liver resection surgery [19, 21–24]. In its turn, the release of MMP-9 can be induced by certain cytokines, such as IL-8 [25].

HGF, produced in liver by non-parenchymal perisinusoidal cells, induces a proliferation of hepatocytes. This could, moreover, foster the migration and differentiation of HSCs in the damaged hepatic tissue [19].

Closely linked to the role of these mediators is that of oxygen and its radicals. *In vitro* studies on the activity of stem cells are generally carried out with concentrations of oxygen of about 20%, while, when carried out *in vivo,* the percentage of this gas is markedly lower. This leads to a transcriptional response that is different from that of experimental models [26].

Ischemia of the tissues appears to induce an increased release of SDF-1. This factor in turn is involved in the metastasising of different types of neoplasia. MMPs and HGF (whose synthesis is regulated by oxygen) also act in the metastatic process, as well as on the normal mobilisation of HSCs of the bone marrow [19, 20, 27–29]. In the present study, chiefly based on clinical data and thus lacking of other laboratory investigations than the CD34+ cell count, these growth factors have been not determined. Obviously, in keeping with the hypothesis we proposed and to confirm it, further studies, including more sophisticated and expensive laboratory parameters such as SDF-1 and HGF, should be planned.

In conclusion, factors that regulate the mobilisation of stem cells from hematopoietic bone marrow are also involved in the processes of cancer development and metastasising. In this light it is possible to argue about the different mobilisation of HSCs, following the common stimulus induced by surgery, in patients affected with malignant and benign liver disease, as it has been demonstrated in our investigation.

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