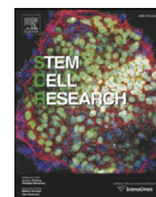
Stem Cell Research 15 (2015) 584–594

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr



Autologous bone marrow stromal cells are promising candidates for cell therapy approaches to treat bone degeneration in sickle cell disease☆

Angélique Lebouvier [a,b,1], Alexandre Poignard [a,c,1], Laura Coquelin-Salsac [a,b], Julie Léotot [a,b], Yasuhiro Homma [d], Nicolas Jullien [a,b], Philippe Bierling [b,c,e], Frédéric Galactéros [c,e], Philippe Hernigou [a,c], Nathalie Chevallier [a,b,2], Hélène Rouard [a,b,*,2]

[a] Université Paris-Est, Faculté de médecine, Laboratoire de "Bioingénierie cellulaire, tissulaire et sanguine", EA3952, Créteil, France
[b] Etablissement Français du Sang d'Ile-de-France, Unité d'Ingénierie et de Thérapie Cellulaire, Créteil, France
[c] AP-HP Hôpital Henri-Mondor – A. Chenevier, Service hospitalier, Créteil, France
[d] Juntendo University, Tokyo, Japan
[e] Inserm UMR955, Créteil, France

ARTICLE INFO

Article history:
Received 22 May 2014
Received in revised form 27 September 2015
Accepted 30 September 2015
Available online 8 October 2015

Keywords:
Bone marrow stromal cells
Sickle cell disease
Osteonecrosis
Bone regeneration

ABSTRACT

Osteonecrosis of the femoral head is a frequent complication in adult patients with sickle cell disease (SCD). To delay hip arthroplasty, core decompression combined with concentrated total bone marrow (BM) treatment is currently performed in the early stages of the osteonecrosis. Cell therapy efficacy depends on the quantity of implanted BM stromal cells. For this reason, expanded bone marrow stromal cells (BMSCs, also known as bone marrow derived mesenchymal stem cells) can be used to improve osteonecrosis treatment in SCD patients. In this study, we quantitatively and qualitatively evaluated the function of BMSCs isolated from a large number of SCD patients with osteonecrosis (SCD-ON) compared with control groups (patients with osteonecrosis not related to SCD (ON) and normal donors (N)). BM total nuclear cells and colony-forming efficiency values (CFE) were significantly higher in SCD-ON patients than in age and sex-matched controls. The BMSCs from SCD-ON patients were similar to BMSCs from the control groups in terms of their phenotypic and functional properties.

SCD-ON patients have a higher frequency of BMSCs that retain their bone regeneration potential. Our findings suggest that BMSCs isolated from SCD-ON patients can be used clinically in cell therapy approaches. This work provides important preclinical data that is necessary for the clinical application of expanded BMSCs in advanced therapies and medical products.

© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Sickle cell disease (SCD) is an autosomal recessive disorder characterized by the presence of abnormal hemoglobin S (Hb S). The polymerization of deoxygenated Hb S causes deformation of red blood cells (RBCs) into less pliable cells called sickled RBCs (Lonergan et al., 2001). These cells are prematurely destroyed at high rates, which lead to anemia. Furthermore, the sickled RBCs induce vascular occlusions that lead to tissue ischemia and infarction (Serjeant, 1997). Severe osteoarticular injuries often occur in patients with SCD. Osteonecrosis induced by a temporary or permanent loss of the blood supply to bone is a common complication that occurs in up to 50% of SCD patients and affects primarily the hip, but other joints and sites can also be affected (Hernigou et al., 2006; Vichinsky et al., 1999). The prevalence of osteoarticular injuries appears to be similar between homozygous patients (Hb SS), heterozygous patients (Hemoglobin S combined with C; Hb SC) and patients with various types of sickle-β-thalassemia (Akinyoola et al., 2009). Joint replacement is a controversial treatment option considering the young age of SCD patients (under 30 years of age) and is associated with several complications: intra-operative bleeding, infections, and loosening or early loss of the prosthesis (Marti-Carvajal et al., 2012). Autologous bone marrow (BM) grafting combined with core decompression is an effective strategy that preserves the native joint (Hernigou et al., 2008). However, SCD patients frequently have multifocal osteonecrosis (sometimes six to eight sites), and the number of osteoprogenitor cells present in BM harvested from the iliac crest is not sufficient to treat all of the lesions during the same procedure (Flouzat-Lachaniette et al., 2009; Hernigou et al., 2008). Given that anesthesia is risky in SCD patients, it would be useful

☆ Disclosure of potential conflicts of interest: The authors indicate no potential conflicts of interest.

* Corresponding author at: Etablissement Français du Sang d'Ile-de-France, Unité d'Ingénierie et de Thérapie Cellulaire, 5 rue Gustave Eiffel, 94017 Créteil cedex, France.
E-mail address: helene.rouard@efs.sante.fr (H. Rouard).

[1] These two authors contributed equally to this work.
[2] Co-last authors.

http://dx.doi.org/10.1016/j.scr.2015.09.016
1873-5061/© 2015 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

to expand osteoprogenitor bone marrow stromal cells (BMSCs, also known as bone marrow-derived mesenchymal stem cells) in vitro, so that several lesions could be treated in the same procedure, thereby reducing the number of procedures requiring anesthesia (Stanley & Christian, 2013). BMSCs represent a promising therapeutic approach for bone tissue engineering because of their differentiation capacity and bone regenerative potential (Lee et al., 2003; Maumus et al., 2011). However, BMSCs also play a role in hematopoïesis; therefore, the changes occurring in SCD may reflect not only a hematopoietic disorder but also abnormalities in the activity of BMSCs (Bianco & Robey, 2004). As described by Kuznetsov and others, genetic defects or micro environmental changes in the BM can alter the number or biological activity of stromal cells (Bianco & Robey, 1999; Kuznetsov et al., 2009). The number of stem cells in the BMSC population can be approximated by measuring the colony-forming efficiency (CFE) (Kuznetsov et al., 2009), and appears to be higher in SCD patients than in normal individuals (Hernigou & Beaujean, 2002). However, the number of these cells has not been thoroughly characterized in a large series of patients, and the biological activity of BMSCs from SCD patients has not been studied in vitro. Furthermore, not all patients with SCD develop osteonecrosis, which suggests that the influence of BMSCs and their therapeutic potential is heterogeneous (Vichinsky et al., 1999). Indeed, BMSCs from some SCD patients seem to have a higher capacity for spontaneous bone repair than those from most SCD patients with bone disorders.

These data collectively suggest that the function of BMSCs is affected in SCD patients. Here, we quantitatively and qualitatively evaluated the function of BMSCs isolated from a large number of SCD patients with osteonecrosis (SCD-ON) compared with control groups (patients with osteonecrosis not related to SCD (ON) and normal donors (N)). A cell therapy approach involving ex vivo expanded BMSCs appears to be a useful method to limit the anesthesia risk to SCD patients and will increase the number of osteoprogenitor cells delivered to the site of osteonecrosis. To determine whether these cells are an innovative conservative treatment of SCD osteonecrosis, we characterized BMSCs from SCD patients and studied their osteogenic capacity in vitro and in vivo.

2. Materials and methods

2.1. BM collection

In the context of a validated cell therapy process in orthopedic surgery, we retrospectively reviewed 340 consecutive patients who underwent osteonecrosis treatment with autologous BM grafting (Hernigou & Beaujean, 2002). One hundred and seventy patients were affected by SCD and osteonecrosis (called in this study "SCD-ON patients"), comprising 85 female and 85 male patients (Age range of patients: 15–40 years; Average age of female patients = 29 +/− 6 years; Average age of male patients = 31 +/− 6 years; Average weight = 73 +/− 15 kg). BM was harvested from these patients outside of a sickle cell vaso-occlusive crisis. The remaining one hundred and seventy patients had osteonecrosis not related to SCD (called "ON patients") and consisted of 85 female and 85 male patients (Age range of patients: 14–40 years; Average age of female patients = 30 +/− 7 years; Average age of male patients = 32 +/− 6 years; Average weight = 65 +/− 12 kg).

BM was harvested under general anesthesia from the iliac crest of patients diagnosed with osteonecrosis. Then, a concentrated buffy-coat of approximately 50 ml was obtained after centrifugation on a Cobe 2991 cell separator (Terumo, Lakewood, Colorado) prior to the BM grafting procedure. The BM samples used in this study corresponded to the samples routinely used for product qualification in the quality control department for cell therapy between 2004 and 2012. Nucleated cells from the BM were counted automatically before buffy-coat concentration using an ABX Pentra 60 C+ (Horiba ABX, Montpellier, France). In addition, in the context of BM allografting in hematology, we retrospectively reviewed BM from 14 normal donors (called "N patients"). Informed consent was obtained from each patient (Approval number from the French research ministry: DC-2009-1049). All work presented was based on groups of patients who were matched in age and sex.

2.2. Platelet lysate (PL) preparation

Platelet apheresis collections performed at the "Etablissement Francais du Sang" (EFS, Rungis, France) were biologically qualified according to the French legislation. The platelet count in each product was measured automatically (with an ABX Pentra 60 C+, Horiba ABX, Montpellier, France). Only samples containing 1×10^9–2.5×10^9 platelets/ml were retained; they were frozen at −80 °C and subsequently used to obtain PL containing platelet-released growth factors. Different batches were obtained (from two to five apheresis collections) to adjust the concentration to 50×10^6 platelets/ml. This concentration of platelets was previously shown to facilitate three-fold faster expansion of BMSCs compared with fetal bovine serum (FBS) (StemCell Technologies, Grenoble, France) (Chevallier et al., 2010).

2.3. BM cell cultures

After automated counting, the nucleated cells from fresh BM were seeded at 2×10^5 cells/cm^2 in tissue culture flasks. A cell sample from fresh BM was used to confirm the BMSCs characteristics, as previously described (Chevallier et al., 2010). The BMSCs were expanded in α-modified Eagle's medium (αMEM) (PAA, Les Mureaux, France) supplemented with 5% PL and 0.5% ciprofloxacine (Bayer Pharma, Puteaux, France). Heparin (2UI/ml, Sanofi-Aventis, Paris, France) was added to avoid clot formation. The cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 °C, and the culture medium was changed twice a week. When the cells reached 80–90% confluence (passage 0; P0), they were detached using Trypsin/EDTA (PAA) and then replated at 1000 cells/cm^2 until 80–90% confluence (passage 1; P1). Figs. 3, 4, and 5 have been done with the same BMSCs samples from ON patients, normal donors (N) and SCD patients (SCD-ON).

2.4. Clonogenic potential

The colony forming efficiency (CFE) assays were performed to evaluate the clonal expansion of BMSCs. Nucleated cells from concentrated BM were seeded at 20 000 and 80 000 cells/cm^2 in duplicate into 25 cm^2 tissue culture flasks with αMEM containing 10% FBS (not heat inactivated; ref. 06,471 from STEMCELL™ Technologies, Grenoble, France) and supplemented with 0.5% ciprofloxacine. After 10 days of culture, the cells were washed with saline solution (NaCl 0.09%) (B. Braun, Melsungen, Allemagne), fixed in methanol (VWR, Fontenay Sous Bois, France) and stained with 7% Giemsa (RAL Technopolis, Bordeaux, France) at pH 7 for 15 min. The number of colonies was counted using an inverted microscope (magnification ×25).

2.5. Colony-forming units-alkaline phosphatase (CFU-ALP+) assay

We measured the number of ALP+ colonies in the BM preparations from three ON patients, three normal donors (N) and four SCD patients (SCD-ON). Nucleated cells from fresh BM were seeded at 20 000 and 80 000 cells/cm^2 in duplicate into 25 cm^2 tissue culture flasks with αMEM containing 10% FBS (not heat inactivated; ref. 06,471 from STEMCELL™ Technologies) and supplemented with 0.5% ciprofloxacine, 50 μM L-Ascorbic Acid-2-phosphate (AA), 10 mM β-glycerophosphate (β-gly) and 0.1 mM dexamethasone (Dex) (Sigma Aldrich, Saint Quentin Fallavier, France). After 10 days of culture, the formation of osteoblast progenitors was detected using an alkaline phosphatase assay, performed according to the manufacturer's specifications (Sigma Aldrich). The total number of colonies was then determined by counterstaining

Download English Version:

https://daneshyari.com/en/article/2093906

Download Persian Version:

https://daneshyari.com/article/2093906

Daneshyari.com