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MESENCHYMAL STROMAL CELLS

Effects of hypoxia on osteogenic differentiation of mesenchymal stromal cells used as a cell therapy for avascular necrosis of the femoral head

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Abstract

Background aims. Avascular necrosis of the femoral head (AVN) occurs as common result of various conditions or develops as a primary entity, with a high freqency in young adults. Because of its tendency toward osteoarthritis requiring total hip arthroplasty, alternative treatments are being advocated, including cell therapy with mesenchymal stromal cells (MSCs). Because osteonecrotic bone is a severely hypoxic tissue, with a 1–3% oxygen tension, the survival and function of multipotent cells is questionable. *Methods.* In this study, the proliferative, immunophenotypic and osteogenic properties of bone marrow (BM)-derived MSCs from a clinical series of patients with AVN were evaluated under *in vitro* conditions mimicking the hypoxic milieu of AVN to verify the rationale for cell therapy. MSCs retrieved from the iliac crest (BM-MSC) were isolated, expanded and induced to osteogenic differentiation under a 2% pO_2 atmosphere (hypoxia) in comparison with the standard 21% pO_2 (normoxia) that is routinely used in cell culture assays. *Results.* Both proliferation and colony-forming ability were significantly enhanced in hypoxia-exposed BM-MSCs compared with BM-MSCs under normoxia. The expression of bone-related genes, including alkaline phosphatase, Type I collagen, and osteocalcin was significantly increased under hypoxia. Moreover, mineral deposition after osteogenic induction was not hampered, but in some cases even enhanced under low oxygen tension. *Conclusions.* These findings support autologous cell therapy as an effective treatment to stimulate bone healing in the hypoxic microenvironment of AVN.

Key Words: *avascular necrosis, bone marrow stromal cells, bone regeneration, hypoxia*

Introduction

Avascular necrosis of the femoral head (AVN) is characterized by cellular death in subchondral bone due to a deficiency in blood supply. This may occur as a consequence of severe trauma, such as in displaced intracapsular fractures of the femoral neck or after hip dislocation, or of a variety of conditions, including sickle cell anemia, hypercortisonism or steroid treatment, radiation therapy, caisson disease and alcohol abuse. In addition, AVN may also occur abruptly in young adults [1]. Necrosis of subchondral bone usually evolves toward articular cartilage damage, eventually leading to secondary osteoarthritis requiring major surgery, including total hip arthroplasty.

Less invasive alternatives have been suggested to prevent the progression of AVN. Among these, core decompression has been used extensively as a mean to decrease intraosseous edema and improve nutrient supply, enhancing and accelerating the natural healing potential of bone tissue before any damage of the overlying cartilage develops [2,3]. However, core decompression alone may often lead to a transient benefit with discouraging failure [4]. It is therefore conceivable that bone regeneration may be promoted by a supply of autologous osteogenic cells at the site of AVN [5,6].

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Bone marrow–derived mesenchymal stromal cells (BM-MSCs) are multipotent progenitor cells of perivascular origin that actively participate in the modulation of bone tissue homeostasis [7]. For therapeutic use, because clonogenic MSCs are relatively rare in human BM (1 in 10 000 nucleated cells), a proper number of MSCs can be obtained by *ex vivo* concentration or by *in vitro* expansion techniques [8]. The actual efficacy of cell therapy for AVN, however, is still controversial because of the harsh conditions at the osteonecrotic site, where ischemia and hypoxia cause apoptosis of bone cells [9]. Moreover, the osteogenic potential of MSCs from patients with AVN has also been debated [5,10]. In particular, it is still unclear whether the hypoxic environment of necrotic bone may have an impact on the proliferation and osteogenic differentiation of transplanted autologous MSCs [11]. In a previous study, Müller *et al.* observed the secretion of angiogenic and osteogenic factors as well as the osteogenic differentiation of BM-MSCs under *in vitro* hypoxia (1% O_2), although the BM aspirates were from pediatric patients with acute lymphoblastic leukemia [12].

To date, substantial understanding of the effects of hypoxia on the differentiation of MSCs toward the osteogenic lineage is missing, likely because of differences in study design, patient population and the source of MSCs. Most studies have shown an inhibited mineralization of osteogenic cells under low oxygen conditions [13–15], whereas some have reported that an osteogenic phenotype can be promoted if MSCs are under hypoxia during the initial steps of differentiation [16–18]. To our knowledge, no data about the performance of MSCs from AVN patients under low oxygen tension are currently available.

The aim of this study was to investigate the influence of hypoxia on the proliferation, clonogenicity and osteogenic potential of BM-MSCs retrieved from patients with AVN. Immunophenotype, proliferation, colony-forming ability, gene expression and bone matrix production were evaluated in BM-MSCs cultured under "low" oxygen tension (2%–3% O_2, hereinafter termed "hypoxia") versus "air" oxygen tension (21% O_2, hereinafter "normoxia"), either in basal medium (standard) or upon osteogenic stimuli. The *in vitro* simulation of a nonlethal hypoxic environment offers a realistic scenario of the micro-environment where MSCs are expected to act following delivery to the femoral head.

Methods

Patients

A randomized clinical trial (ClinicalTrials.gov; identifier NCT01892514) was designed to treat patients with early AVN with autologous BM concentrate (BMC), as a source of BM-MSCs, plus autologous platelet-rich fibrin and either demineralized bone matrix (arm A) or lyophilized bone chips (arm B) as a substrate, following surgical core decompression of the femoral head. The inclusion criteria were as follows: age 18–60 years and AVN without collapse of subchondral bone (i.e., stage IIA–C according to the Association Research Circulation Osseous [ARCO] classification). Patients unable to provide informed consent to treatment, who suffered from local infection or who had active neoplastic disease were excluded. Fourteen patients (nine men and five women, mean age: 36 ± 10 years, range 21–56) with ARCO stage II femoral head (four IIA, eight IIB and two IIC) were recruited and treated for idiopathic (ten) or corticosteroid-induced (four) osteonecrosis. Patient demographic characteristics and clinics are shown in Table I.

Iliac crest BM aspirates were obtained after written informed consent according to the Helsinki Declaration and local ethical committee approval. BM aspirates were obtained by insertion of a Jamshidi needle in the iliac crest. Three to five perforations, spaced at approximately 2 cm from each other, were made to collect 60 mL of BM. All aspirates were pooled in a plastic

Table I. Demographic characteristics and clinics of patients included in the study.

Patient code	Age (y)	Gender	ARCO stage	Etiology	Smoke	Hip
4843	30	M	IIB	Idiopathic	Unknown	L
4857	36	F	IIA	Idiopathic	No	L
4863	21	M	IIB	Steroid induced	No	L
4874	27	M	IIA	Idiopathic	No	R
4887	38	F	IIA	Idiopathic	No	R
4891	43	M	IIC	Idiopathic	Yes	L
4894	24	F	IIB	Steroid induced	Yes	L
4899	27	M	IIC	Idiopathic	Yes	L
4971	56	M	IIB	Idiopathic	No	L
4982	54	F	IIA	Idiopathic	No	R
4988	47	M	IIB	Steroid induced	Unknown	L
4993	35	F	IIB	Idiopathic	No	R
4995	38.9	M	IIB	Steroid induced	No	L
5001	27.7	M	IIB	Idiopathic	Yes	R