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Bone 36 (2005) 74–83

BONE

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Human reaming debris: a source of multipotent stem cells

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Received 27 July 2004; revised 20 September 2004; accepted 20 September 2004
Available online 24 November 2004

Abstract

The biological characteristics of human reaming debris (HRD) generated in the course of surgical treatment of long bone diaphyseal fractures and nonunions are still a matter of dispute. Therefore, the objective of the present investigation has been to characterize the intrinsic properties of human reaming debris in vitro. Samples of reaming debris harvested from 12 patients with closed diaphyseal fractures were examined ultrastucturally and were cultured under standard conditions. After a lag phase of 4–7 days, cells started to grow out from small bone fragments and established a confluent monolayer within 20–22 days. The cells were characterized according to morphology, proliferation capacity, cell surface antigen profile, and differentiation repertoire. The results reveal that human reaming debris is a source of multipotent stem cells which are able to grow and proliferate in vitro. The cells differentiate along the osteogenic pathway after induction and can be directed toward a neuronal phenotype, as has been shown morphologically and by the expression of neuronal markers after DMSO induction. These findings have prompted interest in the use of reaming debris-derived stem cells in cell and bone replacement therapies.

Keywords: Fracture treatment; Reaming; Multipotent stem cells; Transdifferentiation; Neurons

Introduction

In trauma and orthopedic surgery, long bone diaphyseal fractures are routinally treated by intramedullary nailing. Stability of the internal fixation increases when intramedullary reaming precedes the insertion of a nail. Although clinical success is obviously achieved with the reaming procedure [1–5], injudicious use may lead to complications such as disruption of the cortical blood flow [6], thermal necrosis of the cortical bone [7,8], or marrow embolization caused by evolved intramedullary pressures [9].

The intrinsic properties of reaming debris produced in the course of the reaming procedure are still a matter of dispute. It has been suggested that reaming debris is composed of bone marrow, blood, and bone scrapies [10,11]. In this respect, the basic composition of reaming debris appears to be similar to that of cancellous bone routinally harvested from iliac crest grafts. However, because of the elevations of intramedullary pressure and temperature generated by intramedullary reaming [12], human reaming debris (HRD) has been regarded as a necrotic tissue, and negative effects of reaming on fracture healing have been assumed [8,13,14]. On the other hand, it has been suggested that collection of HRD at the fracture site contributes to accelerated callus formation in an osteogenic manner [10,11,15–18] and leads to a short healing time of fractures treated with reamed nails [19–25]. In view of these controversial data, an attempt has been made to elucidate the intrinsic properties of HRD. By means of standard culturing conditions, it has been shown that cells have survived the harsh reaming procedure. Of particular interest

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doi:10.1016/j.bone.2004.09.019

was the observation that these cells revealed some characteristic features of mesenchymal stem cells (MSC).

MSC represent classical mesodermal derivates, they normally give rise to mesenchymal elements [26–30]. In the present investigation of MSC harvested from HRD, their osteogenic potential and their transdifferentiation into neurons have been demonstrated. Neuronal induction has been performed by using a previously published protocol [31]. Several neuronal marker proteins such as neuron-specific enolase (NSE), β-III-tubulin, and neurofilament-H (NF-H) could be identified by immunocytochemistry and Western blot analysis in the newly developed neurons.

Material and methods

Generation of reaming debris

Reaming debris was harvested from 12 adult patients with closed diaphyseal femoral fractures and without additional injuries. The investigations were conducted in accordance with local ethical approval. All patients underwent general anesthesia with endotracheal intubation. Normal routine monitoring was done. In the course of operative fracture treatment, the greater trochanter region was exposed, and the medullary cavity was opened at the trochanteric side. The surgical procedure was performed by intramedullary reaming of the medullary cavity prior to the insertion of a nail. For the reaming procedure, the Howmedica reamer system (Stryker Howmedica Osteonics GmbH, Kiel, Germany) was used. The femora were reamed progressively with reamers of increasing diameter until the medullary cavity was widened up to a diameter of 15 mm. From the point when the reamer head was touching the inner cortical wall, the medullary canal was reamed by taking 0.5-mm steps. Reaming was done with moderate speed and pressure. The reaming debris was harvested from the flutes of the largest reamerheads that were used to remove the inner cortical wall.

Histology of reaming debris

For histology, HRD samples were immersed overnight in 4% paraformaldehyde. In order to cut the specimens at a cryostat (2800 Frigocut, Reichert-Jung, Germany), decalcification of the HRD samples was performed by using 5% EDTA (pH 7.4) for 5 days at 4°C. Then, the samples were cryopreserved overnight in 17% sucrose and were embedded in Tissue Tek (Sakura, USA). Cryosections were cut at a thickness of 15 μm, placed on gelatin-coated slices, and stained with toluidine blue.

Transmission electron microscopy of reaming debris

Immediately after intramedullary reaming, small samples of the debris (weighing approximately 1 g) were removed from the reamer head and were fixed by immersion in Yellow-Fix (4% paraformaldehyde, 2% glutaraldehyde, 0.04% picric acid). After several washes in 0.1 M phosphate buffer (pH 7.2), the specimens were fixed for 2 h in 1% osmium tetroxide (OsO_4), washed carefully and repeatedly in 0.1 M phosphate buffer (pH 7.2), and dehydrated in series in graded ethanol. Subsequently, the samples were embedded in Epon (Serva, Heidelberg, Germany). Polymerization was performed at 60°C for 20 h. Thin sections were cut with a diamond knife (45°, Diatome, Switzerland) on an Ultracut (Reichert-Jung, Germany). Semithin sections (1 μm) were stained with Richardson (1% methylene blue, 1% borax, 1% azure II). Ultrathin sections (80 nm) were counterstained with uranyl acetate and lead citrate (Reichert Ultrostainer, Leica, Germany) and examined in a Zeiss EM 109 transmission electron microscope.

Cell culture of reaming debris

The debris was immediately removed from the reamerhead, and the specimens (weighing approximately 1 g) were placed in F12K medium containing 20% FCS (selected lots, PAA, Austria), 0.05 U/ml of penicillin, and 0.05 μg/ml streptomycin. The culture medium was exchanged three times a week. After 10 days, the cells were transferred into F12K-medium with 15% FCS (selected lots, PAA), 0.05 U/ml of penicillin, and 0.05 μg/ml streptomycin. The cultures were maintained at 37°C in humidified 95% air–5% CO_2 atmosphere. The medium was replaced every 2–3 days. After the cells had grown to 70–80% confluency, they were lifted with 0.25% trypsin, diluted 1:2 or 1:3 and were replated. The procedure was repeated for 12 passages. For immunocytochemistry and toluidine blue staining, cells of passages 1 and 6 were fixed in 4% paraformaldehyde. For toluidine blue staining, the cells were embedded in Epon (Serva) and sectioned at a thickness of 1 μm.

FACS analysis of reaming debris-derived cells

Cells of passages 1 and 6 were detached mechanically or by using 0.05% (w/v) trypsin. Then they were washed in PBS EDTA (2 mM) and were incubated at a final concentration of 1.0×10^6/ml in FACS buffer (CellWash, BD, CA) containing 4 μl of each primary antibody. In order to study the expression of lineage-specific markers, the following monoclonal antibodies (mAb) conjugated with FITC, phycoerythrin (PE), or peridinin-chlorophyll-protein (PerCP) were used for three-color FACS analysis (FACS-Calibur IV, BD): CD34-PE (BD), CD44-FITC (PharMingen, San Diego, CA), CD45-PerCP (BD), CD90-PE (Thy1, BD PharMingen), CD105-FITC (Serotec, Oxford, UK), and C106-PE (Chemicon, Temecula, CA). The cells were incubated at 4°C for 15 min, washed twice in FACS buffer (CellWash, BD), and were investigated by FACS flow cytometry.

Isotype control antibodies (mouse IgG1–antihuman IgG1-FITC, Pharmingen Pharmaceutical, Inc., San Diego,