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Granulocyte-colony stimulating factor enhances bone fracture healing

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ABSTRACT

Background: Circulating mesenchymal stem cells contribute to bone repair. Their incorporation in fracture callus is correlated to their bioavailability. In addition, Granulocyte-colony stimulating factor induces the release of vascular and mesenchymal progenitors. We hypothesized that this glycoprotein stimulates fracture healing, and analyzed the effects of its administration at low doses on bone healing.

Methods: 27 adult male Sprague-Dawley rats underwent mid-femur osteotomy stabilized by centromedullar pinning. In a post (pre) operative group, rats were subcutaneously injected with 5 μg/kg per day of Granulocyte-colony stimulating factor for 5 days after (before) surgery. In a control group, rats were injected with saline solution for 5 days immediately after surgery. A radiographic consolidation score was calculated. At day 35, femurs were studied histologically and underwent biomechanical tests.

Findings: 5 weeks after surgery, mean radiographic scores were significantly higher in the Preop group 7.75 (SD 0.42) and in the Postop group 7.67 (SD 0.52) than in the control group 6.75 (SD 0.69). Biomechanical tests showed femur stiffness to be more than three times higher in both the Preop 109.24 N/mm (SD 51.86) and Postop groups 100.05 N/mm (SD 60.24) than in control 32.01 N/mm (SD 15.78). Mean maximal failure force was twice as high in the Preop group 68.66 N (SD 27.78) as in the control group 34.21 N (SD 11.79). Histological results indicated a later consolidation process in control than in treated groups.

Interpretation: Granulocyte-colony stimulating factor injections strongly stimulated early femur fracture healing, indicating its potential utility in human clinical situations such as programmed osteotomy and fracture.

1. Introduction

Fracture consolidation has long been considered a locoregional process involving mesenchymal progenitor cells derived from the tissues damaged by the trauma (local bone marrow, endosteum, bone tissue, periosteum, muscles). Following a series of cellular and molecular event cascades reminiscent of the embryonic stages of skeletal tissue formation, these cell precursors lead to the regeneration of the initially injured tissue. However, the 2000s saw the discovery of circulating osteoprogenitor cells (Kuznetsov et al., 2001; Labat et al., 2000), now known to contribute to the bone formation and repair process (Otsuru et al., 2007; Otsuru et al., 2008). In physiologic state, these cells represent 1 to 2% of circulating mononuclear cells in adults and nearly 10% in adolescents (Eghbali-Fatourechi et al., 2005). They increase in response to osteogenic requirements: in animals, during an ectopic osteogenesis process, they can transiently rise to 80% of circulating mononuclear cells (Otsuru et al., 2008). This cell pool can contribute up to 10% of the osteoblasts present in a fracture consolidation callus (Kumagai et al., 2008) and as much as 50% of the osteocytes present in an ectopic bone regenerate (Otsuru et al., 2008).

Intravenous injections of blood-derived osteoprogenitor cells stimulate fracture repair (Granero-Molto et al., 2009; Wan et al., 2006). Their rate of incorporation into the callus increases proportionally to their serum bioavailability, until it reaches a plateau. Beyond this value, it remains stable regardless of increases in serum level (Granero-Molto et al., 2009). On the other hand, endogenous circulating

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osteoprogenitor cell bioavailability can be transiently boosted by the pharmacological use of bioactive molecules that trigger the mobilization of their medullary precursors, thereby favoring bone repair (Kumar and Ponnazhagan, 2012; Matsumoto et al., 2008; Toupadakis et al., 2013).

G-CSF is a glycoprotein used in human therapeutics for its ability to mobilize medullary hematopoietic stem cells in systemic circulation. G-CSF also induces the mobilization of vascular stem cells (Minamino et al., 2005) and mesenchymal stem cells (Levesque et al., 2007; Tatsumi et al., 2008; Zhdanov et al., 2007), both of which are involved, to varying degrees, in skeletal tissue repair. In rats, there was a significant increase in $CD34^+$ progenitor cells after five consecutive injections of G-CSF (Herrmann et al., 2018). These cells are capable of differentiating into osteogenic as well as vasculogenic lineages (Sidney et al., 2014).

Surprisingly, few studies have been published on the use of G-CSF as a skeletal tissue repair or regeneration adjuvant (Kaygusuz et al., 2006; X. Wu et al., 2008). When administered topically, G-CSF improves neovascularization and osteogenesis, which leads to regeneration of both critical-size bone defects in a rabbit bone resection model (Ishida et al., 2010) and tendon graft in a ligamentoplasty model (Sasaki et al., 2008). When administered parenterally, G-CSF counterbalances the negative effects of NSAIDs on bone healing, probably by stimulating osteogenesis (Kaygusuz et al., 2006). In a rabbit femoral osteonecrosis model, the combined use of G-CSF and Stem Cell Factor cause increased osteoblast activity and improve local revascularization, leading to more effective regeneration of the necrotic bone tissue volume (X. Wu et al., 2008).

To date, only two studies have focused on the effects of parenteral administration of G-CSF on fracture healing (Bozlar et al., 2005; Herrmann et al., 2018), both finding that G-CSF accelerated bone repair in rats. However, the doses used in these studies were respectively 2.5 (Bozlar et al., 2005) and 5 times higher (Herrmann et al., 2018) than the recommended dose in human clinical practice with healthy subjects. In fact, for donors providing peripheral blood stem cells for recipients of hematopoietic stem cell transplants, recombinant human G-CSF is generally recommended at a dose of 10 µg/kg per day. Even at this dose, side effects are observed, although when the dose is reduced, the side effects decrease (Lambertini et al., 2014). Furthermore, Bozlar et al. (2005) and Herrmann et al. (2018) chose to work only on emergency clinical applications (e.g. fractures, large defects) and did not consider programmed clinical applications (e.g. bone lengthening, tumor removal).

In this work, from a perspective of the eventual therapeutic use of GCSF in humans, we investigated the effects of parenteral administration of a 5 µg/kg per day dose of G-CSF on fracture consolidation in rats. We also investigated G-CSF administration pre-surgery vs G-CSF administration post-surgery. Pre-surgery administration mirrors the human clinical situation of a programmed osteotomy, and post-surgery administration mirrors a fracture situation.

2. Materials and methods

Twenty-seven male *Sprague Dawley* rats (OFA), weighing 500 g and twelve weeks old at time of surgery were used in the experiment. The animals were fed a standard diet ad libitum. They were housed singly in cages in temperature-controlled rooms (22 °C) having a 12 h light cycle. All animal protocols were approved by the University of Aix-Marseille institutional animal care and use committee and the French research ministry (authorization number 02572.02), and performed in the conventional animal house of Marseille Medical Faculty (France).

2.1. Surgical protocol

The surgical model consisted of right femur mid-diaphyseal osteotomy, immediately osteosynthesized by centromedullary pinning. Under general anesthesia consisting of intraperitoneal Ketamine 75 mg/kg and Medetomidine 0.15 mg/kg and under strictly aseptic conditions, the right femur was exposed via a lateral subperiosteal approach. Medial-diaphyseal osteotomy was performed using piezotome. Retrograde centromedullary pinning (2 mm diameter Kirschner wires) was performed by lateral parapatellar arthrotomy. The muscular fascia was closed with separated resorbable stitches and the skin with slow-absorption continuous stitches. Postoperative analgesia and prophylactic antibiotic therapy consisted of an injection of Buprenorphine 0.05 mg/kg and subcutaneous Baytril 10 mg/kg peroperatively, then once per day for 3 days. Rats were followed weekly by radio to check that they were healthy.

2.2. Experimental groups

Rats were randomized to one of three distinct pharmacological procedures. The “Postoperative” group (Postop) with 9 rats were injected subcutaneously with 5 µg/kg per day of G-CSF (FILGRASTIM) for 5 days starting from surgery. The “Preoperative” group (Preop) with 9 rats received identical G-CSF injections for 5 days preoperatively. In the Control group, 9 rats were injected subcutaneously for 5 days with a saline solution, starting immediately after surgery.

One rat died in the control group upon induction of anesthesia. One rat died in the Postop group being found dead one day after surgery. One rat in the Preop group was sacrificed three days after surgery due to splitting of the scar tissue. No locoregional infections or other complications were observed in the other animals. After 35 days of consolidation, the animals were sacrificed by intraperitoneal lethal injections of Sodium Pentobarbital 100 mg/kg.

2.3. Radiographic analysis

X-rays of the femurs subjected to surgery were taken immediately postoperatively, then at days 7, 21 under general anesthesia and at day 35 after the sacrifice. A radiographic consolidation score (An et al., 1999) was calculated from the analysis of the X-rays by two orthopedic surgeons not involved in the study (Table 1) and who analyzed independently each X-ray according to the radiographic scoring system for fracture healing (An et al., 1999). The final score assigned to each X-ray was the mean of the scores of the two surgeons.

2.4. Mechanical analysis

Femurs subjected to surgery (6 femurs from control group, 6 femurs

Table 1
Radiographic scoring system for fracture healing (An et al., 1999).

Category	Score
Periosteal reaction	
Full (across the defect)	3
Moderate	2
Mild	1
None	0
Bone union	
Union	3
Moderate bridge (> 50%)	2
Mild bridge (< 50%)	1
Non-union	0
Remodeling	
Full remodeling cortex	2
Intramedullary canal	1
No remodeling	0
Maximum total score	8