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angiogenesis plays an important role in normal bone development and adult bone healing. Experimental studies have shown that angiogenesis induced by adjunctive modalities, such as angiogenic factors, cytokines, and stem or progenitor cells, contributes to regeneration of bone and repair of fractures.1–3 Recently, it has been shown that autologous bone marrow mononuclear cell (BMMNC) implantation increases collateral vessel formation in ischemic limb models and in patients with limb ischemia.4–6 We present a case of intractable tibia fracture followed by compartment syndrome. In this case, autologous BMMNC implantation promoted angiogenesis, leading to bone regeneration.

**Case Report**

In January, 2004, a 28-year-old man was involved in a traffic accident. On arrival at the hospital, his right tibia and fibula were fractured and acute compartment syndrome had developed rapidly. Fasciotomy was immediately performed to decompress all compartments of his right leg to prevent ischemic damage to muscles, nerves, blood vessels, and bones. Unfortunately, it was not sufficient and many of the arteries collapsed (Fig 1), causing permanent tissue damage. His right toes became gangrenous, requiring partial foot amputation and split-thickness skin grafts were harvested from his left outer thigh to cover the wound. Although an external fixation device was used to stabilize and align the fractured bones, the tibia did not heal and he could not walk on his foot 6 months later (Fig 2A). Angiography showed severe arterial injury and poor collateral vessel formation in the right lower leg (Fig 3A).

There was no option for conventional therapy, and autologous BMMNC implantation for therapeutic angiogenesis and subsequent bone regeneration was therefore performed. Under general anaesthesia, 700 ml of bone marrow was aspirated from the ileum and collected into plastic bags containing heparin. The BMMNC were immediately sorted using a CS3000-plus blood-cell separator (Baxter, Deerfield, USA) and concentrated to a final volume of approximately 50 ml containing \(1.1 \times 10^9\) BMMNC. The cells were then

\[\text{Autologous Bone Marrow Mononuclear Cell Implantation Induces Angiogenesis and Bone Regeneration in a Patient With Compartment Syndrome}\]

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A 28-year-old man developed compartment syndrome in the right lower leg after fracture of the tibia and fibula. Despite fasciotomy, many arteries collapsed and union of the tibial and fibula fractures did not occur. Autologous bone marrow mononuclear cell (BMMNC) implantation for therapeutic angiogenesis and subsequent bone regeneration was performed and 4 weeks later, angiography showed a marked increase in collateral vessels surrounding the tibial fracture, and union was completed 6 months later. BMMNC implantation therapy might provide therapeutic angiogenesis and osteogenesis in patients with compartment syndrome. (Circ J 2006; 70: 1362–1364)

**Key Words:** Angiogenesis; Bone marrow mononuclear cells; Bone regeneration; Compartment syndrome

Fig 1. Angiography and radiography show many collapsed arteries and the decreased blood supply below the fractured tibia and fibula, as well as the severe swelling of the gastrocnemius on arrival at hospital.
implanted into the gastrocnemius muscle around the fractured bone as previously described. Four weeks later, there was marked formation of collateral vessels around the tibial fracture (Fig 3B) and a slight increase in the external callus (Fig 2B). Thereafter, union of the fracture gradually proceeded, and remodeling could be seen. The tibial fracture healed completely and the external fixation device was removed 6 months later (Fig 2C). Furthermore, BMMNC transplantation accelerated wound healing and the open wound on his right foot was completely repaired by partial plastic surgery.

The therapy was approved by the Ethics Committee of the Hiroshima University Graduate School of Biomedical Sciences and the patient gave written informed consent.

Discussion

Fracture of a long bone associated with crushing of structural muscles can often trigger acute compartment syndrome. Although fasciotomy is a useful treatment, delay or insufficient treatment leads to irreversible injury to the muscles, nerves, blood vessels, and bones. There is no option for conventional therapy in cases of poor blood supply in an injured leg. Restoration of bioactivity in the fractured site is thought to be essential for the treatment of a non-union bone fracture. Recently, the effects of various modalities, such as angiogenic factors, cytokines, and stem or progenitor cells, on osteogenesis in animal models have been investigated.

In the present case, despite immediate fasciotomy, collateral vessel formation remained poor and bone union was impaired in the injured leg. At 6 months after onset, bioactivity of the fractured site was almost lost, and there was little possibility of union of the fracture. Therapeutic angiogenesis by BMMNC implantation has recently been investigated in experimental ischemic limb models and in patients with severe peripheral arterial disease. It improves limb ischemic symptoms according to angiography in

Fig 2. Radiographs of the fractured bones before (Panel A), at 1 month after (Panel B), and 6 months after (Panel C) autologous bone marrow mononuclear cell implantation.

Fig 3. Angiographs of the artery in the injured leg before (Panel A) and at 1 month after (Panel B) autologous bone marrow mononuclear cell implantation.

Fig 4. In vitro differentiation of attached cells derived from bone marrow mononuclear cells (BMMNC) into osteocytes. (A) Alizarin red staining after osteogenic differentiation in osteogenic induction medium or control medium. (B) Attached cells derived from BMMNC before osteogenic differentiation. (C) Attached cells after osteogenic differentiation are stained red and form mineralized nodules.
patients with limb ischemia. It is well known that bone fracture healing requires a blood supply, so BMMNC implantation might be useful not only for revascularization, but also for subsequent bone regeneration. Indeed, in the present study clinical and functional healing of bone fracture was achieved after BMMNC implantation. Some possible mechanisms by which BMMNC implantation induces angiogenesis and bone regeneration are postulated. BMMNC include endothelial progenitor cells and other cell populations, including osteoblasts. We did the following experiment to confirm that the BMMNC differentiated into osteocytes in the present patient. The BMCs, including erythrocytes, were seeded at a density of 1 × 10^{6} cells per 35-mm tissue culture dish (Corning, Nagog Park Acton, MA, USA) and maintained in 10 ml Dulbecco's modified minimum essential medium (DMEM) (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA), 100 U/ml penicillin G (Sigma), and 100 μg/ml streptomycin (Sigma) at 37°C in a 5% CO2 incubator. After 4 days in culture, nonadherent cells were removed and fresh medium was added. Attached cells were fed with fresh medium every 3 days. Passages were performed when the cells were approaching confluence! Osteogenic conversion of these cells from the 6th passage culture was identified as previously described. For osteogenic differentiation, cells were seeded at 4 × 10^{4} cells/16-mm well (2.3 × 10^{4} cells/cm²) and maintained in DMEM supplemented with 10% FBS, 10 mmol/L dexamethasone (Sigma), and 50 μg/ml ascorbic acid-2-phosphate (Sigma, osteogenic induction medium). Cultures maintained in the osteogenic induction medium or in the control medium (DMEM supplemented with 10% FBS) were stained with alizarin red on day 21. Mineralized matrix (alizarin red staining) was observed in the osteogenic induction medium but not in the control medium (Fig 4). After BMMNC implantation, endothelial progenitor cells and osteoblasts will differentiate to mature endothelial cells and bone. In addition, the implanted cells might release several angiogenic factors and cytokines, such as fibroblast growth factor, vascular endothelial growth factor, hepatocyte growth factor, and insulin-like growth factor-1, leading to an increase in blood supply and bioactivity in the process of bone regeneration. Interestingly, these cytokines promote bone healing by activation of osteogenesis, resulting in union of the fracture. We speculate that the process of bone healing in this case was mainly by bone regeneration after angiogenesis. However, we cannot rule out the possibility that angiogenesis and bone regeneration occurred at the same time after BMMNC implantation. A non-union bone fracture is defined as one that has not healed after a certain period of time. If non-union is still evident at 6 months post-injury, the bone fracture will remain unhealed. In the present case because the tibial fracture had not healed at 6 months after onset, it is unlikely that the course of bone healing was a natural course. We believe that the union of the bone fracture was largely because of the BMMNC implantation therapy.

In conclusion, it is obvious that increased blood flow helped maintain the structural and functional integrity of peripheral tissue and facilitated bone regeneration in this case. Angiogenesis in cartilage might contribute to bone repair, as well as the increase in blood flow. BMMNC implantation therapy might be a novel treatment for patients with compartment syndrome.

References
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