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Case Report

Treatment of osteonecrosis of the femoral head with multiple drilling and bone marrow mesenchymal stem cells expanded *ex vivo* plus biomolecules derived from platelet-rich plasma: a case report

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Abstract: Osteonecrosis of the femoral head (ONFH) is a debilitating condition that predominantly affects young individuals, resulting in disability and involving significant healthcare costs. Therefore, it is crucial to develop an effective therapeutic strategy to treat this debilitating disease. In this context, autologous bone marrow-derived mesenchymal stem cells (auto-BM-MSCs) have emerged as a promising approach for treating ONFH. In this case report, we applied this therapy to a patient with ONFH and evaluated both its safety and therapeutic benefits. The treatment consisted of the administration of a single dose of 4×10^7 *ex vivo*-expanded auto-BM-MSCs combined with biomolecules derived from platelet-rich plasma. These therapeutic agents were injected into the necrotic zone after accessing it through the technique of multiple small drillings. Subsequently, the progression of ONFH was assessed after 18 months of the auto-BM-MSC administration. Radiographic evaluation showed that the initial femoral head flattening persisted, but no further progression or coxofemoral arthritic changes were observed. Nevertheless, magnetic resonance imaging (MRI) demonstrated a significant improvement in the affected femoral head's area, resulting in a Kerboull angle of 80°, without evidence of flattening or a notable collapse compared to the preoperative condition. Furthermore, the patient exhibited a remarkable functional improvement, as evidenced by a modified Harris hip score of 90 points. The absence of any additional surgery reinforces the positive outcomes achieved through this therapeutic intervention. In conclusion, our case study provides evidence for using the *ex vivo*-expanded auto-BM-MSCs in combination with platelet-rich plasma-derived biomolecules as a viable and safe treatment for ONFH. However, further research and clinical trials are necessary to validate these promising findings.

Keywords: Osteonecrosis, femoral head, mesenchymal stem cells and platelet-rich plasma

Introduction

Osteonecrosis of the femoral head (ONFH) is a debilitating disease caused by the disruption of the vascular supply to the subchondral bone. This disruption leads to the death of osteoblasts and failure of bone marrow, resulting in the collapse of the articular surface of the femoral head and the subsequent development of osteoarthritis [1]. Some of the primary causes of ONFH are corticosteroid usage and excessive alcohol intake, which account for more than 80% of cases [2, 3]. In this context, the prevalence of ONFH is increasing, although it remains unclear whether is due to a substantial rise in cases or an improvement in the methods of diagnosis [4]. In the United States, between 10,000 to 20,000 new cases are diagnosed each year, with a higher incidence in individuals aged 20 to 50 years. In fact, adolescents and adults have a significantly higher risk of developing ONFH compared to children [5]. Also,

approximately 5-12% of patients eventually require total hip replacement [2].

Current treatments for ONFH include both non-surgical and surgical techniques. Non-surgical options comprise protective weight bearing, physical therapy, and drug therapy; while surgical interventions consist of non-vascularized bone grafts, osteotomies, core decompressions, vascularized bone grafts, allografts, and joint replacements [5]. Importantly, in the early stage of the disease, the primary approach is preventing the collapse of the subchondral bone. Nevertheless, when the disease progresses and significant depression occurs, total hip replacement becomes the preferred treatment.

Bone regeneration is a complex physiological process influenced by various factors that regulate bone niche. These factors involve the tissue's complex structure, the soluble microenvironment, the composition and the renewal of extracellular matrix (ECM) insoluble proteins, glycoproteins, microRNAs, as well as cell-cell and cell-ECM interactions [6]. In addition, mesenchymal stem cells (MSCs) play a crucial role in finely regulating bone formation by orchestrating osteogenic differentiation [7]. MSCs have shown promising evidence that indicates their role in facilitating the effective repair of bone fracture, even in diseased microenvironments. As a result, MSCs are the most studied stem cells in pre-clinical and clinical research on skeletal diseases [8].

Given the early onset of ONFH and the limited long-term efficacy of surgical interventions, there is a need for having more effective therapeutic approaches. Several studies have evaluated the clinical effectiveness of several bone marrow products in ONFH patients; for instance, bone marrow aspirate, bone marrow-derived mononuclear cell fractions, or bone marrow containing MSCs [9-11]. Nonetheless, studies involving *ex vivo*-expanded bone marrow-derived MSCs (BM-MSCs) are scarce. In fact, MSCs are noticeable among other stem cell populations due to their easiness to be obtained from healthy allogeneic donors, low immunogenicity (reduced expression of MHC class II constitutive molecules), anti-inflammatory properties, and relative ease of growth and expansion *ex vivo*. In our case report, we assessed the beneficial effects of *ex vivo*-expanded autologous BM-MSCs (auto-BM-MSCs), in combination with biomolecules derived from platelet-rich plasma, for the treatment of a 17-year-old female patient with ONFH.

Case report

A 17-year-old female who has experienced pain in her right inguinal region for a year, which worsens with walking but improved at rest. The patient complained of a limp that increased after 20 minutes of walking, and inability to practice any sports. Upon examination, the patient exhibited an antalgic gait limp but there were no length discrepancies. The patient had full mobility of the hips, including the contralateral side, and experienced groin pain with rotation of the right hip. The dynamic internal rotatory impingement (DIRI) test was positive. There was no gluteal or trochanteric pain, preserved muscle strength, and no distal neurovascular deficit. The patient had a body-mass index of 22 and a pre-surgical Harris hip score of 50.

The patient's medical history did not reveal treatment with steroids or any associated diseases. She underwent sedative physiotherapy and took non-steroidal anti-inflammatory drugs (NSAIDs) without improvement, leading to the decision to attend in our center for medical consultation.

Preoperative X-rays of the hips revealed a radiolucent area with a sclerotic base in the support area of the femoral head (**Figure 1A**, **1B**). Magnetic resonance imaging (MRI) showed a hypointense zone in the femoral head at t1, with slight flattening of the femoral head (**Figure 1C**). In the coronal projection, a hyperintense zone of the femoral head was observed at t2 (**Figure 1D**). Additionally, computed axial tomography (CAT) measured a head collapse of less than 2 mm with a Kerboul angle of 200° (**Figure 1E**, **1F**). The necrosis was classified as ARCO IIIA due to a subchondral fracture with flattening of the femoral head, but with a depression of less than 2 mm. The lesion was considered in an early stage, leading to the diagnosis of idiopathic ONFH.

The patient received into the necrotic zone a single dose of 4×10^7 auto-BM-MSCs that were *ex vivo*-expanded, in combination of biomolecules derived from platelet-rich plasma. This

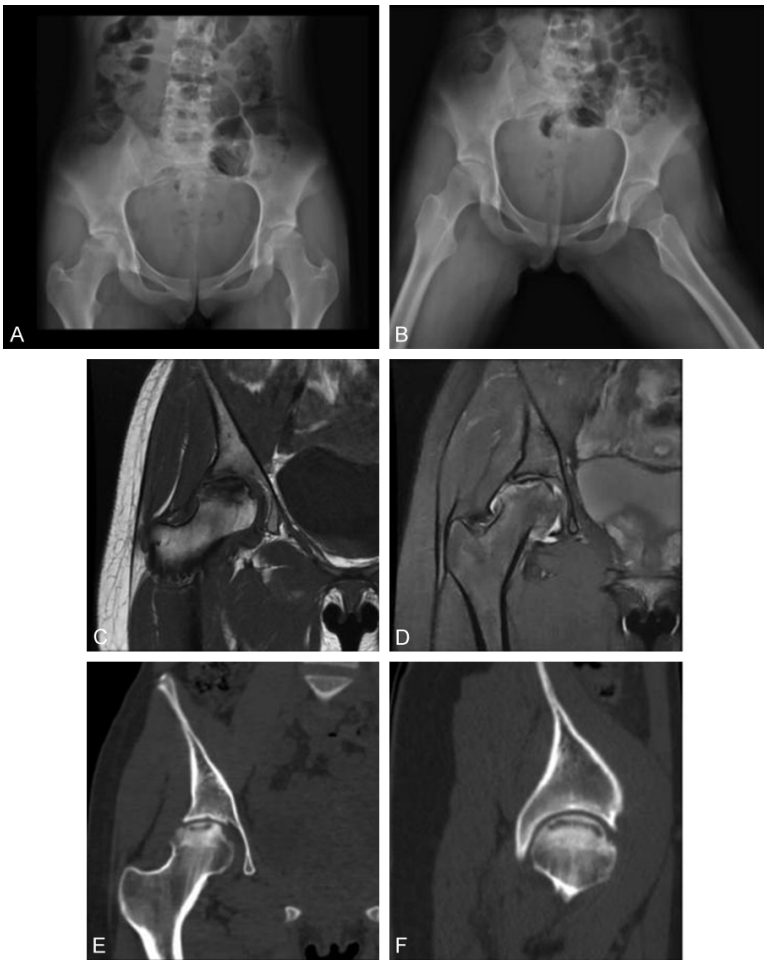

Figure 1. Preoperative X-rays, MRI and CAT images of the hip. A. Anteroposterior X-rays image. B. Lateral X-rays image. C. Coronal slice by MRI on t1. D. Coronal slice by MRI on t2. E. Coronal slice of the hip by CAT. F. Sagittal slice of the hip by CAT.

was done through the canal of a preceding core decompression process using the technique of multiple small drillings, in order to avoid large holes as well as reduce the risk of fractures (**Figure 2**). To enhance the retention of auto-BM-MSCs in the damaged bone area, a bone substitute with tricalcium phosphate and bone wax was immediately placed on the outer cortex after cell administration. The patient was restricted from bearing weight for six weeks after starting full support.

After 18 months of having received the treatment, follow-up X-rays showed persistence of the initial flattening of the femoral head, but without progression or the development of coxofemoral arthritic changes (**Figure 3A**, **3B**). Remarkably, MRI showed a substantial improvement in the area of the femoral head, with a Kerboul angle of 80° and no flattening. There was no substantial collapse of the femoral head compared to the preoperative images (**Figure 3C**, **3D**). Also, the patient showed significant functional improvement, as assessed by the modified Harris hip score of 90 points, and did not require additional surgical procedures.

Discussion

ONFH is a condition that occurs when the blood supply to the joint is interrupted, typically resulting in secondary osteoarthritic changes in young adults. While trauma, alcohol intake, and steroid use are commonly associated with the development of ONFH, the etiology was idiopathic in this case report. Recent efforts have focused on preserving the joints of these patients, who are typically young and active; for example, stem cell-based therapy has shown success in rebuilding the medullary cavity [12, 13].

Unlike other tissues, bone has the ability to completely regenerate rather than heal with a scar after injury [14]. The early stages of fracture healing resemble those of wound healing occurring in other tissues, with an inflammatory step followed by a mesenchymal and angiogenic activation phase. In fact, MSCs are recruited to the injury site and proliferate. In bone regeneration, MSCs differentiate into either chondrocytes or osteoblasts. In detail, osteoblasts deposit bone directly via intramembranous ossification, while chondrocytes multiply, hypertrophy, mineralize, and deposit new bone onto the cartilaginous matrix through endochondral ossification. The final step of this process involves the remodeling of the bone in order to restore both its typical structure and function [15, 16].

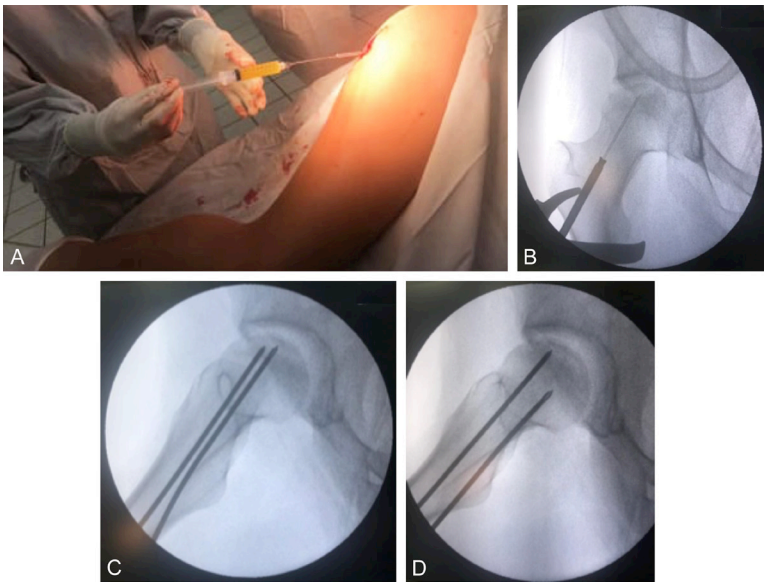

Figure 2. Administration of auto-BM-MSCs plus biomolecules derived from platelet-rich plasma using the technique of multiple small drillings. A. Administration of cell product into the necrotic zone. B. Canal of a preceding core decompression process using the technique of multiple small drillings. C, D. Fluoroscopic images show multiple small-diameter with Steinmann pins 2.5 mm.

Numerous studies have demonstrated the beneficial effects of using auto-BM MSCs in patients with ONFH. Specifically, studies involving the transplantation of autologous bone marrow cells into femoral heads have shown not only a significant reduction in joint deterioration but also an enhanced regeneration of hip bones [17]. While initial investigations used bone marrow-derived mononuclear cells, the latest studies have utilized MSCs derived from bone marrow due to their significant role in fracture repair [18-21]. The results of these studies revealed notable improvement in clinical and radiological parameters, which may be attributed to the following molecular mechanisms of MSCs: (i) adhesion to vascular endothelial cells that express various adhesion molecules, (ii) migration and survival in the necrotic site and (iii) expression of growth factors and chemokines, which leads to an *in-situ* regenerative response [18].

Our case report is the first to deliver *ex vivo*-expanded auto-BM-MSCs combined with biomolecules derived from platelet-rich plasma to a patient with ONFH, through the technique of multiple small drillings. This approach caused minimal morbidity and avoid inducing the development of other fractures or more serious complications. Also, our results are consistent with other studies that demonstrate medullary cavity repair and neovascularization after treating the patients with the cell-base therapy, by inducing both the regeneration of necrotic bone and formation of new tissue [9-11]. In addition, platelet-rich plasma obtained from peripheral blood is an abundant source of growth factors, including transforming growth factor-β1, platelet-derived growth factor, insulin-like growth factor, vascular endothelial growth factor, epidermal growth factor, and basic fibroblast growth factor, among others [20]. As a result, we delivered biomolecules derived from platelet-rich plasma to strengthen MSC activity. This combined therapy yielded effective results, preventing a total hip replacement and improving mobility. Nevertheless, further research is necessary, including phase I and phase II trials with a large sample size from diverse geographic locations, and further follow-up to collect more information on the effectiveness of using *ex vivo*-expanded auto-BM-MSCs combined with platelet-rich plasma to treat patients with ONFH.

Conclusion

Our case report suggests that the administration of *ex vivo*-expanded auto-BM-MSCs combined with biomolecules derived from platelet-rich plasma, into the necrotic zone using the technique of multiple small drillings, is an excellent therapeutic option for ONFH patients.

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Informed consent was obtained from the patient's mother.

Disclosure of conflict of interest

None.

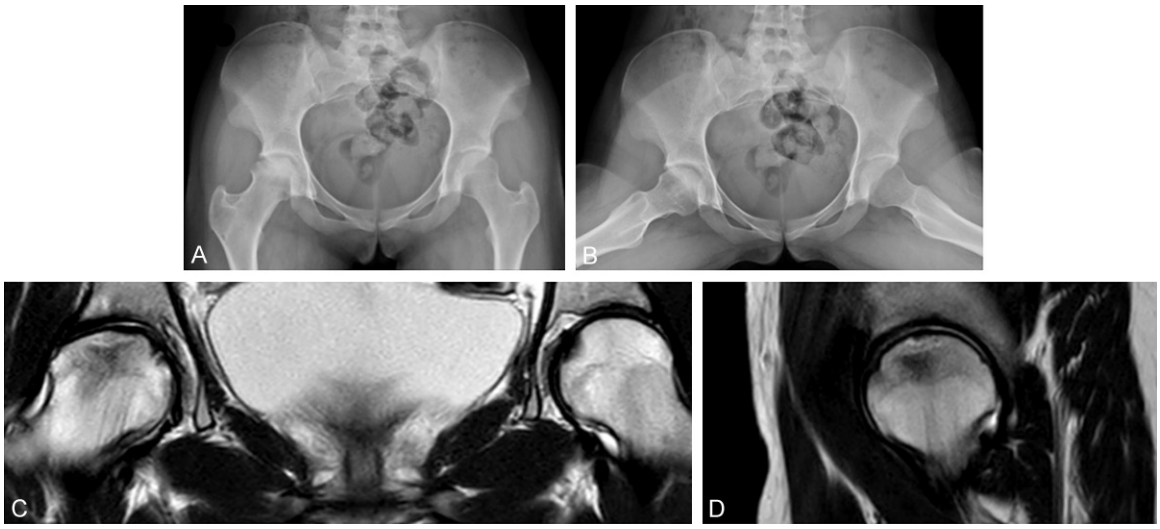

Figure 3. Postoperative X-rays and MRI of the hip. A. Anteroposterior X-rays image. B. Lateral X-rays image. C. Coronal MRI slice. D. Sagittal MRI slice.

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