



Research Article

The Femoral Head of Patients with Hip Dysplasia is not as Osteogenic as Iliac Crest Bone Location

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ABSTRACT

When Total Hip Arthroplasty (THA) is required in a patient with developmental dysplasia of the hip (DDH), bone deficiency in the acetabular roof often remains a problem. The iliac crest (IC) has long been the preferred source of autograft material, but graft harvest is associated with frequent complications and pain. Autologous bone graft can also be obtained from the femoral head (FH) for reconstruction of the acetabulum in hip arthroplasty. However, in certain challenging clinical scenarios, incorporation of the femoral head autograft appears less successful than the iliac crest autograft. The difference in potential for proliferation and osteoblastic differentiation between the two sites has still not been evaluated; therefore, it is not known how to compensate for this difference when it is present. We designed this study to evaluate the number of mesenchymal stem cells (MSCs) in both the iliac crest and femoral head of the same patient. We also determined the best operating room procedure for loading the femoral head with MSCs to achieve equivalent numbers of MSCs as in the IC. Twenty patients (8 men and 16 women) undergoing THA for DDH were enrolled in the study. The mean age was 55.5 years (range 41–65 years). Bone marrow aspirates were obtained from three depths within the femoral head and the aspirates were quantified relative to matched iliac crest aspirates that were obtained from the same patient at the same time. The cell count, progenitor cell concentration (cells/mL marrow), and progenitor cell prevalence (progenitor cells/million nucleated cells) were calculated.

Aspirates of FH marrow demonstrated less concentrations of mononuclear cells compared with matched controls from the iliac crest. Progenitor cell concentrations were consistently lower in FH aspirates compared to matched controls from the iliac crest ($p = 0.05$). The concentration of osteogenic progenitor cells was, on average, 40% lower in the FH aspirates than in the paired iliac crest samples ($p = 0.05$). However, with bone marrow aspirated from the iliac crest, we were able to load the femoral head autograft with sufficient MSCs to obtain the same number as present in an iliac crest. With concentrated bone marrow from the IC, supercharging the femoral autograft with MSCs to numbers above that present in the IC was possible in the operating room, and the number of MSCs supercharged in the femoral head was predictable.

Based on these findings we suggest that FH graft supercharged with BM-MSCs from the IC is comparable to IC graft for osseous graft supplementation especially in THA for patients with DDH.

INTRODUCTION

When Total Hip Arthroplasty (THA) is required in a patient with developmental dysplasia of the hip (DDH), bone deficiency in the acetabular roof often remains a problem. Options for obtaining adequate bone coverage for stable fixation of the acetabular component have included use of a small cup with augmented bone graft prepared from resected femoral head. Bulk bone grafting offers advantages: the cup may be placed in an anatomic position rather than a high one, it provides support for the acetabular component, and if incorporated it provides beneficial bone stock for

any future revision surgery. However numerous authors have reported high rates of autograft resorption, collapse of the bone graft, and socket loosening [1,2], and some authors have found that autografts from the iliac crest (free or vascularized) better integrate with iliac bone [3-5], which indicates that the osteogenic capacity of the femoral head (FH) may be decreased as compared with that of the iliac crest (IC).

No study has analyzed the osteogenic capacity of the femoral head of patient with developmental dysplasia of the hip as compared with that of the iliac crest bone. Adult mesenchymal stem cells (MSCs) are used to enhance the repair of a wide range of diseased or traumatized human tissues [6,7]. When cultured in the presence of specific medium supplements, these cells are osteogenic stem cell precursors. We measured the concentration and the number of MSCs as a marker to evaluate the osteogenic capacity of the femoral head and iliac crest in the same patients.

With the hypothesis that the osteogenic capacity of the femoral head of patient with developmental dysplasia hip osteoarthritis is lower than that of the iliac crest, we evaluated and compared the concentration of MSCs at the two anatomical sites (femoral head and iliac crest) of the same patients and proposed a technique to improve the osteogenic capacity of the femoral autograft in the operating room.

MATERIALS AND METHODS

Subjects

Twenty adult patients (male = 4; female = 16) older than 40 years of age (mean: 55, range: 41–65) were enrolled in the study. All subjects were diagnosed with DDH. These individuals were scheduled for THA in which FH and IC bone marrow were harvested at the time of surgery. The rationale for the investigation and the accompanying risks were discussed with each patient, and an informed consent form approved by the Institutional Review Board of our University Hospital was signed. Patients had no underlying general or hematological disease. Patients were excluded from the study if they had previous iliac crest harvest or previous irradiation of the pelvis. Patients were also excluded if they were being managed with chronic steroid medication, thyroxine, or chemotherapy.

Marrow harvest and collection

The surgical procedure was carried out in a routine fashion, as indicated for the underlying disorder, with no alteration in the surgical approach or technique. Bone marrow was aspirated from the femoral head and the IC of the same patient using a marrow aspiration needle. Bone marrow was collected as previously reported [8,9] from the proximal femur at the beginning of THA. Under general anesthesia, the needle was rinsed with a heparin solution, introduced by hand through the lower aspect of the greater trochanter in three different parts of the femoral head and marrow was aspirated at each site. At the same time bone marrow aspirate was obtained from the iliac crest of the patient to act as the control. With use of an established technique that has been validated in previous studies, bone marrow cells were aspirated directly into 10.0-mL syringes that had been preloaded with heparinized saline solution (1000 units of sodium heparin in 1.0 mL of saline solution). To maximize the number of harvested osteoprogenitor stem cells, 2-mL samples of marrow were collected at 3 sites of 2 cm distance from each other along the IC, and also at 2 cm depth within the iliac crest. A smear was made at the time of each aspiration to confirm the presence of nucleated cells and the quality of the aspirate.

Cell isolation and culture

The number of nucleated cells was counted using a standard Malassez hemocytometer. To measure the number of connective progenitor cells (MSCs) that

were transplanted, we used the Colony Forming Unit – Fibroblast (CFU-F) assay as an indicator of mesenchymal stem cell activity [7-10]. An aggregate sample of the marrow before and after concentration was cultured in vitro in order to determine the concentration of stem cells achieved by the process. Cells were washed once and re-suspended in Hanks balanced salt solution without Ca^{++} or Mg^{++} . Buffy coats were collected after centrifugation of the aspirates at 1200 g for ten minutes. For the CFU-F assay, quadruplicate aliquots of 2×10^6 cells were inoculated in twenty-five square mL tissue culture flasks containing ten mL of culture medium supplemented with 20% fetal calf serum, 1% L-Glutamine, penicillin (100 units/mL) and streptomycin (100 mg/mL). Culture flasks were placed in a humidified incubator with 5% CO_2 and maintained at 37°C . The growth medium was completely renewed every three to four days. Cultures were read for colonies on the 10th day. Fibroblast colonies were Giemsa stained and counted under an inverted microscope at 25X magnification. An aggregate of cells containing more than 50 fibroblasts was scored as a colony. Results were expressed as the mean number of Colony Forming Units (CFUs) per 10^6 bone marrow cells. The fibroblast lineage of the colonies was demonstrated by immunofluorescence staining with antibodies against fibronectin and type I and III collagen. The number of connective-tissue progenitor cells in a sample can be estimated by counting the number of colony forming units (CFUs) expressing alkaline phosphatase activity in culture (CFU-APs). Alkaline phosphatase is an early marker for osteoblastic differentiation of these pluripotent cells.

Three parameters were measured directly or calculated from the results of cell culture: (1) the nucleated-cell count (the number of nucleated cells per 1.0 mL of marrow aspirate), (2) the prevalence of connective-tissue progenitor cells (the number of connective-tissue progenitor cells per 10^6 nucleated cells), and (3) the concentration of connective-tissue progenitor cells (the number of connective-tissue progenitor cells per 1.0 mL of aspirate).

Technique for loading MSCs in the dysplastic femoral head with bone marrow concentrate from the iliac crest

This phase of the study was performed to quantify the volume of bone marrow concentrate from the iliac crest and the quantity of MSCs needed to be loaded into the femoral head at the time of surgery to obtain the equivalent number of MSCs as in the iliac crest. The technique has been previously reported for allografts [11,12]. Briefly, injection through the cartilage was performed several times at different entry points as previously reported for allografts. A 10mL syringe was used to inject the bone marrow concentrate. Bone marrow concentrate injected at different entry points through the cartilage was measured. Injected bone marrow concentrate that flowed outside the femoral head through the neck was also measured. The total volume that remained inside the femoral head was calculated as the difference between the volume that was injected and the volume that flowed outside the femoral head.

RESULTS

The femoral head autografts contained a lower concentration of MSCs than the iliac crest of the same patient

Nucleated cell count: The total number of nucleated cells per 1.0 mL of aspirated marrow was consistently lower in the FH aspirates as compared with paired iliac crest specimen, and the differences were significant. A mean of 11.76 ± 7.8 million cells/mL (range, 5 to 30 million cells/mL) were contained in each 1.0 mL of FH marrow aspirated, compared with 24.1 ± 13.2 million cells/mL (range, 11 to 49 million cells/mL) in the IC marrow. The cell count for iliac crest values was, on the average, about twice as high than the paired FH aspirates ($p = 0.02$).

Prevalence of connective-tissue progenitor cells: The mean prevalence of osteogenic progenitor cells per million cells in the samples from the iliac crest and from the femoral head were evaluated. A mean of 21.9 ± 10.2 (range, 0 to 130) connective-tissue progenitor cells per one million nucleated cells (determined by CFU-F assay) were aspirated from the FH. This value was significantly lower than the mean of 37.4 ± 21.9 (range, 29 to 64) connective tissue progenitor cells per million nucleated cells that were aspirated from the iliac crest. The prevalence of connective-tissue progenitor cells in the FH was a mean of 41% lower than that in the paired iliac crest samples ($p = 0.04$).

Concentration of MSCs per mL of bone marrow: The concentration of osteogenic connective-tissue progenitor cells (MSCs) was calculated for each sample as the product of the nucleated cell count and the prevalence of osteogenic connective-tissue progenitor cells. In the femoral head autograft the number of MSCs per 1.0 mL of bone marrow aspirates averaged 257 ± 116 MSCs/mL. This was significantly ($p < 0.05$) lower than the number of MSCs in the paired iliac crest (average 901 ± 324 MSCs/mL). There was a relationship ($p < 0.05$) between the number of MSCs obtained by bone marrow aspiration in the iliac crest and in the femoral head. On average, the concentration of osteogenic connective-tissue progenitor cells in the IC aspirates was 350% higher than that in the paired FH samples ($p = 0.01$).

In only one patient was the concentration of connective-tissue progenitor cells in the iliac crest aspirates lower than that in the FH aspirates; however, in that patient both the iliac crest and FH progenitor cell numbers exceeded the iliac crest values of the other patients. One other patient demonstrated a severe deficit in connective-tissue progenitor cells within the FH marrow while showing average numbers equivalent to other patients in IC marrow.

With concentrated bone marrow from the iliac crest, the femoral head autograft could be charged with MSCs to the same levels as the iliac crest in the operating room

In absence of bone marrow concentration, a femoral head allograft could not be charged with the same number of MSCs as the number present in an autograft: The mean volume of the femoral heads was 56.1 ± 14.2 mL. The difference in the total number of MSCs in the femoral head as compared to those present in the IC was on average 36 128 MSCs ($(901 \text{ MSCs/mL} - 257 \text{ MSCs/mL}) \times 56.1 \text{ mL}$). The average volume of bone marrow concentrate that can be injected into femoral heads is 5.1 ± 1.47 mL. Therefore, increasing the level of MSCs in the femoral head to the same level as in the iliac crest requires injection of 5 mL of bone marrow concentrate containing 7225 MSCs/mL ($36\ 128 / 5$), which usually can be obtained only with concentrated bone marrow.

With concentrated bone marrow, the femoral head could be charged with a similar or higher number of MSCs than the number present in the iliac crest: When the bone marrow of the IC is concentrated, 10 times the number of MSCs can be injected. By filling the femoral head with 5 mL of concentrated bone marrow 45 050 MSCs (average $901 \pm 324 \text{ MSCs/mL} \times 5 \text{ mL} \times 10$) can be injected which is more than the difference of cell numbers between the FH and IC.

DISCUSSION

The purpose of this investigation was to compare the concentration of MSCs in the femoral head and in the iliac crest in patients with DDH. This study also compared the osteogenic capacity potential of FH graft with that of IC graft. Characteristics of FH bone marrow have not been well quantified although it has previously been considered to be a common source [13-16] of augmenting bone graft material for THA in DDH. Our study confirms the presence of MSCs in the femoral head of patients with

osteoarthritis. However, this study shows that the FH bone marrow contains a lower number of mononuclear cells on average, and lower concentration of MSCs as compared with the number of MSCs present in the iliac crest of the same patient. This lower number may explain the failure of femoral head auto grafts in some circumstances. Based on their experience, Mulroy and Harris [17] described poor experience with bulk autograft fabricated from the femoral head for dysplastic hips describing a high rate of postoperative resorption and secondary collapse of the bone graft. However, considering future potential loosening of the acetabular sockets in association with periprosthetic bone loss, it is necessary in these patients to protect and increase bone stock. To avoid such risks in revision THA, restoration of the acetabular bone defect in primary THA for the dysplastic hip is necessary.

Many clinical papers showed the usefulness of MSC transplantation in terms of its osteogenic potential. The paracrine effect of MSCs is also well described; they secrete factors to promote angiogenesis [17,18] as well as various other growth factors and bioactive molecules. They stimulate cell differentiation, modulate extracellular matrix, and promote bone formation and neovascularization. The low number of MSCs in the femoral head may warrant increasing their number by injecting bone marrow concentrate from the iliac crest in autografts as performed in allografts [11,19] in another study.

Graft resorption in hip revision with or without cement [18,20-24] is probably due to different mechanical factors but also biological factors. The mechanical factors and the revascularization process have been extensively discussed in the literature. After an initial phase of necrosis related to a non-specific inflammatory response, revascularization of the graft occurs. This process is accompanied by osteoclastic resorption of bone. In this respect, a transient but prolonged increase in porosity and a decrease in mechanical strength of a graft is a normal and inevitable consequence of the incorporation process. During the incorporation and remodeling process, the graft should be partially unloaded and protected from mechanical overstress. Careful pathologic assessments of retrieved grafts in humans have shown that the primary mode of union is via external host-derived callus which bridges the host-graft interface and leads to cortical -cortical and cancellous -cancellous bone union [25,26,11,19]. There is a gradual process of creeping replacement which works inward from the host-graft junction toward the middle of the graft at a gradual rate [27-30].

Our study has several limitations. First, it is a purely biologic study and we have no clinical data in the long term to be certain that loading an autograft with MSCs will improve the results. However, our experience with allograft has demonstrated the long term benefits of MSCs injection [15,16], and we propose the same benefits for autografts. Second, we have not assessed the rate or extent of incorporation of reparative tissue into grafted bone and the metabolic condition of the bone in and around the autograft as we have done for allografts loaded with MSCs.

In conclusion, FH bone marrow could contain lower osteoblastic differentiation potential compared with IC, thus effectively providing a lower quality graft augmentation during THA for DDH. The potential benefit of these findings would be to recommend bone marrow aspiration from the ipsilateral IC and concentration the bone marrow to provide an additional source of MSCs for patients requiring hip grafting with FH autograft. The aspiration from the iliac crest, the concentration and the injection can be performed during hip surgery.

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