Bone-marrow-derived mononuclear cells with a porous hydroxyapatite scaffold for the treatment of osteonecrosis of the femoral head

A PRELIMINARY STUDY

We have investigated the effectiveness of the transplantation of bone-marrow-derived mononuclear cells (BMMNCs) with interconnected porous calcium hydroxyapatite (IP-CHA) on early bone repair for osteonecrosis of the femoral head. We studied 22 patients (30 hips) who had osteonecrosis with a minimum follow-up of one year after implantation of BMMNCs. The mean age at surgery was 41 years (18 to 64) and the mean period of follow-up was 29 months (19 to 48). In a control group, cell-free IP-CHA was implanted into a further eight patients (9 hips) with osteonecrosis of the femoral head and the outcomes were compared.

A reduction in the size of the osteonecrotic lesion was observed subsequent to hypertrophy of the bone in the transition zone in the BMMNC group. In three patients in the treatment group progression to extensive collapse was detected. In the control group subtle bone hypertrophy was observed, but severe collapse of the femoral head occurred in six of eight hips.

In this limited study the implantation of BMMNCs and IP-CHA appears to confer benefit in the repair of osteonecrosis and in the prevention of collapse.

Idiopathic osteonecrosis of the femoral head may develop bilaterally in the young patient and joint-preserving procedures such as femoral osteotomy and vascularised bone grafting are appropriate treatment. However, the long-term results of these measures have been variable. Bone-marrow-derived mononuclear cells (BMMNCs) are thought to be one of the most advantageous cell sources for promoting both angiogenesis and osteogenesis. We have previously described the efficacy of the implantation of autologous BMMNCs which was undertaken simultaneously with osteotomy of the contra-lateral hip for osteonecrosis in two patients with bilateral disease. It appears that BMMNC implantation has the potential to accelerate bone repair subsequent to angiogenesis in the presence of osteonecrosis and to avoid the requirement for osteotomy or hip replacement. In this study we have evaluated the short-term results of the transplantation of BMMNCs for bone repair at the site of osteonecrosis of the femoral head.

Patients and Methods
We reviewed retrospectively 22 patients (30 hips) at a minimum follow-up of one year after transplantation of BMMNCs for osteonecrosis (treatment group). There were eight women and 14 men with a mean age at surgery of 41 years (18 to 64). In 14 patients (22 hips) the aetiology was considered to be corticosteroid-induced osteonecrosis, in six (6 hips) alcohol abuse and in two (2 hips) it was idiopathic. The radiological stages and types of osteonecrosis were classified according to the criteria of the Japanese Orthopaedic Association. They are stage 1, no specific findings of osteonecrosis on routine radiography; stage 2, demarcating sclerosis is observed without collapse; stage 3, collapse including crescent sign without joint-space narrowing and stage 4, osteoarthritic changes. Pre-operatively, two hips were classified as stage 1, 25 as stage 2, and three as stage 3A. There was further classification pre-operatively of two hips as type B, 13 as type C-1 and 15 as type C-2. The mean pre-operative volume of osteonecrosis within the femoral head measured by the method of Steinberg, Hayken and Steinberg was 21% (3% to 36%).

The mean follow-up was for 29 months (19 to 45). The contralateral hips were simultaneously treated by transtrochanteric rotational osteotomy in nine, hip replacement in two, intertrochanteric varus osteotomy in one and...
vascularised iliac bone graft in one. One contralateral hip had no treatment.

**Isolation of BMMNCs.** At the beginning of the procedure 700 ml of bone-marrow aspirates were obtained from the iliac crest using a 2 mm needle connected to a 10 ml syringe containing 1 ml of heparinised saline. The aspirates were filtered through 500 μm and 200 μm filters and centrifugal separation was performed with a cell separator (COBE Spectra; Gambro, Tokyo, Japan) to obtain a sample of BMMNCs of approximately 40 ml containing approximately 1 × 10⁹ cells.

**Preparation for BMMNCs grafting.** We used interconnected porous calcium hydroxyapatite (IP-CHA) (Neobone; MMT, Osaka, Japan) as a scaffold, the effect of which has been previously reported.¹⁴ This material is cylindrical in shape and has an interconnected porous pattern with a porous ratio of 75%, a pore size of 150 μm and an interconnected ratio > 90%. This is suitable for a population of BMMNCs which were seeded into the IP-CHA at the time of surgery (Fig. 1a).

**Operative procedure of implantation of BMMNCs.** The treatment was approved by the ethical committee of the hospital and informed consent was obtained from the patients. Under combined spinal and epidural anaesthesia, initially, conventional surgery such as a joint-preserving procedure or total hip replacement (THR) was performed as required on the contralateral hip according to the pre-operative stage and type of osteonecrosis. During this intervention, the harvested BMMNCs were separated by centrifugation in the operating theatre. Subsequently, a 5 cm longitudinal incision was made distally from the greater trochanter which was intended for the transplantation of BMMNCs. The fascia was incised longitudinally and the lateral aspect of the femoral shaft was exposed. Under fluoroscopic control, two guide wires were inserted into the subchondral bone at the location of the osteonecrosis. Holes were then drilled with a 6 mm to 10 mm burr over the guide wire. The size of the IP-CHA cylinder was chosen so that its diameter was 1 mm less than that of the drilled hole. Cell-seeded IP-CHA was then inserted through this aperture (Figs 1b and 1c) and soft-tissue closure was performed in a routine manner. Partial weight-bearing to the cell-seeded hip was allowed after one week with full weight-bearing at three weeks.

As a control group, eight patients (9 hips) were retrospectively reviewed after implantation of cell-free IP-CHA into the site of osteonecrosis following the method described previously. There was one woman and seven men with a mean age at surgery of 49 years (28 to 73). In these patients it was considered that the osteonecrosis was due to corticosteroids in two (2 hips), alcohol abuse in three (4 hips) and to be idiopathic in three (3 hips). All these hips were classified as pre-operative stage 2. One hip was classified as type C-1 and eight as type C-2. The mean pre-operative volume of osteonecrosis was 22% (15% to 55%). The mean follow-up was for 31 months (22 to 51). In the control group the contralateral hips had been treated simultaneously by trochanteric rotational osteotomy in one hip, THR in one hip and a vascularised iliac bone graft in one.

The radiological findings of each group were statistically compared using the Mann-Whitney U test. A value of p < 0.01 was considered significant. The clinical scores using the rating system of Merle d’Aubigné and Postel¹⁵ were evaluated pre-operatively and at the last follow-up in each group. Radiological findings such as the progression of the collapse, the presence of hypertrophy or resorption were evaluated using anteroposterior and frog-leg lateral views. The post-operative volume of osteonecrosis was measured at intervals of six months after surgery. Gadolinium-enhanced MRI was also performed to detect bone repair and re-vascularisation of the osteonecrosis at six and 12 months after surgery.
In order to test the reproducibility of the radiological evaluation, three co-authors (YY, MI, TH) assessed the radiological findings in five randomly selected hips. Each observer measured each hip three times, with an interval of one week between measurements and the mean value was calculated. The data were analysed for intra- and inter-observer variance, and the coefficient of variation was calculated to be less than 5%.

Results

Clinical evaluation. The mean clinical score increased from 14.7 points (13 to 16) pre-operatively to 17.0 points (15 to 18) at the last follow-up in the treatment group except in one hip which required THR at seven months after BMMNC transplantation because of the early progression of collapse. The mean pain score improved from 4.2 points to 5.5 points. In the control group, the mean score reduced from 15.2 points (14 to 17) pre-operatively to 14.2 points (12 to 15) at the last follow-up, and THR was needed in three hips. No intra- or post-operative complications were observed in either group.

Radiological evaluation. In the treatment group, no progression of collapse was observed in 17 hips (56.7%). Mild collapse of less than 2 mm was seen in ten (33.3%) which occurred within one year of surgery and was not progressive. In these 27 hips (90.0%), no or only subtle irregularity was detected at the main load-bearing area of the femoral head, and the post-operative clinical courses were considered to be satisfactory on the basis of the clinical score system. Collapse of more than 2 mm was observed in three hips (10%) within six months after surgery, and one hip required THR at seven months after the initial procedure. In the control group all nine hips had some degree of collapse. Progression of collapse of more than 2 mm was seen in six hips and THR was required in three within two years of surgery.

In the treatment group, at three to six months after surgery, bone hypertrophy in the transition zone progressed in 28 of the 30 hips and the size of the osteonecrotic defect gradually decreased (Fig. 2). The mean volume of osteonecrosis decreased to 12% (1.5% to 24%) at six months, 9.0% (0.86% to 22%) at 12 months and 7.7% (0.61% to 16%) at 18 months in hips with no or only mild collapse (Fig. 3). In the control group, subtle bone hypertrophy was detected between 12 and 18 months after surgery. However, the initial observation of this radiological change was noted in the already collapsed femoral head.

In the treatment group, partial bone resorption at the site of osteonecrosis was observed post-operatively in 14 hips.
on plain radiography or CT. Bone resorption was detected from two to six months after surgery and gradually decreased after 12 months (Fig. 4). In the control group, such early bone resorption was not observed in any cases.

Post-operative MRI detected enhancement around the IP-CHA and transition zone at six months in 20 of the 30 hips (Fig. 5), and partial enhancement of the osteonecrosis adjacent to the transition zone at 12 months in 17.

**Discussion**

Surgical intervention is often recommended in the treatment of osteonecrosis of the femoral head and mainly involves THR or joint-preserving surgery. Core decompression is one of the least invasive procedures which may be undertaken, but the results are open to debate since they have not been evaluated according to the location or the volume of osteonecrosis. Free vascularised bone grafting has been reported to show favourable longer term results for early-stage small osteonecrotic lesions, although possible morbidity at the donor site should not be overlooked. Transplantation of mesenchymal cells with beta-tricalcium phosphate ceramics has been combined with this procedure in an attempt to improve the post-operative results.

BMMNCs have considerable potential for angiogenesis due to the presence of endothelial precursor cells in the fraction. Additionally, this fraction could contain osteogenic progenitor cells. It has been reported that BMMNCs may have a role in the production of osteoblasts, and the presence of mesenchymal stem cells or endothelial precursor cells in BMMNCs may release angiogenic factors. In a rabbit model we have investigated the efficacy of BMMNCs for capillary formation at osteonecrotic sites, and have evaluated the quality of the fraction by flow cytometric analysis. CD34-positive cells, which include haematopoietic and endothelial precursor cells, were detected more commonly in the BMMNC fraction than in the total bone marrow or peripheral blood.

We consider that it is important to use IP-CHA as a scaffold for BMMNCs and to graft the cell-seeded IP-CHA at the anterolateral edge of the lesion of osteonecrosis in order to prevent early progression of the collapse of the femoral head. The effectiveness of our procedure should be apparent from 12 to 18 months after surgery. Not only cell transplantation, but also cell distribution, are thought to be important to avoid the progression of collapse. Our current results reveal that bone repair at the lateral side of the femoral head, including the transition zone and subchondral bone, progresses after treatment with BMMNCs. It is estimated that revascularisation along the transition zone is acceler-
We have described the apparent effectiveness of implantation of BMMNCs with IP-CHA in the treatment of patients with osteonecrosis of the femoral head in the short term. Longer term follow-up on larger numbers of patients will be required before this method can be introduced into general orthopaedic practice.

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References


