THE EVALUATION OF A MESENCHYMAL STEM CELL–HYDROXYAPATITE COMPOSITE GRAFT FOR POSTERIOR SPINAL FUSION

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Introduction

Posterior spinal fusion is performed to treat a variety of spine related conditions. Autologous bone is the gold standard graft, but donor site complications are common and supply is limited. Bone marrow derived mesenchymal stem cells (MSC’s) have the ability to differentiate into a variety of mesenchymal tissues including bone. The objective of this study was to use a hydroxyapatite - MSC (HA-MSC) composite graft material for posterior spinal fusion in a rabbit model to achieve spinal fusion rates and histological graft integration equivalent to that obtained with the use of autologous iliac crest graft.

Materials and methods

The study was performed in accordance with the Home Office Animals (Scientific Procedures Act) 1986 (UK). The HA-MSC composite graft was prepared by aspiration of bone marrow from the iliac crest of rabbits prior to spinal fusion. The nucleated bone marrow cells were cultured in medium comprising Dulbecco’s modified Eagle’s medium (Sigma-Aldrich), 10% bovine foetal calf serum and 1% penicillin / streptomycin (Sigma Aldrich). After reaching confluence colonies of MSC’s which were adherent to the culture flask were detached using trypsin. 3x10^3 stem cells were seeded onto 5 grams of HA granules which were cultured for a further 7 days prior to implantation.

Bilateral posterior L4-L5 interlamina spinal fusion was performed using an HA-MSC composite graft (4 Rabbits), hydroxyapatite granules (6 rabbits) or autologous bone graft obtained from the iliac crest (6 rabbits).

Rabbits were sacrificed at 5 weeks. Fusion was assessed by manual palpation of the specimens by 2 independent observers. There was agreement between observers for all specimens.

Specimens were processed for non decalcifying histology. Quantitative histological analysis of cartilage, fibrous tissue and bone in the mid portion of the graft was performed using image analysis software (Zeiss KS300). Comparisons of fibrous tissue and bone formation between groups was analysed using a one way analysis of variance with the Games Howel post hoc test. Comparison of cartilage formation was made using the Mann Whitney U test and fusion rates were compared using the Fisher’s exact test.

Results

Three of four of the HA-MSC grafts fused successfully compared to 5 of 6 of the autologous bone grafts and 0 of 6 of the HA control grafts. The fusion rate was significantly higher in the iliac crest and HA-MSC groups than the HA control group (p<0.05).

In both the HA control and HA stem cell composite grafts there was ingrowth of new bone and encasement of HA granules by new trabecular bone at the graft – host interface.

Within the mid region of the grafts there was bone formation in 2 of 4 fusion masses in the HA-MSC group comprising 26% and 45% of tissue in the area examined (Fig. 1). In contrast bone formation was seen in the centre of one of six HA fusion masses and amounted to only 2% of tissue.

In the autologous bone graft groups intramembranous bone formation with the deposition of osteoid seams on trabecular bone was the dominant mechanism of bone formation. In contrast to successful fusions, bone graft fragments in the graft that did not unite were surrounded by dense fibrous tissue. Comparing HA-MSC composite and the HA control groups, there was no significant difference in average percentage area of new bone, cartilage or fibrous tissue within the central region of the grafts. There was a higher mean percentage area of new bone formation within the autologous bone graft (27%) than the HA control group (0.3%), p<0.02 games howel post hoc test (Fig. 2).

More fibrous tissue was seen in the HA graft than autologous bone graft (p<0.005). 89 % of tissue in the centre of the HA graft was fibrous compared to 14 % in the autologous bone graft group.

Discussion

In this study we have assessed the efficacy of an HA-MSC composite graft material for spinal fusions and compared results with autologous bone graft and hydroxyapatite. With respect to achieving fusion we found the MSC–HA composite to be as effective as autologous graft and superior to hydroxyapatite. Hydroxyapatite when used alone was ineffective for posterior spinal fusion.

On histological analysis a positive finding to support the osteogenic potential of the stem cell loaded hydroxyapatite granules was the presence of moderate amounts of enchondral new bone aligned within the central regions of the graft away from the graft host interface in 2 of 4 fusion masses. In contrast the HA control grafts only supported significant amounts of bone formation at the pheriphery of the graft, adjacent to the host bed. This would suggest that the HA granules do not have significant inherent osteogenic capacity. When interpreting the results of this study factors which may influence outcomes should be considered. These include the effects of motion at the fusion bed, variability in the preparation of spinal fusion bed and the relatively small number of animals in the HA-MSC group.

Reference