

The Impact of ABCB5+ Mesenchymal Stem Cells on the Osteogenic Potential of Human Bone Marrow Mesenchymal Stem cells (MSCs)

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Background

For the treatment of segmental long-bone defects and non-unions, the diamond concept is a well-established treatment algorithm and reflects the global gold standard due to its good clinical results. One of its crucial pillars is the use of autologous osteogenic cells (Bone marrow mesenchymal stem cells BM-MSCs), harvested from the iliac crest or the medullary cavity of long-bones (Reaming Irrigation Aspiration - RIA) while these reservoirs are limited, the harvest is associated with a significant donor site morbidity and the cells can be of poor quality. Hence, an autologous mesenchymal stem cell source with a high osteo-regenerative potential would be ideal as it would be unlimited available and has a no donor site morbidity. ABCB5+ cells are a new discovered skin-derived mesenchymal stem cells which are capable of osteogenic differentiation and beyond that also exhibit strong immunomodulatory and anti-inflammatory properties to support bone regeneration. Here, we present for the first time results from the in-vitro evaluation of the osteo-regenerative potential using ABCB5+ MSCs as a single-cell source and in a co-culture setting with BM-MSCs.

Methods

To evaluate the osteogenic potential of ABCB5+ MSCs alone as well as their influence on the osteogenic differentiation of BM-MSCs in a co-culture setting, Experiment A was performed: BM-MSCs from n=3 healthy human donors were co-cultured in 35mm petri dishes in monolayers with commercially available ABCB5+ stem cells using the following ratios (ABCB5+/BM-MSC [%]): 100/0, 90/10, 50/50, 0/100. The total cell number was always 80,000 per petri dish. The cells were cultured for 3 weeks in two groups. Group 1 Osteogenic differentiation (OM): DMEM low-glucose medium either containing the supplements β -glycerol phosphate, L-ascorbic acid and dexamethasone and Group 2 Non-osteogenic control group (CTRL) not containing any osteogenic supplements but just the basal cell culture media (control groups, CTRL).

In parallel, Experiment B was performed just using the corresponding amounts of BM-MSCs from experiment A without any ABCB5+ MSCs to validate that the results seen in experiment A really came due to the presence of ABCB5+ cells: BM-MSCs (n=3) were seeded using the following number of cells: 8,000; 40,000; 80,000. Cell culture conditions were identical to experiment A.

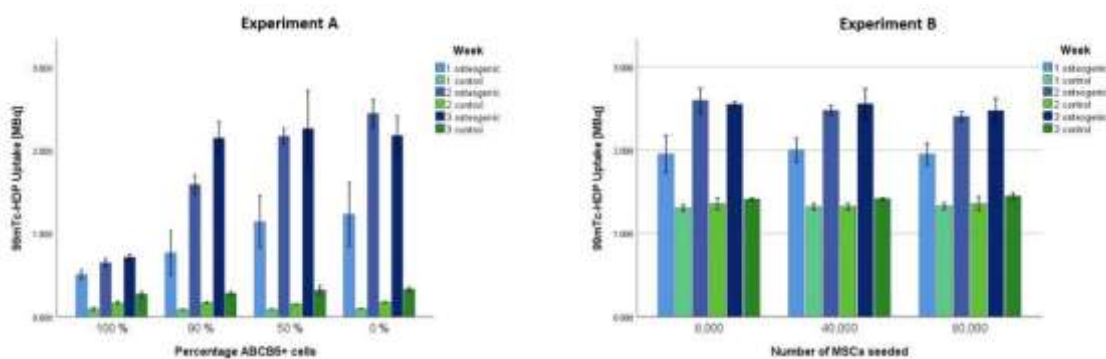
Both experiments were conducted in triplicates and after 1, 2 and 3 weeks the corresponding cell cultures were terminated to evaluate the osteogenic differentiation after these different timepoints. The petri dishes were then air dried and incubated for 30 min with 5 MBq of the radioactive tracer ^{99m}Tc-HDP which binds selectively to newly formed hydroxyapatite. This uptake can be quantified using an activimeter which counts the amount of bound radioactivity to the formed hydroxyapatite layer and therefore reflects the osteogenic potential in vitro. After incubation, the dishes were washed and the remaining activity ("uptake") was measured using such an activimeter. Statistical analysis was performed using one-factorial ANOVA ($\alpha=0.05$).

Results

Experiment A: All osteogenic groups showed a significantly higher uptake of the tracer compared to their corresponding control groups at all three timepoints (1,2,3 weeks) while this uptake increased over time for the ratios 100 % ABCB5+ (week 1: 0.51; week 3: 0.71 MBq) and 90 % (0.77 and 2.15 MBq) but reached a steady state after 2 weeks for 50 % (week 2: 2.17; week 3: 2.26 MBq) and 0 % (2.44 and 2.18 MBq). These increases were statistically significant for 90 % ABCB5+ for all weeks and 50 % ABCB5+ as well as 0 % for the increase between week 1 and 2. Furthermore, in week 1 there was no significant difference between the different cell ratios, whereas in week 2 100 % ABCB5+ (0.65 MBq) and 90 % (1.59 MBq) were significantly different from each other and 50 % (2.17 MBq) and 0 % OM (2.44 MBq). In week 3, only 100 % ABCB5+ showed a significantly lower uptake (0.71 MBq), whereas the other three OM were very similar in uptake (around 2.2 MBq).

Experiment B: All osteogenic groups showed a significantly higher uptake than their corresponding control groups. For every number of cells seeded, the uptake increased significantly between week 1 and 2 and then stagnated. Within each week, there was no difference between the OM (e.g. week 3: 8,000 – 2.55 MBq; 40,000 – 2.56 MBq; 80,000 – 2.47 MBq).

The osteogenic groups of experiment B showed a slightly higher uptake than the osteogenic groups of experiment A after 1 and 2 weeks, whereas there could no significances be shown for the timepoint week 3. The control groups of experiment B showed always a significantly higher uptake than those of experiment A.



Discussion

Overall, it can be stated that up to 90 % of a BM-MSCs in a cell culture can be replaced by ABCB5+ MSCs without any negative effect on osteogenic potential after 3 weeks. Thus, allogenic ABCB5+ cells present themselves as a promising addition to autologous MSCs, as they create an optimal micro milieu for bone regeneration due to their anti-inflammatory effects, while a small amount of BM-MSCs is still needed for successful osteo-regeneration. The higher uptake of the OM compared to the control groups show a successful osteogenesis. Osteogenesis is generally slower under the presence of ABCB5+, however, after 3 weeks there is no significant difference between any groups as long as they contain MSCs. Compared to that, a cell culture containing only ABCB5+ cells cannot perform a sufficient osteogenesis. Generally, MSC seem to reach their maximum osteogenic potential quicker in the absence of ABCB5+ cells. This is not necessarily an effect of cell density, since it can be equally observed for 50 % and 0 % ABCB5+ co-cultures, where cell density is identical. The significantly higher uptake in the control groups of experiment B indicate, that ABCB5+ presence might also stop spontaneous differentiation of MSCs. However, this effect needs to be further examined since it could not be shown for the 0 % ABCB5+ co-culture.