

The effect of AdBMP-2 on ovine osteoblasts and BMSC including a comparison to human and murine cells

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INTRODUCTION: Osteoinductive cytokines like Bone Morphogenetic Protein-2 (BMP-2) are able to accelerate fracture healing by stimulating differentiation into the osteoblastic lineage. However the rapid proteolysis and the need for a carrier matrix are relevant disadvantages for clinical application of recombinant proteins. Gene transfer offers an attractive technique that allows in situ production of growth factors. Direct administration of adenoviral vector encoding hBMP-2 showed enhanced bone formation in small animal models, but failed to accelerate fracture healing using a sheep model.

Human BMP-2 enhances osteoblastic activity and differentiation of bone-marrow derived mesenchymal stem cells (BMSC) into osteoblasts in human, murine and lapine cells, but there is no data available for ovine osteoblasts and BMSC.

This study evaluates the effect of an adenoviral vector encoding hBMP-2 on ovine osteoblasts and BMSC including a comparison to human and murine cells with respect to osteoblastic differentiation.

METHODS: For primary culture of osteoblastic cells, trabecular bone was harvested from iliac crests of adult White Mountain Sheep, from the calvaria of Swiss Wistar rats and from human femur head (after approval by the Swiss Ethic Committee). The cells were isolated enzymatically. The BMSC were isolated from bone-marrow taken from ovine iliac crests or murine femora followed by Ficoll-gradient.

Cells were cultured to confluence and then subcultured in 24-well plates, 30000 cells per well. After 24h, cells were transfected with a recombinant, first generation type 5 adenoviral vector encoding human BMP-2 (AdBMP-2) using a concentration of 10^7 to 10^8 particles per well (50 pfu/cell). For control, cells were treated with recombinant BMP-2 (50ng/ml), were transfected with an empty vector or left untreated. After 3days an osteogenic medium containing 0,1mM ascorbic acid and 1mM β -glycerophosphate was added.

For evaluation BMP-2 was measured in the media (ELISA, R&D), alkaline phosphatase activity (ALP) (Sigma) was determined at day 4, 8 and 14, mineralization was tested by incorporation of

radioactive calcium (Ca^{45}) at day 21. Total DNA was measured 4 and 14 days after transfection by fluorometric method to normalize the results. All analyses were performed in triplicates.

RESULTS: Four days after transfection with Ad.BMP-2 ovine osteoblasts and BMSC produce $138.4 \pm 106.8 \text{ ng/ml}$ and $65.2 \pm 2.5 \text{ ng/ml}$ BMP-2 respectively. After Ad.BMP-2 treatment the alkaline phosphatase activity increases over time with highest values at day 14 (osteoblasts: 6.1-127.8-fold, BMSC: 2.7-42.8-fold increase compare to control) and an increase of calcium incorporation (osteoblasts: 1.1-163.2-fold, BMSC: 0.9-6.4-fold). The total DNA content decreased after transfection (Ad.BMP-2 and empty vector), in the BMSC more than in the osteoblasts, representing a lower cell number. No significant changes in ALP and Ca^{45} were observed when cells were treated with empty vector or rhBMP-2. Large interindividual differences were found in all parameters. ALP did not correlate to age of donor or amount of BMP-2 in the media.

The human and murine cells showed the same tendency in ALP, calcium incorporation and DNA content. The amount of BMP-2 produced after transfection was lower in the murine and human cells compared to ovine cells.

DISCUSSION & CONCLUSIONS: The present study used an adenoviral gene transfer approach to show that human BMP-2 enhances the activity of ovine osteoblast and differentiation of ovine BMSC into the osteoblastic lineage. Decreased cell number after transfection might indicate vector toxicity. No effect was seen after treatment with recombinant BMP-2 albeit the added amount of BMP-2 was similar to the concentration seen after transfection. This might be due to higher biological effectiveness when a gene transfer approach is used. The comparison between the three different species did not show significant differences after treatment with Ad.BMP-2 or rhBMP-2, although the comparison is limited due to difficulties to culture cells identically. However, human BMP-2 is able to stimulate bone formation in species different to humans.