The Effects of rhPDGF-BB Alone and Combination with rhBMP-2 to Promote the Osteogenic Differentiation of Human Mesenchymal Stem Cells

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Introduction

There is a compelling need to develop an alternative to the autograft and allograft that matches the complex biological profile of signaling molecules of the osseous wound healing cascade.(1) Platelet-derived growth factor (PDGF) and bone morphogenetic protein (BMP) are pre-eminent biological cues in osseous wound healing. PDGF is a potent mitogen and chemoattractant promotes wound healing and bone formation in bone fractures.(2) BMP is an osteoblast differentiating factor with a rich history of clinical efficacy for bone healing.(3) Consequently, the dual delivery of recombinant molecules PDGF-BB and BMP-2 to a bone healing site would be consistent with the endogenous osseous wound healing cascade. However, prior to the design of the dual delivery composition, it is necessary to determine the cellular responses of temporal and sequential administration of specific doses and combinations of rhBMP-2 and rhPDGF-BB on proliferation and osteogenic differentiation of human mesenchymal stem cells (hMSCs).

Materials and Methods

Human mesenchymal stem cells (hMSCs, Lonza, Walkersville, MD) were used to determine cell responses to designated combinations of rhBMP-2 (100 ng/mL) and rhPDGF-BB (50 ng/mL) with specific timing and sequence of administration (Table 1). The hMSCs were plated at a density of 20,000 cells/well in each well of 24-well tissue culture plates. After 24 h of incubation at 37 °C, the media in each well was replaced with hMSC basal media supplemented with 100 nM dexamethasone, 50 µg/mL ascorbic acid and 10 mM β-glycerophosphate and the cells were cultured up to 21 days in a 95% humidified atmosphere containing 5% CO_2. In vitro outcomes were determined by measuring cell proliferation, alkaline phosphatase (ALP) activity, calcium content as well as von Kossa staining. All assays were done in quartets; data were reported as means +/- standard deviations and were analyzed by analysis of variance and *post hoc* orthogonal contrasts with $p \leq 0.05$.

Results and Discussion

There was a quantitative increase in the number of hMSCs following administration of rhPDGF-BB up to 14 days of culture. However, it is noteworthy that rhPDGF-BB alone promoted ALP activity as compared to the cells without rhPDGF-BB. In combination with rhBMP-2, sequential administration of rhPDGF-BB and rhBMP-2 in groups D and E significantly increased ALP activity as well as extracellular calcium deposition as compared either to rhBMP-2 (group A) or rhPDGF-BB alone (group B) or rhBMP-2 and rhPDGF-BB together (group C).

Table 1. Treatment groups for combination of rhBMP-2 and rhPDGF-BB

Groups	rhBMP-2		rhPDGF-BB	
	Days 1-7	Days 7-21	Days 1-7	Days 7-21
A	+	+	-	-
B	-	-	+	+
C	+	+	+	+
D	+	+	+	-
E	-	+	+	-
F	-	+	-	+
G	-	-	+	-
H	-	+	-	-
I	-	-	-	-

Notes: + and - denotes either presence or absence of PDGF and BMP.

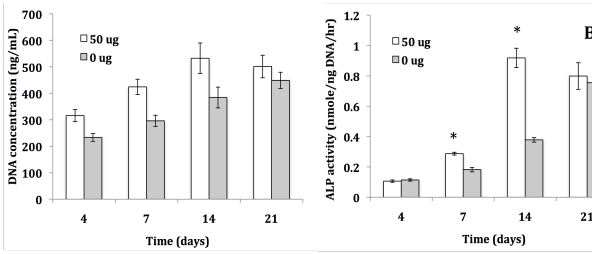

Figure 1. (A) DNA concentration and (B) ALP activity of hMSC in the presence of exogenous rhPDGF-BB up to 21 days. All data are reported as the mean ± standard deviation for n=4. * represents significant difference from the control (absence of rhPDGF-BB) ($p \leq 0.05$). THE HISTOGRAM IS TOO SMALL

Conclusions

The data indicate that sequential administration of rhPDGF-BB and rhBMP-2 appeared to promote osteogenic differentiation and mineralization of hMSCs. Moreover, ALP activity and mineralization were significantly increased with rhPDGF-BB either alone or in combination with rhBMP-2.

References

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