Inhibition of the ROCK signalling pathway in mouse osteoblasts

I.Simfia[1], M.C.Voisin[1], C.Mullen[1], L.M. McNamara[1]
[1]Centre for Biomechanics Research, Biomedical Engineering, NUI Galway
i.simfia1@nuigalway.ie

Abstract

Drug treatments that target mechanotransduction proteins have gained interest as therapeutic targets for cancer, such as inhibitors of MMP and ROCK biochemical signalling. Although osteoporosis has been linked to alterations in mechanotransduction of bone cells, such approaches have never been investigated as therapies for osteoporosis. The objective of this research is to investigate the use of a pharmacological ROCK inhibitor to target the mechanobiological response of bone cells in osteoporotic bone.

1. Introduction

Osteoporosis reduces overall bone mass causing bone fragility. Recent studies have shown that bone tissue composition is altered at the microscopic level. These changes in bone composition might be explained by alterations in bone cell biology, in particular the mechanobiological responses [1]. Recent findings indicate that the small GTPase RhoA and its effector protein ROCKII regulate fluid-flow-induced osteogenic differentiation of murine mesenchymal stem cells [2]. Drug treatments that target mechanotransduction proteins have gained interest as therapeutic targets for cancer, such as inhibitors of MMP [3] and ROCK biochemical signalling [4, 5]. However, such approaches have never been investigated as therapies for osteoporosis. The objective of this research is to investigate the use of a pharmacological ROCK inhibitor to target the mechanobiological response of bone cells in osteoporotic bone to prevent bone loss.

2. Methods

Preliminary experiments have been conducted to examine the *in vitro* viability of mouse osteoblastic cells (MC3T3-E1) following exposure to Y27632, a pharmacological agent that inhibits the ROCK signalling pathway involved in the mechanobiological responses of bone cells [6]. MC3T3-E1 cells were seeded at a density of 10^4cells/ml and cultured *in vitro* in α-MEM (supplemented with 10% FBS and 2% Penicillin Streptomycin and 1% L glutamine 200mM) as follows: (1) cells in control media [7]; (2) cells were incubated in a Y27632 (Sigma; 10 µM), for exposure periods of 1hr (with recovery of 24hrs), 1 day and 4 days. After inhibition, cells were analyzed for their viability, DNA content and Alkaline Phosphatase (ALP) activity.

3. Results

The results exhibited an increase in DNA content post inhibition of the ROCK pathway using Y27632 after 1 hour of incubation. No significant difference was seen in ALP activity of the treated cells. There was a slight decrease in cell viability following 1 day of exposure. However there was no adverse affect on the viability or osteogenic differentiation of MC3T3-E1 cells following exposure for 4 days.

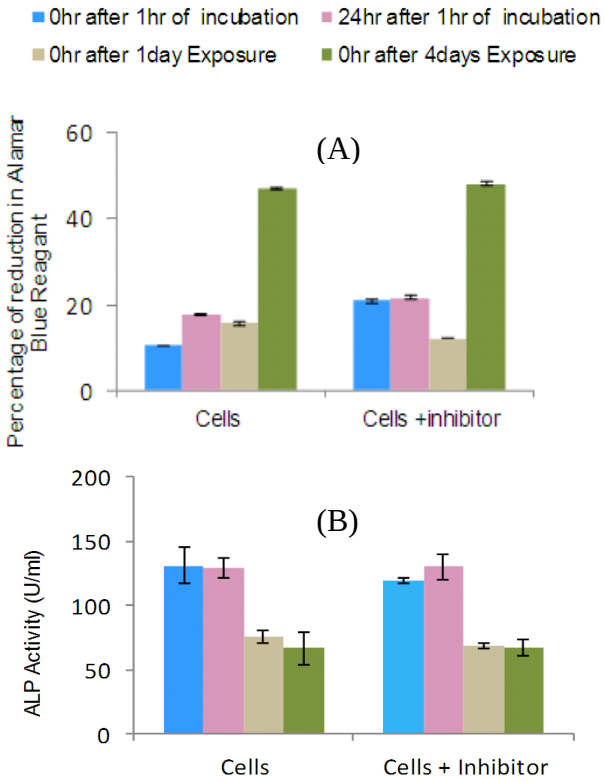

Figure1: Showing the effect on viability (A) and ALP activity (B) after treating the MC3T3-E1 with ROCK signalling pathway inhibitor, Y27632.

4. Discussion and Conclusions

The serum half-life of Y27632 is 12-16 hours [8, 9], and based on the findings of the 4 day exposure study, it is proposed that the cells completely recovered from the effects of the inhibitor once it was all degraded. Ongoing studies are investigating cell necrosis and apoptosis and matrix/mineral production with varying inhibitor concentrations. Future studies will investigate the inhibition of mechanoresponsiveness during estrogen deficiency using parallel plate flow chambers.

5. References

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