In vitro effects of G-Protein Coupled Receptor 120 agonist on osteoblast differentiation and activity in osteosarcoma cells

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Introduction

- Bone is metabolically active tissue that is continuously repaired by a process known as bone remodeling. Osteoclasts are responsible for the resorption (breakdown) of bone while osteoblasts form new bone. These cells work together to maintain the structure and integrity of bone. An imbalance between bone formation and resorption may lead to bone diseases. Osteoporosis is a bone disorder characterized by a decrease in bone mass and strength.
- The mitogen-activated protein kinase (MAPK) pathways play a role in osteoblast differentiation to regulate the activity of transcriptional factors such as runt-related transcription factor 2 (Runx2) responsible for osteoblast specific gene upregulation.
- G-protein coupled receptor 120 (GPR120) has gained attention as a target for the development of drugs for several human diseases because of its effects on inflammatory-associated diseases, however, the exact mechanisms of the processes are not fully understood.

The aim of the study was to investigate whether TUG-891, a GPR120 agonist, could regulate the differentiation and activity of osteoblasts through attenuating MAPK signalling pathways *in vitro* using MG-63 osteosarcoma cells.

Methods

- The effects of TUG-891 (0.01-100 µM) on cell viability in undifferentiated MG-63 cells containing osteogenic medium was tested using resazurin assay.
- The alkaline phosphatase (ALP) activity assay was used to measure the enzymatic activity of MG-63 cells exposed to TUG-891 (100 µM) for 7, 14 and 21 days of osteoblast differentiation.
- The ability of MG-63 cells to produce calcified extracellular matrix was tested using Alizarin Red S staining.
- Western Blotting was used to interrogate ERK and AKT signalling pathways.

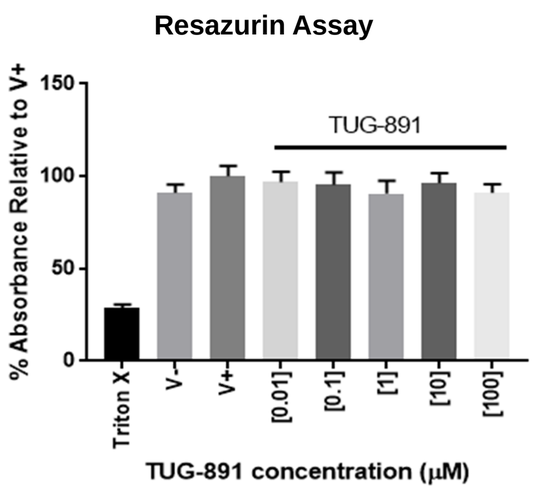

Figure 1: Effect of TUG-891 on cell viability in undifferentiated MG-63 cells. TUG-891 showed no significant changes in cell viability at any of the tested concentrations. Triton X-100 was used as control of cell death. V- = negative control with cells only. V+= positive control containing 0.1% DMSO.

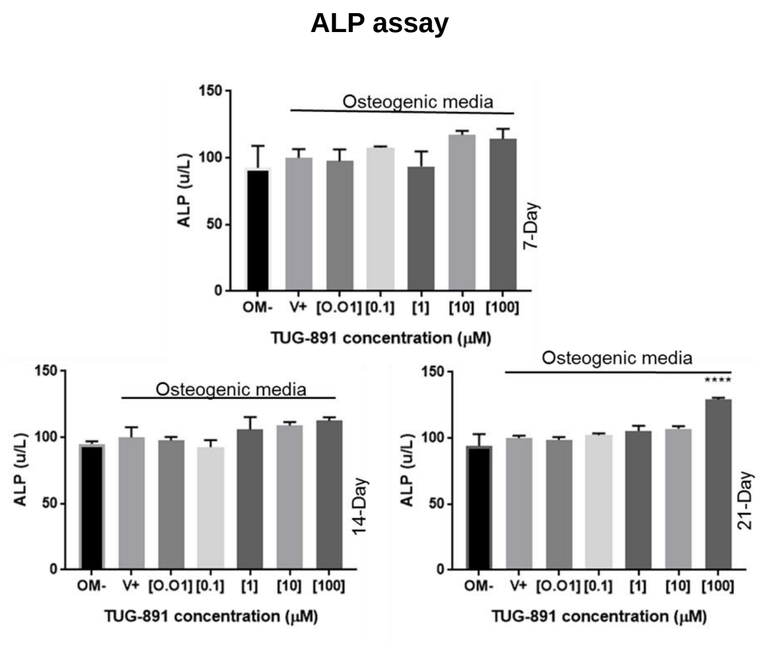

Figure 2: Effect of TUG-891 on osteoblast differentiation of MG-63 cells. The cells were seeded (10 000 cells per cm^2) and treated with OM and increasing concentrations of TUG-891 [0.01-100 µM] for 7-21 days. Thereafter, they were incubated in ALP buffer and ALP activity was measured. 100 µM TUG-891 increased ALP activity after 21 days (*** P<0.0001).

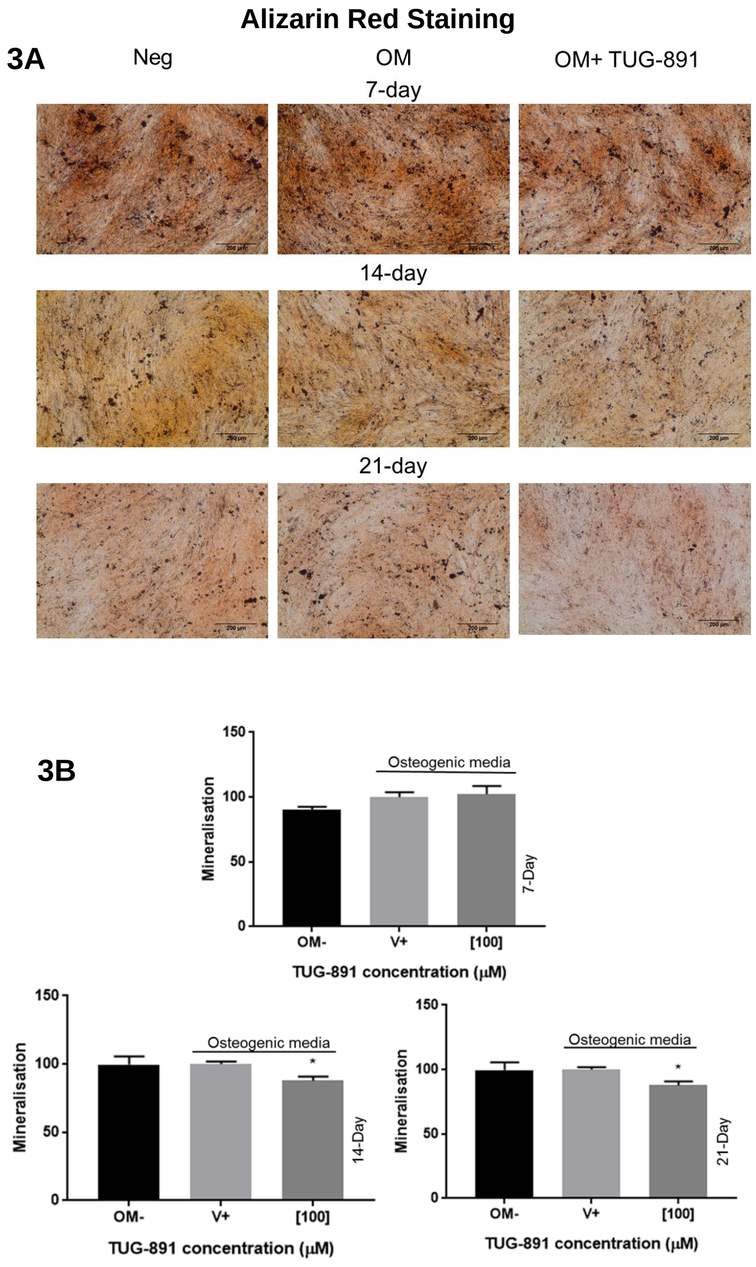

Figure 3: Effect of TUG-891 on osteoblast mineralisation in differentiated MG-63 cells. A. 10 000 MG-63 cells per cm^2 were seeded and exposed to osteogenic medium (OM) and TUG-891 (100 µM). At endpoint cells were stained with 2% (w/v) Alizarin red S and photographs were taken (scale bar: 200µm).
B. Alizarin red S stain was eluted and neutralised with 10% acetic acid and ammonium hydroxide, respectively. Mineralisation was measured relative to V+= cells containing osteogenic medium, at 405 nm wavelength and showed a significant decrease (100 µM) after 14 and 21 days *P < 0.05.

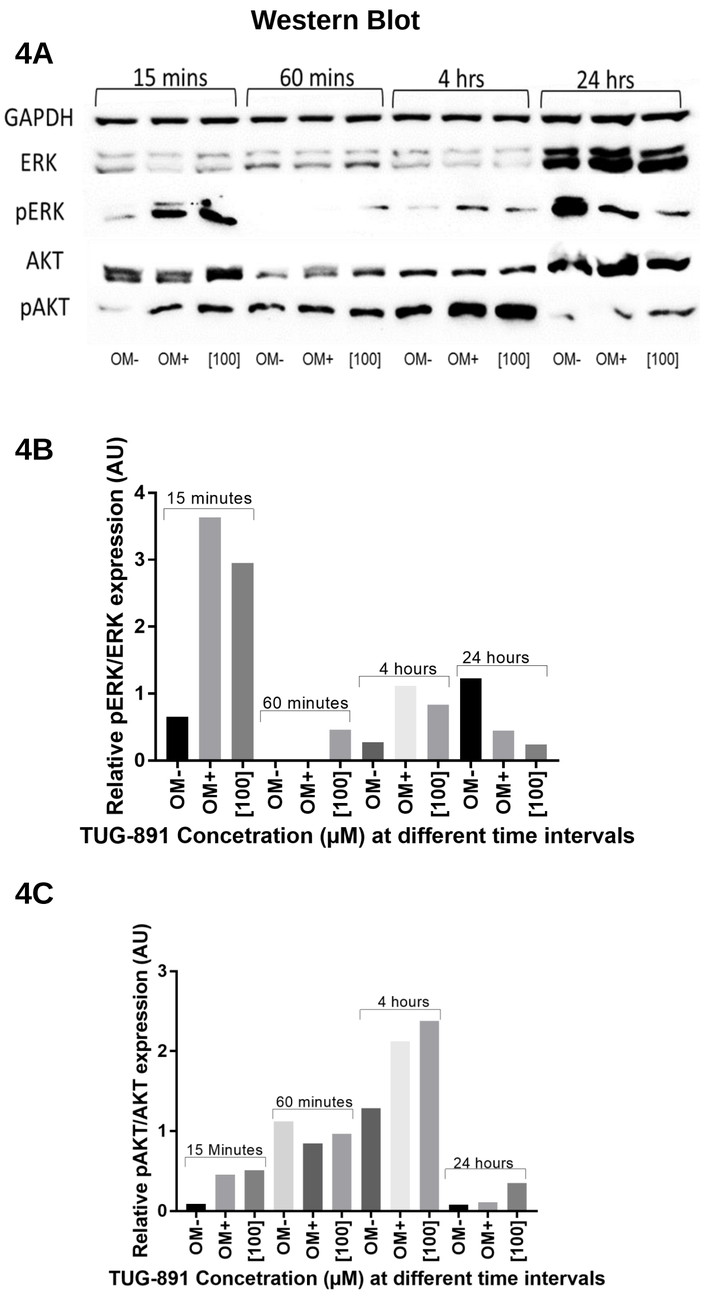

Figure 4: Effect of TUG-891 on pERK/ERK, pAKT/AKT expression. A. Protein bands for western blotting showing the expression of pERK/ERK and pAKT/AKT in MG-63 cells after exposure to TUG-891 (100 µM) at 15min, 60min, 4h and 24h.
B and C. Band densities were quantified using Image J software. Results showed that TUG-891 caused pERK/ERK expression to increase after 15 minutes, decrease after 60 minutes and thereafter, gradually increasing after 4 and 24 hours, pAKT/AKT continued to increase from 15 minutes to 4 hours then decreased after 24 hours.

Conclusion

- TUG-891 had no significant effect on cell viability at the tested concentrations when compared to the vehicle control.
- TUG-891 showed an increase in ALP activity after 21 days while on the other hand, alizarin red S staining showed a significant reduction in osteoblast mineralisation after 14 and 21 days of treatment when compared to the vehicle control, probably due to MG-63 cells not mineralising properly.
- TUG-891 showed phasic signalling of pERK/ERK and pAKT/AKT at different time points.
- This study suggests that GPR120 activation can increase ALP activity to increase differentiation of osteoblasts leading to bone synthesis.
- Future studies will evaluate how TUG-891 affects protein expression when GPR120 is silenced as well as osteoblastic gene expression to further illustrate the mechanisms of action of TUG-891 on osteoblast formation and activity.

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Acknowledgements: This study has no conflicts of interest.
The study was funded by the National Research Foundation of South Africa and Thuthuka Fund - Grantholder Dr. A.E Kasonga.

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