Sustained Delivery of Glycogen Synthase Kinase Inhibitor AR28 Is Critical for Osteogenic Selectivity in MSCs

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Introduction

Wnt/β-catenin signaling is critical to bone formation but decreased in myriad bone diseases and impaired healing of osseous injuries.[1,2] Agonists of Wnt signaling, such as glycogen synthase kinase-3 beta (GSK3β) inhibitors, have been shown to increase bone formation in both clinical and animal models.[3,4] However, careful control of dose and availability of GSK3β inhibitors is critical to realize osteogenic effects. Herein, the GSK3β inhibitor AR28 was investigated for doses and dosing regimens that achieve osteoprogenitor cell stimulation *in vitro*. To develop a drug delivery approach for identified AR28 doses and regimens, AR28 was loaded into nanoparticles (NPs) and released with control via degradable hydrogel depots and validated for osteogenic potential *in vitro*.

Materials and Methods

Osteoprogenitor cells (C3H10T1/2) were transfected with TOPflash/FOPflash plasmids and treated with AR28 for 24 h (0-10 μM). β-catenin activity was measured by luciferase and normalized by DNA quantification. Continuous treatment was achieved by treating cells with AR28 for 7 d, and doses that increased β-catenin activity were tested with human bone marrow-derived mesenchymal stem cells (MSCs). Singular doses (S-2 μM, S-10 μM) over 24 h and continuous doses (C-0.29 μM, C-1.43 μM) over 7 d were explored. Synthesized diblock copolymers of poly(styrene-alt-maleic anhydride)-block-poly(styrene) (PSMA-b-PS)[5-7] were self-assembled into ~51 ± 2 nm NPs and loaded with AR28 (loading capacity of 7.6% wt AR28/wt NP). To provide sustained release, AR28-NPs were photoentrapped within hydrolytically degradable hydrogels composed of poly(ethylene glycol)-poly(lactide)-dimethacrylate (PEG_{91}-PLA_1-DM) (10 wt%) Gel degradation and subsequent release of AR28-NPs were analyzed over time using mass loss and HPLC analyses, respectively. MSCs were treated with hydrogel-released AR28-NPs via a transwell system continuously for 7 days to approximate low-dose treatments of 0.29 μM based on drug loading and release kinetics. Osteogenic potential of AR28 treatments were evaluated using alkaline phosphatase (ALP) and alizarin red stains and gene expression. Data are expressed as mean ± standard deviation (n>6). Statistical differences were analyzed using 2-way ANOVA with Tukey's post hoc analysis ($p \leq 0.05$).

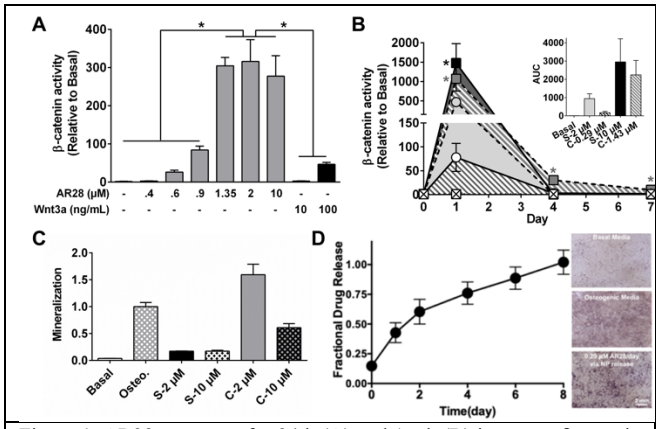

Figure 1. AR28 treatment for 24 h (A) and 1 wk (B) increases β-catenin activity and mineralization (C) in osteoprogenitor cells. These effects are mirrored by hydrogel depot NP-mediated AR28 release and effects on ALP expression (D).

Discussion of Results

AR28 induced potent β-catenin activation with maximal response 24 h post-treatment (Fig 1A). There were ~300-fold increases in β-catenin activity relative to untreated controls and ~60-fold increases in activity relative to Wnt3a positive controls. However, AR28 had no long-term effects, as there were no significant differences between treated and untreated cells after 48 h. Thus, continuous dosing was investigated, whereby sustained β-catenin activity of 3-fold and 16-fold increases after 4 days and 2-fold and 7-fold increases after 7 days were observed using C-0.29 μM and C-1.43 μM AR28 (Fig 1B). Quantification of mineralization by alizarin red stain identified a robust increase in osteogenesis, with C-0.29 μM AR28 inducing a ~1.5-fold increase in mineralization relative to positive controls (Fig 1C). In contrast, S-2 μM AR28 decreased mineralization, demonstrating the need for a drug delivery system to control AR28 release in bone. A28 was loaded into NPs and entrapped in degradable PEG hydrogels, which were designed to approximate zero-order release. Gels were used to continuously treat MSCs and showed similar osteogenic effects on MSCs, as evidenced by ALP staining (Fig. 1D).

Conclusions

It is necessary to stimulate osteoprogenitor cells in bone to augment bone formation to treat bone disease and injury. In particular, β-catenin agonists have great potential but require careful control over doses and exposure times to ensure reproducible therapeutic efficacy. This work explored the agonist AR28 for stimulating osteogenesis. Continuous dosing using hydrolytically degradable hydrogel depots that achieved pseudo zero-order release enhanced osteogenic markers, including ALP.

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