Characterization & Role of the β-Adrenergic System in Regulating Aortic Calcification

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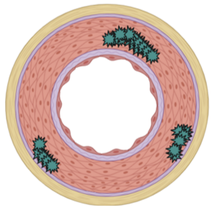
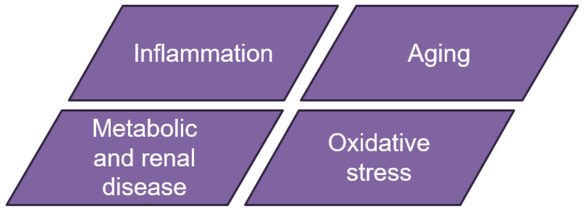
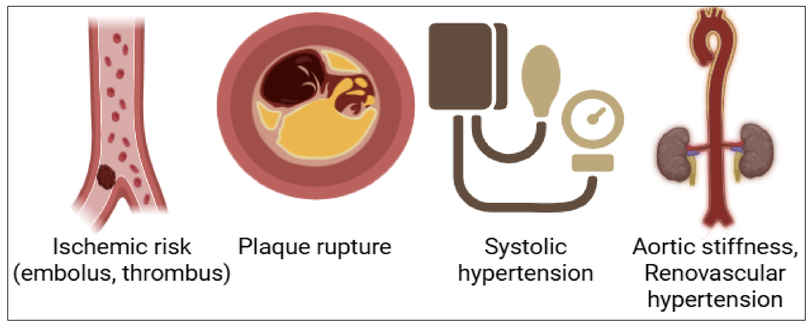
Introduction



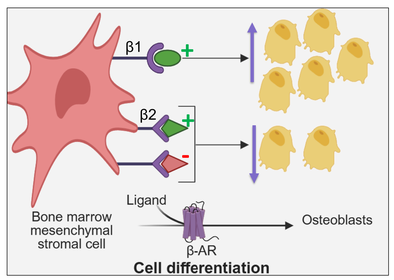
Calcium phosphate crystals (hydroxyapatite) in arterial wall



Aortic calcification is a pathological feature of systemic vascular disease[1].

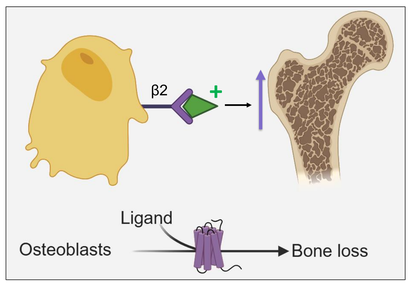


Calcification significantly contributes to cardiovascular morbidity and mortality[2].



β2-adrenergic receptor (β2-AR) signaling regulates bone remodeling through calcium deposition[3], but its role in vascular calcification, particularly in aortic smooth muscle cells (SMC)[4,5], is poorly understood.

We hypothesize that β2-AR activation will inhibit the osteogenic differentiation of human aortic SMCs and promote the maintenance of a contractile phenotype



Methods

HASMCs were cultured in growth media or osteogenic media (OST) for 21 days to induce calcification, with or without treatment using β-AR ligands (Table I). Calcification and phenotype were assessed through immunocytochemistry (ICC) staining for β1-ARs, β2-ARs, the osteogenic marker osteopontin (OPN), and the contractile marker alpha-smooth muscle actin (aSMA). Alkaline phosphatase (ALP) activity was measured with an ALP enzyme assay (Figure 1).

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Drug	Mechanism	Concentration	Vehicle
Isoproterenol	β1 & β2 agonist	1 uM	DMSO
Salmeterol	β2 agonist	1 uM	
CGP 20712 A	β1 antagonist	0.3 uM	
ICI 118,551	β2 inverse agonist	0.3 uM	

Table I: β-AR ligands used for cell treatment

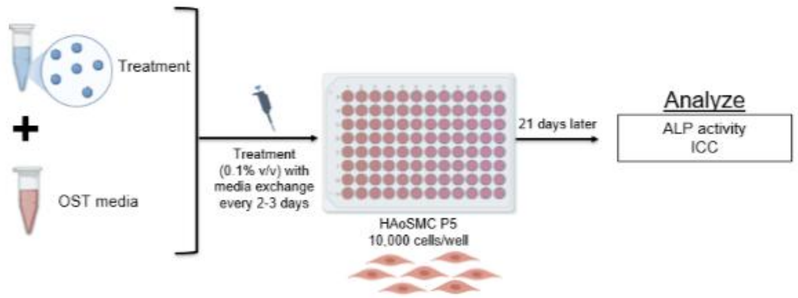

Figure 1: Experimental design

Results

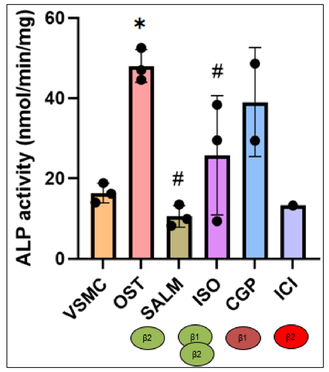

Figure 2: ALP activity after differentiation with treatments

OST-treated cells showed elevation of ALP activity compared with growth medium, confirming successful induction of calcification (Figure 2).
Salm and ISO each reduced ALP activity relative to the osteogenic control, demonstrating a protective effect of β-agonism (Figure 3).
CGP-20712A and ICI-118551 applied alone produced no change in ALP activity.

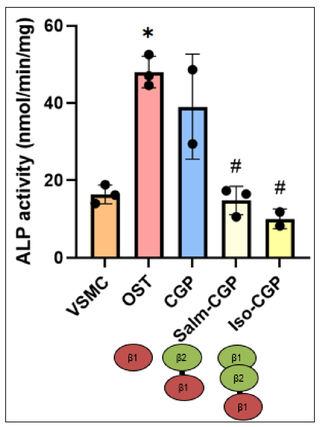

Figure 3: ALP activity after differentiation with co-treatments

Co-administration of CGP-20712A with either Salm or Iso did not diminish the agonist-mediated reduction in ALP(Figure 4), indicating that β1 blockade does not interfere with the anti-calcific response.

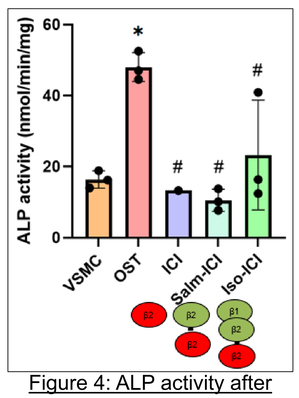

Figure 4: ALP activity after differentiation with co-treatments

Combining ICI-118551 with either agonist failed to restore ALP to osteogenic levels (Figure 4), suggesting that inverse agonism at β2 does not negate the protective signaling elicited by Salm or Iso under these conditions.

Results cont'd

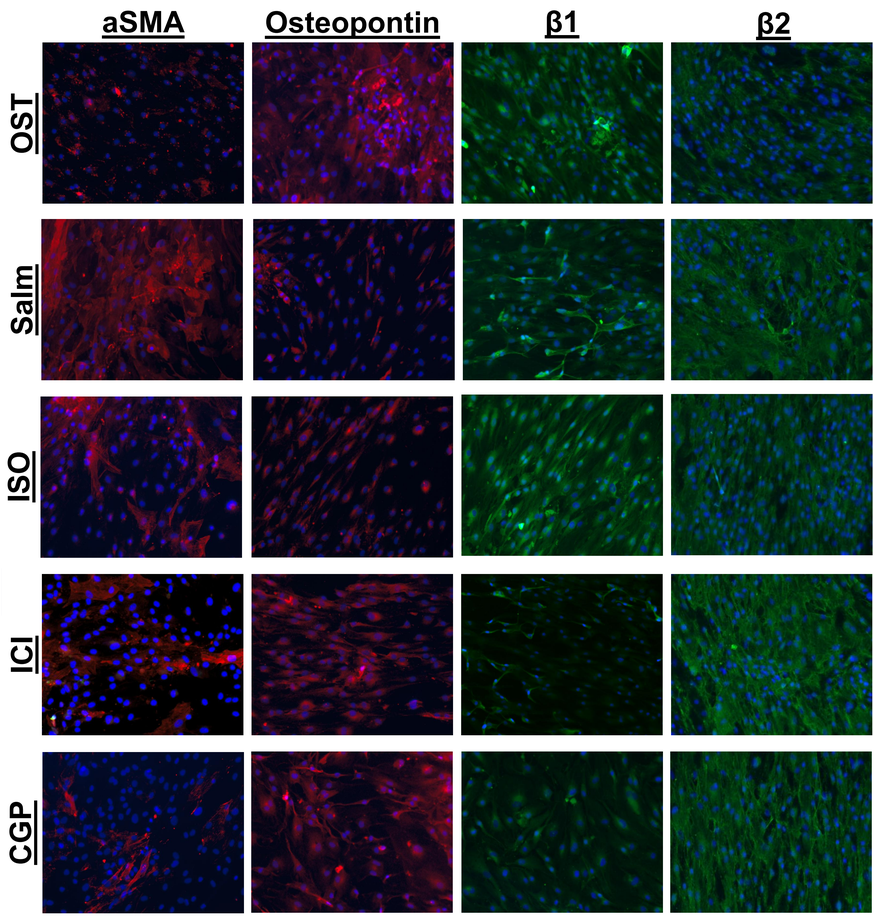

Figure 5: Immunocytochemistry of HASMCs after 21-day differenation in osteogenic (OST) media. Cells were cultured in OST media alone or with OST media and a treatment (row labels -- Salmeterol, Salm; Isoproterneol, Iso; ICI, ICI118,551; CGP20712A, CGP).

DAPI: Blue; Marker (column labels): Red or Green

Conclusions

- β-AR stimulation suppresses osteogenic differentiation of HASMCs, and this effect persists despite β1 blockade or concurrent β2 inverse agonism.
- Non-canonical or biased β2 signaling, or alternative β-AR–independent pathways, may underlie the anti-calcific response.
- Ongoing studies should map downstream signaling through cAMP production and evaluate contractility to clarify therapeutic prospects for β-AR–targeted modulation of vascular calcification.

References

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