183 NOVEL GLYCOMIMETICS INHIBIT GLY-LDL INDUCED SMOOTH MUSCLE CELL CALCIFICATION VIA CREB

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Advanced glycated end-products (AGEs) are known drivers of cardiovascular complications such as vascular calcification, which is currently untreatable, and are increased in diabetic subjects in part due to oxidative stress and poor glycaemic control. Our previous studies using smooth muscle cells (SMCs) isolated from patients with peripheral arterial disease (PAD) have implicated a number of signalling pathways involved in their osteogenic differentiation *in vitro*, including OPG/RANK. We have also shown that alterations in the cell surface glycocalyx regulates cell function, suggesting that non-sugar glycosaminoglycan mimics can potentially modulate cell phenotypes.

We aim to investigate how modified LDL and PAD serum affects the progression of calcification of SMCs *in vitro* and whether this pathology can be prevented by novel glycosaminoglycan mimics, using qPCR, ELISA, Alkaline phosphatase (ALP) activity, and Western blotting.

Gly-LDL (10 μg/ml) increased an early marker of calcification (ALP activity) at 4 days and enhanced calcification at 21 days, compared to controls ($p<0.05$) as shown by alizarin red staining. PAD serum accelerated calcification, which was apparent after 10 days ($p<0.05$). The glycomimetics significantly inhibited both gly-LDL and PAD serum-induced mineralisation, and reduced gly-LDL induced ALP activity at day 4 ($p<0.05$). In the gly-LDL-treated SMCs, secreted levels of osteocalcin (OCN), a promoter of osteogenic differentiation, were reduced when treated with the glycomimetics, whereas osteopontin (OPN) and osteoprotegerin (OPG), inhibitors of calcification, were increased compared to gly-LDL alone. A similar trend was observed in PAD serum-treated SMCs, with glycomimetics reducing OCN and increasing OPN secretion. A phospho-kinase array analysis of gly-LDL-treated SMCs was performed to identify underlying mechanism of action. Gly-LDL increased phosphorylation of cyclic AMP response element-binding protein (CREB), TOR, and the SRC proteins LYN, YES and CHK-2 compared with untreated control, which was attenuated with the addition of the glycomimetics. A number of upstream activators of CREB were targeted using known pharmacological inhibitors in SMCs treated with gly-LDL. The MEK inhibitor U0126 accelerated calcification, increasing both ALP activity and expression of receptor for AGEs (RAGE), a key receptor implicated in vascular calcification, compared to gly-LDL alone, suggesting that MEK may be a mediator of mineralisation process via phosphorylation of CREB.

Glycomimetics have potential as an anti-calcification strategy, inhibiting mineralisation in SMCs induced by both gly-LDL and patient serum *in vitro*. The protective effect of glycomimetics against calcification may occur via regulation of CREB phosphorylation and subsequent modulation of downstream osteogenic markers, including upregulation of OPN and OPG and reduction of OCN, leading to the development of therapeutics to treat vascular calcification.

184 UROTENSIN II INDUCES CARDIOMYOCYTES HYPERTROPHY VIA ACTIVATION OF THE MAPK AND CAMKII SIGNALLING PATHWAYS

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Introduction The normal concentration of circulating Urotensin II (UII) is elevated in heart failure (Ng et al. 2002). UII has been shown to be involved the development of pathological cardiac hypertrophy (Tzanidis et al. 2003). The aim of this study is to investigate the cellular mechanism by which UII results in ventricular hypertrophy.

Methodology Adult Rat Ventricular Myocytes (ARVMs) were isolated from male Wistar rats by enzymatic digestion and placed into primary culture. ARVMs were cultured in six wells plate for up 48 hours. To induce hypertrophy, cells were treated with UII (200 nM) or phenylephrine (10 μM), and hypertrophy quantified as Length/Width (L/W) ratio from photomicrographs. Results Cultured AVRMs exposed to either phenylephrine or UII developed hypertrophy in a time-dependent manner, with a significant reduction in L/W ratio after incubation with UII for 24 hours in comparison with control group (4.25 ± 0.06, $n=362$ vs. 4.45 ± 0.06, $n=335$) ($p<0.05$) or 48 hours (3.99 ± 0.06, $n=209$ vs. 4.53 ± 0.10, $n=126$) ($p<0.0001$).Treatment of AVRMs with phenylephrine induced a similar level of hypertrophy to that seen by UII, to alteration in the morphology of myocytes, there was a significant reduction in L/W ratio after incubation with phenylephrine for 24 hours in comparison with control group (4.08 ± 0.05, $n=338$ vs. 4.45 ± 0.06, $n=335$) ($p<0.05$) or 48 hours (3.77 ± 0.08, $n=141$ vs. 4.53 ± 0.10, $n=126$) ($p<0.0001$). To study the role of ERK1/2, P38 and CaMKII signalling in the UII-induced hypertrophy, cultured ARVMs were treated with either 5μM of ERK1/2 inhibitor (PD 184352), 10 μM of P38 inhibitor (SB 202190) or 5 μM CaMKII (KN-93) for 30 min prior to stimulation with UII (200 nM). Inhibition of all three signalling pathways completely blocked the UII-induced hypertrophy after 48 hours; from (3.87 ± 0.05, $n=420$) in UII group to (4.64 ± 0.08, $n=195$) (UII + PD) ($p<0.0001$) and from (3.87 ± 0.05, $n=420$) in UII group to (4.86 ± 0.09, $n=212$) (UII + SB) ($p<0.0001$). Inhibition of CaMKII from (3.39 ± 0.04, $n=386$) in UII group to (4.14 ± 0.06, $n=223$) (UII + CaMKII) ($p<0.0001$).

Conclusion These data show that the ERK1/2, p38 and CaMKII signalling pathways are involved in the hypertrophic response to UII. I am currently using Western Blot to confirm the involvement of ERK1/2, P38 and CaMKII pathways.

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185 MISALIGNMENT OF TRANSCATHETER AORTIC VALVE IMPLANTS WITH THEIR DESTINATION AORTIC ROOT RESULTS IN DIMINISHED VALVE PERFORMANCE

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