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Novel small molecule SIRT1 activators attenuate vascular calcification in an in vitro diabetic model

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Introduction / Purpose

Vascular calcification, involving activation of osteogenic regulators and transcription factors in conjunction with loss of mineralization inhibitors, is triggered by inflammation in metabolic disorders such as diabetes and kidney disease. This sinister pathology is associated with significant morbidity and mortality and its increasing prevalence and the growing recognition that it can be modulated, provides the impetus for further study. Recent evidence demonstrates the beneficial role of Sirtuin 1 (SIRT1), an NAD+-dependant deacetylase, in insulin sensitivity and glucose homeostasis, and suggests a link between hyperphosphatemia and SIRT1 downregulation. In the current study, we aimed to investigate the therapeutic role of SIRT1 activation in the prevention of vascular calcification.

Methods

To mimic diabetes, we incubated human coronary artery smooth muscle cells (hCASMCs) for up to 21 days, in either low or high physiologically relevant glucose levels, in the presence of osteogenic media containing β-glycerophosphate and calcium chloride. SIRT1 was inhibited by Sirtinol and activated by SRT1720, both at sub-toxic concentrations and confirmed via cellular staining, qPCR and western blot analysis. hCASMC calcification was confirmed via Alizarin red staining, alkaline phosphatase activity, qPCR and Western blot analysis.

Results

hCASMCs, cultured in high glucose osteogenic conditions, in the presence of the SIRT activator, SRT1720, showed i) a significant decrease in ALP activity (n=3) (p > 0.05) at day 4, which was sustained until day 7, ii) a 3-fold reduction in alizarin red staining at day 21 (n=4), iii) a decline in the osteogenic transcription factor RUNX2 mRNA expression to a tenth of its control levels (n=4) (p>0.05), and iv) reduced RUNX2 protein by a half (n=2). Conversely, when SIRT1 was inhibited with Sirtinol, there was i) a significant increase in ALP activity (n=3) (p > 0.05), ii) an increase in alizarin red staining after 21 days in both osteogenic and control treatments (n=3) (p > 0.05), iii) significantly increased mRNA expression of RUNX2; (n=3) (p>0.01) and iv) supported by increased RUNX2 protein expression (n=2).

Conclusions

This study demonstrates that use of the small molecule SRT1720, which activates SIRT1, attenuates deposition of a calcified matrix in hCASMCs grown in diabetic conditions, via reduction of osteogenic markers. The data suggest an essential role of SIRT1 in protection against vascular calcification, which may be compromised in the diabetic patient.
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