Regulation of Vascular Calcification In Rat Aortic Smooth Muscle Cells By Nitric Oxide

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Introduction: Vascular calcification (VC) results in abated elasticity of arterial lumen and hemodynamic changes that increase the incidence of cardiovascular related events in renal and diabetic subjects. Inducible nitric oxide synthase (iNOS) and VC independently and/or in combination, could cause detrimental changes to the vessel wall that may lead to accelerated calcification. Furthermore, iNOS expression and induced nitric oxide (NO) production in calcifying smooth muscle cells may promote VC (Zaragoza et al., 2006). However, contradicting data suggest that NO rather than being pro-calcific, may be protective towards VC (Kanno et al., 2008). Thus, it is not entirely clear what the role of iNOS/NO is in the development of VC. Studies have therefore been initiated to understand the link between induced NO synthesis and the development and progression of VC in normal smooth muscle cells.

Methods: Rat aortic smooth muscle cells (RASMCs) were cultured to ~ 90% confluency. Cells were activated by LPS (100µg ml⁻¹), IFN-γ (100U ml⁻¹) or in combination for 24 hours in the absence and presence of the calcification inducers: calcium chloride (CaCl₂; 7 mM), β-glycerophosphate (β-GP; 7 mM) or a combination of the two (calcification buffer). Total nitrite, protein and calcium levels were quantified utilising the Griess assay, BCA assay, and calcium DICA-500 assay kit (Bioassay, Cambridge, UK) respectively. Calcification was confirmed microscopically by staining cells with alizarin red dye. Fourier transform infrared spectroscopy (FTIR) was performed to identify the chemical compositions of calcified plaques. All measurements were taken 5 days after initiating each experiment. One way ANOVA followed by a post hoc Dunnett’s test were used to determine statistical significance.

Results: Treatment of cells with LPS alone induced nitrite production, iNOS expression and calcification which were significantly higher (p<0.05) than in controls. These effects were enhanced by CaCl₂ and by calcification buffer (CaCl₂ and β-GP) but not by β-GP. In contrast, IFN-γ failed to cause any significant changes in NO and iNOS above control levels but appear to induce calcification which was again enhanced by CaCl₂ and by calcification buffer (CaCl₂ and β-GP) but not by β-GP. The combination of LPS and IFN-γ produced trends similar to that seen with LPS but much enhanced. FTIR analysis of the plaques formed showed the presence of hydroxyapatite nature (1085-1020 cm⁻¹) which together with the positive staining obtained with the alizarin red dye confirms the presence of calcification.

Conclusion: These preliminary findings suggest that NO may indeed play a fundamental role in calcification, enhancing mineralization of smooth muscle cells. More importantly, the expression of iNOS and subsequent production of NO appears to be enhanced under conditions that favour calcification and these together may contribute to enhanced calcification with potential detrimental consequences in vivo. These observations now need to be further examined to establish a strong pivotal link between the NO/iNOS and calcification, as well as identify the potential underlying mechanisms that may be involved.

References
Kanno et al, Cardiovascular research, 77(1), 221-230, 2008.