Pharmacological inhibition of plasma membrane calcium ATPASE 4 improves VEGF-induced angiogenesis

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Ischaemic cardiovascular diseases are the leading cause of death worldwide and are often associated with partial or full occlusion of the blood vessel network in the affected organs. Therapeutic angiogenesis provides a valuable tool for treating cardiovascular diseases by stimulating the growth of new blood vessels from pre-existing ones. The pro-angiogenic factor Vascular Endothelial Growth Factor (VEGF) has been identified as a crucial regulator of angiogenesis through activation of the calcineurin/Nuclear Factor of Activated T-cells (NFAT) signalling pathway. We have previously reported a novel role for the Plasma Membrane Calcium ATPase 4 (PMCA4) as a negative regulator of angiogenesis via interaction with calcineurin. Aurintricarboxylic acid (ATA) has been recently identified as a PMCA4-specific inhibitor. We hypothesise that pharmacological inhibition of PMCA4 with ATA will enhance VEGF-mediated angiogenesis.

Here, we show that treatment of endothelial cells with nanomolar concentrations of ATA notably enhances calcineurin/NFAT signalling, and the subsequent expression of the VEGF-induced, NFAT-dependent, pro-angiogenic protein RCAN1.4. Targeting PMCA4 with ATA reduces the level of membrane-associated calcineurin, and the amount of calcineurin co-precipitated with PMCA4 in immunoprecipitation assays, indicating that ATA promotes disruption of the PMCA4/calcineurin interaction. ATA mediated inhibition of PMCA4 also enhances endothelial cell motility, and both in vitro and in vivo blood vessel formation. Low concentrations of ATA do not have any deleterious effects on the viability of endothelial cells or the development of zebra fish embryos, highlighting the potential clinical use of ATA, at low concentrations, to improve blood vessel formation in patients with ischaemic cardiovascular diseases.