Furthermore 3 mM Pi treatment increased glucose uptake (98%, $p<0.05$) and Glut-1 mRNA expression (1.47 fold, $p<0.001$). Glycolysis converts glucose to pyruvate which is subsequently converted to either (i) acetyl-CoA by the pyruvate dehydrogenase complex (PDH) or (ii) lactate by lactate dehydrogenase (LDH). Notably, decreased VSMC calcification was observed in cells treated with sodium dicholoroacetate, an inducer of PDH activity (1 mM; 40%; $p<0.01$) and citric acid, synthesised in the mitochondria from acetyl CoA (1 mM; 72%, $p<0.001$). Treatment with the LDH inhibitor sodium oxamate (20 mM) or sodium lactate (50 mM) to induce pyruvate production also inhibited VSMC calcification (68% and 53% respectively, $p<0.05$). Activation of the Wnt pathway – an established regulator of Warburg metabolism – using the selective GSK3 inhibitor CHIR99021 (1 nM) significantly increased VSMC calcification (417%, $p<0.001$). However, co-treatment with sodium oxamate (20 mM) significantly blunted the pro-calcification effect of CHIR99021 (69%, $p<0.01$).

Conclusion Together these data suggest that arterial calcification requires glucose metabolism through a mechanism involving Wnt signalling. Interruption of the glycolysis pathway may therefore represent a novel therapeutic target for clinical intervention.

224 VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR INHIBITION INDUCES VASCULAR DYSFUNCTION VIA REDOX-SENSITIVE PROCESSES

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Vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR) inhibitors, used as anti-angiogenic drugs to treat cancer, induce severe hypertension. Molecular mechanisms whereby VEGF inhibitors cause hypertension are unclear, but nitric oxide (NO) and oxidative stress may be involved. We questioned whether reactive oxygen species (ROS), important regulators of vascular function in hypertension, also play a role in VEGF inhibitor-induced vascular dysfunction. Human microvascular endothelial cells (HMECs) were stimulated with vatalanib (VEGFR inhibitor) and gefitinib (EGFR inhibitor). Normotensive male SV-129 mice (8 weeks old) were treated with Vatalanib (100 mg/Kg/day) or Gefitinib (100 mg/Kg/day). Vascular reactivity was performed mesenteric arteries using wire myograph and blood pressure was measured by tail-cuff method. Phosphorylation of eNOS was assessed by immunoblotting. ROS were measured by amplex red, lucigenin and nitrotyrosine elisa. TBARS levels were measured by lipid peroxidation assay kit and catalase activity by amplex red. Nox and antioxidant enzymes mRNA was analysed by qPCR. No changes in blood pressure were observed in animals treated with vatalanib on this dose. However acetylcholine (ACh)-induced vasodilatation was impaired in those mice and phosphorylation of eNOS activation site (Ser1177) was decreased, while no changes were observed after exposure of HMECs to gefitinib. Hydrogen peroxide (H_2O_2) levels were reduced in HMECs stimulated with vatalanib and in aorta and heart from vatalanib-treated mice. This effect was followed by an increase in catalase activity and a decrease in Nox 4 mRNA expression while Nox5 mRNA levels were increase by vatalanib. VEGF inhibition also increased peroxynitrite ($ONOO^-$) levels in aorta and kidney and increased plasma TBARS levels. In kidney vatalanib increased H_2O_2 and O_2^- production which was followed by a decrease in catalase activity and Nrf2 nuclear translocation. Finally mRNA levels of antioxidant enzymes in HMECs, kidney and heart were decreased after exposure to vatalanib. Gefitinib only increased catalase activity and $ONOO^-$ levels in heart as well as decreased Nrf2 nuclear translocation in kidney from mice. In conclusion, our data identify novel mechanisms whereby VEGFR inhibition modulates NO signalling, antioxidant defences and ROS production in tissues and endothelial cells. These molecular processes may contribute to reduced vasorelaxation and may play a role on VEGFRI-induced hypertension.

225 THE ROLE OF THE DNA DAMAGE RESPONSE IN VASCULAR CALCIFICATION

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Introduction Vascular calcification is a hallmark of vascular ageing, and associated with vascular smooth muscle cell (VSMC) death, phenotype modulation and maladaptation. However, it remains unclear how the initial stress signals link to these downstream cellular events. Emerging evidence and our *in vitro* data suggest that stress may drive vascular calcification through elevated levels of DNA damage, a key factor driving cellular ageing.

Methods and Results We investigated the effects of different DNA damage signalling pathway inhibitors (ATM, ATM/ATR and PARP1) on the progression of vascular calcification. Using comet assays and western blot, we found elevated levels of DNA damage in calcified VSMCs and that inducing DNA damage accelerated rates of calcification. Chemical inhibition or siRNA knockdown of ATM, ATM/ATR or PARP signalling reduced or delayed calcification and prevented cells undergoing calcification-associated phenotype changes including osteo/chondrogenic differentiation. Prevention was associated with down-regulation of senescence and inflammatory markers suggesting the senescence associated secretory phenotype (SASP) acts to potentiate VSMC calcification.

Conclusion Taken together, these *in vitro* data suggest DNA damage signalling is involved in the pathological regulation of calcification. Therefore, interventions that reduce DNA damage, promote DNA damage repair, or modulate DNA damage