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A short-term treatment with a Mek1/2 inhibitor promotes myocardial arteriogenesis and perfusion in vivo: focus on cardiac mural cells

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Background: Arteriogenesis is crucial for heart recovery after ischaemia, but cellular and molecular mechanisms able to foster this phenomenon are still poorly characterised.
Purpose: To discover novel pro-arteriogenic approaches by exploiting cardiac mural cells endowed with arteriogenic capacity: pericytes (PCs) and vascular smooth muscle cells (VSMCs).
Methods and results: We derived human and murine CD31neg CD34pos cardiac PCs (cPCs) from myocardial samples of adult subjects and confirmed the pericyte phenotype and function *in vitro*. We discovered that the withdrawal of EGF and bFGF from the culture media induces the differentiation of cPCs into contractile VSMCs. Molecular investigations of pathways associated with the two factors showed that the Mek1/2-Erk1/2 signalling exerts an inhibitory transcriptional control on contractile VSMC genes. Screening of compounds able to interfere with this pathway revealed that PD0325901 - a potent Mek1/2 inhibitor (MeKi) tested in clinical trials for the treatment of cancer - activates the VSMC phenotype in cPCs. We observed a similar effect on coronary artery VSMCs. Next, we interrogated the effect of PD0325901 on cardiac arteriogenesis *in vivo*. Adult C57BL6/J mice were given the MeKi 10 mg/kg/day or vehicle (DMSO), orally for 14 days (n=11/group). At the endpoint, echocardiographic evaluation of left ventricle (LV) function and dimensions (n=6/group) showed no difference in comparison with the respective baseline in both groups. Effective inhibition of Mek1/2 in the heart of PD-treated mice was confirmed by the reduced immunostaining for the phosphorylated form of Erk1/2. The MeKi cardiotoxicity was ruled out by assessment of cardiomyocytes and vascular cells apoptosis (Tunel) and plasmatic levels of cTn-I. Histological analyses of the hearts (n=5/group) showed an increase in small arterioles (diameter < 20μm) density in the LV of PD-mice compared with the DMSO group (16.4 vs 11.7 art/mm^2). No change was observed for the capillary density. The drug promoted the maturation of VSMCs within both small and large (> 20μm) arterioles, as shown by the higher ratio between the areas of the vascular wall occupied by the mature contractile marker SM myosin heavy chain and the synthetic/early contractile marker alpha-SM actin (αSMA). The PD treatment reduced the fraction of small arterioles covered with a CD34pos layer (53% vs 70% of total arterioles), along with a lower ratio between the areas occupied by adventitial CD34pos cells and αSMApos VSMCs, suggesting a contribution of cPCs to the arteriolar remodelling. Finally, the drug improved the LV myocardial perfusion in the PD- vs the DMSO-group (6.8 vs 5.3 ml/min/g of LV tissue, n=6/group).
Conclusions: We show that a short treatment with a Mek1/2 inhibitor stimulates myocardial arteriogenesis and perfusion without either inducing cardiotoxicity or deteriorating heart function. This may be a novel, intriguing approach to promote therapeutic arteriogenesis.