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INTERFERENCE WITH AKT SIGNALLING IN DYSLIPIDEMIA DIMINISHES MYOCARDIAL INFARCTION AND PROMOTES SURVIVAL BY INHIBITING OXIDATIVE STRESS.

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[1]Lining Ma, [2]Bethany A Kerr, [2]Xiaoxia Z West, [3]Nikolay L Malinin, [2]Malory E Weber, [3]Liang Ding, [4]Payaningal R Somanath, [2]Eugene A Podrez, [3]Tatiana V Byzova, [1]Lining Ma. *[1]Department of Molecular Cardiology, Joseph J. Jacobs Center for Thrombosis and Vascular Biology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, 44195, USA; [2]Cardiovascular Department, Hainan Provincial People's Hospital, Hainan, China; [3]Clinical and Experimental Therapeutics, College of Pharmacy, University of Georgia, Augusta, GA, 30912, USA; [4]Clinical and Experimental Therapeutics, College of Pharmacy,University of Georgia, Augusta, GA, 30912, USA*

Objectives Cardiovascular disease resulting in myocardial infarction (MI) is the leading cause of mortality in developed countries; however, the mechanisms controlling this complex disorder remain elusive. Since Akt activation was shown to be increased in human and mouse atherosclerotic lesions and failing hearts, we hypothesised that Akt signalling might contribute to several aspects of the pathogenesis of cardiovascular disease.

Methods C57BL/6, ApoE$^{-/-}$ (C57BL/6 background), and SR-BI$^{+/-}$ApoE$^{-/-}$ (mixed C57BL/6xS129 background) mice were purchased from Jackson Laboratories (Bar Harbor, ME). SR-BI$^{+/-}$ApoE$^{-/-}$ mice were backcrossed to C57BL/6 background for 10 generations. Akt1$^{-/-}$ mice were generated as previously described) and backcrossed to the C57BL/6 background for 10 generations. SR-BI$^{-/-}$ApoE$^{-/-}$ (DKO) mice were generated by intercrossing SR-BI$^{+/-}$ApoE$^{-/-}$ mice. SR-BI$^{-/-}$ApoE$^{-/-}$Akt1$^{-/-}$ (TKO) mice were generated by first crossing Akt1$^{-/-}$ mice with SR-BI$^{+/-}$ApoE$^{-/-}$ mice. Mice were sacrificed at 42 days of age for most experiments. Immunoblotting

Hearts were homogenised in sample buffer. Proteins were detected with anti-Akt1, anti-phospho-Akt, anti-phospho-GSK-3α/β or anti-GSK-3β antibodies. Cardiac function was evaluated with a Sequoia C256 (Acuson) and M mode analysis was performed to measure ejection fraction (EF) and fraction of shortening (FS). Mice were sacrificed at 6 weeks of age when cardiac dysfunction was just becoming apparent to assess the role of Akt1 deletion in cardiac pathogenesis. Hearts were cut at the mid-level transversely, as reported previously, and embedded in paraffin. Serial sections (8 μm) were cut and stained with H&E or Masson's trichrome to quantify fibrosis. Five images were taken of four sections per Masson's trichrome stained heart on an Olympus BX51 microscope. Cardiac fibrosis was quantified by measuring the total stained area the total area of the heart using Image Pro.

Atherosclerotic lesions were quantified by en face aortic coverage measured by computer-assisted planimetry. Aortae were cut open longitudinally, stained with Oil red O, and digitally scanned.

VCAM-1 expression was assessed in endothelial cells, as demonstrated by CD31 staining, in the aortic arch and descending thoracic aorta by en face staining, followed by laser-scanning confocal microscopy (Leica TCS-SP) as previously described.

Hearts were embedded in OCT freezing medium and sectioned at 7 μm. Sections were then fixed in 4% PFA and analysed for TUNEL staining using the In Situ Cell Death Detection Kit.

Dihydroethidium (DHE, Invitrogen) staining for superoxide was carried out as previously described.

Results Akt1 deletion under dyslipidemia alleviates cardiac dysfunction (EF37.67%±6.60 in TKO vs 21.94%±3.78 in DKO, FS 39.25% ±3.23 in TKO vs 16.22%±3.09 in DKO), diminishes MI size (3.78% ±0.864 in TKO vs 22.02%±5.926 in DKO), and, most importantly, prolongs lifespan (44.23±7.43 days in DKO vs 51.43±6.29 days in TKO). TKO mice exhibit reduced atherosclerosis. While dyslipidemia was equal, ROS generation and consequent oxidised lipid accumulation was dramatically reduced in TKO. Simultaneously, Akt1 deletion diminished CD36 expression, the main oxidised lipid receptor responsible for foam cell formation.

Conclusions Interference with Akt activation improves survival during dyslipidemia by reducing oxidative stress and oxidised lipid

responses thus providing a protective effect. Normalisation or prevention of Akt overactivation during atherogenesis might be beneficial for the treatment of atherosclerosis and heart failure.