Basic science

BS1 REPROGRAMMING HUMAN MACROPHAGES WITH DRUG X: POTENTIAL MECHANISMS FOR STABILISATION OF ATHEROSCLEROTIC PLAQUES

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Introduction Ischemic stroke is commonly caused by large artery atherosclerosis. Patients with a high atherosclerotic burden (stenosis) and inflamed or ulcerated plaque are at increased risk of early recurrent ischaemic events. Treatment with anti-inflammatory agents may therefore reduce stroke incidence and recurrence in patients with this condition, but in vitro human studies of the possible mechanisms are lacking. We hypothesised that an anti-inflammatory compound (denoted drug X*) alters the expression of macrophage specific genes, including scavenger receptors in in vitro models of human carotid plaque macrophages.

Methods CD14+ monocytes were collected from whole blood donations (volunteers) and differentiated to monocyte derived macrophages (MDMs). Macrophage polarisation with M1 (100 ng/ml LPS + 20 ng/ml INF-γ) and M2a (20 ng/ml IL-4) markers was performed in complete media supplemented with 3 different clinically relevant concentrations of drug X.

Gene expression (RT-qPCR) analysis (n=10) was carried out in the differentiated hMDMs to test for macrophage specific genes and scavenger receptors. Western Blot analysis (n=7) and immunofluorescence stain (IF) (n=5) was performed on drug X- treated MDMs to validate changes in the expression of Oxidized Low Density Lipoprotein Receptor 1 (OLR1). The physiological effect of drug X was also tested on oxLDL (25 μg/ml) uptake by macrophages (n=5).

Results Our data suggest that drug X may re-program macrophages to a less inflammatory state (an M2 state) by significantly altering Mannose Receptor C-type 1 (MRC1) gene expression (p:0.0183). Drug X also alters the expression level of Macrophage Scavenger Receptor 1 (MSR1) gene, provoking the pacification of the M1 state and resulting in a beneficial effect on LDL uptake (p: 0.0845). Using OLR1 gene expression as a readout, in both M1 and M2a macrophages, the average OLR1 expression was reduced by treatment with drug X (p: 0.0003). This implies that M1 macrophages can bind less oxLDL which could reduce the formation of atherosclerotic plaque in the vessel wall. OLR1 protein expression was also reduced (p:0.0003) following 24-hour exposure of the macrophages to drug X. In functional tests, oxLDL uptake by drug X treated hMDMs was reduced by 44% (p:0.003).

Conclusions The gene expression of macrophage specific genes and scavenger receptors in human M1 and M2a macrophages was significantly altered following 24-hour exposure to clinically relevant concentrations of drug X in vitro. Drug X reduced 'active' and pathogenic cell behaviours e.g., lipid uptake and promoted the polarisation to the M2 state.

These data demonstrate that human macrophages can be reprogrammed to a less pathogenic state in vitro and they provide a potential mechanism for the effectiveness of drug X in the stabilisation of carotid atherosclerotic plaques in humans.

* Drug X is used for ongoing IP/patent assessment

Conflict of Interest No

BS2 MECHANOSENSOR PIEZO1 IN SKELETAL MUSCLE PERICYTES

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Introduction Pericytes (PC) are mural cells which are present in blood microvessels. They play an important part in the formation and stabilization of new blood vessels. They are essential for vascular development and cardiovascular homeostasis. They wrap around the endothelial cells (EC) and endothelial-pericyte communication is both via physical contact and paracrine signalling.[1] Piezo1, a cation-permeable pore-forming ion channel protein, has recently been described as mechanosensors in many mammalian cells including EC. Shear stress sensing by endothelial Piezo1 is essential for angiogenesis and development of regular vascular architecture during embryogenesis.[2] Moreover, endothelial Piezo1 regulates blood pressure and exercise-induced mesenteric vasoconstriction.[3] PC of skeletal muscle respond to exercise and contribute significantly to angiogenesis and myogenesis. Certain types of exercise can increase and others can reduce or maintain the PC numbers and coverage.[4 5 6] As both EC and PC are prone to mechanical stress produced by blood flow and pressure, we hypothesise that Piezo1 may play important roles in PC physiology too. Hence, we aim to evaluate the expression and functions of Piezo1 in PC using in vivo and in vitro models and experiments.

Methods We have developed a novel transgenic mouse model – tamoxifen-inducible PC-specific knockout of Piezo1 (PP-/-). We developed a method to isolate PC from mouse skeletal muscle (skmPC) and obtained human brain vascular PC (HBVP) from commercial sources. Expression and functionality of Piezo1 was analysed by RT-PCR and Ca2+ influx assay. Using Yoda1 (Piezo1 agonist) and Yoda1 analogues as pharmocological tools and Piezo1-specific siRNA for gene silencing, we performed functional experiments to elucidate the role of Piezo1 in PC.

Results We confirmed the purity of PC population using flow cytometry and PC specific antibody markers. The flow cytometric analysis showed >98% of isolated mouse skeletal muscle PC in culture. We found different populations of primary PC and HBVP express functional Piezo1, demonstrated by Yoda1-induced Ca2+ influx. RT-PCR, Ca2+ influx assay and gene-rescue experiments confirmed the PC-specific knockdown of Piezo1 in our transgenic mouse model. Piezo1 mRNA expression was reduced via siRNA-mediated silencing of Piezo1 in HBVP. Transcriptomics analysis showed chemical activation of Piezo1 leads to a perturbance in interleukin (IL)-mediated signaling in HBVP. We found that Piezo1 activation leads to downregulation of IL33, IL1RAP and VCAM-1 mRNA, upregulation of IL1RL1, ICAM-1 mRNA levels. Complementary work on murine skmPC confirmed the role of Piezo1 activation in IL signalling pathways.

Conclusions In summary, we demonstrated that the primary skmPC and HBVP express functional Piezo1. Activation of