cardiomyopathy and suggests exciting new targets for BH4-based therapeutics.

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HUMAN OXIDISED PHOSPHOLIPID MACROPHAGES HAVE HIGH LIPOPROTEIN HANDLING CAPABILITIES WITHOUT READILY FORMING UNWANTED FOAM CELLS

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Rationale Cardiovascular disease is the leading cause of death world-wide. Macrophages are crucial in regulating the plaque environment, especially the lipid content. However, current characterisation of macrophage phenotypes lipoprotein handling capacity is conflicting and incomplete. We hypothesised that lipoprotein handling differed among distinct macrophage phenotypes due to differential gene and protein expression. We tested this using a range of functional, gene and protein expression assays.

Methodology Monocytes were isolated from healthy donor blood by gradient centrifugation and magnetic selection, then differentiated into macrophages over 7 days using M-CSF. Macrophages were polarised over 24 hour by IFN γ +LPS, IL-4, IL-10, oxPAPC (oxidised phospholipid) and CXCL4, respectively. Unpolarised macrophages were used as controls.

Foam cell formation was determined by Oil-Red-O staining and acLDL uptake was detected by flow cytometry. Cholesterol content and efflux were measured using colorimetric and fluorescent assays. RNA expression was determined by RNA-seq and qRT-PCR and cell surface protein expression was measured by flow cytometry.

Results IFN γ +LPS macrophages did not readily form foam cells (0.23 compared to unpolarised) or process lipoprotein particles, whereas IL-4 and IL-10 polarised macrophages displayed the highest capacity in foam cell formation (1.02 and 1.08 compared to unpolarised) and lipoprotein handling. OxPAPC macrophages exhibited lipoprotein processing capabilities similar to IL-4 and IL-10 macrophages, but did not readily form foam cells (0.22 compared to unpolarised). CXCL4 macrophages displayed intermediate foam cell formation (0.84 compared to unpolarised) and lipoprotein handling capabilities.

Differences in foam cell formation and lipoprotein uptake correlated directly to specific scavenger receptor gene and protein expression. Only IFN γ +LPS macrophages had significantly reduced expression of key internal lipoprotein processing genes (q 0.0001). Cholesterol efflux correlated directly to specific ABC transporter protein, but not RNA expression.

Conclusions. *In vitro* human macrophage phenotypes differ in foam cell formation and lipoprotein handling capabilities that are associated with differential key gene and protein expression.

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THERAPEUTIC RESOLUTION OF PULMONARY ARTERIAL HYPERTENSION (PAH) BY NOVEL SMALL MOLECULE NATURAL PRODUCTS

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Heterozygous germline mutations in the gene encoding type II bone morphogenetic protein receptor (BMPRII) underlie the majority (~80%) of the familial form of pulmonary arterial hypertension (HPAH).¹ PAH is a devastating cardiovascular disorder caused by narrowing of blood vessels in the lungs. We earlier demonstrated that mutations in *BMPR2* impinge upon the BMP signalling pathway and potentiate the TGF- β ² signalling leading to abnormal proliferation and apoptosis resistance of endothelial and pulmonary arterial smooth muscle cells (PASMCs).^{2,3} No cure for this disorder is known. Traditional therapies aim to improve cardiopulmonary function and were established before recognising the involvement of substantial genetic components of PAH. Hence, there is an urgent need to identify novel compounds capable of providing therapeutic intervention prior to or following the onset of disease.

High-throughput cell based BMP-responsive screens were carried out, which identified several natural compounds as hits. The hit compounds were optimised through a medicinal chemistry programme. These compounds were tested in a number of cell based experiments including reporter assays, gene expression studies and western blot analyses. The compounds CRT-01, CRT-02 and CRT-03 enhanced BMP signalling in BMP responsive reporter assays. Moreover, these compounds were able to induce BMP responsive *id1* gene expression and increased the phosphorylation of SMAD1/5 proteins. Furthermore, these compounds inhibited excessive proliferation of PASMCs harbouring a pathogenic *BMPR2* mutation. Taken together, this study identified novel compounds eliciting pro-BMP effects which may have experimental and clinical applications in PAH.

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