THE ANTI-ATHEROGENIC ROLE OF SPHINGOSINE 1-PHOSPHATE AND SPHINGOSINE 1-PHOSPATE RECEPTOR 3IN THE REVERSE CHOLESTEROL TRANSPORT

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Introduction: Sphingosine 1-phosphate (S1P) is a bioactive lysosphingolipid involved in the regulation of different biological responses including inflammation, immunity and cardiovascular function. S1P is an integral constituent of High-density Lipoprotein (HDL) particles and exerts its biological activity by binding to its G protein-coupled receptors; interestingly, S1P has been proposed to contribute to many of the cardiovascular and atheroprotective effects of HDL. Despite that, the molecular mechanisms underlying the anti-atherogenic effects of S1P are still partially known. To date, we have no direct evidence connecting the role of sphingosine 1-phosphate receptor 3(S1P3) in cellular and systemic cholesterol handling. This study aims to investigate whether the anti-atherogenic activity of S1P is related to the regulation of lipid metabolism through the modulation of reverse cholesterol transport (RCT), a well-known anti-atherogenic process.

Materials and methods: We evaluated the role of S1P3receptor in a mouse model overexpressing S1P3in myeloid lineage (S1P3-Lyz). ATP-binding cassette (ABC) transporters A1and G1gene and protein expression in murine peritoneal macrophages (MPM) were quantified through RT-qPCR and Western Blot analysis. ABCA1- and ABCG1-mediated cholesterol efflux was evaluated in control (C57BL/6) and S1P3-Lyz MPM through a radioisotope technique, using different mouse plasma concentration (0,1% and 2% v/v) and HDL (12,5mg/ml) as cholesterol acceptors. In vivo RCT was measured through a radioisotope technique by injecting ^{3}[H] cholesterol-enriched MPM isolated from both C57BL/6and S1P3-Lyz mice in C57BL/6recipient.

Results: S1P3-Lyz MPM displayed an increased ABCG1protein and gene expression compared to C57BL/6, while no differences were observed in ABCA1. Accordingly, ABCG1-mediated cholesterol efflux to mouse plasma was higher in S1P3-Lyz MPM compared to C57BL/6MPM; similarly, acetylated LDL-loaded S1P3-Lyz MPM displayed an higher cholesterol efflux to plasma acceptors compared to C57BL/6MPM. Finally, S1P3-Lyz MPM incubated with plasma together with a selective S1P3antagonist (TY52156, 10μM) displayed a reduced cholesterol efflux compared to non-treated S1P3-Lyz MPM. In vivo total RCT resulted higher in S1P3-Lyz group, as ^{3}[H]-Cholesterol found in plasma, liver and faeces was higher compared to C57BL/6group.

Discussion and conclusion: These results showed that endogenous S1P, through the interaction with its receptor S1P3on myeloid cells, positively modulates cholesterol metabolism by improving RCT. For these reasons, S1P-S1P3axis may represent a potential pharmacological target for the modulation of cholesterol homeostasis.