ROLE OF SPHINGOSINE 1-PHOSPHATE AND ITS RECEPTORS S1P1 AND S1P3 IN THE REVERSE CHOLESTEROL TRANSPORT

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Background and Aim. Sphingosine 1-phosphate (S1P) is an integral constituent of High-Density Lipoprotein (HDL) particles and has been proposed to contribute to many of the cardiovascular and atheroprotective effects of HDL. Indeed, S1P is a bioactive lysosphingolipid, which binds to its 5 specific G protein-coupled receptors, particularly expressed in cardiovascular system. To date, we have no direct evidence connecting endogenous S1P with cellular and systemic cholesterol handling. This study aims to investigate the role of endogenous S1P in the modulation of reverse cholesterol transport (RCT), a relevant physiological anti-atherogenic process.
Materials and Methods. We evaluated the role of S1P receptors employing a transgenic mouse model overexpressing S1P1 or S1P3 receptor in myeloid lineage (S1P1-Lyz or S1P3-Lyz, respectively). In vivo RCT was measured through a radioisotope technique by injecting 3[H]-Cholesterol-enriched MPM isolated from both CTRL and S1P1-Lyz or S1P3-Lyz mice in C57BL/6 recipient. Cholesterol efflux from cultured MPM was evaluated in CTRL and S1P1 and S1P3-Lyz MPM through a radioisotope technique, adding HDL (12,5µg/ml) or murine plasma (0,5% and 2% v/v) as cholesterol acceptors.
Results and Conclusions. Mice injected with S1P1-Lyz MPM displayed an increased 3[H]-Cholesterol elimination in faeces compared to CTRL MPM-injected mice (0,38%±0,009 vs 0,30%±0,01; p<0,001). Upon incubation with acetylated LDL, S1P1-Lyz MPM are characterized by an increased cholesterol efflux to plasma compared to CTRL MPM (5,42%±0,16 vs 4,33%±0,38; p<0,001). S1P1-Lyz MPM stimulated with LXR/RXR agonists showed an increased cholesterol efflux to HDL (5,24%±0,77 vs 4,34%±0,38; p<0,05). In vivo total RCT resulted higher in S1P3-Lyz MPM injected mice compared to CTRL MPM-injected group, as 3[H]-Cholesterol found in plasma (0.99%±0.32 vs. 0.60%±0.12; p<0.05), liver (2.66%±0.41 vs 1.99%±0.35; p<0.01) and faeces (0.99%±0.19 vs 0.66%±0.10; p<0.01) was higher in the former. Consistently, acLDL-loaded S1P3-Lyz MPM displayed an increased cholesterol efflux to HDL (8.47%±0.63 vs 5.93%±0.46; p<0.001) and mouse plasma compared to CTRL (8.04%±0.43 vs 10.49%±1.2; p<0,001, and 22.99%±1.2 vs 37.09%±5.43; p<0,001, to 0.1% and 2% mouse plasma, respectively). Similarly, S1P3-Lyz MPM stimulated with LXR/RXR agonists showed an increased cholesterol efflux to HDL (7,45%±1,36 vs 5,31%±0,37; p<0,01) and plasma (6,17%±1,17 vs 3,76%±0,46; p<0,01) compared to CTRL MPM. Endogenous S1P, through the interaction with its receptors S1P1 and S1P3 on macrophages, positively modulates cholesterol metabolism by improving RCT, thus exerts a potentially anti-atherogenic function in vivo.

ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES IN LIVERS OF KNOCK-IN MOUSE MODELS BY MICROARRAY TECHNOLOGY

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The natural variant of human apoA-I, i.e. the apoA-IMilano (A-IM), is the result of a point mutation, with an arginine to cysteine substitution at position 173. Carriers of this mutation exhibit hypertriglyceridemia with markedly reduced HDL and apoA-I plasma levels, a condition generally associated with a high risk of premature coronary disease. Evaluation of the cardiovascular status in A-IM carriers, compared with control subjects from the same kindred, did not reveal any evidence of increased CVD. In addition, whether A-IM may impart a "gain of function" compared to wild-type apoA-I still an open question. This study was aimed at investigating intrinsic differences in the livers of mice expressing human apoA-I or A-IM, by using the Affimetrix GeneChip Mouse Gene ST system. To this aim, previously generated A-I (A-I k-in) or A-IM knock-in mice (A-IM k-in) were crossed with transgenic mice expressing human apoA-II but lacking of murine apoA-I (hA-II) to generate hA-II/A-I k-in, and hA-II/A-IM k-in, respectively. hA-II/A-IM k-in mice were characterized by lower HDL cholesterol and A-I/A-IM plasma levels and by higher triglyceride concentrations compared to both hA-II/A-I k-in and A-IM k-in mice. The expression of 871 genes was significantly altered between the hA-II/A-I k-in and hA-II/A-IM k-in mouse lines, of which 373 up- and 498 down-regulated in hA-II/A-I k-in compared to hA-II/A-IM k-in mice. 1018 differentially expressed genes, 434 up- and 584 down-regulated, were instead found in A-IM versus hA-II/A-IM k-in animals. Comparison of the up-regulated genes by Venn diagrams revealed 46 genes in common to hA-II/A-I k-in, hA-II/A-IM and A-IM k-in mice. Among these, the Elovl6 gene, a key lipogenic enzyme, has been discovered. Protein association networks (STRING database) of Elovl6 highlighted that A-IM could be associated with a modulation of fatty acid (FA) metabolism (i.e. FAs synthesis and catalysis) and biosynthesis of unsaturated FAs.