High density lipoproteins exert pro-inflammatory effects on macrophages via passive cholesterol depletion and PKC-NF-kB/STAT1-IRF1 signaling

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Background: Membrane cholesterol is known to modulate a variety of cell signaling pathways and functions. While cholesterol depletion by High-Density Lipoproteins (HDL) has potent anti-inflammatory effects in various cell types, its effect on inflammatory responses in macrophages remain ill defined.

Methods & Results: Pre-incubation of human and murine macrophages in vitro with human reconstituted (apolipoproteinA-I/phosphatidylcholine) or native HDL significantly decreased LPS-induced anti-inflammatory IL-10 production, while the opposite was observed for the pro-inflammatory mediators IL-12 and TNF. We show that these effects are mediated by passive cholesterol depletion and lipid raft disruption, without involvement of ABCA1, ABCG1, SR-B1 or CD36. These pro-inflammatory effects are confirmed in vivo in peritoneal macrophages from ApoA-I transgenic mice, which have high circulating HDL levels. In line, innate responses required for clearance of P. aeruginosa bacterial infection in lung were compromised in mice with low HDL levels. Native and reconstituted HDL enhances Toll Like Receptor-induced signaling by activating protein kinase C (PKC), since inhibition of PKC ablated the observed HDL effects. Using microarray analysis and macrophages from NF-kB luciferase mice, we observed that HDL induces NF-kB activation. Western blot and ChIP-PCR analyses showed that in particular the p65 subunit was activated. Using specific knock-out mice for the upstream activation pathways, we show that the observed HDL effects are independent of the upstream kinases IKK, NIK and CKII. Furthermore, using STAT1 knock-out mice we observed that also STAT1 is involved in the pro-inflammatory HDL effects on IL-10 and IL-12 secretion. On the other hand, using pharmacological inhibitors, we show that HDL enhances ADAM protease activity, thereby mediating TNF release.

Conclusion and Clinical Relevance: HDL exerts pro-inflammatory effects on macrophages via passive cholesterol depletion by activation of PKC, NF-kB and STAT1. These pro-inflammatory activities on macrophages could at least partly underlie the disappointing therapeutic potential of HDL raising therapy in current cardiovascular clinical trials.

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Homocysteine accelerated the formation of THP-1 macrophages-derived foam cells and cholesterol disorder via regulating the expressions of LXRa, ABCA1 and ABCG1

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Background: Atherosclceorsis(AS) is a chronic disease with accumulation of excessive cholesterol in the arterial intima. The formation of macrophage-derived foam cells plays a critical role in the pathogenesis of AS. Large quantities of evidence-based medical studies have proved that homocysteine(Hcy) is an independent risk factor of AS. Liver X receptor alpha(LXRa) can keep cholesterol homostasis, and ATP-binding cassette transporters A1and G1(ABCA1,ABCG1) are target genes of LXRa, which prevent macrophage-derived foam cells formation by mediating the active transport of intracellular cholesterol.

Purpose: To evaluate the potential effects of Hcy on cholesterol efflux of THP-1 macrophage-derived foam cells and the expressions of LXRa,ABCA1 and ABCG1,and verify the cholesterol efflux by using LXRa agonist to clarify the underlying mechanisms.

Methods: THP-1 monocytes were cultured and differentiated into macrophages with PMA.Then macrophages were induced by Hcy at 0,50,100,200 umol/L with ox-LDL at 100mg/L for 24h to become foam cells. Positive CD14 was detected by flow cytometry to examine the percentage of macrophages. Hcy at 0umol/L was considered as control group. The formation of foam cells were observed by Oil Red O staining. The foam cells counting was calculated by software. The intracellular total cholesterol(TC),-free cholesterol(FC) and cholesterol ester(CE) were quantified as cholesterol efflux with kits.RT-qPCR and Western blot were performed to analyze the mRNA and protein expressions of LXRa,ABCA1 and ABCG1. Finally,LXRa agonist was used to verify cholesterol efflux again.

Results: Compared with control group,the CD14 positive result showed that Hcy groups had more foam cells($P<0.05$). Increased Hcy promoted the cholesterol accumulation in foam cells. Large quantities of red lipid droplets appeared in foam cells. The result of foam cells counting showed statistical difference between control and Hcy groups ($P<0.05$). And CE/TC in Hcy groups were higher than the control group ($P<0.05$). Besides, the mRNA and protein of LXRa, ABCA1 and ABCG1 were lower than the control group ($P<0.01$). And Hcy at 100umol/L had most significant difference in the above results ($P<0.01$). Finally,the LXRa agonist group(5ug/ml T0901317+100umol/L) reversed the effects of Hcy on cholesterol efflux ($P<0.05$).

Conclusions: Hcy can increase the accumulation and reduce the efflux of cholesterol in foam cells.Inhibition of LXRa-ABCA1/ABCG1 pathway may be a potential mechanim of Hcy induced disorder of cholesterol metabolism,which can provide a new insight to the scientific research and clinical work of AS.

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Protein components of HDL as markers of cardiovascular damage in patients with arterial hypertension

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Purpose: HDL (High-density lipoproteins) are atheroprotective. The mechanisms of HDL-mediated atheroprotection are underlain by anti-atherogenic biological activities of HDL, and do not necessarily correlate with HDL-Cholesterol (HDL-C) concentration. Atheroprotective activities of HDL are thought to be mediated in part by both ApoA1 within the core lipoprotein particle and an assortment of HDL-associated proteins. HDL particle enriched in paraoxonase-1 (PON1), an atheroprotective protein, have been linked to the antioxidant, anti-inflammative, and lipid cargo-carrying functions of HDL. PON1 has been shown to help HDL prevent the accumulation of lipid peroxides in oxidized LDL (low-density lipoproteins), inactivate bioactive oxidized phospholipids, stimulate HDL-mediated eNOS-dependent NO production, and enhance cholesterol efflux from cholesterol-laden macrophages. Myeloperoxidase (MPO), like PON1, both binds to HDL and is mechanistically linked to oxidative stress and atherosclerosis. The aim of this study was to evaluate the protein components of HDL in patients with arterial hypertension (AH).

Methods: The study included 63 patients (mean age 61 years) with AH 2 grade. As control group we enrolled 24 healthy persons (mean age 59 years). Level of carbonyl oxidation protein products (COPPs) in serum, HDL and LDL+VLDL fractions, activities of PON1 and MPO, degree of oxidative modification of LDL, and level of C-reactive protein (CRP) were evaluated in all subjects. Lipid parameters were measured in serum, such as total cholesterol, triglycerides, LDL-C, HDL-C.

Results: AH subjects demonstrated higher level of COPPs in serum, HDL and LDL+VLDL fractions and degree of oxidative modification of LDL in comparison with healthy persons. Decrease of PON1 activity and increase of MPO activity were observed in patients with AH in comparison with healthy persons. The levels of total cholesterol, LDL-C and HDL-C were within the normal range in patients with AH. CRP was also within the values characteristic of healthy individuals.

Conclusions: The accumulation of carbonyl oxidation protein products in blood, HDL and LDL eventually results in oxidative modification of HDL and LDL, and loss of its functional properties. The activity of HDL-associated enzymes (PON1 and MPO) is the most informative indicator of functional state of HDL, and not the level of HDL-C. Changes of MPO and PON1 activity may serve as a useful marker of dysfunctional HDL. Our evaluation showed a significant decrease of PON1 activity and increase of MPO activity that may contribute to the HDL oxidation, irrespective of HDL-C levels. A more sensitive marker of inflammation can serve as MPO activity, while the level of CRP would remain within the normal range, as has been shown in our research work. Demonstrated changes in the functional state of HDL, in our opinion, create a predisposition to development and progression of atherosclerosis in patients with AH.