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Cholesterol Efflux Potential and Anti-inflammatory Properties of HDL following Treatment with Niacin or Anacetrapib

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Abstract

Objective—This study examines the effects of treatments with niacin or anacetrapib (an inhibitor of cholesteryl ester transfer protein (CETP)) on the ability of HDL to promote net cholesterol efflux and reduce Toll-like receptor-mediated inflammation in macrophages.

Methods and Results—18 subjects received niacin 2g daily for 4 weeks, 20 subjects received anacetrapib 300mg daily for 8 weeks and 2 groups of 4 and 5 subjects, respectively received placebo. HDL samples were isolated by PEG precipitation or ultracentrifugation, tested for ability to promote cholesterol efflux in cholesterol-loaded THP-1 or mouse peritoneal macrophages, or used to pre-treat macrophages followed by LPS exposure. HDL cholesterol levels were increased by 30% in response to niacin and by ~100% in response to anacetrapib. Niacin treatment increased HDL-mediated net cholesterol efflux from foam cells primarily by increasing HDL concentration, whereas anacetrapib treatment increased cholesterol efflux both by increasing HDL concentration and by causing increased efflux at matched HDL concentrations. The increased efflux potential of anacetrapib-HDL was more prominent at higher HDL cholesterol concentrations (> 12μg/ml), associated with an increased content of lecithin:cholesterol acyltransferase (LCAT) and apolipoprotein E (apoE) and completely dependent on expression of ATP binding cassette transporters, ABCA1 and ABCG1. Potent anti-inflammatory effects of HDL were observed at low HDL concentrations (3–20 μg/ml) and were partly dependent on expression of ABCA1 and ABCG1. All HDL preparations showed similar anti-inflammatory effects, proportionate to HDL cholesterol concentration.

Conclusions—Niacin treatment caused a moderate increase in the ability of HDL to promote net cholesterol efflux while inhibition of CETP via anacetrapib led to a more dramatic increase in association with enhanced particle functionality at higher HDL concentrations. All HDLs exhibited potent ability to suppress macrophage TLR4-mediated inflammatory responses, in a process partly dependent on cholesterol efflux via ABCA1 and ABCG1.

Introduction

New therapies for raising HDL might be an effective way to reduce the significant burden of residual cardiovascular disease in patients treated with current lipid lowering therapies.[1–3] However, current approaches for raising HDL are limited. While niacin has been shown to reduce atherosclerotic cardiovascular disease in the Coronary Drug Project,[4] and probably to

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reduce atherosclerotic plaque in the coronary and carotid arteries in the FATS and Arbiter studies,[5,6] its ability to raise HDL cholesterol levels is often associated with troublesome side-effects such as flushing. New approaches to reduce niacin-induced flushing such as the combination of niacin with PGD_2 receptor (DP_1) antagonists are currently under evaluation.[7] Inhibitors of cholesteryl ester transfer protein (CETP) such as torcetrapib or anacetrapib, can produce higher elevations of HDL levels than niacin, up to 100%, as well as LDL lowering.[8–10] However, the failure of the CETP inhibitor torcetrapib in a large clinical trial (ILLUMINATE) has called this approach into question.[11,12] It is not known whether the excess of cardiovascular events and deaths occurring in subjects receiving torcetrapib was due to mechanism-related defects such as production of dysfunctional HDL or off-target toxicity. Although major off target toxicity of torcetrapib, involving increased BP and hyperaldosteronism, has been identified, it remains uncertain whether these or related toxicities,[13] were responsible for the adverse outcome.[3,4,14] Consequently, there has been great interest in developing assays or biomarkers that may reflect the underlying anti-atherogenic functions of HDL.[15]

A principal antiatherogenic property of HDL is thought to be its ability to promote cholesterol efflux from macrophage foam cells.[1, 3,16,17] We and others recently reported that large CE-rich HDL particles accumulating in CETP deficient or torcetrapib-treated subjects showed increased ability to promote cholesterol efflux from macrophage foam cells.[18–20] Central to these observations was the key role of LCAT and apolipoprotein E (apoE) present at high levels in CETP deficient and torcetrapib-treated subjects driving net cholesterol efflux in an ABCG1 dependent fashion.[18,19] The increased cholesterol efflux potential of HDL from torcetrapib-treated subjects that was observed at higher doses and higher HDL levels,[19] appeared to correlate with the finding that subjects receiving torcetrapib who had the largest increase in HDL levels on therapy also had significant regression of coronary atherosclerosis as assessed by IVUS. [21]

In addition to its role in promoting cholesterol efflux, HDL also exhibit anti-inflammatory properties in endothelial cells and macrophages that could contribute to its athero-protective effects.[3,17,22] Although multiple different mechanisms are likely to be involved, anti-inflammatory effects may in part be related to ABCA1 and ABCG1-mediated cholesterol efflux since cholesterol accumulation in the plasma membrane of *Abca1*$^{-/-}$, *Abcg1*$^{-/-}$ and *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ macrophages has been shown to increase signaling of Toll-like receptors enhancing the inflammatory response to LPS or other TLR ligands in an NF-κB dependent fashion.[23–26] Murphy et al.,[27] recently showed that anti-inflammatory effects of apoA-1 in macrophages were likely mediated by cholesterol efflux via ABCA1, but potential involvement of macrophage ABC transporters in anti-inflammatory effects of HDL particles have not been assessed.

The present study was undertaken to compare the cholesterol efflux potential of HDL accumulating in subjects treated with niacin or a new class of CETP inhibitor, anacetrapib, currently being assessed in phase 3 clinical trials.[10] A secondary goal of the study was to determine the consequences of these treatments on the anti-inflammatory potential of HDL in macrophages and the role of ABCA1 and ABCG1 transporters in mediating these effects.

Materials and Methods

For details, see the online data Supplement.

Study subjects

This study was conducted using archive plasma samples from 3 clinical trials (2 with niacin and 1 with anacetrapib). The niacin samples were from 2 different studies, the details of

which have been published previously.[28,29] Samples from a total of 22 dyslipidemic subjects were analyzed (18 subjects received Niaspan(tm) for 8–16 weeks with the last 4 weeks being at 2g/day, and 4 subjects received placebo for 8 weeks). Patients in these studies were permitted to be on stable background lipid-modifying therapy in addition to study medication. The anacetrapib samples were from a dose-ranging study in patients with primary hypercholesterolemia or mixed hyperlipidemia, the details of which have been previously published.[10] Samples from a total of 25 subjects were analyzed (20 subjects received anacetrapib at doses of 300mg daily and 5 subjects received placebo for 8 weeks). Blood was collected before and after treatment to allow comparison between each individual subject receiving treatments.

Results

Patient groups and HDL responses

Niacin treatment increased plasma concentrations of HDL by 28% compared to either baseline or placebo treatment (Supplemental Fig. IA) while 8-weeks anacetrapib treatment raised HDL cholesterol by approximately 100% (Supplemental Fig. 1B). In the niacin group, two subjects showed no change in HDL-C; these subjects were included in all analyses. At baseline, the different groups did not show significant differences with respect to demographic and lipid characteristics (Supplemental Table. I). Three independent pools of plasma from subjects receiving daily niacin before and after treatment, four pools of plasma from subjects receiving 300mg anacetrapib daily for 8 weeks before and after treatment and one pool of placebo control for each condition were made to isolate HDL particles by sequential density ultracentrifugation in order to have sufficient material to analyze HDL-2 protein composition (Supplemental Fig. II). Immunoblot analysis of PAFAH in individual pooled HDL-2 samples from patients before and after niacin or anacetrapib treatment did not reveal any differences (Supplemental Fig. IIA and IIC). The level of apoA-I was also not significanty changed in the different groups as expected because samples were matched for total protein content and apoA-I is the major protein of HDL (Supplemental Fig. IIA and IIC). By contrast, anacetrapib-HDL2 exhibited a significant mean 1.4-fold increase in apoE and LCAT proteins compared to control HDL-2 (Supplemental Fig. IIC and IID). These findings are consistent with the increased cholesterol ester content of anacetrpib-HDL2 (Supplemental Fig. IID) and with earlier findings using HDL samples from subjects with homozygous CETP deficiency or treatment with higher doses of torcetrapib (120mg).[8,18,19] Interestingly, similar changes were not observed for niacin-HDL (Supplemental Fig. IIA and IIB)

Effects of niacin and anacetrapib on HDL-mediated cholesterol efflux

We evaluated the cholesterol efflux potential of HDL particles isolated from subjects receiving niacin and anacetrapib. Dose response curves for cholesterol mass efflux mediated by HDL in PEG supernatants from niacin-treated subjects using 3 different concentrations of pooled HDL (i.e, 12, 36 and 72μg/mL cholesterol) showed similar levels of cholesterol efflux as control, pre-treatment HDL samples (Fig. 1A). By contrast, the dose response curve for cholesterol efflux using 3 different concentrations of pooled control and anacetrapib-HDL (i.e, 12, 36 and 72μg/mL cholesterol) showed increased cholesterol efflux compared to pre-treatment HDL, as reflected by increased cholesteryl ester (CE) and total cholesterol (TC) accumulation in media (Fig. 1B). As observed previously,[19] the increment over control for cholesterol efflux by anacetrapib-HDL tended to increase with increasing HDL concentration. At higher cholesterol concentration (i.e, 72μg/mL) there was also a significant increase in free cholesterol (FC) efflux with anacetrapib-HDL compared to control-HDL (Fig. 1B). The comparison between control-HDL at 36μg/mL cholesterol with anacetrapib-HDL at 72μg/mL cholesterol also allowed us to estimate an approximately 2.4-

fold increase in the efflux potential of anacetrapib-HDL matched by volume compared to control-HDL (Fig. 1B). To further test for any potential dysfunctional effects of niacin on HDL particles, we next performed a dose response curve for cholesterol efflux matched by sample volume. This showed increased cholesterol efflux for the niacin-HDL samples compared to controls that was significant at the two higher sample volumes, primarily reflecting increased CE accumulation in media (Fig. 1C).

To confirm these observations carried out in pooled samples, we measured cholesterol mass efflux using 18 individual determinations for the niacin group and 20 individuals determinations for the anacetrapib group. In Fig. 2, placebo, control and niacin-HDL were added to media for 8 hours using samples matched by volume. Niacin-HDL caused a significant 25% increase in total cholesterol (TC) accumulation in the media compared to the pre-treatment control HDL, while there was no change in cholesterol efflux for HDL samples obtained from individuals before or after placebo treatment (Fig. 2A and 2B). When control and anacetrapib-HDL were added at the same cholesterol concentration (50μg/mL), anacetrapib-HDL exhibited a significant 4-fold increase in CE accumulation in media and a significant 1.6-fold increase in TC efflux (Fig. 3). These findings suggested that both anacetrapib-HDL and niacin-HDL showed increased cholesterol efflux potential but the effect is more dramatic for the anacetrapib HDL and is likely to reflect both increases in particle functionality as well as concentration, while the latter reflects primarily an increase in HDL concentration.

Role of ABCA1 and ABCG1 in mediating net cholesterol efflux to control, niacin and anacetrapib-HDL

To assess the role of ABCA1 and ABCG1 in mediating the increased cholesterol efflux potential of anacetrapib- or niacin-HDL, we first carried out dose-response curves for both sets of samples, matched either by volume or by HDL-cholesterol concentration using WT and Abca1$^{-/-}$Abcg1$^{-/-}$ mouse peritoneal macrophages (Fig 4). These studies used [^{3}H]-cholesterol to measure efflux. Since both ABCA1 and ABCG1 are unidirectional cholesterol transporters,[30] the difference in percentage efflux between WT and Abca1$^{-/-}$Abcg1$^{-/-}$ cells is a direct measure of ABC transporter dependent net cholesterol efflux. These studies confirmed observations above, i.e. increased cholesterol efflux potential for niacin-HDL when samples were matched by volume and not concentration, and also showed increased cholesterol efflux potential for anacetrapib-HDL matched by either volume or concentration. The latter effect was not as marked as in the earlier study (Fig 1) probably because a lower range of HDL concentrations was used in Fig 4. Interestingly, the increased cholesterol efflux potential of both niacin- and anacetrapib HDL was completely dependent on expression of ABCA1 and ABCG1. We also confirmed a major role of ABCG1 in mediating the efflux potential of anacetrapib-HDL and the key role of LCAT in this process (Supplemental Fig. III).[18,19] Residual [^{3}H]-cholesterol efflux levels in *Abcg1*$^{-/-}$ and *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ cells, probably comprising components of both exchange and net efflux, were very similar for all HDL samples. Thus, macrophage ABCG1 and to a lesser extent ABCA1 have a key role in control-, niacin- and anacetrapib-HDL promoted cholesterol efflux and are required for increased cholesterol efflux potential of niacin- and anacetrapib-HDL.

Effects of niacin and anacetrapib-HDL on inflammatory responses mediated by toll-like receptor 4

We next compared the ability of the different HDL preparations to modulate the macrophage inflammatory response to TLR4 activation induced by lipid A treatment. To avoid potential confounding effects of direct binding of lipid A to HDL, we used a protocol in which cholesterol-loaded macrophages were pre-treated with HDL (matched by concentration or

volume), extensively washed then challenged with a lipid A stimulus. The inflammatory response was monitored by measuring the mRNA levels of a battery of cytokines and chemokines. In order to assess the potential dependence of anti-inflammatory effects of HDL on ABCA1 and ABCG1, we carried out experiments in WT and *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ macrophages. Results for niacin-HDL are shown in Fig 5 and for anacetrapib HDL in Fig 6. Treatment of cells with lipid A induced an inflammatory response as shown by increased mRNA expression of TNFα, MIP1α, MIP-2 and IL-6 in wild-type cells. As reported previously,[26] this response was exaggerated in *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ macrophages. All HDL samples showed a similar ability to suppress inflammatory responses, and the only significant difference observed was increased suppression of inflammation in WT macrophages by anacetrapib-HDL for samples matched by volume (Fig 6B and supplemental Fig IV), likely reflecting the marked increase in HDL concentration in these samples. As expected from the cholesterol efflux experiments (Fig 4), this ability was lost in *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ macrophages. Interestingly, HDL samples were still able to suppress inflammatory responses in *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ macrophages but in comparison to WT macrophages, higher concentrations or volumes of HDL were required to bring the inflammatory responses in *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ macrophages back towards the baseline (pre-lipid A) values.

Discussion

This study was undertaken to assess the functionality of HDL accumulating in subjects treated with extended release niacin or the CETP inhibitor, anacetrapib. Both niacin- and anacetrapib-HDL were functional in supporting cholesterol efflux from macrophage foam cells and in inhibiting inflammatory responses induced by toll-like receptor 4. However, only anacetrapib-HDL led to higher levels of cholesterol efflux when matched for particle cholesterol content. Increased functional capacity per particle was driven by an increase in media CE formation, and probably reflects an increased content of apoE and LCAT in anacetrapib-HDL. Anacetrapib-HDL also exhibited an increased ability to reduce the inflammatory response induced by toll-like receptor 4 ligand when samples were matched by volume. Although our macrophage assay only reflects one particular set of anti-inflammatory functions of HDL, these findings may help to allay concerns that CETP inhibitors induce formation of a pro-inflammatory HDL particle. On a mechanistic level our studies show for the first time that anti-inflammatory effects of HDL in macrophages are likely mediated in part by cholesterol efflux via ABCA1 and ABCG1.

While HDL from torcetrapib treated subjects only modestly increased macrophage cholesterol efflux at the 60 mg dose used in the ILLUMINATE study,[19] the efflux potential of HDL at the 300 mg anacetrapib dose appears to be larger. One likely reason is that the 300mg dose probably causes complete inhibition of CETP activity, as judged by the fact that HDL raising and LDL lowering of ancetrapib is equivalent to that produced by homozygous genetic CETP deficiency.[10,31] Niacin also increased cholesterol efflux potential but only when samples were matched by volume, reflecting the approximately 30% increase in HDL cholesterol levels without any increase in apoE and LCAT protein levels in HDL-2. While these subjects were also on lipid lowering therapies, the rise in HDL cholesterol levels has been previously attributed to a direct effect of Niacin.[5–7] By contrast, increased apoE and LCAT protein levels were observed in anacetrapib-HDL2 correlating with the finding of increased HDL particle cholesterol efflux potential. We cannot exclude that the storage of the samples may have modestly exaggerated these effects.[32] However, in the present study, HDL were isolated from subjects with triglycerides in the normal range and the increase in apoE in CETP-deficient HDL was originally shown in fresh unfrozen samples.[33] In earlier studies, we showed that removal of the apoE rich fraction from CETP deficient HDL greatly reduced its cholesterol efflux potential.[18] Similar differential effects on macrophage

cholesterol efflux were also seen comparing 120mg torcetrapib that induced increased amounts of apoE-rich HDL in most subjects and 60mg torcetrapib that did not in most subjects.[19] These results point to a role of apoE acting in conjunction with LCAT, possibly facilitating expansion of the CE core of HDL and permitting ongoing cholesterol efflux.[34–37] We also noted that the increased efflux potential of anacetrapib-HDL required the activity of ABCA1 and ABCG1 over a range of HDL cholesterol concentrations. The increased cholesterol efflux potential was more apparent at higher cholesterol concentrations (>12μg/mL). This could perhaps reflect dissociation of apoE from HDL particles in diluted samples.[38] Alternatively, it could reflect the relatively high apparent Km values for ABCG1-mediated cholesterol efflux (~30–50 μg phospholipid/ml),[30] and the dependence of the increased efflux potential on activity of ABCG1.[18]

Accumulating evidence suggests that by removing excess free cholesterol from macrophages, HDL exerts anti-inflammatory effects.[39, 40] Our findings suggest that HDL from subjects treated with either niacin or anacetrapib has the same ability as control-HDL to lower inflammation-induced by toll-like receptor 4 (TLR4) in macrophage foam cells when matched by HDL cholesterol concentration. This anti-infllammatory effect of HDL occurred at low HDL cholesterol concentrations consistent with a recent report from Murphy et al.[27] It is not clear why anti-inflammatory properties of HDL occur at such low HDL concentrations. One possibility is that pro-inflammatory receptors such as MD2-TLR4 complexes are very sensitive to small changes in membrane cholesterol content, perhaps reflecting disruption of lipid rafts.[26,27] Interestingly, maximum anti-inflammatory effects of anacetrapib-and other HDLs required activity of ABCA1 and ABCG1. Overall, anti-inflammatory effects of HDL in this study correlate well with cholesterol efflux data. Thus, over the concentration range where HDL exerted increasing anti-inflammatory effects (3 to 20 μg/ml), there were parallel increases in cholesterol efflux and both anti-inflammatory effects and cholesterol efflux were reduced in *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ macrophages. All types of HDL particles exerted anti-inflammatory effects in proportion to their cholesterol efflux properties in the same concentration range. Anacetrapib-HDL were more efficient anti-inflammatory agents matched by volume but not by concentration, mirroring the effects on cholesterol efflux in this low HDL concentration range. Together, this suggests that a major anti-inflammatory mechanism of HDL relates to cholesterol efflux via ABC transporters. HDL is still anti-inflammatory at higher HDL concentrations in *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ cells, probably reflecting less efficient passive cholesterol efflux mechanisms,[16] or possibly other anti-inflammatory properties of HDL.[41] Our findings showing that anti-inflammatory effects of HDL are partly mediated by cholesterol efflux via ABCA1 and ABCG1 links two previous sets of independent observations showing that HDL exhibit anti-inflammatory properties in macrophages,[27] and that activities of ABCA1/ABCG1 are anti-inflammatory.[24–26]

The ability of HDL and ABCA1/ABCG1 to suppress inflammation mediated by Toll receptor signaling is likely relevant to atherogenesis. Seminal studies have indicated roles of TLR4 and Myd88 in atherogenesis in hypercholesterolemic animal models.[42,43] There are likely be other anti-inflammatory effects of niacin and anacetrapib that were not assessed in this study. For instance, HDL may exhibit anti-inflammatory effects in endothelial cells by reducing the expression of VCAM-1 and ICAM-1 and leading to suppression of monocyte adhesion and transmigration across endothelium.[44–47] HDL may become pro-inflammatory in such assays, possibly reflecting accumulation of oxidized lipids or altered protein cargo.[48–51] McGrath et al., recently proposed a novel ant-inflammatory effect of HDL mediated by an upregulation of 3β-hydroxysteroid-δ 24 reductase (DHCR24) that inhibits the nuclear factor-kappa B (NF-κB) binding site.[52] Other effects include the modulation of the protein content of paraoxonase (PON), glutathione peroxidase or other complement proteins directly in HDL particles.[41,48] At least, PAFAH protein content in HDL-2, known to exert anti-

inflammatory property such as inhibition of the biological activity of minimally oxLDLs and and associated reduction in dendritic cell migration,[53,54] was unchanged by either niacin or anacetrapib treatment in this study.

In the ILLUMINATE study that used the CETP inhibitor torcetrapib to increase HDL levels, there was an unexpected excess of deaths from acute sepsis.[3,14] Although this result could reflect off-target toxicity of torcetrapib, it is also possible that the HDL-mediating dampening of the acute phase response during sepsis, as reflected in its anti-inflammatory properties measured in this study, could lead to an adverse outcome.

To summarize, our data indicate that there was no evidence for an adverse effect of niacin or anacetrapib on HDL-mediated cholesterol efflux or anti-inflammatory responses using our assays. Whether such findings can be translated into clinical benefit can only be answered by ongoing randomized clinical trials involving niacin, anacetrapib and dalcetrapib.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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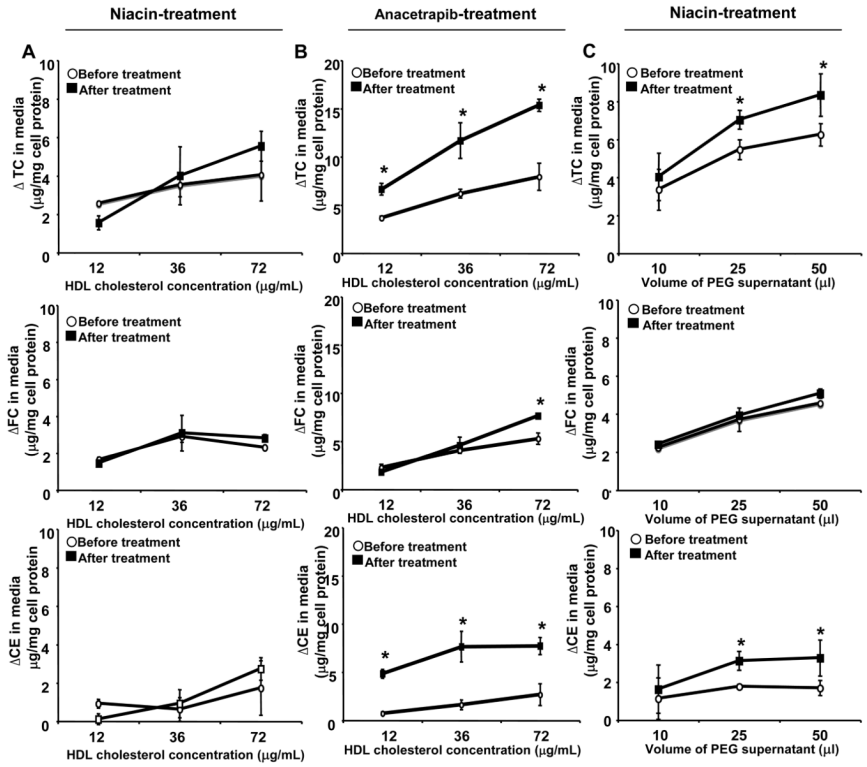

Figure 1.

Dose-response curve for cholesterol efflux induced by similar concentration or volume of control and PEG-HDL post post-niacin and –anacetrapib treatment. THP-1 macrophage were treated with 50μg/mL acLDL and 3μmol/L TO901317 for 24h. Then, increased concentrations of pooled PEG-HDL (12, 36, 72μg/mL cholesterol) from niacin treatment (A) and anacetrapib treatment (B) or increased volumes of pooled control PEG-HDL (34±4mg/dL cholesterol) and niacin-PEG-HDL (45±4mg/dL cholesterol) (C) were added to media for 6h before TC, FC and CE mass analysis. Values are means±SEM of an experiment performed in triplicate. **P*<0,05, significant difference vs control PEG-HDL.

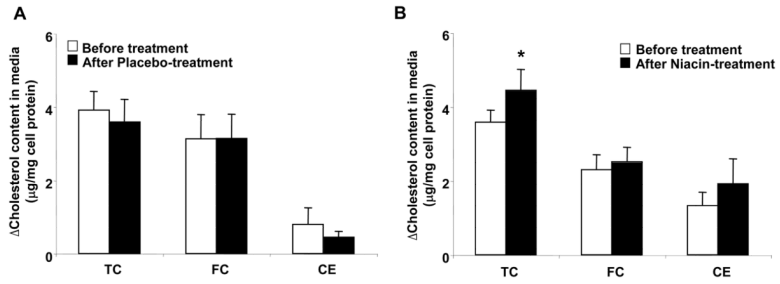

Figure 2.

Effect of niacin-PEG-HDL on cholesterol efflux from human THP-1 macrophage foam cells treated with 50μg/mL acLDL and 3μmol/L TO901317 for 24h. The TC, FC, and CE mass in media were determined 6h after incubation of 25μl of placebo PEG-HDL (35±4mg/dL and 35±2mg/dL cholesterol before and after treatment, respectively) (A) or niacin-PEG-HDL (34±4mg/dL and 45±4mg/dL cholesterol before and after treatment, respectively) (B) in 500μl of media. Values are means±SEM of 5 individuals determinations for niacin placebo groups and 18 individuals determinations for niacin group. *P<0,05, significant difference vs control PEG-HDL.

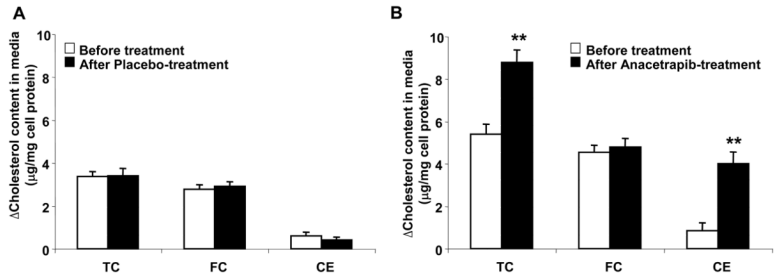

Figure 3.

Effect of anacetrapib-PEG-HDL on cholesterol efflux from human THP-1 macrophage foam cells treated with 50μg/mL acLDL and 3μmol/L TO901317 for 24h. The TC, FC, and CE mass in media were determined 6h after incubation of similar concentration of PEG-HDL (50μg/mL cholesterol) from placebo (C) or anacetrapib group (D) before and after 2 week treatment. Values are means±SEM of 5 individuals determinations for anacetrapib placebo groups and 20 individuals determinations for anacetrapib group. ***P*<0.001, significant difference vs control PEG-HDL.

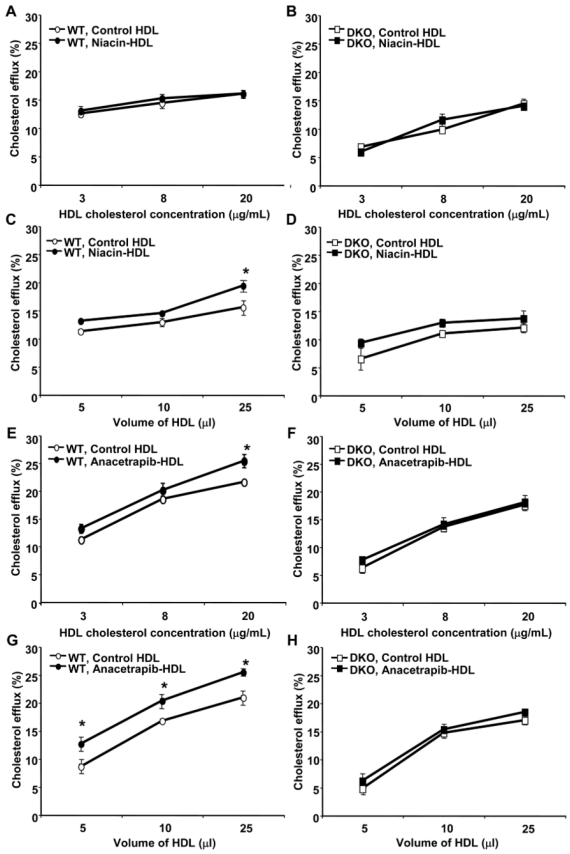

Figure 4.

Dose response curve for cholesterol efflux induced by similar concentration or volume of ultracentrifugated control and HDL-2 post-niacin and -anacetrapib treatment in WT and *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ macrophages. Bone-marrow-derived macrophages from WT and *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ mice were treated for 16h with 50μg/mL acLDL and 2μCi/mL of [3H]-cholesterol. Then, increased concentrations of pooled HDL-2 (3, 8, 20μg/mL cholesterol) from niacin treatment (A and B) and anacetrapib treatment (E and F) or increased volumes of pooled control HDL-2 (34±4mg/dL and 38±2mg/dL cholesterol for control niacin and control anacetrapib, respectively), niacin-HDL-2 (45±4mg/dL cholesterol, C and D) and anacetrapib-HDL-2 (81±4mg/dL cholesterol, G and H) were added to 500μl media for 3h before isotopic cholesterol efflux. Control, niacin and anacetrapib-HDL significantly increased cholesterol efflux along with enhanced concentration or volume of HDL. Values are means±SEM of an experiment performed in triplicate. **P*<0.05, significant difference vs control HDL-2.

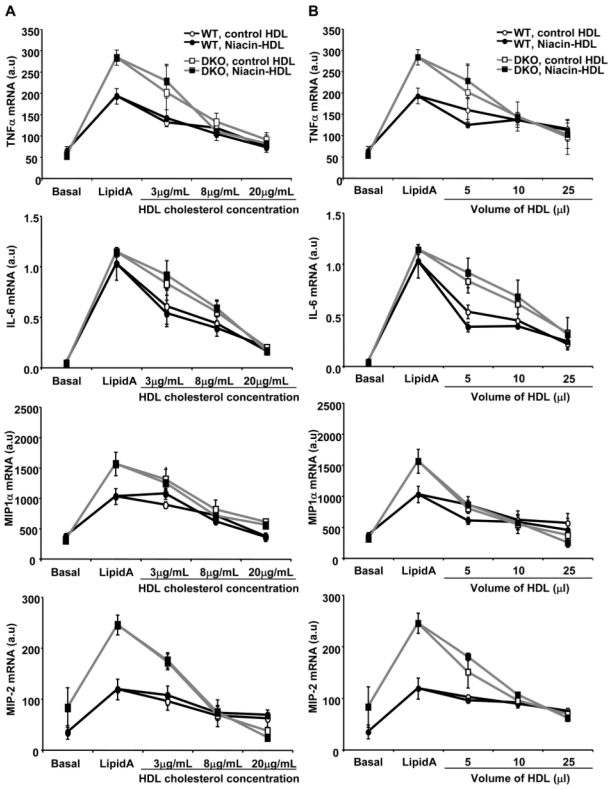

Figure 5.

Dose response curve for suppression of toll-like receptor 4-mediated inflammation induced by similar concentration or volume of ultracentrifugated control and niacin-HDL in WT and *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ macrophages. Bone-marrow-derived macrophages from WT and *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ mice were treated for 16h with 50μg/mL acLDL. Then, increased concentrations of pooled HDL-2 (3, 8, 20μg/mL cholesterol) from niacin treatment (A) or increased volumes of pooled control HDL-2 (34±4mg/dL cholesterol) and niaicn-HDL-2 (45±4mg/dL cholesterol) (B) were added to 500μl of 0.2%BSA media for 3h before LipidA treatment for 3h more hours as described in the Methods. At the end of the incubation, inflammatory transcript levels (TNFα, IL-6, MIP1α and MIP2) were quantified and normalized to m36B4. Results are means±SEM and expressed as arbitrary units (a.u) from an experiment performed in triplicate. **P*<0,05, significant difference vs control HDL-2.

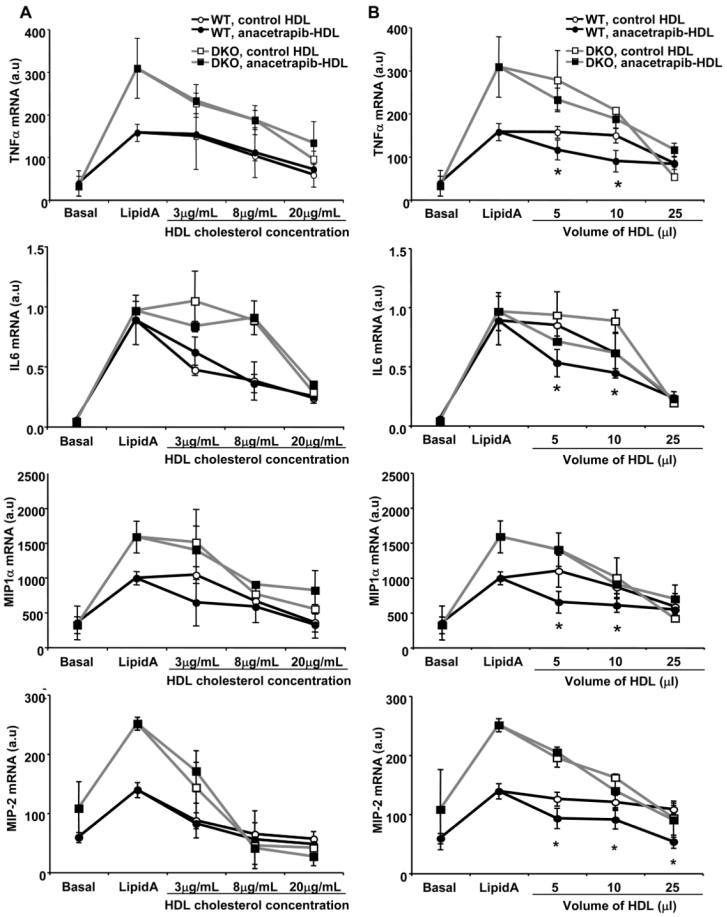

Figure 6.

Dose response curve for suppression of toll-like receptor 4-mediated inflammation induced by similar concentration or volume of ultracentrifugated control and anacetrapib-HDL in WT and *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ macrophages. Bone-marrow-derived macrophages from WT and *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ mice were treated for 16h with 50μg/mL acLDL. Then, increased concentrations of pooled HDL-2 (3, 8, 20μg/mL cholesterol) from 300mg anacetrapib treatment (A) or increased volumes of pooled control HDL-2 (38±2mg/dL cholesterol) and anacetrapib-HDL-2 (81±4mg/dL cholesterol) (B) were added to 500μl of 0.2%BSA media for 3h before LipidA treatment for 3h more hours as described in the Methods. At the end of the incubation, inflammatory transcript levels (TNFα, IL-6, MIP1α and MIP2) were quantified and normalized to m36B4. Results are means±SEM and expressed as arbitrary units (a.u) from an experiment performed in triplicate. **P*<0,05, significant difference vs control HDL-2.