Epoxycholesterol Impairs Cholesteryl Ester Hydrolysis in Macrophage Foam Cells, Resulting in Decreased Cholesterol Efflux

Mireille Ouimet, Ming-Dong Wang, Natalie Cadotte, Kenneth Ho, Yves L. Marcel

Objective—Strategies to inhibit or reverse cholesterol accumulation in macrophages have been shown to be atheroprotective. Notably, the administration of LXR agonists upregulates key players in the reverse cholesterol transport pathway, including the ABCA1 and ABCG1 transporters. However, the effects of natural LXR activators, oxysterols, on lipid-laden macrophages remains elusive.

Methods and Results—We assessed the ability of 2 oxysterols, 22(R)-hydroxycholesterol (22-OH) and 24(S),25-epoxycholesterol (epoxycholesterol), to promote cholesterol efflux to apoA-I from LDL- and modified LDL-labeled and loaded macrophages and thus rescue the phenotype associated with the accumulation of cellular cholesterol in these cells. In macrophages labeled with LDL-derived cholesterol, epoxycholesterol treatment enhances ABCA1-mediated cholesterol efflux. In contrast, in AcLDL-loaded macrophages, epoxycholesterol treatment decreases cholesterol efflux to apoA-I, despite a dramatic increase in the expression of ABCA1 in response to epoxycholesterol treatment. We show that the decreased efflux is attributable to impaired cholesterol mobilization from lipid droplets, resulting from decreased cholesteryl ester hydrolysis activity.

Conclusion—Epoxycholesterol impairs cholesteryl ester hydrolysis activity in macrophage foam cells, thus reducing the availability of cholesterol for efflux to cholesterol acceptors. (Arterioscler Thromb Vasc Biol. 2008;28:1144-1150)

Key Words: macrophage • epoxycholesterol • cholesteryl ester hydrolysis • cholesterol • ABCA1

Whereas low density lipoprotein (LDL) endocytosis is a regulated process, the uptake of modified LDL via macrophage scavenger receptors1 or via macropinocytosis2 lacks feedback inhibition by intracellular cholesterol accumulation. The internalization of modified LDL, such as oxidized LDL (OxLDL), acetylated LDL (AcLDL), or aggregated LDL (AgLDL), leads to the formation of foam cells,3,4 which are a major component of atherosclerotic lesions.

The liver X receptors (LXRs) α and β, members of the nuclear receptor family of transcription factors, are key regulators of whole body lipid homeostasis. LXR-β is expressed ubiquitously whereas LXR-α is predominantly expressed in tissues important in lipid metabolism including liver, adipose tissue, and macrophages.5,6 The naturally occurring oxysterols 22(R)-hydroxycholesterol (22-HC) and 24(S),25-epoxycholesterol (epoxycholesterol) are endogenous LXR ligands and have been shown to be potent physiological activators of both LXRs.7,8 LXR function by forming an obligate heterodimer with the retinoid X receptor (RXR), and this complex drives LXR-dependent transactivation of multiple genes involved in cellular cholesterol homeostasis, including ABCA1, ABCG1, and apoE in the lipid efflux pathway, which normally prevent cholesterol accumulation as intracellular lipid droplets.9

Given that ABCA1-mediated cholesterol efflux to lipid-poor apolipoprotein A-I (apoA-I) is the preferred efflux pathway of the macrophage foam cell,10 we sought to promote this pathway in cholesterol-loaded cells using 22-HC and epoxycholesterol. Oxysterols have been shown to be beneficial for enhancing cholesterol efflux6,11–13 and preventing foam cell formation,14 but it is not established whether they are similarly effective when macrophages become cholesterol-loaded. Large quantities of excess lipoprotein-derived cholesterol are esterified by the ER-resident protein, acyl-coenzyme A (CoA):cholesterol acyl transferase (ACAT) and stored as cholesteryl esters (CE) in cytoplasmic lipid droplets.15 Unesterified cholesterol can be released from the lipid droplets via CE hydrolysis, and subsequently effluxed to a cholesterol acceptor or reesterified by ACAT.16

Unexpectedly, we found that whereas 22-HC enhanced cholesterol efflux from AcLDL-loaded macrophages, epoxycholesterol promoted cholesterol efflux only in unloaded or LDL-labeled macrophages. Despite inducing a dramatic increase in ABCA1 protein, epoxycholesterol impaired CE
Epoxycholesterol Reduces Lipid Efflux From Foam Cells

Ouimet et al

Molecular and Cellular Biology

hydrolysis in lipid droplets of macrophage foam cells and reduced efflux to apoA-I, resulting in a net increase of CE and exacerbation of the foam cell phenotype.

Materials and Methods

Materials

Oxysterols: 22(R)-hydroxycholesterol, 22(S)-hydroxycholesterol, and 24(S),25-epoxycholesterol (Steraloids Inc). DMEM and RPMI 1640 medium (Invitrogen/Gibco), and Penicillin/Streptomycin (P/S) (Cambrex Bio Science). Fetal bovine serum (FBS), bovine serum albumin (BSA), and phosphol 12-myristate 13-acetate (PMA) were purchased from Sigma. Radioactive compounds: [1,2-3H]-Cholesterol, [5-3H(N)]mevalono-lactone-Rs, [3H]-Oleate, and [3H]-Acetic acid (PerkinElmer Life Sciences). Human recombinant apoA-I was produced as previously described.19 The Sandoz 58 to 035 ACAT inhibitor was a kind gift from Novartis (Basel, Switzerland).

Cell Culture

Bone marrow–derived macrophages (BMDM): hematopoietic stem cells were flushed from the femurs of C57Bl6 mice (Jackson Laboratories) and differentiated into mature macrophages by incubation in DMEM media supplemented with 10% FBS, 1% P/S, and 15% L929-conditioned medium for 7 days. Monocyte-derived macrophages (MDM): mononuclear cells were isolated from the blood of normolipemic volunteers, and differentiated to macrophages, as previously described.19,20 THP-1 human monocytes (ATCC) were cultured in complete growth medium (RPMI 1640) supplemented with 10% FBS, 0.05 mmol/L 2-mercaptoethanol, and 1% P/S), and were differentiated by treatment with PMA (100 nmol/L) for 7 days.

Lipoprotein Preparation

Plasma was collected from normolipemic volunteers. LDL and HDL lipoproteins were isolated by sequential density ultracentrifugation, as previously described.18 THP-1 human monocytes (ATCC) were differentiated for 24 hours, then treated with oxysterols overnight. Cells were washed with PBS, scrapped with lysis buffer (Tris-EDTA-EGTA+Complete protease inhibitor; Roche) and mechanically homogenized. Total protein samples (25 μg per well) were electrophoresed on a precast 8% SDS-polyacrylamide gel (Invitrogen) and transferred to nitrocellulose membranes at 125V for 4 hours. Membranes were incubated overnight with anti-ABCA1 (1:500, Novus Biologicals), anti-ABCG1 (1:2500, Novus Biologicals), anti-β-Actin (1:500, BioLegend), or anti–Heat Shock Protein 60 (HSP60) (1:500, Sigma). An anti-rabbit secondary antibody conjugated with horseradish peroxidase (Amersham Biosciences) and SuperSignal West Pico Chemiluminescent Substrate (Pierce) were used for detection.

Statistical Analysis

Results are shown as mean±SEM, and all experiments were run in triplicates. The statistical significance of the differences between groups was determined using Student t test with or without Welch correction or 1-way ANOVA with Tukey post test using GraphPad InStat v.3.06 statistical analysis software (GraphPad Software Inc).

Results

Epoxycholesterol Promotes Cholesterol Efflux to Lipid-Poor ApoA-I in LDL-Treated But Not in AcLDL-Loaded Macrophages

Bone marrow–derived macrophages (BMDM) were labeled with 3H-mevalonate in the presence of oxysterols for 48 hours, after which efflux of de novo synthesized cholesterol to apoA-I was measured. In unloaded macrophages (not preincubated with lipoproteins), both 22-HC and epoxycholesterol increased efflux of newly synthesized cholesterol (Figure 1A). Epoxycholesterol, which has a higher affinity for LXR than 22-HC,26 was a stronger inducer of efflux of newly synthesized cholesterol to apoA-I. The observed increase in cholesterol efflux was not attributable to an increase of cholesterol synthesis in response to treatment with oxysterols, because total cholesterol synthesis was decreased in cells treated with oxysterols (data not shown). This is in agreement with the well-documented downregulation of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase by oxysterols.27-29

The effect of oxysterols on cholesterol efflux in macrophage foam cells has not been well characterized. Here in BMDM foam cells generated by incubating with AcLDL, the net cellular cholesterol level (μg/mg cell protein) was 8.7-fold higher compared to unloaded macrophages (488±18 versus 56±5). As expected, because of the feedback inhibition of the LDL receptor, the cholesterol content of LDL-labeled macrophages was similar to that of unloaded macro-
phages (63 ± 9 compared to 56 ± 3). Surprisingly, epoxycholesterol decreased cholesterol efflux to apoA-I in AcLDL-loaded foam cells, whereas 22-HC had a stimulatory effect (Figure 1B). This efflux reduction was also observed in macrophages treated with oxLDL and agLDL, 2 other well-known foam cell inducers, but not in LDL-treated macrophages. Epoxycholesterol not only decreased cholesterol efflux in murine macrophage foam cells, but also in human THP-1 macrophages (Figure 1C) and in primary human monocyte-derived macrophages (MDM; Figure 1D) loaded with AcLDL-derived cholesterol.

In unloaded or LDL-treated macrophages, epoxycholesterol stimulated cholesterol efflux, suggesting that the reduction of efflux in response to epoxycholesterol is specific to the macrophage foam cell (Figure 1B). Indeed, when macrophages were loaded with increasing amounts of AcLDL-derived cholesterol (Figure 2A), epoxycholesterol concomitantly reduced cholesterol efflux to apoA-I proportionally to the amount of AcLDL internalized into the cell (Figure 2B).

Induction of ABCA1 and ABCG1 Expression by Epoxycholesterol Treatment in AcLDL-Loaded Macrophages Is Not Associated With Enhanced ABCA1- and ABCG1-Mediated Cholesterol Efflux

In AcLDL-loaded BMDM, we observed a dose-dependent decrease of cholesterol efflux to lipid-poor apoA-I in response to epoxycholesterol treatment (Figure 3A). Another member of the ATP-binding cassette (ABC) family, ABCG1, has previously been described as a LXR-responsive gene13,30 and has an established role in the efflux of cholesterol to high density lipoprotein (HDL) acceptors.31,32 Interestingly, increasing doses of epoxycholesterol also correlated with a decrease in cholesterol efflux to HDL in AcLDL-loaded BMDM (Figure 3B), indicating that epoxycholesterol does not uniquely affect the ABCA1-mediated pathway. Rather, epoxycholesterol seems to impair cholesterol efflux regardless of the cholesterol acceptor.

The decline of cholesterol efflux occurred in spite of the upregulation of the transporters implicated in these processes. Expression of both ABCA1 (Figure 3C) and ABCG1 (Figure 3D) was induced by epoxycholesterol, in a dose-dependent fashion, and translocation of the transporters to the plasma membrane was not hindered (data not shown).

**Epoxycholesterol Impairs the Mobilization of Cholesterol**

Excess cellular cholesterol is stored as CE, which can be mobilized for efflux by CE hydrolase activity. We therefore evaluated the effects of 22-HC and epoxycholesterol on CE stores. In unloaded BMDM, epoxycholesterol dramatically increased the proportion of newly synthesized cholesterol in CE pools (Figure 4A). Epoxycholesterol also promoted the accumulation of CE in BMDM incubated with LDL, AcLDL, OxLDL, and AgLDL (Figure 4B). In contrast, treatment with 22-HC did not induce a comparable rise in cellular CE. The relative changes in CE levels shown in Figure 4A and 4B

![Figure 1. Epoxycholesterol promotes cholesterol efflux in unloaded but not in lipid-laden macrophages. Efflux of newly synthesized cholesterol (A), and lipoprotein-derived cholesterol (B, C, D) to lipid poor apoA-I in murine (A, B) and human (C, D) macrophages, in response to treatment with oysterols. **P<0.0001, *P<0.001 compared to control.](http://atvb.ahajournals.org/)

![Figure 2. Epoxycholesterol-mediated decrease in cholesterol efflux is inversely proportional to the increasing macrophage cholesterol load. Increasing amounts of intracellular cholesterol (A) leads to inversely proportional decreases in cholesterol efflux (B). Results are expressed as a fold-change of the efflux of the epoxycholesterol-treated cells relative to controls. P<0.0001 (1-way ANOVA).](http://atvb.ahajournals.org/)
were indeed representative of net CE accumulation, as confirmed by direct measurement of esterified cholesterol cellular content (Figure 4C and 4D). Additionally, epoxycholesterol enhanced cellular CE mass in THP-1 macrophages in both unloaded and AcLDL-loaded cells (Table).

Because the accumulation of CE was specifically attributable to epoxycholesterol treatment, we speculated that this might be implicated in the impairment of cholesterol efflux from epoxycholesterol-treated macrophage foam cells. One of two scenarios (or perhaps a combination of both) could be proposed: Epoxycholesterol promotes the esterification of cholesterol via stimulation of ACAT, or epoxycholesterol decreases CE hydrolysis. In both, availability of free cholesterol for efflux to apoA-I would be decreased. To distinguish between the two, we performed a series of experiments in the presence of an ACAT inhibitor, which was added at key times during the experiments.

First, the ACAT inhibitor was added during treatment with epoxycholesterol, after the cells had been incubated with 3H-cholesterol-AcLDL for 24 hours. Subsequently, efflux to apoA-I was measured for 5 hours. Whereas the ACAT inhibitor-treated cells displayed increased cholesterol efflux, the addition of epoxycholesterol increased but failed to restore efflux to apoA-I (Figure 5A), indicating that the ability of epoxycholesterol to reduce cholesterol efflux in macrophage foam cells is largely independent of cholesterol esterification.

Next, the ACAT inhibitor was administered during labeling of BMDM with LDL- or AcLDL-derived 3H-cholesterol for 6 hours, as well as during treatment with epoxycholesterol (12 hours) and efflux to apoA-I (5 hours). Under these conditions, cholesterol esterification in the ER for storage in the lipid droplet was inhibited, and essentially

---

**Table. Cholesterol and Cholesteryl Ester Levels in Human THP-1 Macrophages**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Total Cholesterol (µg/mg cell protein)</th>
<th>Cholesteryl Ester (µg/mg cell protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unloaded</td>
<td>Control 102 ± 6</td>
<td>3 ± 1</td>
</tr>
<tr>
<td></td>
<td>22-HC 97 ± 5</td>
<td>5 ± 2</td>
</tr>
<tr>
<td></td>
<td>Epoxy 132 ± 3***</td>
<td>49 ± 2***</td>
</tr>
<tr>
<td>AcLDL-loaded</td>
<td>Control 386 ± 23</td>
<td>138 ± 7</td>
</tr>
<tr>
<td></td>
<td>22-HC 384 ± 24</td>
<td>139 ± 4</td>
</tr>
<tr>
<td></td>
<td>Epoxy 450 ± 24*</td>
<td>177 ± 11**</td>
</tr>
</tbody>
</table>

THP-1 macrophages were incubated for 24 hours in medium containing AcLDL (50 µg/mL) or not. Cells were treated with 22(R)-hydroxycholesterol or 24(S),25-epoxycholesterol (10 µmol/L) for 12 hours, after which cellular cholesterol mass was measured by fluorometric assay.

*P<0.05, **P<0.01, ***P<0.005, or ****P<0.0001.
all of the lipoprotein-derived $^3$H-cholesterol remained unesterified (Figure 5B). We observed an increase in both LDL- and AcLDL-derived cholesterol efflux in response to epoxysterol treatment (Figure 5C), demonstrating that epoxysterol does not hinder efflux of unesterified cholesterol, and mediates its effect downstream of cholesterol esterification in the ER.

Finally, to directly assess the degree of CE hydrolysis in response to epoxysterol treatment, BMDM were incubated with unlabaled AcLDL in the presence of $^3$H-oleate for 24 hours, such that AcLDL-derived cholesterol could be esterified to the radio-labeled oleate. Subsequent treatment with epoxysterol in the presence of an ACAT inhibitor and apoA-I resulted in equivalent CE cellular content as epoxysterol treatment alone (Figure 5D), therefore allowing us to conclude that epoxysterol treatment impairs mobilization of cholesterol from the CE pools. Specifically, epoxysterol decreases CE hydrolysis from the lipid droplet, and reduces the availability of free cholesterol for efflux to lipid-poor apoA-I or HDL.

Discussion

Because activated LXRs induce the expression of numerous genes involved in the reverse cholesterol transport pathway, notably ABCA1 and ABCG1, they have become attractive targets for pharmacological treatment of atherosclerosis. Whereas the therapeutic potential of LXR activation has been demonstrated by the targeted disruption of LXR in macrophages in athero-susceptible mice, systemic LXR activation demonstrated by the targeted disruption of LXR in macrophages, leads to a reduction of cholesterol efflux and accumulation of CE in lipid droplets. We thus conclude that epoxysterol reduces cholesterol availability for efflux via downregulation of CE hydrolysis. The exact identity of the enzymes that catalyze CE lipolysis in macrophages remains ambiguous. Whereas the presence of the hormone-sensitive lipase (HSL) in human macrophages is controversial, a neutral CEH gene was cloned from THP-1 and PBMC libraries, the overexpression of which enhanced both ABCA1- and ABCG1-mediated efflux. We have investigated whether epoxysterol regulates the expression of this human CEH in THP-1 macrophages. Our results indicate that epoxysterol does not decrease cholesterol efflux via transcriptional regulation of CEH expression in human macrophages (supplemental Figure IA, available online at http://atvb.ahajournals.org).

Interestingly, we were able to detect HSL mRNA in THP-1 macrophages using the primers designed by Reue et al. However, we did not observe any significant change in HSL mRNA in our treated versus untreated cells (supplemental Figure IB). Hence, changes in HSL expression at the transcriptional level cannot explain the decrease in CE hydrolysis measured in epoxysterol-treated cells. It is, however, unlikely that HSL plays a major role in the CE hydrolysis in these THP-1 macrophages given that its mRNA levels were strikingly lower, by as much as 20 000-fold, compared to CEH mRNA. Conversely, as one early study has shown, for certain hydrolases there is little correlation between mRNA levels and lipolytic activity.

It remains to be determined how epoxysterol decreases CE hydrolysis in lipid-loaded macrophages. We do know that this occurs independently of LXR activation, because the simultaneous treatment of lipid-loaded THP-1 cells with epoxysterol and an LXR antagonist does not increase cholesterol efflux, and the addition of an LXR synthetic agonist in conjunction with epoxysterol fails to rescue the efflux defect (supplemental Figure II). Although epoxysterol does not reduce CE lipolysis in lipid-loaded macrophages, CE hydrolysis in response to epoxysterol treatment in macrophage foam cells occurred despite the significant upregulation of the ABCA1 and ABCG1 transporters (Figure 3C and 3D), in agreement with earlier reports of oxysterol stimulation of ABCA1 and ABCG1 expression. Interestingly, it has previously been shown that in macrophage foam cells 7-ketocholesterol impairs cholesterol efflux to apoA-I, compared with macrophages loaded with AcLDL alone, thus indicating that this oxysterol also inhibits reverse cholesterol transport in AcLDL-loaded macrophages.

CE stored in cytoplasmic lipid droplets must be mobilized by enzymes with CE hydrolytic activities before export of cholesterol from the cell via ABCA1- and ABCG1-mediated efflux. We have found that epoxysterol decreases cholesterol efflux downstream of the ER-resident enzyme responsible for the esterification of cholesterol (Figure 5A through 5C). When esterification of cholesterol for storage in the lipid droplets was inhibited via the administration of an ACAT inhibitor, epoxysterol enhanced efflux of both LDL- and AcLDL-derived cholesterol. In contrast, epoxysterol treatment in the presence of the ACAT inhibitor, after macrophage lipid-loading, leads to a reduction of cholesterol efflux and accumulation of CE in lipid droplets. We thus conclude that epoxysterol reduces cholesterol availability for efflux via downregulation of CE hydrolysis.
macrophages via transcriptional downregulation of CEH or HSL, the possibility of altered posttranslational regulation of these enzymes remains to be investigated, as well as the contribution of other candidate enzymes for CE hydrolysis in these macrophages. It is worth to note that other lipid droplet associated proteins, such as perilipin and adipocyte differentiation-related protein (ADRP), were reported to influence lipid hydrolysis by regulating the accessibility of the hydrolytic enzymes to lipids within the droplet core. The examination of the effects of epoxycholesterol on these proteins is underway.

Potential LXR agonists for therapeutic intervention in atherosclerosis need to be selected with extreme caution. As shown here, an increase in the expression of ABCA1 and ABCG1 transporters does not necessarily translate into enhancement of cholesterol efflux from the macrophage. We have observed that once the macrophage becomes cholesterol-loaded, its response to oxysterols differs than that observed in unloaded and LDL-loaded macrophages. Our results indicate that 22-HC is better capable of promoting cholesterol efflux in lipid-laden macrophages, whereas epoxycholesterol appears to impair this process. In conclusion, epoxycholesterol elicits cholesterol efflux from noncholesterol loaded cells but aggravates the phenotype of the macrophage foam cell. Hence, epoxycholesterol may have quite different effects on the prophyllaxis versus regression of atherosclerosis.

Acknowledgments
We thank Vivian Franklin for expert technical support and Drs R. McPherson and R. Milne for critical reading of this manuscript.

Sources of Funding
This work was supported by grants from Canadian Institutes of Health Research and the Heart and Stroke Foundation of Ontario to Y.L.M. M.O. was the recipient of a Heart and Stroke Foundation of Ontario Master’s Studentship Award.

Disclosures
None.

References


Epoxycholesterol Impairs Cholesteryl Ester Hydrolysis in Macrophage Foam Cells, Resulting in Decreased Cholesterol Efflux
Mireille Ouimet, Ming-Dong Wang, Natalie Cadotte, Kenneth Ho and Yves L. Marcel

Arterioscler Thromb Vasc Biol. 2008;28:1144-1150; originally published online March 27, 2008;
doi: 10.1161/ATVBAHA.107.157115
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/28/6/1144

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2008/05/21/ATVBAHA.107.157115.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org/subscriptions/
Supplementary Material to:

Epoxycholesterol impairs cholesteryl ester hydrolysis in macrophage foam cells, resulting in decreased cholesterol efflux

Mireille Ouimet, Ming-Dong Wang, Natalie Cadotte, Kenneth Ho and Yves L. Marcel
Supplementary Figure 1. CEH and HSL mRNA levels in THP-1 macrophages. THP-1 macrophages were incubated for 24h in medium (1% FBS, 1% P/S, RPMI 1640) containing AcLDL (50µg/mL) or not. Cells were washed, and equilibrated in 2mg/mL BSA RPMI containing 22(R)-hydroxycholesterol or 24(S),25-epoxycholesterol (10µM) for 12h. Cells were washed twice with PBS, and mRNA was extracted and measured by quantitative real-time PCR. Expression of the human macrophage cholesteryl ester hydrolase (CEH) and hormone sensitive lipase (HSL) were normalised to that of the internal control GAPDH. The two-tailed p-value from student’s $t$ test from oxysterol-treated cells compared to their respective controls (unloaded and AcLDL-loaded cells) is $<0.0001$ (**) or $<0.05$ (*), or from AcLDL-loaded oxysterol-treated cells versus unloaded control cells $<0.005$ (#).
Supplementary Figure 2. Administration of an LXR antagonist does not affect cholesterol efflux in epoxycholesterol-treated macrophages. Human THP-1 macrophages were incubated for 24h with AcLDL (50µg/mL) containing ³H-cholesterol. Cells were washed, and treated overnight with 22(R)-hydroxycholesterol (10µM), 22(S)-hydroxycholesterol (10µM), or both (10µM each) (A), 24(S),25-epoxycholesterol (10µM), 22(S)-hydroxycholesterol (10µM) or both (10µM each) (B), or 24(S),25-epoxycholesterol (10µM), T901713 (5µM) or both (10µM Epoxy, 5µM T901713) (C). Cholesterol efflux was measured in the presence or absence of human apoA-I (50µg/mL). Efflux was calculated as a percentage of total medium count relative to the total amount of radioactivity incorporated by the macrophages for the control and epoxycholesterol-
treated cells, as described under Methods. The two-tailed p-value from student’s t test from treated cells compared to control is <0.0001 (\textasteriskcentered), or oxysterol alone compared to oxysterol + 22(S)-hydroxycholesterol (or oxysterol + T901713) is <0.001 (\texthash).