Expression of cholesterol-7α-hydroxylase in murine macrophages prevents cholesterol loading by acetyl-LDL

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Abstract **Unlike macrophages, the hepatic parenchymal cells express cholesterol-7α-hydroxylase (CYP7A1) which regulates the conversion of cholesterol into bile acids, the major quantitative pathway maintaining cholesterol homeostasis. We examined if CYP7A1 expression in RAW 264.7 macrophages could prevent the accumulation of cholesterol when they were incubated with acetyl-LDL. A single cell clone (designated as 7αRAW cells) that stably expresses rat CYP7A1 displayed similar rates of growth as non-transfected RAW cells. The CYP7A1 enzymatic activity expressed by microsomes obtained from 7αRAW cells was similar to that expressed by microsomes obtained from mouse liver. Incubating non-transfected RAW with increasing amounts of acetyl-LDL caused a parallel accumulation of cholesterol, whereas 7αRAW cells displayed a complete resistance to cholesterol accumulation. 7αRAW cells displayed increased expression of both ABCA1 mRNA (3.1-fold, $P < 0.001$) and ABCG1 mRNA (2.2-fold, $P < 0.01$), whereas the expression of scavenger receptor class A mRNA was unchanged. 7αRAW cells also displayed small but significant increases in the rate of efflux of [^{3}H]cholesterol to both delipidated apolipoprotein A1 and to HDL. Thus, CYP7A1 expression in RAW cultured macrophages prevented the accumulation of cholesterol from acetyl-LDL via both increased metabolism and efflux of cholesterol.**—Moore, G. L., and R. A. Davis. **Expression of cholesterol-7α-hydroxylase in murine macrophages prevents cholesterol loading by acetyl-LDL.** ***J. Lipid Res.*** **2002.** 43: **629–635.**

Supplementary key words atherosclerosis • bile acids • cholesterol-7α-hydroxylase • lipoproteins

The liver specific enzyme cholesterol-7α-hydroxylase (CYP7A1) controls the rate-limiting step in the pathway through which cholesterol is converted into bile acids (1, 2). In mammals, this pathway accounts for the majority of cholesterol that is metabolized and removed from the body (3). The ability to metabolize and excrete cholesterol in the form of biliary cholesterol and bile acids may explain how the liver maintains cholesterol homeostasis. Transgenic expression of CYP7A1 in McArdle rat hepatoma cells (4) and in the livers of mice (5, 6) decrease the accumulation of cholesterol and cholesterol esters. These findings support the importance of the cholesterol-bile acid pathway in the maintenance of cholesterol homeostasis.

The accumulation of cholesterol in arterial wall macrophages (7) is intimately associated with the development of atherosclerosis (8). Since the formation of atherosclerosis is greatly enhanced by functional loss of the LDL receptor, cholesterol uptake by macrophages must occur through alternative receptors/pathways (9). Modification of LDL through alteration of lysine residues of apolipoprotein B (apoB) reduces its binding and uptake by the LDL receptor, causing it to be taken up by macrophages via other receptors (10, 11). Macrophages express several high affinity lipoprotein receptors [members of the scavenger receptor family: SRA (12), CD36 (13), SR-B1 (14)] and other receptor families (e.g., CD68) (15). Since unlike the LDL receptor, whose expression is tightly controlled by cellular cholesterol levels (16), scavenger receptors are not downregulated by cellular cholesterol levels. The lack of "negative-feedback" regulation of the expression of many of the lipoprotein receptors expressed in macrophages contributes to their susceptibility to cholesterol accumulation.

Macrophages can both metabolize and excrete as a means to maintain cholesterol homeostasis. The formation of 27-hydroxycholesterol from cholesterol occurs in many cells (16), including macrophages (17, 18). The secretion by macrophages of 27-hydroxycholesterol into blood and its subsequent uptake by the liver and conversion into bile acids may play an important role in preventing cholesterol accumulation (17). Macrophages express the ATP-cassette binding proteins ABCA1 (19) and ABCG1 (20). Studies of ABCA1 suggest that it can actively transfer free cholesterol to an acceptor such as apoA-1 (21). In other studies, it has been reported that ABCG1 facilitates the transfer of free cholesterol and phospholipid to HDL and that this transfer can be inhibited by an antisense probe to the coding region of ABCG1 (20). Additional findings show that the expression of ABCA1 and ABCG1 by macrophages is in-

Abbreviations: CMV, cytomegalovirus; CYP7A1, cholesterol-7α-hydroxylase; DEX, dexamethasone; NADPH, oxygen oxido reductase (EC 1.14.13.17).

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duced by acetyl-LDL cholesterol loading and supports their potential role in preventing cholesterol accumulation and foam cell formation (20, 22).

In the studies reported here, we examine the proposal that expression of the liver-specific enzyme CYP7A1 in macrophages would enhance their ability to prevent cholesterol accumulation. Recent studies show that stable expression of a CYP7A1 plasmid transgene in non-hepatic CHO cells increased both the expression of ABCA1 and the excretion of cholesterol (23). Thus, we anticipated that expressing CYP7A1 in macrophages might prevent the accumulation of cholesterol from the uptake of acetyl-LDL. The results show that while expression of CYP7A1 in macrophages has no adverse effect on cell growth and viability, it completely blocks the accumulation of cholesterol from acetyl-LDL.

MATERIALS AND METHODS

Cell culture and transfection

All tissue culture supplies, chemicals, and radioactive chemicals were obtained from suppliers, as described (24). Cells were cultured in DMEM with 25 mM HEPES modification (Life Technologies, Gaithersburg, MD) and contained 10% Defined FBS (Hyclone, Logan, UT). RAW 264.7 cells were obtained from ATCC (Rockville, MD). 7αRAW cells were obtained by transfecting RAW cells with an expression plasmid (pcDNA37α), which contained the coding sequence of the rat CYP7A1 (25) ligated into pcDNA3 (Invitrogen, San Diego, CA), as described (5). 7αRAW cells were screened for resistance to G418 (400 μg/ml) and then single cell cloned. Cells with stable expression of CYP7A1 were designated as the "7αRAW" cells. G418 was removed prior to plating and growth (to 80% confluence).

CYP7A1 enzyme activity

Cells were harvested at 80–90% confluence. Following disruption by nitrogen decavitation, microsomes were prepared by ultracentrafugation, as described (23). The enzyme activity of CYP7A1 was determined by HPLC using [4^{14}C]cholesterol (Perkin Elmer NEN Dupont, Boston, MA) (26, 27).

HMG-CoA reductase enzyme activity

Cells were harvested at 80–90% confluence and cell extracts were assayed for HMG-CoA reductase (28). Each assay was performed in duplicate from three individual culture dishes of cells. Protein concentrations were determined by dye binding assay (Bio-Rad, Hercules, CA).

Quantitation of free and esterified cholesterol

Cells were washed with PBS and then scraped into tubes. A portion of the cells was assayed for protein. To another portion, stigmasterol (Sigma, St. Louis, MO) was added as an internal standard. The cells were extracted with hexanes-isopropanol 3:2 (v/v) and stored under nitrogen until use. Prior to injection, the samples were resuspended in isopropanol. Each sample was injected into a Hewlett Packard (Palo Alto, CA) gas chromatograph and cholesterol mass was quantitated (29).

Western blot analysis

Western blotting was performed as described (5). Following SDS-PAGE (1–15% gradient), the gels were electroblotted onto nitrocellulose membranes. The non-specific binding sites of the membranes were blocked using 10% defatted dried milk, followed by addition of the appropriate primary antibody. The relative amount of primary antibody bound to the proteins on the nitrocellulose membrane was detected with the species-specific horseradish peroxidase-conjugated IgG. Blots were developed using the electrochemiluminescence (ECL) detection kit (Amersham, Arlington Heights, IL).

Northern analysis

Cells were seeded at 2×10^6 cells per 150 mm plate in 10% FBS HEPES buffered DMEM, in triplicate for 48 h. Cells were washed with ice cold PBS, and poly(A)$^+$ RNA was isolated from cells by a modification of the guanidinium isothiocyanate method, as described (27, 30, 31). The poly(A)$^+$ RNA was blotted by capillary action onto Zeta-probe nylon support (Bio-Rad) and the RNA fixed by ultraviolet crosslinking (Stratagene, San Diego, CA). The blot was pre-hybridized and then hybridized with 1–5 $\times$ 10^6 cpm/ml ^{32}P nick-translated cDNA probes. After hybridization and washing, Northern blots were exposed to phosphor screens of a Molecular Dynamics PhosphorImager (27, 31).

Transcription run-on assays of nuclei

Cells, cultured as described above, were harvested by scraping the cells from the plate and pelleting the cells in a Beckman GPR centrifuge at 1,000 rpm using a swinging bucket rotor for 5 min at 4°C. The cell pellet was washed with cold, sterile PBS, 2.5 ml of hypotonic lysis buffer (10 mM Tris, pH 7.4, 1 mM KCl, 3.0 mM $MgCl_2$) were added and then brought up to 5 ml of hypotonic lysis buffer with 0.3% NP40, and the tubes were incubated on ice for 5 min. The resulting nuclei were pelleted at 500 rpm for 5 min at 4°C. They were washed in 5 ml of hypotonic lysis buffer with 0.3% NP40, pelleted for 5 min at 500 rpm, and the supernatant removed. The wash was repeated using hypotonic lysis buffer without NP40. The nuclei were flash frozen in liquid nitrogen and stored at −70°C in 100 μl of nuclear storage buffer (40% glycerol, 50 mM Tris, 5 mM $MgCl_2$, 0.1 mM EDTA, pH 8.3).

The nuclear run-on transcription buffer contained 30% glycerol, 2.5 mM DTT, 1 mM $MgCl_2$, 70 mM KCl, 0.25 mM each of GTP, CTP, ATP, and 100 mCi of [^{32}P]UTP (3,000 Ci/mmole). The transcription reaction was run for 15 min at 26°C and stopped by adding EDTA, tRNA, and guanidinium isothiocyanate-RNA isolation mix. After the ^{32}P-labeled nuclear RNA was isolated and precipitated, incorporation was estimated by binding to DE81 paper (Whatmann) and equal counts of each reaction hybridized to nylon-bound unlabeled cDNAs for CYP7A1 and β-actin. Hybridization was performed at 65°C in 0.015 M Tris, 0.7 M NaCl, 0.015 M EDTA, Denhardt's, 2% (w/v) $Na_4P_2O_7$, 0.2% SDS, and tRNA to a final concentration of 1 mg/ml, pH 7.5. After washing, the blots were exposed to a phosphorimager (Molecular Dynamics).

Determination of cholesterol efflux

Cells were seeded at 100,000 cells per well in 96-well dishes and incubated overnight at 37°C with 5% CO_2 and humidity. The next day, media was changed to fresh HEPES buffered DMEM containing the following conditions: 1% FBS containing 2 μCi/ml [^{3}H]cholesterol (Perkin Elmer NEN Dupont, Boston, MA), and 1% FBS containing 2 μCi/ml [^{3}H]cholesterol and 100 μg protein/ml human acetyl-LDL. The dishes were incubated for 24 h. Media changed to contain 0.2% fatty acid free BSA (Sigma, St. Louis, MO) to equilibrate cholesterol pools for 24 h (41). Media was changed again and cells were expose to 20 μg protein/ml apoA-1 (Calbiochem, San Diego, CA) or 500 μg protein/ml human HDL to stimulate cholesterol efflux for 24 h. Media and alkaline lysed cell samples were counted separately by scintillation counting and total cellular protein concentration was deter-

mined by a dye binding assay (Pierce, Rockford, IL). The results are reported as the percent of total [^{3}H]cholesterol.

Statistics analysis

Results are given as mean ± SD. Statistical analysis was determined using Student's *t*-test. Significance was determined using double-tailed *P*.

RESULTS

Stable expression of CYP7A1 expression in RAW macrophages

RAW 264.7 mouse macrophages were transfected with a mammalian expression plasmid (pcDNA7αL) that contained the coding region of rat CYP7A1 and 632 bp of the 3′ untranslated region (UTR) inserted in a pcDNA3 mammalian expression vector. Cells were selected for resistance to the neomycin analog G418. Northern blot analysis of poly(A)-enriched RNA isolated from three separate G418 resistant single cell clones showed no detectable CYP7A1 mRNA (data not shown). However, ^{32}P-labeled nucleotide run-on assays using nuclei obtained from the cells transfected with pcDNA7αL showed that ^{32}P-labeled CYP7A1 mRNA was produced (**Fig. 1A**, lanes 3 and 4). In contrast, nuclei obtained from non-transfected RAW cells did not produce any detectable ^{32}P-labeled CYP7A1 mRNA (Fig. 1A, lanes 1 and 2). Nuclei obtained from both groups of cells produced ^{32}P-labeled β-actin mRNA (Fig. 1). These data suggest that the lack of detectable CYP7A1 mRNA in cells transfected with pcDNA7αL might have been caused by its rapid degradation. Detailed analysis of the 3′UTR of rat CYP7A1 mRNA revealed several AUUUA elements, which have been implicated in regulating mRNA stability [for individual localization of each AUUUA element in rat CYP7A1 see Fig. 1 in reference (32)]. One canonical AUUUA element was present within the 594 bp of the remaining 3′UTR. To examine the possibility that this AUUUA element may have rendered the CYP7A1 transcript too unstable to allow translation, we deleted the 594 bp 3′UTR of the rat CYP7A1 mRNA which contained the entire AUUUA element (32) and examined if it would allow stable expression of CYP7A1 mRNA. The resulting plasmid (i.e., pcDNA37αS) had all but 83 bp of the 3′UTR removed, lacked AUUUA elements, and was tested using transient transfection assays. Preliminary experiments showed that 48 h after this plasmid was transfected into RAW cells, CYP7A1 mRNA was detected via Northern blot analysis (data not shown). Single cell clones of G418 resistant RAW cells transfected with pcDNA37αS were also obtained and characterized. As shown in Fig. 1B, CYP7A1 mRNA was detected by Northern blot analysis in RAW cells transfected with pcDNA37αS, whereas there was no CYP7A1 mRNA detected in RAW cells transfected with the pcDNA37αL. Hereafter the RAW cells expressing CYP7A1 are referred to as 7αRAW cells.

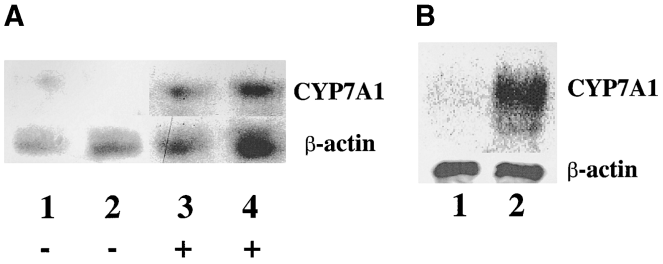

Fig. 1. Transcription and expression of cholesterol-7α-hydroxylase (CYP7A1) mRNA. Nuclear run-on assay (A) and Northern blot analysis (B) of RAW cells transfected with plasmids containing the coding region of rat CYP7A1 and 632 bp of the 3′UTR (pcDNA37αL) or the coding region of rat CYP7A1 and 86 bp of the 3′UTR (pcDNA37αS). Cells were grown to 80% confluence in 10% FBS HEPES-buffered DMEM and harvested for preparation of nuclei or mRNA. A: Lanes 1 and 2 represent wild-type RAW nuclei, whereas lanes 3 and 4 represent RAW cells transfected with pcDNA37αL. B: Lane 1 represents RAW cells transfected with pcDNA37αL, whereas lane 2 represents RAW cells transfected with pcDNA37αS.

Expression of CYP7A1 enzyme activity in 7αRAW cells

To examine if 7αRAW cells express CYP7A1 enzyme activity, we disrupted cells by nitrogen decavitation and isolated microsomes as described (33). The level of CYP7A1 enzyme activity present in microsomes from non-transfected RAW cells was not significantly different than background (**Fig. 2**). In contrast, microsomes obtained from 7αRAW cells displayed an activity (9.8 ± 3.6 pmol/min/mg protein), which was similar to the activity present in microsomes from the livers of chow-fed mice sacrificed at the mid-dark period of a 12 h light cycle (9.6 ± 4.4 pmol/min/mg protein) (Fig. 2).

7αRAW cells display growth and viability similar to RAW cells

To ensure that the expression of CYP7A1 in macrophages was not associated with impaired viability or growth, we compared the growth rates of 7αRAW cells with non-transfected RAW cells (**Fig. 3**). The growth rates between the cell types were similar and the effect seen in 7αRAW cells indicate that CYP7A1 enzymatic expression was not toxic to the cells.

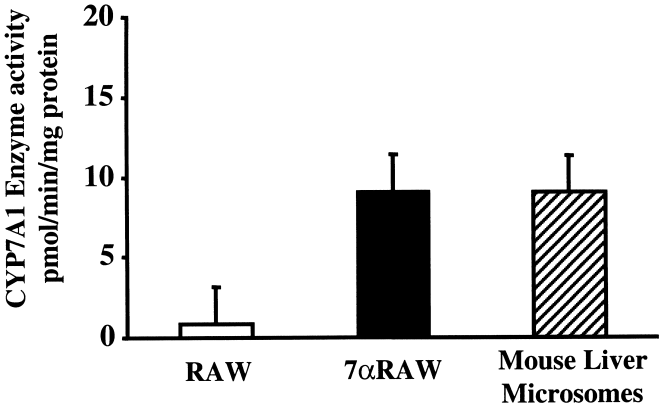

Fig. 2. CYP7A1 enzyme activity. Livers from mice fed a chow diet sacrificed during the mid-dark period were obtained and used for the preparation of microsomal membrane fractions. RAW and 7αRAW cells were grown to 80% confluence in 10% FBS HEPES buffered DMEM and used for the preparation of microsomal membrane fractions. CYP7A1 enzyme activities for three separate plates of cells or three separate mice are presented as the mean ± SD. Open column, non-transfected RAW cells; closed column, 7αRAW cells; and hatched column, mouse liver.

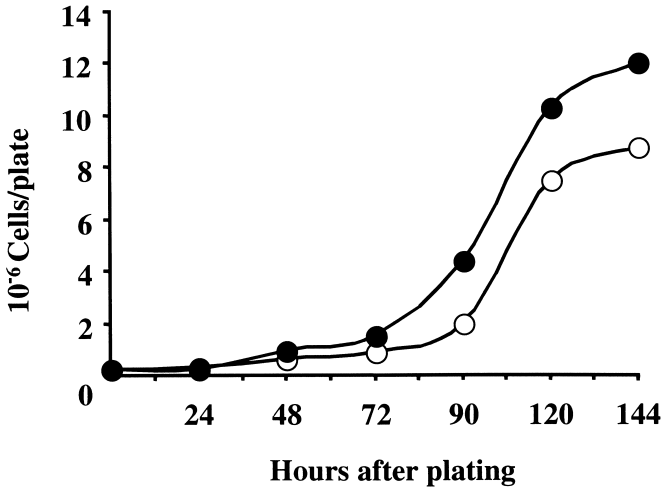

Fig. 3. Growth of cells is not affected by the expression of CYP7A1. Cells were grown to 80% confluence in 10% FBS HEPES-buffered DMEM. Cells were counted with a hemocytometer. Values represent the mean of three separate wells of cells for RAW (open circles) and 7αRAW (closed circles). Each value has been divided by 10^6.

Expression of CYP7A1 reduces the cellular accumulation of cholesterol from acetyl-LDL

Expression of CYP7A1 in RAW cells caused a marked a 70% reduction in the cellular concentration of esterified cholesterol (**Fig. 4B**). Moreover, expressing CYP7A1 in RAW cells had a dramatic impact on preventing the accumulation of cholesterol derived from human acetyl-LDL (Fig. 4). While increasing amounts of acetyl-LDL caused non-transfected RAW cells to accumulate both free and esterified cholesterol in a manner proportional to the amount of acetyl-LDL added, 7αRAW cells displayed a completed resistance to cholesterol accumulation (Fig. 4). The accumulation of cholesterol in RAW cells incubated with human acetyl-LDL has been reported (34). Interestingly, adding a small amount (50 μg/ml) of human acetyl-LDL to the cultured medium of 7αRAW caused a >50% decrease in free cholesterol, whereas there was no effect on esterified cholesterol concentrations. The esterified cholesterol concentrations increased in 7αRAW cells as the human acetyl-LDL concentration increased. Yet, even at the highest acetyl-LDL concentration examined (i.e., to 500 μg/ml), the esterified cholesterol concentration in 7αRAW cells is still ~45% less than that in non-transfected RAW cells incubated without acetyl-LDL (Fig. 4). These data clearly support the proposal that the expression of CYP7A1 in RAW macrophages prevents the accumulation of cholesterol from acetyl-LDL.

Expression of CYP7A1 in RAW cells increases the activity of HMG-CoA reductase

The enzymatic activity of HMG-CoA reductase displayed by 7αRAW cells was about 2-fold greater than the activity displayed by non-transfected RAW cells (**Fig. 5**). It is likely that the increased activity of HMG-CoA reductase displayed by 7αRAW cells is due to the decreased cellular content of cholesterol (Fig. 4). These findings are consistent with other studies that showed that expression of CYP7A1 in rat hepatoma cells (5) and in the livers of transgenic mice (6) both decreased cellular cholesterol content and increased the abundance of HMG-CoA reductase mRNA.

Expression of CYP7A1 in RAW cells increases the expression of SRA, ABCA1, and ABCG1 mRNAs

The abundance of SRA, ABCA1, and ABCG1 mRNA relative to β-actin mRNA was determined in replicate dishes of 7αRAW cells and non-transfected RAW cells (**Fig. 6**). While the relative abundance of SRA mRNA was slightly greater in 7αRAW cells compared with non-transfected cells, this increase was not statistically significant ($P > 0.05$). However, the relative abundance of ABCA1 mRNA (3.1-fold, $P < 0.001$) and ABCG1 mRNA (2.2-fold, $P <$

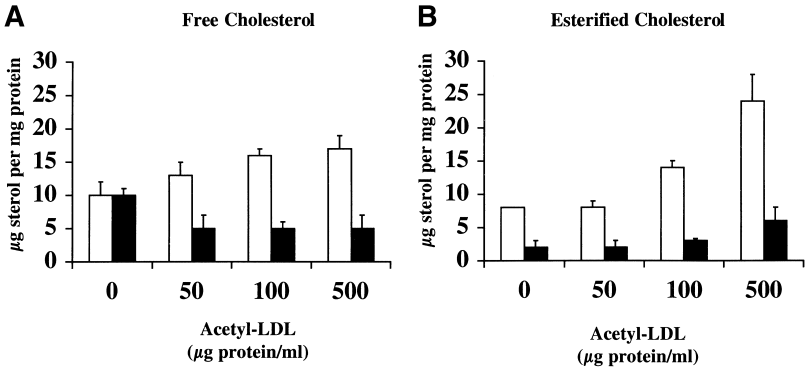

Fig. 4. Expression of CYP7A1 in RAW cells maintains cellular cholesterol homeostasis. Free (A) and esterified (B) cholesterol mass in RAW (open columns) and 7αRAW cells (closed columns). Both cell types were grown to 80% confluence in 10% FBS HEPES-buffered DMEM. The medium was changed for overnight incubation to HEPES-buffered DMEM with the following conditions: 10% FBS alone and 10% FBS containing 50, 100, and 500 μg/ml protein acetyl-LDL for 24 h. Cells were harvested and the amount of free and esterified cholesterol was determined by GLC. Values represent the mean ± SD of three separate wells of cells. Cellular free cholesterol content was significantly decreased in culturing conditions that contained acetyl-LDL in 7αRAW cells compared with RAW cells ($P \leq 0.01$). Cellular esterified cholesterol content was significantly decreased under all culturing conditions in 7αRAW cells compared with RAW cells ($P \leq 0.01$).

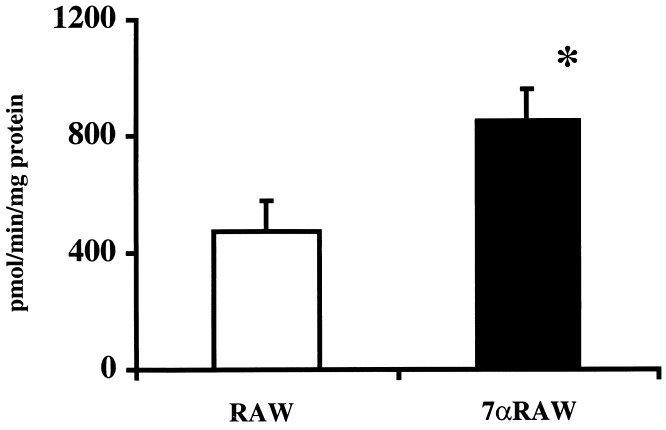

Fig. 5. HMG-CoA Reductase enzyme activity in RAW and 7αRAW cells. RAW (open column) and 7αRAW (closed column) cells were grown to 80% confluence in HEPES buffered DMEM with 10% FBS. The medium was changed for overnight incubation to HEPES buffered DMEM with 10% FBS alone. Results are expressed as the mean ± SD. for quadruplicate dishes of cells. There were significant increases in enzyme activity under this culturing condition in 7αRAW cells compared with RAW cells ($P \leq 0.05$).

0.01) were both significantly increased in 7αRAW cells compared with non-transfected cells.

Expression of CYP7A1 increases cholesterol efflux

The increased expression of both ABCA1 mRNA and ABCG1 mRNA by 7αRAW cells suggested the possibility that increased efflux of cholesterol out of the cells might contribute to the relative resistance of these cells to cholesterol loading by acetyl-LDL (Figs. 3 and 6). To examine this possibility, we measured the ability of apoA-1 (21, 35) and human HDL (20) to facilitate the efflux of [^{3}H]cholesterol from 7αRAW cells and non-transfected RAW cells (**Fig. 7**). Both groups of cells were incubated with acetyl-LDL (100 μg/ml) and [^{3}H]cholesterol for 24 h. The medium was changed to serum-free medium containing either delipidated human apoA-1 (20 μg/ml) or human HDL (500 μg/ml) for 24 h, after which cells and medium were extracted and the amount of [^{3}H]cholesterol in each extract was determined. Adding delipidated apoA-1 to the serum-free medium of both groups of cells had a small ~25% increase in the efflux of cellular cholesterol (from 1.7% to 2.1%, data not shown). Moreover, 7αRAW cells display significant ~50% increases in the efflux rates of [^{3}H]cholesterol facilitated by apoA-1 ($P < 0.025$) and HDL ($P < 0.01$) (Fig. 7). Interestingly, the rate of efflux [^{3}H]cholesterol in the presence of HDL was ~20-fold greater than the rate displayed in the presence of delipidated human apoA-1 (Fig. 7). These experiments were repeated two additional times (separated cell preparations) and similar results were obtained (i.e., there were a small, ~20–50%, but significant increases in the efflux rates of [^{3}H]cholesterol facilitated by apoA-1 and HDL).

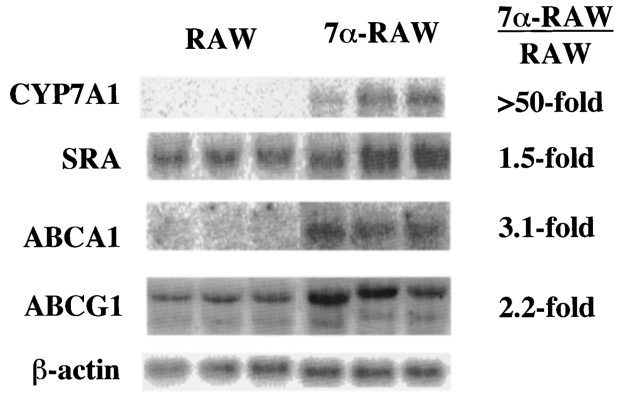

Fig. 6. The relative abundance of mRNAs encoding CYP7A1, Acetyl-LDL Receptor (SRA), ABCA1, and ABCG1. RAW cells and 7αRAW cells were plated and grown to 80% confluence. Cells were harvested and poly(A) mRNA was isolated. The mRNA was Northern blotted and hybridized to ^{32}P-labeled cDNAs, as indicated. Lanes 1–3, are from three separate plates of RAW cells; lanes 4–6 are from three separate plates of 7αRAW cells. The 7αRAW-RAW ratios for each mRNA relative to β-actin are indicated.

DISCUSSION

Our data show for the first time that expression of the liver specific enzyme CYP7A1 in cultured mouse RAW macrophages increases their ability to maintain cholesterol homeostasis even when cultured in the presence of supraphysiological concentrations of acetyl-LDL (i.e., 500 μg/ml). Furthermore, when characterizing the 7αRAW cell phenotype, the mechanism responsible the efficient maintenance of cholesterol homeostasis is caused by: *1*) the enzymatic conversion of cholesterol to 7α-hydroxycholesterol at rates that approximate those of mouse liver (Fig. 2) and *2*) increased cholesterol efflux facilitated by both apoA-1 and HDL (Fig. 7). Thus, as a result of increased cholesterol metabolism and efflux, 7αRAW cells were completely refractory to cholesterol loading via acetyl-LDL (Fig. 4).

The finding that the expression of CYP7A1 in RAW cells increases the activity of HMG-CoA reductase (Fig. 6) suggests that the decreased cellular content of cholesterol displayed by 7αRAW cells is not caused by decreased cholesterol biosynthesis. Rather, these data support the concept that enzymatic expression of CYP7A1 causes parallel changes in HMG-CoA reductase (1).

In the absence of plasma lipoproteins, 7αRAW cells display a 75% reduction in the cellular content of cholesterol esters (Fig. 4). These data indicate that the resistance of 7αRAW cells to cholesterol ester accumulation is caused by the ability of these cells to metabolize and excrete endogenous cholesterol. Clearly, the increased capacity of 7αRAW cells to metabolize and excrete cholesterol in a manner that maintains cholesterol homeostasis is exhibited to a greater degree when cells are challenged with acetyl-LDL. While non-transfected RAW cells accumulated both free and esterified cholesterol in proportion to the amount of acetyl-LDL that was present in the culture medium, 7αRAW cells displayed a complete resistance to cholesterol accumulation (Fig. 4).

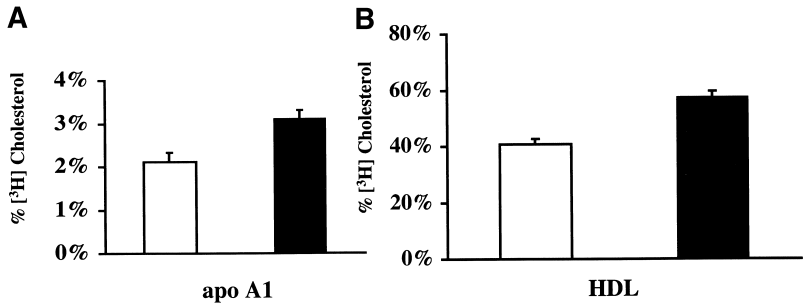

Fig. 7. [^{3}H]Cholesterol efflux from cultured macrophages. RAW (open columns) and 7αRAW (closed columns) cells were exposed to the presence of 100 μg/ml protein acetylated LDL in 10% FBS HEPES buffered DMEM for 24 h. The media was then removed and 0.2% fatty acid free BSA in HEPES-buffered DMEM was added to equilibrate cholesterol pools for 24 h. The media was removed again and fresh 0.2% BSA HEPES-buffered DMEM was added containing one the following acceptors: (A) 20 μg/ml delipidated human apoA-1; or (B) 500 μg/ml human HDL_3. Cells were incubated 24 h longer and the radioactivity was determined in medium and cells. Cholesterol efflux is presented as the percent of the total radioactivity recovered from the cells and medium. Quadruplicate values represent the mean ± SD.

Unexpectedly, adding even small amounts of acetyl-LDL to the culture medium of 7αRAW cells caused a marked decrease in the content of free cholesterol (Fig. 4). The most likely explanation for this effect is that acetyl-LDL acts as an acceptor for cholesterol efflux. Additional findings that show 7αRAW cells displaying increased rates of [^{3}H]cholesterol efflux (Fig. 7) provides further support for this proposal. Furthermore, the findings showing that the expression of both ABCA1 and ABCG1 mRNA were significantly increased ~2- to 3-fold in 7αRAW cells, compared with nontransfected RAW cells, suggest the possibility that these gene products may have contributed to the increased [^{3}H]cholesterol efflux. ABCA1 facilitated cholesterol efflux appears to require an acceptor such as apoA-1, other apolipoproteins, and in some cases HDL (21, 36, 37). Recent studies suggest that ABCG1 might use HDL as an acceptor that facilitates cholesterol efflux (20). HDL also acts as an efficient acceptor for the diffusion-mediated efflux of cholesterol out of the cell (38). Our findings showing that HDL-facilitated [^{3}H]cholesterol efflux was 20-fold greater than delipidated human apoA-1-facilitated [^{3}H]cholesterol efflux may be explained by its ability to facilitate "active" efflux (via ABCA1 and ABCG1) as well as diffusion-mediated efflux.

Our combined data support the concept that expression of CYP7A1 in cultured macrophages renders them resistant to cholesterol accumulation by acetyl-LDL by increased metabolism via 7α-hydroxylation and by increased efflux of cholesterol. The ability of CYP7A1 to contribute to the resistance of RAW macrophages to cholesterol accumulation shown by these studies is consistent with other studies showing that transgenic hepatic expression of CYP7A1 increases the ability of the liver parenchymal cell to resist cholesterol accumulation (5,6). There is one distinct difference between the macrophage and the liver parenchymal cell in their response to the expression of CYP7A1: unlike 7αRAW cells, liver cells expressing a CYP7A1 transgene do not display increased expression of ABCA1 or ABCG1 mRNA (6). This difference may relate to the ability of the liver to excrete cholesterol from the body via the biliary system. The liver parenchymal cell is a polar cell having a distinct apical membrane that provides a route for the biliary excretion of bile acids and cholesterol. The hepatic basolateral membrane provides a route for the secretion of lipoproteins into blood. Expression of CYP7A1 by liver parenchyma cells increases the biliary excretion of cholesterol (6). The recent finding showing that the biliary excretion of cholesterol is unaffected in ABCA1$^{-/-}$ mice (39) suggests that biliary cholesterol excretion occurs independently of ABCA1.

Recent studies using bone marrow transplanted LDL receptor$^{-/-}$ mice show that LXRα-mediated induction of ABCA1 gene expression by macrophages is associated with increased cholesterol efflux and decreased atherosclerosis (40). Based on the results of our studies, we predict that expression of CYP7A1 in macrophages in vivo may also reduce atherosclerosis by decreasing cholesterol accumulation.

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