Cholesterol enrichment enhances expression of sterol-carrier protein-2: implications for its function in intracellular cholesterol trafficking

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Abstract Cholesterol enrichment of vascular smooth muscle cells, as occurs under conditions of hypercholesterolemia and atherosclerosis, is accompanied by specific changes in cholesterol metabolism and in intracellular cholesterol trafficking. Sterol-carrier protein-2 (SCP_2), an intracellular lipid binding protein, enhances the activation of enzymes involved in cholesterol metabolism. It may also enhance cholesterol efflux by regulating the size of the "fast" cholesterol pool available for efflux to high density lipoproteins. However, a definitive role for SCP_2 in arterial cholesterol metabolism is unclear. Therefore, we examined the expression of SCP_2 (13.1 kD), SCPx (58 kD), and p30 (30.8 kD) in cultured arterial smooth muscle cells under conditions of cholesterol enrichment. We found that SCP_2, SCPx, and p30 are localized principally in the cytosolic fraction, with lesser amounts associated with the nuclear/peroxisomal fraction; the expression of SCP_2 protein and mRNA, but not SCPx, is increased after exposure of smooth muscle cells to cationized LDL. In contrast to the increased expression of SCP_2, the expression of p30 decreases after cholesterol enrichment of smooth muscle cells. Coupled with previous studies demonstrating enhanced cholesterol efflux from cholesterol-enriched smooth muscle cells in response to high density lipoproteins, our results suggest that increased expression of SCP_2 may partly mediate the cholesterol trafficking process.–**Kraemer, R., K. B. Pomerantz, S. Kesav, T. J. Scallen, and D. P. Hajjar.** Cholesterol enrichment enhances expression of sterol-carrier protein-2: implications for its function in intracellular cholesterol trafficking. *J. Lipid Res.* 1995. **36:** 2630-2638.

Supplementary key words cholesterol • cholesteryl ester • smooth muscle cells • atherosclerosis • gene expression • mRNA • catalase • SCPx • p30

The amount and distribution of intracellular cholesterol is under tight regulatory control. The majority of free cholesterol is located in the plasma membrane, while the endoplasmic reticulum and mitochondria membranes have lower free cholesterol content (1). This observation is significant as the endoplasmic reticulum is a major site of active free cholesterol synthesis. Cholesterol is also synthesized in peroxisomes. Thus, the transport of free cholesterol from the endoplasmic reticulum to the plasma membrane or other target organelles must be very efficient in order to maintain their steady-state free cholesterol content.

The mechanism by which cells maintain asymmetric intracellular cholesterol distribution is currently unknown. However, recent studies have implicated a role for specific lipid-binding proteins in intravesicular membrane cholesterol transport (2–5). One of these proteins, sterol-carrier protein-2 (SCP_2), a 13 kD protein derived from a 0.8 kb mRNA, is highly expressed in liver (6, 7). The protein is located principally in peroxisomes (due to a C-terminal peroxisomal sequence (8)), but it is also found in the mitochondria (due to a N-terminal mitochondrial sequence (8)), on the outer membrane of the endoplasmic reticulum, and in the cytosol (7, 9–13). In addition, a second SCP_2-like protein, termed SCPx, is a 58.6 kD protein transcribed from a 2.2 kB mRNA. It is found in hepatic tissue (14). SCPx possesses the SCP_2 sequence at its C-terminus as well as 3-oxoacyl coenzyme A thiolase activity (15). In contrast to SCP_2, SCPx is restricted to peroxisomes (12, 16). SCPx and SCP_2 are derived from a single gene that undergoes alternative splicing and polyadenylation to yield four mRNA species (14–20). Pulse-chase experiments have shown that SCP_2 is synthesized as a 14.5 kD protein on cytoplasmic free polyribosomes, and is then post-translationally transported to peroxisomes, where it is processed to its mature form of 13 kD (21). In steroidogenic cells, SCP_2 levels may be augmented by cyclic AMP (22).

Abbreviations: SCP_2, sterol-carrier protein-2; ACAT, acyl-CoA:cholesterol acyltransferase; cLDL, cationized low density lipoprotein; HDL, high density lipoprotein; FCS, fetal calf serum; SCPx, sterol-carrier protein-x; ITS, insulin, transferrin, and selenium.

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The exact function of SCP_2 is not fully understood. In vitro assays have shown that SCP_2 facilitates exchange of sterols between phospholipid vesicles or membranes (3, 23–28), which is believed to be its principal function in vivo; in vitro, it can participate in the transfer of other lipids. The in vivo significance of those transfers remains unclear. Interestingly, SCP_2/SCPx can also regulate the formation of cholesterol from its precursors (29, 30) and other isoprenoids (31), enhance free cholesterol esterification through acyl-CoA:cholesterol acyltransferase (ACAT) (8, 32), and it can increase the conversion of cholesterol to other steroids in mitochondria (17). Thus, the concept that SCP_2 mediates directional inter-organellar cholesterol flux and other aspects of cholesterol metabolism suggests that it may also mediate alterations in cholesterol trafficking under pathological conditions.

Two lines of evidence support the hypothesis that alterations in SCP_2 may occur under conditions involving cellular lipid accumulation. First, SCP_2 was recently found in rat aorta (32) and was implicated in the regulation of ACAT activity in vascular smooth muscle cells (32). Second, atherosclerosis is associated with increased ACAT activity due to increased free cholesterol content and increased enzyme mass (33–36). These observations suggest that SCP_2 may modulate ACAT activity in lipid-loaded cells. The smooth muscle cell-derived foam cell is a major component of the atherosclerotic lesion (37). However, the influence of cholesterol enrichment on SCP_2 expression in the smooth muscle cell has not yet been evaluated. In this study, we have assessed the role of cholesterol enrichment on the levels of SCP_2 in vascular smooth muscle cells in order to examine its potential role in intracellular trafficking in smooth muscle cell-derived foam cells. We used a previously established model of the smooth muscle cell-derived foam cell, where there is a 2- and 10-fold increase in free and esterified cholesterol, respectively, after incubation with modified LDL. Cellular changes that accompany these alterations in lipid content include decreased prostaglandin synthesis (38), decreased activities of cholesteryl ester-metabolizing enzymes (39), and increased basic fibroblast growth factor production (all pro-atherosclerotic events) (40). We demonstrate herein that cholesterol enrichment leads to increased expression of SCP_2 mRNA and protein in smooth muscle cells. Our findings provide a basis for increased free cholesterol trafficking in lipid-loaded cells.

EXPERIMENTAL PROCEDURES

Materials

β-Mercaptoethanol was purchased from Sigma (St. Louis, MO); N,N-dimethyl-1,3-propanediamine was purchased from Kodak Chemicals (Rochester, NY); 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl was purchased from Aldrich Chemicals (Milwaukee, WI). Fetal bovine serum, M-199, penicillin/streptomycin, fungizone (amphotericin B), and glutamine were purchased from GIBCO (Grand Island, NY); insulin, transferrin, and selenium (ITS) were bought from Collaborative Research (Bedford, MA).

Rat liver S_{303} supernatant was prepared as previously described (41). This supernatant contains both the 13 kD form of SCP_2 and SCPx (a 60 kD protein which has SCP_2 at its carboxyl terminus) (41). The anti-rat SCP_2 antibody is an immunoaffinity purified antibody and was prepared as described previously (8). Protogel polyacrylamide was purchased from National Diagnostics (Atlanta, GA). Ammonium persulfate and TEMED were purchased from Bio-Rad (Hercules, CA). The enhanced chemiluminescence kit was bought from Amersham (Arlington Heights, IL). Rat liver SCP_2 cDNA is a 672 base pair Eco R1 fragment in the PRS1 plasmid. It encodes the 13.1 kD form of SCP_2 and hybridizes with 0.8, 1.4, 2.1, and 2.7 kilobase mRNAs (42). Murine glyceraldehyde phosphate dehydrogenase cDNA was kindly provided by Dr. D. DeWitt of Michigan State University (East Lansing, MI). [^{32}P]dCTP was purchased from Amersham. The random hexamer primer extension kit was purchased from Boehringer Mannheim.

Preparation of plasma LDL and cationized proteins

LDL (1.019–1.063 g/ml) was isolated from pooled donor human plasma by preparative ultracentrifugation (43). LDL was cationized by incubation with the adduct N,N-dimethyl-1,3-propanediamine using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl as the catalyst (38). Lipoproteins were then dialyzed in PBS, concentrated by ultracentrifugation, and stored at 4°C after membrane sterilization. There was minimal oxidation of the lipoproteins as native LDL and cationized LDL contained less than 1 nmol of tetramethoxypropane equivalents/mg of protein as compared with 10–25 nmol of tetramethoxypropane equivalents/mg of protein found in oxLDL prepared by dialyzing native LDL against 5 μM $CuSO_4$ for 24 h at 4°C (40).

Isolation and culture of rat aortic smooth muscle cells

Smooth muscle cells were grown from medial explants of rat thoracic aortas and cultured as previously described (44). Smooth muscle cells were identified by the appearance of the characteristic hill and valley morphology and immunohistochemical staining using antibodies directed against smooth muscle cell actin (45). Cells were maintained in M-199 supplemented with 10%

fetal bovine serum, 50 μg/ml penicillin/streptomycin, 1 μg/ml fungizone, and 2 mM glutamine (culture media) and were used up to passage 16. Smooth muscle cells were grown to confluence in 100-mm diameter petri dishes. Cells were cholesterol-enriched by incubation with culture media containing cLDL (75–100 μg of protein/ml) for 7 days with one change of media (38). Control cells were cultured in media alone.

Western analysis

Normal and cholesterol-enriched smooth muscle cells were washed twice with PBS and lysed in 2.5 M urea, 1% SDS, 63 mM Tris-HCl, pH 6.8, 2.5% glycerol plus phenyl methyl sulfonyl fluoride (1 mM), aprotinin (1 μg/ml), and leupeptin (10 μg/ml). The cells were incubated for 1 h at 4°C, then triturated through a 23-gauge needle. In some experiments, the cells were cultured overnight in serum-free media containing 12.5 μg/ml ITS (ITS media) prior to being lysed. Protein was measured by the method of Lowry et al. (46). Cell proteins were separated by 15% SDS-PAGE, and transferred to polyvinylidenedifluoride membrane (Immobilon-P). Western analysis was performed using an anti-SCP_2 antibody, which was detected using an anti-rabbit horseradish peroxidase-labeled secondary antibody and the ECL chemiluminescence with minor modifications (N.O. Davidson, personal communication). Briefly, after transfer, immunoblots were washed 3 times with PBS/Tween 20 (0.3%). The blots were then stained with India ink (1:1000 dilution), and then blocked with 5% nonfat dry milk (in TBS (20 mM Tris, 137 mM NaCl, pH 7.5)/Tween 20 (0.05%)). After incubating the blots with anti-SCP_2 antibody (1:5000), the blots were washed with 5% nonfat dry milk, followed by washing with TBS (10 mM Tris, 150 mM NaCl, pH 7.4). After incubation with the secondary antibody, the blots were washed and then exposed to chemiluminescence reagents, followed by autoradiography, and quantified by laser densitometry.

Metabolic labeling and immunoprecipitation

Rat smooth muscle cells were plated and enriched with cholesterol as before. The cells were rinsed with PBS, then cultured overnight in ITS media. The cells were then treated with Trans-^{35}S-label™(New England Nuclear, 50 μCi/ml). After 3 h, the cells were either harvested (0 h) or washed 3 times with PBS and cultured for 24 h in ITS media and then harvested. Cells were lysed in PBS containing 1% Na-deoxycholate/0.1% SDS plus PMSF (1 mM), leupeptin (10 μg/ml), and aprotinin (1 μg/ml), followed by sonication (47). Similar amounts of radioactivity for each sample (as determined by TCA precipitation) were diluted 1:4 in PBS containing 1 mM EDTA, 0.5% Nonidet P-40 plus PMSF (48), and incubated with protein A-Sepharose, followed by incubation with the anti-SCP_2 antibody (0.9 μg/ml) overnight at 4°C. Control incubations were performed using non-immune rabbit IgG. Antigen-antibody complexes were immunoprecipitated using protein A-Sepharose. Immunoprecipitated proteins were separated by 15% SDS-PAGE. After the proteins in the gel were fixed and incubated with Enhance solution (National Laboratories, Atlanta, GA), the gel was dried and subjected to autoradiography and quantified by laser densitometry.

Subcellular distribution studies

Subcellular fractions were prepared by differential centrifugation. After cholesterol enrichment, cells were washed with ice-cold PBS and harvested into Tris buffer (25 mM Tris, 1 mM EDTA) plus protease inhibitors. After sonication, the lysates were centrifuged at 1000 rpm for 5 min to isolate nuclei and peroxisomes. The supernatant was then centrifuged at 100,000 *g* for 1 h. The supernatant was collected (cytosol) and the membranes were resuspended in Tris buffer. Proteins in the whole cell lysate, nuclei and peroxisomes, cytosol, and membrane fractions were then separated by SDS-PAGE and analyzed for SCP_2 content by Western analysis. Catalase activity was quantified as previously described (49). Briefly, either purified catalase (Sigma, St. Louis, MO), cell lysates, or subcellular fractions were diluted up to 50 μl in phosphate buffer (167 mM; pH 7.3) and incubated with 950 μl 82.1 mM $NaBO_3$, pH 6.8, for 5 min at 37°C. The reaction was terminated by the addition of 1 ml 2 N H_2SO_4. The remaining $NaBO_3$ was then titrated with 50 mM $KMnO_4$. Cellular catalase activity was quantified based on the standard curve generated using purified catalase and expressed as units of activity/mg of protein. One unit of catalase activity degrades 1 μmole of H_2O_2/min at pH 7.0 at 25°C, as described by Sigma. Statistical significance between catalase activity in control and cholesterol-enriched smooth muscle cells was determined by a paired Student's *t*-test with significance determined at a P level of < 0.05.

Isolation of RNA and Northern analysis

Total RNA was isolated from guanidinium isothiocyanate/2% β-mercaptoethanol lysates by centrifugation through a $CsCl_2$ cushion (50). RNA (10–20 μg) was electrophoresed in 1% denaturing agarose gels containing formaldehyde and ethidium bromide, transferred to Zetaprobe followed by cross-linking using a UV Stratalinker (Stratagene; La Jolla, CA), hybridized with a ^{32}P-radiolabeled SCP_2 cDNA in 50% formamide for 16–24 h at 43°C (Appligene, Pleasanton, CA), washed under high stringency conditions, visualized by autora-

diography, and quantified by laser densitometry. mRNA levels for SCP_2 were normalized to mRNA levels for GAPDH.

RESULTS

Western analysis was performed to determine whether SCP_2 was present in cultured arterial smooth muscle cells. A 13 kD protein that migrates with an SCP_2 standard (purified SCP_2 and rat liver S_{303}) was detected in smooth muscle cell lysates (**Fig. 1**). The related 58.6 kD protein, SCPx, which contains the entire SCP_2 protein at its carboxy terminus, and attendant thiolase activity, was also detected. Incubation of blots with non-immune IgG did not show either SCP_2 or SCPx (results not shown). This is the first demonstration that SCP_2 and SCPx are present in subcultured populations of arterial smooth muscle cells. Another related protein, p30, originally described in hepatic cells (8, 51), was also found in smooth muscle cell lysates.

We next determined whether cholesterol enrichment altered SCP_2 levels, as determined by Western analysis. Smooth muscle cells were cholesterol-enriched by treating them with cationized LDL (75–100 μg/ml) for 7 days, as previously described (38). Cholesterol enrichment increased SCP_2 protein 2- to 3-fold, but did not significantly alter SCPx protein levels (Fig. 1). In contrast to SCP_2, p30 levels were decreased 2- to 3-fold by cholesterol enrichment of smooth muscle cells.

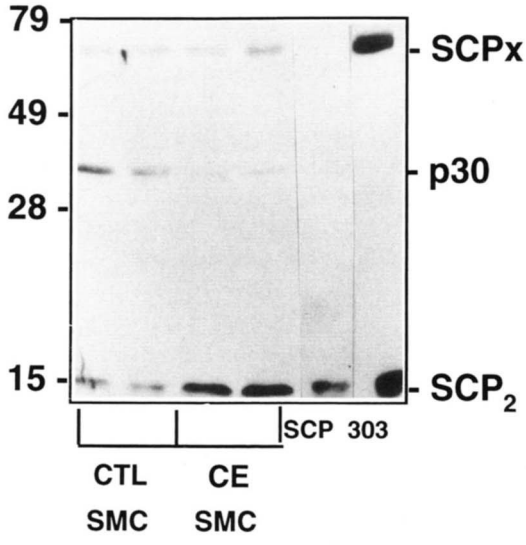

Fig. 1. Smooth muscle cells possess SCP_2 and SCPx protein. Rat aortic smooth muscle cells were lysed and proteins were separated by SDS-PAGE. After transfer to Immobilon, Western analysis was performed as described. Figure represents two of four similar experiments, two replicates per experimental group; CTL-SMC, control smooth muscle cells; CE-SMC, CE-enriched smooth muscle cells.

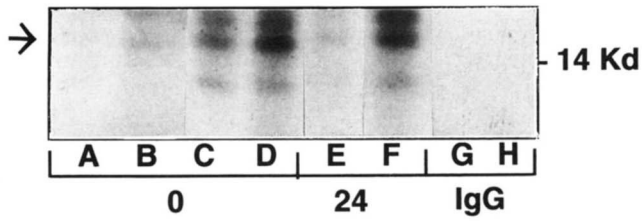

Fig. 2. Immunoprecipitation of metabolically labeled SCP_2 from control and cholesterol-enriched smooth muscle cells. Control and cholesterol-enriched smooth muscle cells were labeled with Trans-^{35}S-label™ The cells were lysed and SCP_2 was immunoprecipitated with the anti-SCP_2 antibody. Antigen–antibody complexes were immunoprecipitated with protein A-Sepharose, and immunoprecipitated proteins were separated by 15% SDS-PAGE and detected as described. SCP_2 migrated as a doublet at approximately 15 kD (arrows). This represents one of two experiments.

To confirm the increased synthesis of SCP_2 by cholesterol enrichment and to examine the rate of degradation of SCP_2 in smooth muscle cells, cells were labeled for 3 h with Trans-^{35}S-label™ and were either harvested immediately or were harvested after a 24-h incubation in the absence of the radiolabeled amino acids. SCP_2 and its related proteins were immunoprecipitated using anti-SCP_2 antibody (**Fig. 2,** lanes A–F). Control immunoprecipitations were also performed using nonimmune IgG (Fig. 2, lanes G–H). Two radiolabeled bands migrating at approximately 15 kD were immunoprecipitated from lysates from both control and cholesterol-enriched smooth muscle cells (Fig. 2, lanes A–D). These bands were not present in the lysates immunoprecipitated with nonimmune IgG. Moreover, there was a 2- to 3-fold increase in the intensity of both bands in lysates from cholesterol-enriched smooth muscle cells. The lower band corresponds to the migration of the single band at 15 kD corresponding to SCP_2 in both smooth muscle cells and S303 supernatants (see arrow). The higher molecular weight band may be the proform of the final 13 kD product of SCP_2, as has been previously described (21, 22). A precursor form of SCP_2 is encoded by the mouse cDNA in which there is an additional 15 amino acid presequence on the N-terminus that has been shown to target proteins to the mitochondria (8, 51). However, this band was not detectable by Western analysis (Figs. 1 and 3), suggesting that the conversion of the proform to the mature form of SCP_2 is very rapid, and the higher molecular weight band that appears after immunoprecipitation may represent a nonspecific protein that is immunoprecipitated by the SCP_2 antibody. Alternatively, the conformation of the proform may be such that it is not detectable by Western blot analysis, and no conclusions about the rate of conversion from the proform to the mature form can be made at this time. There was no change in the synthesis of SCPx, similar to what was observed by Western analysis (results

not shown). No clear bands were observed at 30 kD (results not shown). SCP_2 levels remained the same in both control or cholesterol-enriched smooth muscle cells 24 h after pulse labeling (Fig. 2, lanes E and F), suggesting that SCP_2 has a slow turnover rate in smooth muscle cells.

Experiments were then performed to determine the subcellular distribution of SCP_2 and SCP_2-related proteins within smooth muscle cells, and to assess whether cholesterol enrichment would alter this distribution. Subcellular fractionization by ultracentrifugation was performed to separate the nuclear/peroxisomal, cytosolic, and membrane fractions from control and cholesterol-enriched smooth muscle cells, which were then analyzed for SCP_2 levels by Western analysis (**Fig. 3**). In control cells, SCP_2 was present principally in the cytosolic fraction with smaller amounts found in the nuclear/peroxisomal and fractions. The membrane fraction contained very little SCP_2. p30 and SCPx were also found predominantly in the cytosolic fraction; however, there was more SCPx and p30 localized to the membrane fraction compared to SCP_2. Cholesterol enrichment increased SCP_2 levels in both the nuclear/peroxisomal and in the membrane fractions 3- to 4-fold, while having a smaller effect on the levels of SCP_2 in the cytosolic fractions. p30 levels were decreased in the cytosolic fraction by 2-fold, but did not change significantly in the other fractions. SCPx levels did not change appreciably in the membrane and cytosolic fractions, but decreased 2- to 3-fold in the nuclear/peroxisomal fraction.

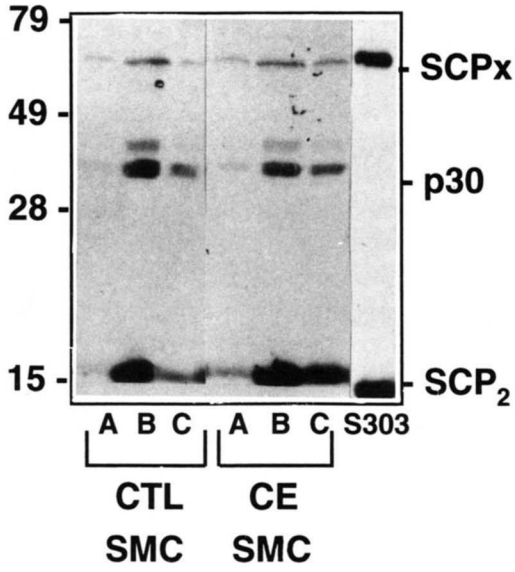

Fig. 3. Subcellular localization of SCP_2: influence of cholesterol enrichment. Subcellular fractions from control (CTL-SMC) and cholesterol-enriched smooth muscle cells (CE-SMC) were prepared as described. Proteins in each fraction were separated by SDS-PAGE, transferred to Immobilon, and subjected to Western analysis. A, membrane; B, cytosol; C, nuclear/peroxisomal. This is one of three separate experiments.

Peroxisomes are very fragile and can easily be damaged during cell preparation. Therefore, catalase activity, an enzyme present in peroxisomes, was assessed to evaluate any peroxisomal contamination in the subcellular fractions. In two of three experiments, catalase activity was highest in the cytosolic fractions of both control and cholesterol-enriched smooth muscle cells, followed by activity in the nuclear/peroxisomal fraction. In two of three experiments, little or no catalase activity could be detected in the membrane fraction. These results are presented in **Table 1.** These results suggest that some of the SCP_2 present in the cytosol is most likely due to peroxisomal damage during the cellular fractionization procedure. Interestingly, cholesterol enrichment increased catalase activity approximately 2-fold in both the nuclear/peroxisomal and cytosolic fractions, with little or no change in the membrane fraction. In whole cell lysate, catalase activity was increased 2-fold in smooth muscle cells after cholesterol enrichment ($P < 0.05$, Table 1).

Northern analysis was performed to determine whether increases in SCP_2 protein in cholesterol-enriched smooth muscle cells were due to the increased content of the steady-state level SCP_2 mRNA. Cholesterol enrichment increased the ratio of the steady-state levels of the 1.3 kb species mRNA of SCP_2 relative to GAPDH by 2- to 3-fold (**Fig. 4**). Interestingly, the 2.2 kb mRNA that codes for SCPx was not observed by Northern analysis using RNA from either control or cholesterol-enriched smooth muscle cells. Thus, cholesterol enrichment increases both SCP_2 protein and mRNA levels.

DISCUSSION

Cholesterol accumulation in the vascular wall, as occurs in atherosclerosis, is associated with decreased lysosomal and cytoplasmic cholesteryl ester hydrolase activities (39, 52) and increased ACAT activity (33–36, 38), which results in an expansion of the cholesteryl ester pool (38, 39, 53). As cholesterol undergoes non-facilitated and facilitated transport between intracellular organelles, these observations also suggest that cholesterol enrichment may alter the mass or activities of the proteins responsible for mediating facilitated intracellular cholesterol trafficking. While the mechanisms that promote such cholesterol trafficking are controversial and not well defined, there is evidence to support a role of SCP_2 in these processes.

SCP_2 facilitates the exchange of sterols between phospholipid vesicles or membranes (3, 23–28) and enhances free cholesterol esterification through ACAT (8, 32). It is most highly expressed in the liver, but recent studies have also described its expression in whole rat aorta (32).

TABLE 1. Catalase activity in control (CTL-SMC) and cholesterol-enriched (CE-SMC) smooth muscle cells

	CTL-SMC				CE-SMC				Fold-Incr.
Exp. No.	N/P	C	M	L	N/P	C	M	L	
		× 10^{-3} units/μg protein				× 10^{-3} units/μg protein			
1	0	1.1	0	0	0	1.4	0	0	
2	5.0	1.7	0	2.2	1.3	3.1	6.0	5.0	2.3
3	1.5	2.0	1.9	5.4	9.0	3.6	1.3	13.9	2.6
4				6.0				14.0	2.3
5				5.0				9.0	1.8

Catalase activity is measured as described. One unit of catalase will degrade 1 μmole of H_2O_2/min at pH 7.0 at 25°C. Fold increase is the activity of catalase in lysates from cholesteryl ester-enriched smooth muscle cells versus control smooth muscle cells; N/P, nuclear peroxisomal fraction; C, cytosolic fraction; M, membrane; L, whole cell lysate.

(32). The results described in the present investigation demonstrate for the first time the expression of SCP_2 in cultured vascular smooth muscle cells by Western analysis and metabolic labeling studies.

Western analysis using the SCP_2 antibody also detected a protein migrating at approximately 30 kD. This protein was previously described in in vitro translation studies using a 1.7 kB for SCP_2 derived from a rat liver cDNA library (51) and in both normal fibroblasts and fibroblasts from patients with peroxisomal deficiencies (5, 54). It is unclear whether this protein represents a precursor to SCP_2 or whether it has an independent function. In contrast to Western analysis, we were unable to detect a protein migrating at 30 kD in metabolic labeling studies, using the SCP_2 antibody to immunoprecipitate labeled protein. There are two potential reasons for this: *1)* p30 is a protein whose synthesis is distinct from SCP_2, and is not sufficiently labeled during the short labeling time; and *2)* p30 is rapidly degraded or converted to another protein, possibly SCP_2.

Subcellular fractionization followed by Western analysis revealed that SCP_2 was localized principally in the cytosol, with lesser amounts associated with peroxisomes/nuclei. This subcellular pattern of SCP_2 was also observed in sonicated homogenates of whole rat aorta (32). However, immuno-electron microscopic studies of other cell types and tissues have revealed that SCP_2 is localized primarily to peroxisomes, cytosol, and membrane fractions of cells (7, 9–13). In addition, sonication releases SCP_2 and catalase (a peroxisomal protein) from the peroxisomes. As catalase activity of sonicated cells was present mostly in the cytosol, our data suggest that some of the SCP_2 present in the cytosol is derived from peroxisomes.

Using a model of the smooth muscle cell-derived foam cell previously established in our laboratory (38), we demonstrate herein that cholesterol enrichment of arterial smooth muscle cells increases the expression of SCP_2 proteins. This occurs commensurate with an increase in the mRNA for SCP_2 (but not SCPx), suggesting that the regulation of each of these proteins is specific. Western analysis indicates that p30 levels significantly decrease in cholesterol-enriched smooth muscle cells while SCP_2 levels increase. Thus, it is possible that p30 is a precursor for SCP_2 and that an increased conversion of p30 to SCP_2 contributes to increased levels in cholesterol-enriched smooth muscle cells. Interestingly, a 1.7 kB transcript that encodes the 30.8 kD form of SCP_2 was not detected (51). Similarly, cholesterol enrichment of rat peritoneal macrophages increased the expression of SCP_2 mRNA and protein (55), suggesting that cholesterol and its derivatives may regulate SCP_2 expression.

In previous studies of arterial SCP_2 it was demonstrated that SCP_2 may stimulate ACAT activity (32). Cholesterol enrichment of smooth muscle cells also displays significantly increased cholesterol esterification rates (38). At that time, we reasoned that the increased cholesterol esterification in cholesteryl ester-enriched smooth muscle cells was due to an increase in the levels of free cholesterol, secondary to the hydrolysis of LDL-derived cholesteryl ester (38). However, results of this study suggest that cholesterol enrichment may also stimulate ACAT activity by increasing the formation of SCP_2, as well as increasing free cholesterol.

Further insight into the significance of increased SCP_2 levels in cholesterol-enriched smooth muscle cells may be derived from two studies examining the influence of peroxisomal dysfunction on cholesterol synthesis and intracellular cholesterol trafficking. First, SCP_2-deficient fibroblasts that still expressed p30 and SCPx had no alterations in intracellular cholesterol trafficking. This suggests that the higher molecular weight forms of SCP_2 may participate in cholesterol trafficking (54). In a second study, the transport of newly synthesized cholesterol was delayed in fibroblasts that were naturally deficient in SCP_2 and those made deficient using anti-sense therapy (5). Thus, SCP_2, or its related proteins, may increase the rate of intracellular direc-

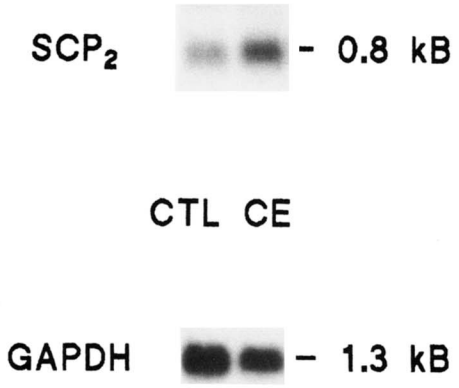

Fig. 4. Northern analysis of steady state levels of SCP_2 mRNA in smooth muscle cells: influence of cholesterol enrichment. mRNA levels were normalized to glyceraldehyde phosphate dehydrogenase (GAPDH). This represents one of three duplicate experiments.

trafficking, without affecting net cholesterol transport.

High density lipoprotein (HDL)-mediated cholesterol efflux occurs via a specific HDL-receptor through a protein kinase C-dependent pathway (56–58). However, the HDL-dependent cholesterol efflux may also be facilitated by intracellular lipid binding proteins, including SCP_2 (59). Thus, SCP_2 may function to increase the availability of cholesterol to this pool by enhancing the rate of exchange of sterols from other intracellular sites. This appears significant as HDL increased cholesterol efflux from cholesterol-enriched smooth muscle cells but not control smooth muscle cells (39). As cholesterol enrichment of smooth muscle cells also increases membrane-associated SCP_2, our results indicate that SCP_2 may participate in HDL-mediated efflux from the smooth muscle cell-derived foam cell.

Thus, the function of SCP_2 in arterial smooth muscle cells may be to facilitate intracellular cholesterol trafficking, particularly under conditions of cholesterol enrichment. As early human atherosclerotic lesions are composed principally of smooth muscle cells, while later lesions contain both smooth muscle cells and macrophages (37), these results, coupled with increased SCP_2 expression in smooth muscle cell and macrophage-derived foam cells, support a role for SCP_2 in intracellular cholesterol trafficking.

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