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Research Article

Opposite pattern of MDR1 and caveolin-1 gene expression in human atherosclerotic lesions and proliferating human smooth muscle cells

B. Batetta [a], M. F. Mulas [a], P. Petruzzo [b], M. Putzolu [a], R. R. Bonatesta [a], F. Sanna [a], A. Cappai [b], G. Brotzu [b] and S. Dessì [a,*]

[a] Dipartimento di Scienze Biomediche e Biotecnologie, Sezione di Patologia Sperimentale, Università degli Studi di Cagliari, Via Porcell 4, 09124 Cagliari (Italy), Fax + 39 70 662 574, e-mail: sdessi@vaxca1.unica.it
[b] Dipartimento di Scienze Chirurgiche e Trapianto D'Organo, Università degli Studi di Cagliari (Italy)

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Abstract. Cholesterol esterification and smooth muscle cell (SMC) proliferation are the crucial events in the development of atherosclerotic lesions. The objective of this study was to analyse cholesterol esterification and the expression of MDR1 (multidrug resistance), ACAT (acyl-CoA:cholesterol acyltransferase) and caveolin-1 genes in atherosclerotic and healthy vascular walls, in SMCs obtained from atherosclerotic lesions and saphenous veins. Results demonstrated higher levels of cholesterol esters, ACAT and MDR1 mRNAs and lower levels of caveolin-1 mRNA in atherosclerotic segments compared to adjacent serial sections of the same artery and the corresponding non-atherosclerotic arteries from cadaveric donors. SMCs isolated from atherosclerotic plaques manifested an increased capacity to esterify cholesterol and to grow at a faster rate than SMCs isolated from saphenous veins. In addition, when SMCs from atherosclerotic plaques were cultured in the presence of progesterone, a potent inhibitor of cholesterol esterification, significant growth suppression was observed. An increase in ACAT and MDR1 expression and a concomitant decrease in caveolin-1 expression were also observed in SMCs isolated from atherosclerotic arteries as early as 12 h after serum stimulation. An opposite pattern was found when SMCs were treated with progesterone. These findings support the idea that cholesterol esterification plays a role both in early atherogenesis and in clinical progression of advanced lesions and raise the possibility that the cholesterol ester pathway might directly modulate the proliferation of SMCs.

Key words. Atherosclerosis; cholesterol esterification; SMC; ACAT; MDR1; caveolin-1.

Cholesterol esterification, via acyl-CoA:cholesterol acyltransferase (ACAT), occurs according to the availability of cholesterol substrate in endoplasmic reticulum (ER). A model has recently been proposed whereby a p-glycoprotein encoded by the (multidrug resistance) MDR1 gene is required for transport of free cholesterol from the plasma membrane to the ER, thus suggesting an important role for MDR1 in the process of cholesterol esterification [1, 2]. Data obtained in our laboratory have underlined a positive correlation between cholesterol esterification, ACAT and MDR1 gene expression and the rate of cell proliferation [3, 4]. We suggested that the MDR1 gene may contribute towards regulating the rate of cell growth and division by modulating intracellular cholesterol ester levels.

Another protein that interacts with cholesterol trafficking is caveolin-1. In an opposite direction to p-glycoprotein

* Corresponding author.

pumping, caveolin-1 transports cholesterol from the ER to the plasma membrane [5, 6] and mediates the efflux of free cholesterol derived either from de novo synthesis or low density lipoproteins. Caveolin-1 has also been reported to play a role as a negative regulator of a number of signalling molecules involved in cell growth and mitogenesis, and has accordingly been proposed to function as a putative tumour suppressor gene [7–10]. A recent study suggests that at least some of the effects exerted by caveolin on signal transduction are indirect and may be mediated through its influence on cholesterol transport [11].

Cholesterol esterification of vascular smooth muscle cells (SMCs) is a key event in both early atherogenesis and the clinical progression of advanced lesions. Over the last few years, a variety of ACAT inhibitors showing beneficial effects of reduced atherosclerosis in experimental animals have been identified [12]. The mechanism by which progression of atherosclerosis is reduced following inhibition of cholesterol esterification is still unclear and whether cholesterol esters are simply an 'innocent bystander' or play a direct causal role in the progression of the disease is still debated. Together with cholesterol ester accumulation, SMC proliferation is an important contributor to the progression of atherosclerosis [13]. Therefore, the question is raised as to whether modifications of the cholesterol ester cycle may mediate the progression of vascular disease through their influence on SMC proliferation.

In the present study, the expression of genes implicated in the cholesterol ester cycle, particularly ACAT, MDR1 and caveolin 1, were investigated in the wall of different arterial sites and saphenous veins as well as during the growth of SMCs from atherosclerotic arteries and saphenous veins. The aim was to establish whether the modulation of cholesterol esterification may affect SMC proliferation and modify the progression of atherosclerotic disease, and whether a link exists between the roles of caveolin in cholesterol trafficking and signal transduction.

Materials and methods

Artery and vein samples

Healthy segments of internal carotid artery (n = 6), abdominal aorta (n = 6), iliac artery (n = 4), superficial femoral artery (n = 4), profunda femoral artery (n = 4) and internal mammary artery (n = 3) were obtained from six multiorgan cadaveric donors (two female and four male), ranging in age from 32 to 60 years.

Samples of human atherosclerotic lesions and the adjacent segments of internal carotid artery (n = 3), abdominal aorta (n = 5), iliac artery (n = 2) and superficial femoral artery (n = 2) were obtained from 12 patients undergoing surgical intervention for occlusive carotid disease, aortic aneurysm or thrombosis, and severe chronic leg ischaemia. Patients were mostly male (9/12) in the age range 52–78 years. In addition, tissue samples of saphenous veins (n = 11) and arteriovenous fistulae were obtained from patients undergoing saphenous vein stripping (age range 42–70 years) and from patients (2 females, 41 and 80 years old) suffering from chronic renal failure.

All studies had been approved by the local Ethical Committee and informed consent was obtained from patients. Within 1 h of excision, samples were placed in sterile boxes containing Eurocollins solution, stored at 4 °C and transported to the laboratory where they were cleaned of adventitial tissue and the endothelial layer, washed, weighed and stored at – 80 °C until biochemical and molecular analyses were performed.

All pathologic specimens used in this study contained atherosclerotic lesions characterised histologically as advanced lesions. Multiorgan donors did not present symptomatic and/or macroscopic atherosclerotic lesions.

Isolation of cells from tissue

Arterial-derived SMCs were isolated from human atherosclerotic tissue obtained from the abdominal aorta of three patients undergoing surgery for aortic aneurism. Vein SMCs were isolated from human samples of saphenous veins obtained from three patients undergoing saphenous vein stripping. The adventia and media of abdominal aortas were separated with the assistance of surgical loops (magnification ×2). The separation of advential from intimal-medial layers was confirmed through immunostaining with α-SM actin antibody of random samples (not shown). For saphenous veins, the adventitial layer was stripped off, and only the media was used for cell isolation.

All tissues were transported to the laboratory in sterile Ca^{2-} and Mg^{2+}-free phosphate-buffered saline (PBS) and cultured within 12 h of surgery. All dissections were performed in PBS supplemented with a fourfold mixture (100 U/ml penicillin, 100 µg/ml streptomycin).

All tissues were minced into small pieces (1 × 1 mm). During the first week, cells were cultured at 37 °C, 5% CO_2, 80% humidity in MEM 199 supplemented with 20% foetal calf serum (FCS). When a monolayer was reached, cells were removed by trypsin-EDTA (0.05–0.02%) and cultured in 25-cm^2 flasks in MEM-199 supplemented with 10% FCS, 1% penicillin-streptomycin and 10 µg/ml of gentamycin at 37 °C, 5% CO_2 air. The medium was changed every 2 days. After 4 weeks, when cell density reached confluence, SMCs were removed with 0.025% EDTA and 0.05% trypsin and subcultured with fresh growth medium. Cultured SMCs grew in a typical 'hill and valley' formation. At passage one, the cells were characterised with a monoclonal antibody against smooth muscle α-actin and were subcultured to be used between passages two to four. Cells were plated at a density of 5000 cells/cm^2 in six-well plates and then

incubated for 48 h in MEM 199 containing 0.2% FCS to synchronise cells at a quiescent state. For all experiments, quiescent cells were stimulated to proliferate by adding FCS (10%). SMCs isolated from atherosclerotic tissue were incubated in the presence or absence of 10 µM progesterone (Sigma).

Cell proliferation and ^{3}H-thymidine incorporation

At the times indicated, quiescent cells supplemented with 10% FCS were washed with PBS, harvested with trypsin/EDTA (0.05%/0.02%) and the cell number was counted using a Coulter counter. For the thymidine incorporation studies, cells were labelled with ^{3}H-thymidine at 2.5 µCi/ml during the last 24 h of incubation, and radioactivity incorporated into the DNA was determined by trichloroacetic acid precipitation of the cell lysate.

Cholesterol esterification

Cholesterol esterification was evaluated by incubating cells for 5 h in medium containing [1-^{14}C]oleic acid (Dupont, NEN; 55 mCi/mmol), bound to bovine serum albumin (BSA). To prepare the [^{14}C]oleate-BSA complex, 100 µCi of [1-^{14}C]oleic acid in ethanol was mixed with 1.4 mg KOH and the ethanol evaporated. PBS (1.5 ml) without Ca^{2+} and Mg^{2+} containing 4.24 mg BSA (essentially fatty acidfree; Sigma) was added and the mixture shaken vigorously. This solution was added to each well at a final concentration of 2 µCi/ml. After incubation, cells were washed with PBS and extracted with acetone. Lipid subclasses were separated by thin layer chromatography (TLC) as described above and incorporation of [^{14}C]oleate into cholesterol esters was measured.

Tissue lipid analysis

Atherosclerotic and control arterial and vein samples were homogenised in 9 vol 0.01 M potassium phosphate buffer (pH 7.4) and lipids extracted with chloroform/methanol 2:1 v/v. They were then separated by TLC on kiesegel plates (Merck, Germany) using a solvent system containing n-heptane/isopropyl ether/formic acid (60:40:2, v/v/v). Cholesterol ester (CE) bands were identified by comparison with standards which ran simultaneously on one side of the plate, using iodine vapour. Cholesterol mass was determined by Bowman and Wolf's method [14] using cholesterol palmitate as standard.

RT-PCR analysis

Total RNA was isolated from arterial specimens using the guanidine isothiocyanate phenol-chloroform extraction method [15]. RNA integrity was evaluated by agarose gel electrophoresis, and RNA yield was quantified spectrophotometrically and A_{260}/A_{280} ratios determined.

Equal amounts of total RNA (1 µg) were reverse transcribed into cDNA using the random-hexamer method. cDNA was subsequently amplified by PCR in the presence of specific primers, according to the instructions provided by the manufacturer (GeneAmp RNA PCR Kit; Perkin-Elmer Cetus).

PCR was performed using the following primers and conditions. For MDR1 [16], 5′CCCATCATTGCAATAGCAGG3′, 5′GTTCAAACTTCTGCTCCTGA3′ (167-bp fragment); 94 °C for 30 s, 55 °C for 60 s, and 72 °C for 60 s for 30 cycles. For ACAT [17], 5′AGCAGAGGCAGAGGAATTGA3′, 5′GCACACCTGGCAAGATGGAG3′ (466-bp fragment); 95 °C for 30 s, 58 °C for 50 s, and 72 °C for 60 s, for 40 cycles. For caveolin-1 [5], 5′GAGCGAGAAGCAAGTGTACGA3′, 5′ACAGACGGTGTGGACGTAGAT3′, (360-bp fragment); 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 120 s, for 30 cycles. For L7 [18], 5′CCTGAGGAAGAAGTTTGCCC3′, 5′CTTGTTGAGCTTCACAAAGGTGCC3′ (285-bp fragment); 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 45 s, for 25 cycles. Preliminary experiments demonstrated that using these PCR conditions, the amount of product amplification to RNA relationship was linear for the cycle number used over the range 500–1500 ng total RNA. Working with these conditions, PCR products separated on agarose and stained with ethidium bromide were characterised by a major band of the predicted size (data not shown).

Blotting analysis

During the PCR reaction, the non-radioactive label digoxigenin-11-dUTP (DIG; Boheringer Mannheim) was incorporated and immunodetected with anti-DIG F_{ab} fragments conjugated to alkaline phosphatase and visualised with the chemiluminescence substrate CSPD. Enzymatic dephosphorylation of CSPD by alkaline phosphatase led to light emission at a maximum wavelength of 477 nm which was recorded on X-ray films. The DNA fragments were separated by electrophoresis on agarose and then blotted onto a nylon membrane for 16 h in 10 × SSC. The blot was exposed to X-ray film for 2–10 min. A Kodak Digital Science Band Scanner Image Analysis System containing HP ScanJet, ID Image Analysis Software assessed the intensity of the bands in the autoradiograms.

The overall procedure was standardised by expressing the amount of PCR product for each target mRNA relative to the amount of product formed for L7, a ribosomal protein which is encoded by a non-inducible cell cycle-independent gene.

Since a low yield of PCR products is often obtained when cDNA segments are coamplified with an internal standard gene in the same tube [19], the relative levels of gene expression were determined by comparing the PCR products of the target cDNA and L7 gene processed in separate tubes.

Statistical analysis

Results were analysed using a non-parametric Mann-Withney test. For all statistical analyses, the level of significance was set at $P < 0.05$.

Results

In the present study, we examined lipid accumulation and the expression of genes correlated with cholesterol metabolism, namely ACAT, MDR1 and caveolin 1 mRNAs, in the wall of different arterial sites in both normal and atherosclerotic vessels, and in saphenous veins and therapeutic arteriovenous fistulae.

Lipid content

As expected, accumulation of cholesterol esters was dramatically higher in atherosclerotic plaques compared to both adjacent serial sections and the corresponding non-atherosclerotic arteries from cadaveric donors used as controls (fig. 1). However, a significantly higher cholesterol ester content was also seen in the control arterial walls of arteries prone to atherosclerosis, such as internal carotid artery, abdominal aorta, iliac artery and superficial femoral artery when compared to resistant arteries, i.e. profunda femoral artery and internal mammary artery, and saphenous veins (fig. 1). Lipid analysis performed on the walls of two arteriovenous fistulae revealed an enhancement of cholesterol ester content similar to that observed in samples from atherosclerotic arteries (fig. 1).

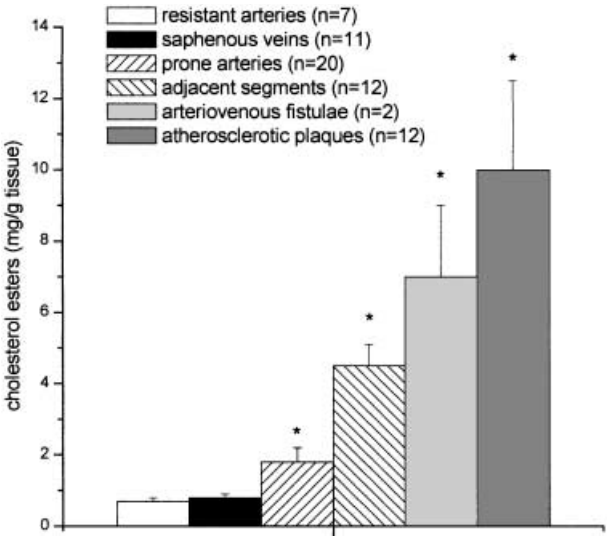

Figure 1. Cholesterol ester content in normal and pathological vessels. Bar graphs show mean + SE cholesterol ester content of normal prone and resistant arteries, atherosclerotic plaques and the adjacent segments, saphenous veins and arteriovenous fistulae. * Statistically significant ($P < 0.05$) compared with resistant arteries and saphenous veins.

RT-PCR analysis

Subsequently, the presence of MDR1 and ACAT mRNA in the arterial and venous specimens was assessed (figs. 2, 3). The small amounts of some arterial specimens led us to apply RT-PCR for these studies. The specificity of RT-PCR was validated in a previous study [20]. Lesions studied were predominantly advanced plaques with characteristic fibrous caps, lipid-laden macrophages, intimal hyperplasia and lymphocytes distributed throughout the lesions.

In all specimens of atherosclerotic tissue studied, the expression of MDR1 and ACAT genes was higher (fig. 2) than in both adjacent serial sections and the corresponding non-atherosclerotic arteries from cadaveric donors. In addition, very low expression of MDR1 and ACAT mRNAs was observed in arteries resistant to atherosclerosis and in saphenous vein walls, while a dramatic increase in MDR1 and ACAT mRNA levels was detected in two arteriovenous fistulae (fig. 3).

A membrane p-glycoprotein, encoded by the MDR1 gene, is required for transport of free cholesterol from the plasma membrane to the ER, where cholesterol is esterified by ACAT [1, 2] demonstrating that MDR1 is involved in the cholesterol esterification process. Conversely, rapid transport of cholesterol from ER to the cell surface is mediated by a membrane-associated protein, caveolin-1, suggesting a central regulator role for this protein in intracellular cholesterol efflux [6, 21]. Therefore the present study also evaluated caveolin-1 mRNA levels.

Caveolin-1 mRNA was abundant in both normal arterial tissues and in the adjacent segments of arteries of the plaques compared to atherosclerotic lesions (fig. 2). Interestingly, very low caveolin-1 mRNA was observed in arteriovenous fistulae compared to saphenous vein and to resistant atherosclerosis arteries (fig. 3).

Time-dependent changes in the cholesterol ester pathway during replication of plaque SMCs

To establish whether cholesterol esterification is associated with the cell cycle progression of SMCs, we studied time-dependent changes in the cholesterol esterification pathway during cell replication of SMCs isolated from atherosclerotic lesions. Vascular SMCs isolated from saphenous veins were used as controls as they usually do not present atherosclerotic lesions.

Quiescent SMCs from atherosclerotic lesions were stimulated to grow by adding 10% FCS, and cell number and ^{3}H-thymidine incorporated into DNA were analysed at different periods of time following stimulation in the presence and absence of 10 µM progesterone. Progesterone was chosen because it is a well-known inhibitor of cholesterol esterification, and is also able to modulate MDR1 activity (1, 2). Progesterone exerts its inhibitory effect by blocking transport of cholesterol substrate from

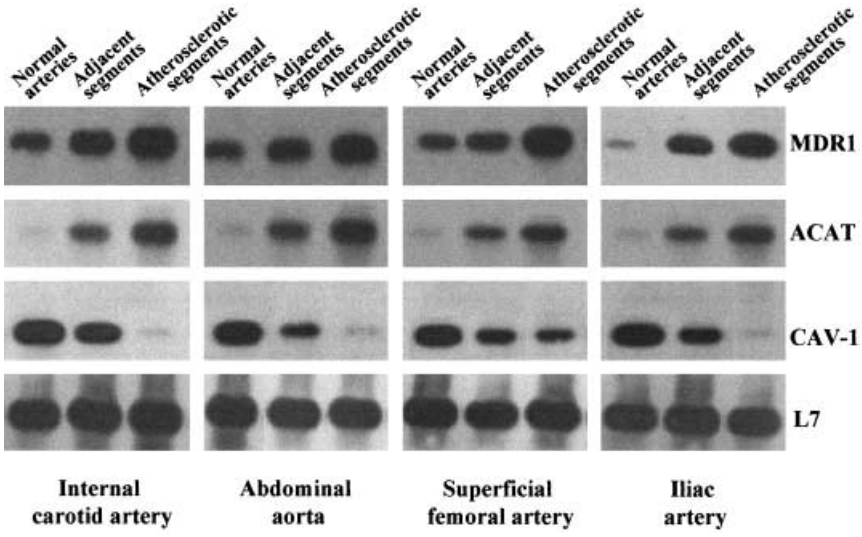

Figure 2. Autoradiograms of MDR1, ACAT, caveolin-1 and L7 gene expression (mRNA levels) of atherosclerotic and adjacent segments, and normal arteries. Autoradiograms are representative of 20 normal arteries (6 internal carotid arteries, 6 abdominal aortas, 4 iliac arteries, 4 superficial femoral arteries) and 12 atherosclerotic segments and relative adjacent segments (3 internal carotid arteries, 5 abdominal aortas, 2 iliac arteries and 2 superficial femoral arteries).

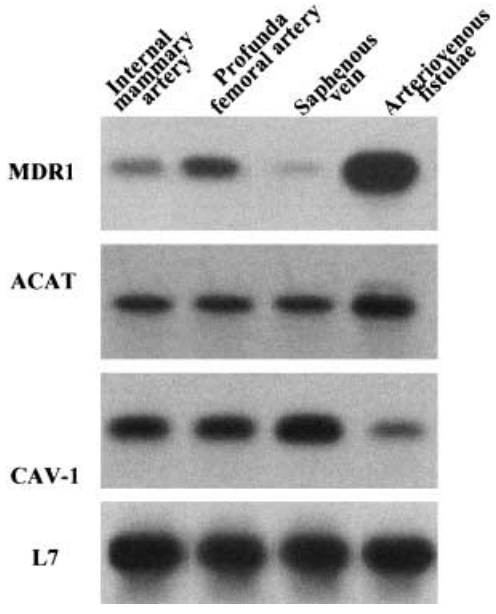

Figure 3. MDR1, ACAT and Caveolin-1 (CAV-1) mRNA levels in arteries resistant to atherosclerosis (profunda femoral artery and internal mammary artery), saphenous veins and arteriovenous fistulae. Autoradiograms are representative of 7 normal arteries (4 profunda femoral arteries and 3 internal mammary arteries), 11 saphenous veins and 2 arteriovenous fistulae.

the plasma membrane to the ER, the site of cholesterol esterification by ACAT.

As shown in figure 4A, untreated SMCs from atherosclerotic lesions grew faster than both SMCs of saphenous veins and atherosclerotic lesions treated with progesterone. The rate of ^{3}H-thymidine incorporation was consistently higher throughout the experiment in untreated SMCs from atherosclerotic lesions compared to SMCs from saphenous veins and atherosclerotic lesions treated with progesterone (fig. 4B). In untreated SMCs, ^{3}H-thymidine incorporation began to increase 24 h after serum stimulation, reached a peak at 48 h, decreased at 72 h and increased again until 120 h (fig. 4B). The significant reduction in ^{3}H-thymidine incorporation at 72 h is probably due to the fact that at this point, the majority of cells were not in the S phase of the cellular cycle. The reduction of ^{3}H-thymidine incorporation mediated by progesterone reflected a reduction in cell number, as confirmed by parallel experiments performed with the Coulter counter (fig. 4A, B).

Changes in ^{3}H-thymidine incorporation were accompanied by similar changes in the levels of [^{14}C]oleate incorporated into cholesterol esters, which began to increase at 12 h, reached maximal levels at 24 h, declined at 48 h and increased again at 72 h (fig. 5). These data demonstrate that the cholesterol esterification pathway is activated prior to the onset of DNA synthesis, supporting the concept that cholesterol esterification may play a role in the regulation of cell cycle progression of SMCs from atherosclerotic lesions. Accordingly, both saphenous vein SMCs and progesterone-treated SMCs from atherosclerotic lesions, which grow more slowly than untreated SMCs, also had consistently lower levels of cholesterol esterification at all time points considered (fig. 5).

Subsequently, we analysed MDR1 and ACAT mRNA levels during growth of SMCs from atherosclerotic lesions in the presence or absence of progesterone (fig. 6). mRNA levels of ACAT and MDR1 were already increased 12 h after serum stimulation in untreated SMCs. At this time, mRNA levels of caveolin-1 were much lower

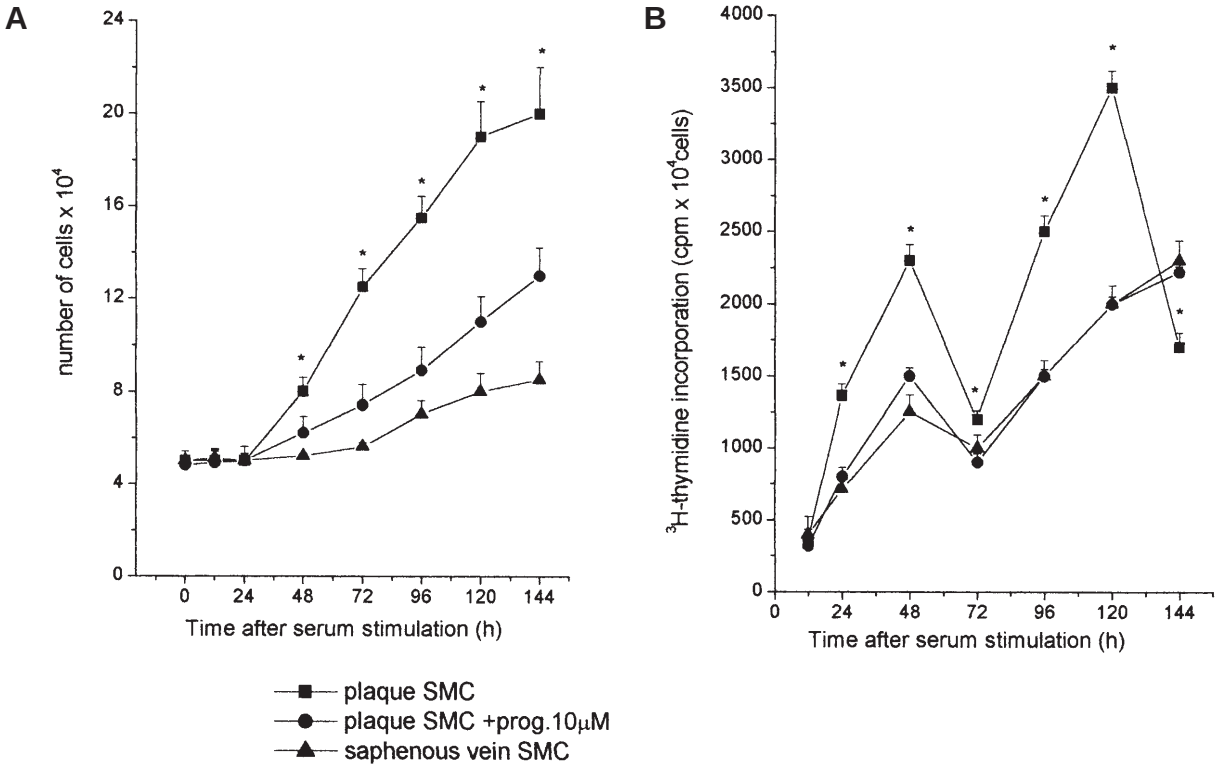

Figure 4. Time course of cell growth (*A*) and ^{3}H-thymidine incorporation (*B*) into saphenous vein SMCs and plaque SMCs in the presence or absence of progesterone. Quiescent cells were seeded at a density of 5000 cells/cm^2 and stimulated to grow by adding 10% FCS. At the indicated times, cell number was determined with a Coulter counter and corrected for viability by Trypan Blue exclusion. Cells were incubated with ^{3}H-thymidine 24 h before each time point (with the exception of 12 h) and harvested every 24 h for a total period of 144 h as described in Materials and methods. Data are the mean + SE of three independent experiments, each performed in triplicate. *Statistically significant ($P < 0.05$) compared with progesterone treated-plaque SMCs and saphenous vein SMCs.

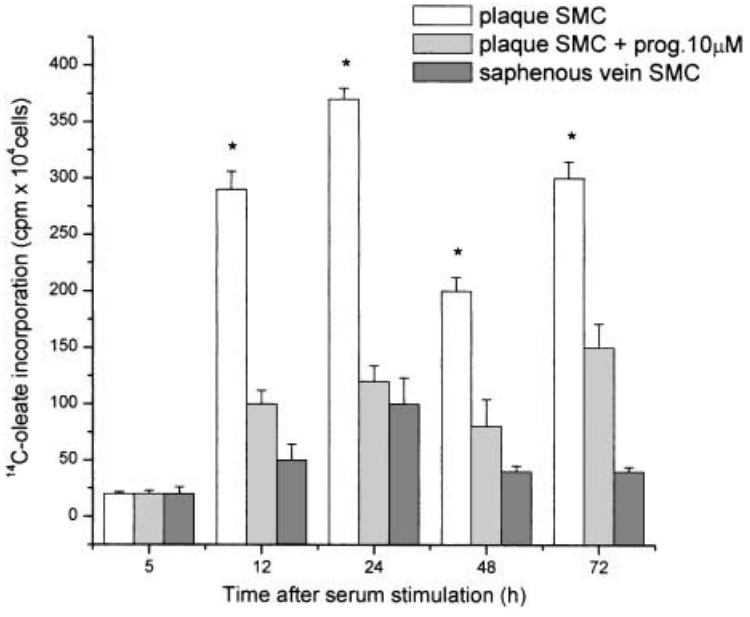

Figure 5. Time course of [^{14}C]oleate incorporation by saphenous vein SMCs and plaque SMCs in presence or absence of progesterone. Quiescent SMCs were stimulated to grow by adding 10% FCS and [^{14}C]oleate 5 h before each time point and harvested every 24 h for a total period of 144 h as described in Materials and methods. Data are the mean + SE of three independent experiments, each performed in triplicate. *Statistically significant ($P < 0.05$) compared with progesterone treated-plaque SMCs and saphenous vein SMCs.

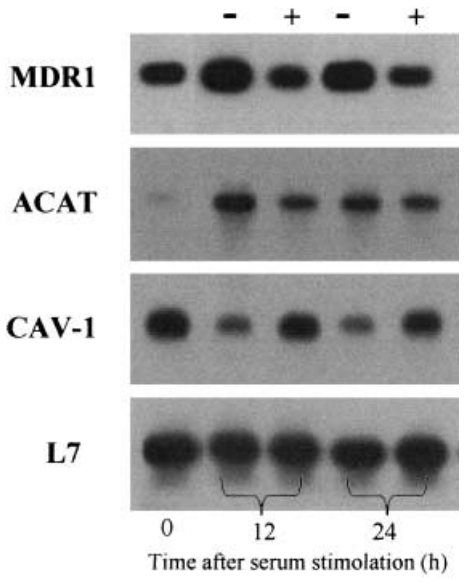

Figure 6. Effect of progesterone on MDR1, ACAT and Caveolin-1 (CAV-1) mRNA levels during the growth of plaque SMCs. Quiescent plaque SMCs were stimulated to grow by adding 10% FCS in the presence (+) and in absence (–) of progesterone (10 µM) and harvested at 12 and 24 h. Shown are representative autoradiograms of MDR1, ACAT, caveolin-1 and L7 gene expression of three independent experiments, each performed in triplicate .

than those observed in unstimulated SMCs. An opposite pattern was observed when SMCs were treated in the presence of progesterone.

Discussion

In the development of atherosclerotic lesions, cholesterol esterification and abnormal proliferation of vascular SMCs are known to be key events [13]. Although atherosclerosis affects only particular sites of the vascular tree, the cause is only partially understood. In addition, whether cholesterol ester accumulation represents simply an epiphenomenon or has a pathophysiological function in atherosclerosis remains unclear. Moreover, we do not know whether cholesterol esters and vascular SMC proliferation correlate during the progression of atherosclerotic lesions. To explore these topics, we first evaluated the cholesterol ester content and expression of related ACAT and MDR1 genes in human arteries prone and resistant to atherosclerosis and in veins. We demonstrated the presence of higher levels of cholesterol ester and ACAT and MDR1 mRNAs in atherosclerotic arteries compared to both adjacent serial sections and the corresponding non-atherosclerotic arteries from cadaveric donors. In addition, higher levels of cholesterol ester and ACAT and MDR1 expression were observed in control arteries prone to atherosclerosis compared to both resistant arteries and veins. These results suggest that different biological properties might explain, at least in part, the minor susceptibility of veins and some arteries, such as internal mammary artery and profunda femoral artery, to the development of atherosclerotic lesions. Accordingly, Oemar et al. [22] found that human connective tissue growth factor, a novel cysteine-rich, secreted polypeptide is highly expressed in SMCs of advanced atherosclerotic lesions. In contrast, in normal arteries, they were unable to detect human connective tissue growth factor expression. They concluded that human connective tissue growth factor may represent a novel factor expressed at high levels specifically in advanced lesions and may play a role in the development and progression of atherosclerosis.

Another interesting finding of the present study was that caveolin-1 was highly expressed in vein walls and in arteries not prone to atherosclerosis, while very low mRNA levels of caveolin-1 were detected in arteries prone to atherosclerosis and particularly in the arteriovenous fistulae and in atherosclerotic lesions. Of particular note is that caveolin-1 expression showed an opposite pattern to ACAT and MDR1 gene expression and to cholesterol ester accumulation in all vessels studied.

Caveolin-1 and MDR1 have recently been reported to have inverse implications in the sterol metabolism of normal cells [1, 6]. The MDR1 gene is involved in the process of cholesterol esterification, being required for transport of free cholesterol from the plasma membrane to the ER. In contrast, caveolin-1 is required for the translocation of cholesterol from the ER to the plasma membrane and is involved in cholesterol efflux [5, 6]. Although the molecular mechanism involved in free cholesterol efflux by caveolin-1 is not yet known, this protein has been postulated to be capable of mediating or facilitating the export of free cholesterol via the class B, type I scavenger high-density lipoprotein receptor (SR-B1) [23]. If caveolin-1 should possess such a capability, the fact that veins and resistant arteries express higher levels of caveolin-1 suggest that this protein, by limiting cholesterol ester accumulation inside the cells, may prevent early stages of the atherosclerotic process in these vessels.

In addition to its role in cholesterol binding and transport, caveolin-1 may modulate signal transduction [8–10]. The mechanisms underlying these signalling events are currently unknown and caveolin-1 has been speculated to bind signalling receptors and second-messenger effectors involved in cell division, such as the MAPK cascade, via a cytoplasmic ‘scaffolding domain’. Caveolin-1 can positively regulate growth arrest in normal and transformed cells [24, 25], suggesting that one functional consequence of the up-regulation of caveolin may be a slower rate of proliferation.

In the current study, we also found that the proliferative activity of SMCs from atherosclerotic lesions induced by

serum was preceded by an increased ability of cells to esterify cholesterol. The increased cholesterol esterification in these cells was accompanied by a higher expression of genes involved in cholesterol esterification, such as ACAT and MDR1, and by a lower expression of caveolin-1. In addition, when SMCs from atherosclerotic plaques were cultured in the presence of progesterone, a potent inhibitor of cholesterol esterification, significant growth suppression was observed. An increase in ACAT and MDR1 expression and a concomitant decrease in caveolin-1 expression was also observed in SMCs isolated from atherosclerotic arteries as early as 12 h after serum stimulation. An opposite pattern was observed when SMCs were treated with progesterone. These findings add further support to the idea that cholesterol esters play an important role both in early atherogenesis and in the clinical progression of advanced lesions, and also raise the possibility that the cholesterol ester pathway might directly modulate the proliferation of SMCs. Cholesterol esters and the genes correlated with cholesterol esterification might be important in growth rate regulation, providing a rapid supply of cholesterol for membrane biogenesis as well as playing a direct role in the balance of the growth regulator factors.

In addition, these findings suggest the possibility that the effect of caveolin-1 on signal transduction is indirect and may be, albeit partially, mediated through its influence on the regulation of intracellular cholesterol levels. The mechanisms underlying these important aspects are currently under investigation in our laboratory.

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