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Lycopene regulation of cholesterol synthesis and efflux in human macrophages☆

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Abstract

Hypercholesterolemia is one of the most important risk factors for atherosclerosis, and tomato lycopene has been suggested to have beneficial effects against such a disease, although the exact molecular mechanism is unknown. We tested the hypothesis that lycopene may exert its antiatherogenic role through changes in cholesterol metabolism. Incubation of THP-1 cells with lycopene (0.5–2 μM) dose-dependently reduced intracellular total cholesterol. Such an effect was associated with a decrease in reduction of 3-hydroxy-3-methylglutaryl coenzyme A reductase expression and with an increase in ABCA1 and caveolin-1 (cav-1) expressions. In addition, lycopene enhanced RhoA levels in the cytosolic fraction, activating peroxisome proliferator-activated receptor gamma (PPARγ) and liver X receptor alpha expressions. Concomitant addition of lycopene and the PPARγ inhibitor GW9662 or lycopene and mevalonate blocked the carotenoid-induced increase in ABCA1 and cav-1 expressions. These results imply a potential role of lycopene in attenuating foam cell formation and, therefore, in preventing atherosclerosis by a cascade mechanism involving inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase, RhoA inactivation and subsequent increase in PPARγ and liver X receptor alpha activities and enhancement of ABCA1 and cav-1 expressions.
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Keywords: Lycopene; Cholesterol; HMG-CoA reductase; ABCA1; Caveolin-1; THP-1 cells

1. Introduction

Arterial macrophages are faced with the task of internalizing and metabolizing atherogenic lipoproteins, an event that challenges cellular cholesterol homeostasis [1,2]. Cholesterol homeostasis is maintained through the coordinated regulation of pathways mediating cholesterol uptake, storage, *de novo* synthesis and efflux, and it is likely that the deregulation of these signals promotes foam cell formation [3,4]. The committed step in the biosynthesis of cholesterol and isoprenoids is catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which promotes the deacylation of HMG-CoA to mevalonate [5,6]. The activity of HMG-CoA is shown to be sensitive to negative regulation by both sterols and nonsterol products of the mevalonate pathway [7,8]. This pathway produces numerous bioactive signaling molecules, including farnesyl pyrophosphate and geranylgeranyl pyrophosphate (GGPP). Members of the RhoA GTPase family are major substrates for posttranslational modification by geranylgeranylation [5,9], a process essential for its proper membrane localization and activation [10]. RhoA GTPase functions as a molecular switch, cycling between an active GTP-bound state and an inactive GDP-bound state, thereby mediating cellular responses through their association with numerous effector molecules, including kinases [11]. In particular, it has been recently demonstrated that HMG-CoA reductase inhibition, through reduced RhoA activation, resulted in decreased phosphorylation and enhanced the activity of nuclear hormone receptor peroxisome proliferator-activated receptor gamma (PPARγ) [5]. Because PPARγ is also negatively regulated through phosphorylation [12], it has been reported that RhoA could also modulate PPARγ activation and the expression of PPARγ target genes. Furthermore, it has been demonstrated that the nonsterol mevalonate intermediate GGPP can directly antagonize liver X receptor (LXR) activity and indirectly inhibit the expression of LXR-responsive genes by reducing Rho protein activation [13,14]. Key targets of PPARγ and LXRα activation are the ATP-binding cassette (ABC) proteins, such as ABCA1, which has been reported to control the rate-limiting step in cellular cholesterol and phospholipid efflux to apoA1 [15], and caveolin-1 (cav-1), which has been reported to play a role by favoring cholesterol association with lipid for efflux [16]. ABCA1 and cav-1 expressions are up-regulated by agonists of PPARγ and LXRα through a transcriptional cascade ultimately dependent on the activation of LXRα

Abbreviations: ABC, ATP-binding cassette; cav-1, caveolin-1; GGPP, geranylgeranyl pyrophosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LXR, liver X receptor; PMA, phorbol 22-myristate 13-acetate; PPAR, peroxisome proliferator-activated receptor; THF, tetrahydrofuran.

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[17,18]. It has been recently reported that PPARγ agonists blocked macrophage foam cell formation induced by oxidized lipoproteins through enhanced ABCA1-mediated cholesterol efflux in an LXR-dependent manner [19]. According to this, statins are competitive inhibitors of HMG-CoA reductase that decrease cholesterol biosynthesis and are widely used for cholesterol-lowering therapy and prevention of atherosclerosis-related events [6]. The potential for tomato and/or tomato products to prevent atherosclerosis is currently under investigation, and a lot of emphasis has been placed on elucidating the role of lycopene, the most abundant carotenoid in ripe tomato, and its mechanism of action. The potential antiatherogenic role of lycopene has been ascribed mainly to its antioxidant capacity, which is related to the prevention of low-density lipoprotein oxidation [20]. Recently, a new mechanism involving regulation of cholesterol metabolism by carotenoids has been evoked [21]. This seems to be sustained by the observation that β-carotene and lycopene share similar initial synthetic pathways with cholesterol, which is synthesized in animal but not in plant cells. In spite of this body of evidence, at the moment, studies investigating directly the role of lycopene and other carotenoids on cholesterol pathways are very few. β-Carotene has been reported to regulate the expression of HMG-CoA reductase in rat liver [22], and both lycopene and β-carotene have been demonstrated to inhibit macrophage HMG-CoA reductase activity [23]. In addition, evidence showing that lycopene may act as a hypocholesterolemic agent in a human trial, in which dietary supplementation with lycopene resulted in a significant reduction in plasma low-density lipoprotein cholesterol concentrations, has been reported [24]. Starting on the concept that excess of any end product in the mevalonate pathway could regulate the production of other products of the same synthetic pathway, in this study, we have investigated the possibility that lycopene may modify the levels of HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, and the localization of RhoA protein in human macrophages. This, in turn, may alter RhoA activation, inducing the modulation of molecules, such as PPARγ and LXRα, and regulating the levels of ABC proteins, such as ABCA1, and those of cav-1. Our results clearly demonstrate that macrophage enrichment with lycopene results in the suppression of cellular cholesterol synthesis and efflux.

2. Materials and methods

2.1. Cell culture

THP-1 cells (American Type Culture Collection, Rockville, MD, USA) were grown in Dutch-modified RPMI (Sigma, Milan, Italy) without antibiotics and supplemented with 10% fetal bovine serum, nonessential amino acids, 2 mM glutamine and 1 mM sodium pyruvate. Cells were maintained in log phase by seeding twice a week at a density of 3×10^8 cells/L at 37°C under 5% CO_2/air atmosphere. Phorbol 22-myristate 13-acetate (PMA; P8139 from Sigma) was added at a final concentration of 150 nM for 48 h to differentiate THP-1 monocytes in macrophages. The differentiation-inducing dose of 150 nM PMA for 48 h was determined in preliminary dose–response experiments (data not shown), and it was in the range reported in the literature [25]. The criteria for differentiation of THP-1 cells were cell adherence, changes in cell morphology and changes in the cell surface marker expression profile (integrin, CD4 and MHC class II antigen) that is associated with the macrophage phenotype (data not shown). After differentiation, medium was removed and replaced with fresh medium containing 1% fetal bovine serum and 0.2% bovine serum albumin. Lycopene (LycoRed Natural Products Industries, Be'er Sheva, Israel) was delivered to the cells using tetrahydrofuran (THF) as a solvent. The solvent used in lycopene experiments contained 0.025% butylated hydroxytoluene to avoid the formation of peroxides. The stock solutions of lycopene were prepared immediately before each experiment. From the stock solutions, aliquots of lycopene were rapidly added to the culture medium to give the final concentrations indicated. The amount of THF added to the cells was not greater than 0.5% (v/v). Control cultures received an amount of solvent (THF) equal to that present in lycopene-treated ones. No differences were found between cells treated with THF and untreated cells in terms of cell number and viability. After the addition of lycopene, the medium was not further replaced throughout the experiments. Experiments were routinely carried out on triplicate cultures. At the times indicated, cells were harvested and quadruplicate hemocytometer counts were performed. The trypan blue dye exclusion method was used to evaluate the percentage of viable cells.

2.2. Intracellular cholesterol assay

The analysis of total intracellular cholesterol was performed as indicated by Chen and Chen [26]. Cholesterol was extracted using an Ultra-Turrax (on ice, 1 min, 12,000 rpm) from 10×10^6 in 1 ml of methanol (containing 0.1% butylated hydroxytoluene) and 3 ml of hexane and centrifuged at 4000*g* for 2 min. After centrifugation, the hexane layer was transferred into another tube and was evaporated (N_2, 30°C±1°C) to dryness. The extraction procedure with 1 ml of *n*-hexane and Ultra-Turrax was performed three times on each sample to ensure total removal of cholesterol. The hexane layers were evaporated in consecutive steps in the same tube and then dissolved in 200 μl of hexane. A 20-μl aliquot was analyzed by HPLC equipped with a spectrophotometer (Perkin-Elmer 295-LC, Perkin-Elmer, Norwalk, CT, USA) at 234 nm. A CN-bonded column, 25×0.46-cm cartridge format (Discovery-Cyano, Supelco, Bellefonte, PA, USA) and 5-μm particle size, was used. A 2-cm cartridge precolumn (Discovery-Cyano), packed with the same material of the column, was used. The mobile phase was hexane-2-propanol (95:5, v/v) at a flow rate of 1 ml.

2.3. Western blot analysis of HMG-CoA, PPARγ, LXRα and ABCA1 and Cav-1 expressions

Cells (10×10^6) were harvested, washed once with ice-cold phosphate-buffered saline and gently lysed for 30 min in ice-cold lysis buffer (1 mM $MgCl_2$, 350 mM NaCl, 20 mM Hepes, 0.5 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 1 mM Na4P2O7, 1 mM PMSF, 1 mM aprotinin, 1.5 mM leupeptin, 1 mM Na_3VO_4, 20% glycerol and 1% NP40). Cell lysates were centrifuged for 10 min at 4°C (10,000*g*) to obtain the supernatants, which were used for Western blot analysis (Mini-PROTEAN 3 Electrophoresis System, Catalog No. 165-3301, and Mini Trans-Blot Electrophoretic Transfer Cell, Catalog No. 170-3930; Bio-Rad Laboratories, Milan, Italy). The anti-HMG-CoA reductase (clone C-18, Catalog No. SC-27580) (dilution 1:500), anti-PPARγ (clone H-100, Catalog No. SC-7196) (dilution 1:500), anti-LXRα (clone C-19, Catalog No. SC-1201) (dilution 1:500), anti-ABCA1 (clone AB.H10, Catalog No. SC-58219) (dilution 1:500), anti-cav-1 (clone N-20, Catalog No. SC-894) (dilution 1:500) and anti-β-actin (clone C-4, SC-47778) (dilution 1:500) monoclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The blots were washed and exposed to horseradish peroxidase-labeled secondary antibodies (Amersham Pharmacia Biotech, Arlington Heights, IL, USA) for 45 min at room temperature. The immunocomplexes were visualized by the enhanced chemiluminescence detection system (ECL Western Blotting Detection Reagents, Catalog No. RPN2106, Amersham, Milan, Italy) and quantified by densitometric scanning using Gel.DOC Imaging Station equipped with a dedicated software (QuantityOne, Bio-Rad Laboratories).

2.4. Analysis of RhoA cellular localization

THP-1 macrophages (100-mm dishes) were incubated in the absence or presence of lycopene (5% lipoprotein-deficient serum). Cells were scraped into 500 μl of lysis buffer [10 mM Tris–HCl, 10 mM NaCl, 3 mM $MgCl_2$ and 0.5% (octylphenoxy)polyethoxyethanol] containing aprotinin (100 units/ml), *N*-acetyl-leucyl-leucyl-norleucinal (2 μg/ml), leupeptin (0.1 mM), phenylmethylsulfonyl fluoride (2 mM), pepstatin (5 μg/ml) and benzamidine (250 μg/ml) and homogenized by 15 passes through a 25-ga needle. The postnuclear supernatant was obtained by centrifugation (500*g*, 4°C, 10 min). The postnuclear fraction was further spun at 100,000*g* for 30 min (4°C, Beckman TLA 120.2 rotor) to obtain a pellet of the cell membrane fraction and cytosol fraction in the supernatant. The pellet was suspended in membrane resuspension buffer (10 mM Tris–HCl, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA and 1% SDS) containing the protease inhibitors. Proteins from the cytosol and membrane (20–100 μg) fractions were mixed with SDS loading buffer and subjected to SDS-PAGE on 12% gel. Proteins were transferred electrophoretically to nitrocellulose membranes, which were blocked (16 h, 4°C) with 5% (w/v) nonfat dried milk in phosphate-buffered saline. Membranes were incubated with a monoclonal antibody for RhoA (RhoA, clone C-15, SC-32039, Santa Cruz Biotechnology), followed by incubation with a peroxidase-conjugated anti-mouse IgG antibody (Santa Cruz Biotechnology). RhoA was detected using BM chemiluminescence blotting substrate (Roche Diagnostics). Quantification analysis of the developed films was performed using an imaging densitometer (GS-700, Bio-Rad Laboratories, Mississauga, Ontario, Canada).

2.5. Reverse transcription polymerase chain reaction of PPARγ

Total RNA was extracted from tissue samples using Trizol according to the manufacturer's protocols (Invitrogen Life Technologies, Paisley, UK); the RNA was eluted in DEPC-treated water (0.01% DEPC) and stored at −80° until reverse transcription polymerase chain reaction (RT-PCR) analysis. Nucleic acid concentrations were measured by spectrophotometry (Hewlett-Packard HP UV/Vis 8450 spectrophotometer).

RT-PCR assay was performed using the two-step method. For the first step of RT, we used a QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) with 500 ng of total RNA as template RNA, following the manufacturer's procedure. For the second step of PCR reactions, we employed QuantiTect SYBR Green Kits (Qiagen) and QuantiTect Primer Assays (Qiagen) for human β-actin and PPARγ according to the

manufacturer's protocol described for the LightCycler real-time thermal cycler (Roche). PCR data obtained by the LightCycler software were automatically analyzed by Relative Quantification Software (Roche) and are expressed as the target/reference ratio. Our approach was based on calibrator-normalized relative quantification including correction for PCR efficiency.

2.6. Statistical analysis

Three separate cultures per treatment were utilized for analysis in each experiment. Values are presented as the mean±S.E.M. One-way ANOVA was used to determine differences between different concentrations of lycopene or different times of treatment in Figs. 1A and C; 2B and C; 3B, C and E; and 4 C and D. When significant values were found ($P<.05$), post hoc comparisons of means were made using Fisher's test. Differences were analyzed using Minitab Software (Minitab, State College, PA, USA).

3. Results

3.1. Effects of lycopene on intracellular cholesterol content and on HMG-CoA reductase expression

To evaluate whether lycopene was able to modulate cholesterol metabolism in human macrophages, we first measured the intracellular levels of total cholesterol in THP-1 cells treated with different concentrations of lycopene for 24 h. The carotenoid strongly decreased total cholesterol content in a dose-dependent manner (Fig. 1A). To investigate the effects of lycopene on cholesterol synthesis in THP-1 macrophages, we measured the expression of HMG-CoA reductase, which promotes the deacylation of HMG-CoA to mevalonate. The treatment with lycopene reduced the expression of HMG-CoA reductase in a dose-dependent (Fig. 1B) and time-dependent (Fig. 1C) manner. They were clearly evidenced at 3 h of treatment.

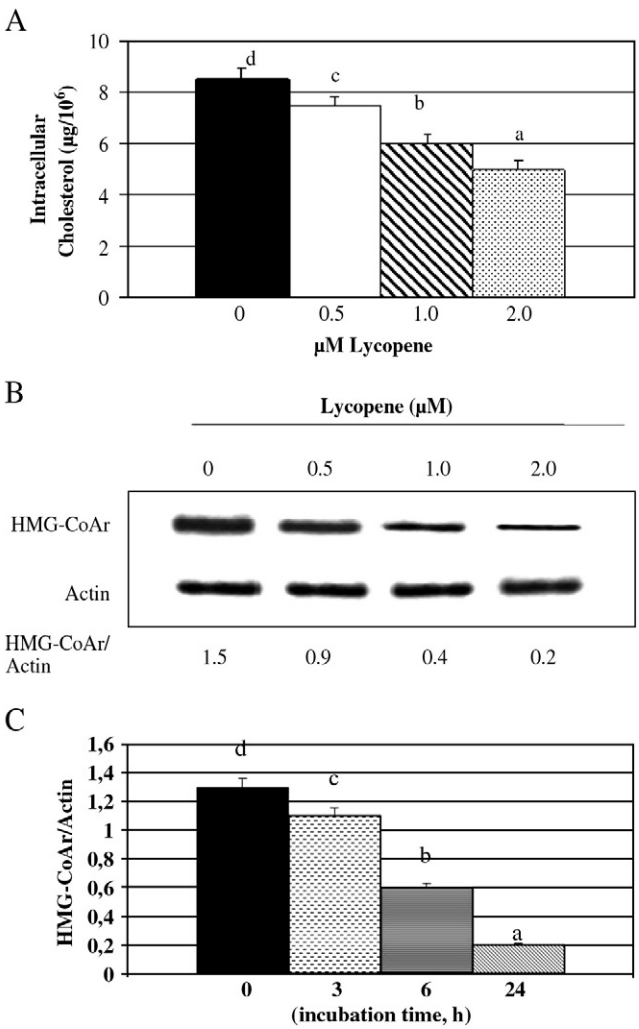

Fig. 1. Effects of lycopene on cholesterol levels (A) and HMG-CoA reductase expression (B and C) in THP-1 cells. (A and B) Dose-dependent effects: cells were treated with varying concentrations of lycopene for 24 h. (C) Time-dependent effects: cells were treated with 2 μM lycopene for the times indicated. Values are expressed as the mean ±S.E.M., $n=4$. In (A) and (B), values not sharing the same letter are significantly different ($P<.05$, Fisher's test).

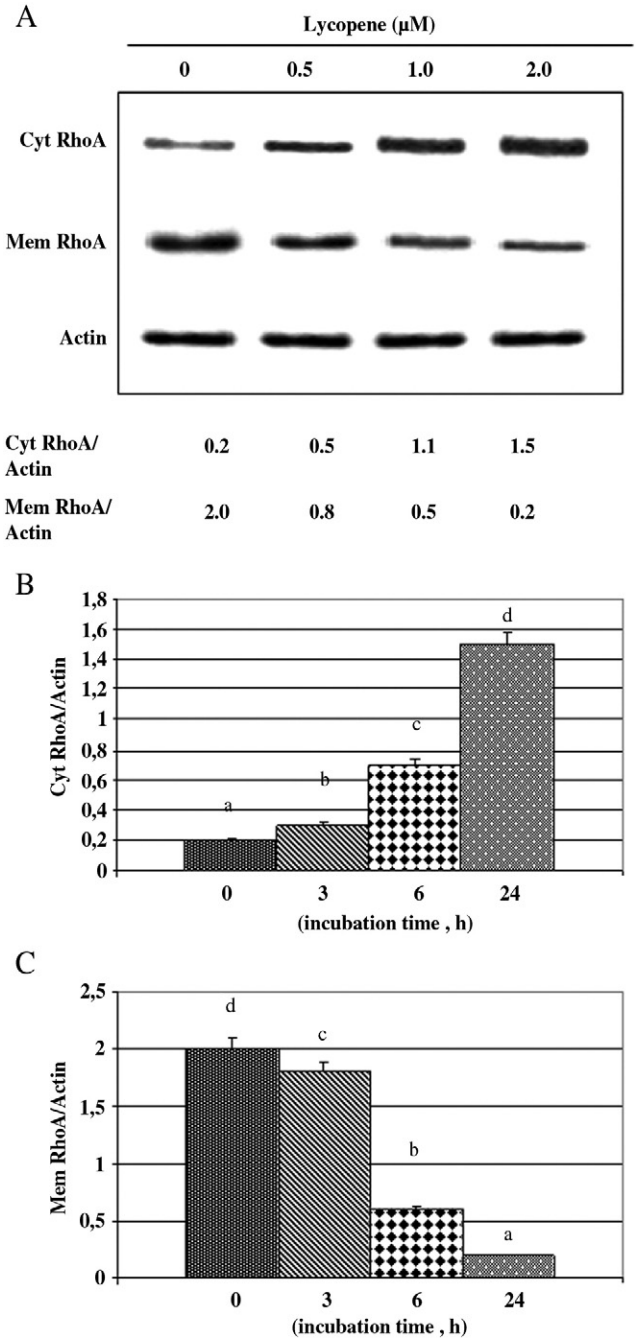

Fig. 2. Effects of lycopene on cytosol and membrane RhoA expression in THP-1 cells. (A) Dose-dependent effects: cells were treated with varying concentrations of lycopene for 24 h. (B and C) Time-dependent effects: cells were treated with 2 μM lycopene for the times indicated. Values are expressed as the mean±S.E.M., $n=5$. In (B) and (C), values not sharing the same letter are significantly different ($P<.05$, Fisher's test).

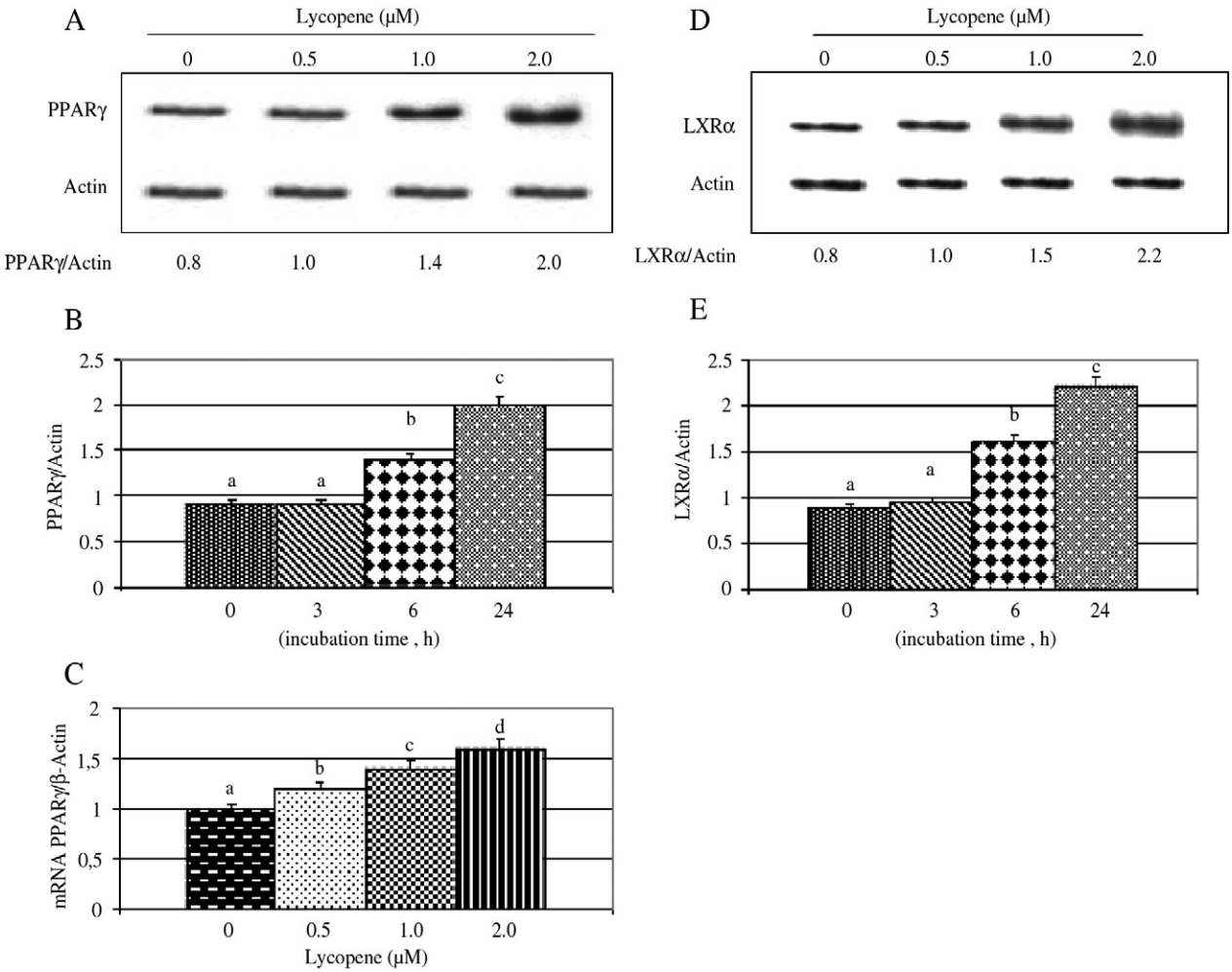

Fig. 3. Effects of lycopene on PPARγ (A–C) and LXRα (D and E) levels in THP-1 cells. In the dose-dependent experiments (A, C and D), lycopene was added for 24 h; in the time-dependent experiments (B and E), the carotenoid was added at a concentration of 2 μM. Values are expressed as the mean±S.E.M., $n=3$. In (B), (C) and (E), values not sharing the same letter are significantly different ($P<.05$, Fisher's test).

3.2. Effects of lycopene on RhoA expression

In addition to depleting cells of intracellular cholesterol, the inhibition of HMG-CoA reductase by lycopene may also reduce the intracellular pool of nonsterol mevalonate metabolites and may alter the prenylation of GTPase proteins, including RhoA. In particular, this protein is preferentially geranylgeranylated [9]. One determinant of RhoA activity is its translocation from the cytosol to cell membranes, including the plasma membrane. To determine whether lycopene reduced RhoA membrane translocation, we measured the relative amount of RhoA protein in the cytosol and in the isolated cell membranes (Fig. 2A). A 24-h treatment of THP-1 cells with lycopene dose-dependently decreased the translocation of RhoA to the cell membrane, whereas RhoA protein expression in the cytosol was increased. Both these effects were also dependent on time (Fig. 2B and C). They were clear at 3 h of treatment.

3.3. Effects of lycopene on PPARγ and LXRα activation

Inhibition of RhoA activity by lycopene may result in changes in PPARγ expression. To test this hypothesis, we performed Western blot and RT-PCR to examine changes in the PPARγ protein and mRNA expressions following lycopene treatment. As shown in Fig. 3, lycopene significantly enhanced PPARγ protein expression in a dose-dependent [panel (A)] and time-dependent [panel (B)] manner in THP-1 cells. A similar increase was observed in PPARγ mRNA levels [panel (C)]. A 24-h treatment with 2 μM lycopene increased the expression levels of PPARγ protein and mRNA by about 2- and 1.6-fold, respectively. Such an effect started at 6 h of incubation with lycopene. LXRα is known to be influenced by cholesterol derivatives and by RhoA translocation. Thus, we measured the effects of lycopene on LXRα expression [panel (D)]. A 24-h treatment with the carotenoid at a concentration of 2 μM significantly increased LXRα expression in a dose-dependent manner. Similarly to PPARγ, such an effect started at 6 h of lycopene treatment [panel (E)].

3.4. Effects of lycopene on ABCA1 and cav-1 expressions

Key targets of PPARγ and LXRα activation are the ABC proteins, including ABCA1, and the caveolin family proteins, including cav-1. ABCA1 controls the rate-limiting step in cellular cholesterol and phospholipid efflux to apoA1. On the other hand, some studies show that cav-1 expression is associated with an enhancement of cholesterol efflux [16]. In particular, it may play a role by increasing cholesterol association with lipid rafts, from which it can move laterally to nonraft domains for efflux. Corresponding with an intracellular cholesterol reduction and with an increased PPARγ–LXRα activation, lycopene treatment was also associated with a dose-dependent increase in both ABCA1 expression (Fig. 4A) and cav-1

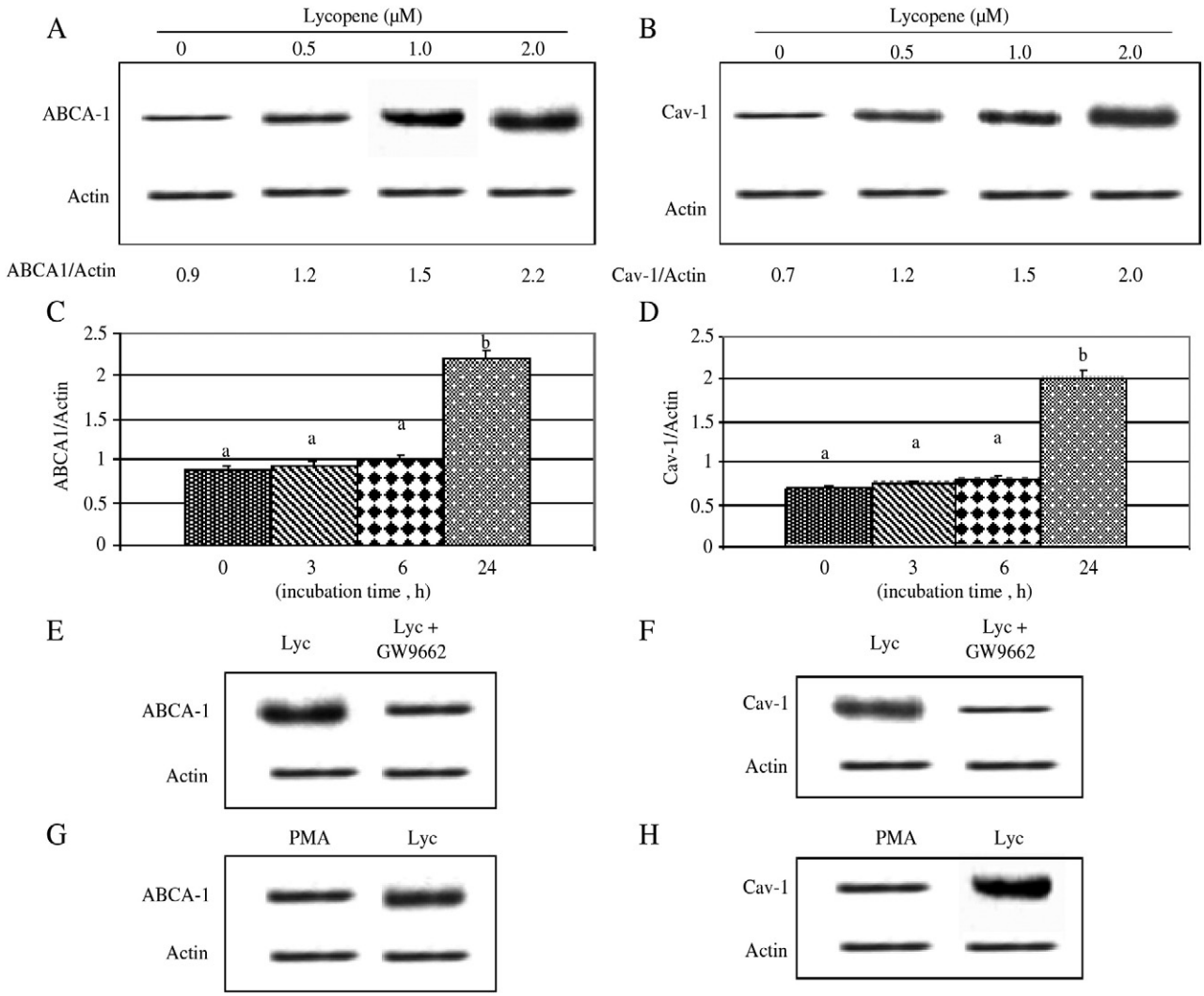

Fig. 4. Effects of lycopene on ABCA1 (A, C, E and G) and cav-1 (B, D, F and H) levels in THP-1 cells. In (A) and (B), lycopene was added for 24 h. In (C) and (D), the carotenoid was added at a concentration of 2 μM. In (E) and (F), lycopene (2 μM) was added for 24 h, after preincubation with the PPARγ inhibitor GW9662 (10 μM). In (G) and (H), lycopene (2 μM) or PMA (150 nM) was added for 24 h. Values are expressed as the mean±S.E.M., $n=3$. In (C) and (D), values not sharing the same letter are significantly different ($P<.05$, Fisher's test).

expression (Fig. 4B), as observed on Western blotting, with ABCA1 and cav-1 protein increases of about 2.2- and 2-fold, respectively, following a 24-h lycopene treatment at 2 μM. Both proteins increased after a 24-h treatment with lycopene (Fig. 4C and D). A 24-h preincubation with 10 μM GW9662, which is an irreversible antagonist of PPARγ, completely prevented the increase in both ABCA1 (Fig. 4E) and cav-1 (Fig. 4F) induced by lycopene, suggesting that their activation by the carotenoid required an increase in PPARγ levels. The effects of lycopene on ABCA1 and cav-1 were not simply due to differentiating properties of the carotenoid, since PMA, added for 24 h, was not able to modify the levels of the two proteins at a concentration of 150 nM, normally used for differentiating THP-1 cells (Fig. 4G and H).

3.5. Effects of mevalonate addition on lycopene-induced RhoA translocation and PPARγ, ABCA1 and cav-1 expressions

The important role of the inhibition of the mevalonate pathway in the regulation of cholesterol efflux by lycopene is clearly demonstrated by our observation that the carotenoid was ineffective in cytosol translocation of RhoA (Fig. 5A) and in increasing PPARγ (Fig. 5B), ABCA1 (Fig. 5C) and cav-1 (Fig. 5D) expressions in the presence of mevalonate, as observed in THP-1 cells treated with 2 μM lycopene and 100 μM mevalonate for 24 h.

4. Discussion

In this work, we have reported on a novel molecular mechanism of lycopene in the control of atherosclerosis. We showed that the carotenoid may reduce intracellular levels of cholesterol in human macrophages. This effect was accompanied by a decrease in the expression of HMG-CoA reductase and by an enhancement of the expressions of ABCA1 and cav-1. The last event occurred through a cascade involving RhoA inactivation and PPARγ and LXRα activation.

The committed step in the biosynthesis of cholesterol and isoprenoids is catalyzed by HMG-CoA reductase, which promotes the deacylation of HMG-CoA to mevalonate [5,6]. This pathway produces numerous bioactive signaling molecules, including farnesyl pyrophosphate and GGPP, which regulate transcriptional and posttranscriptional events that affect various biological processes, including changes in proteins involved in cholesterol metabolism

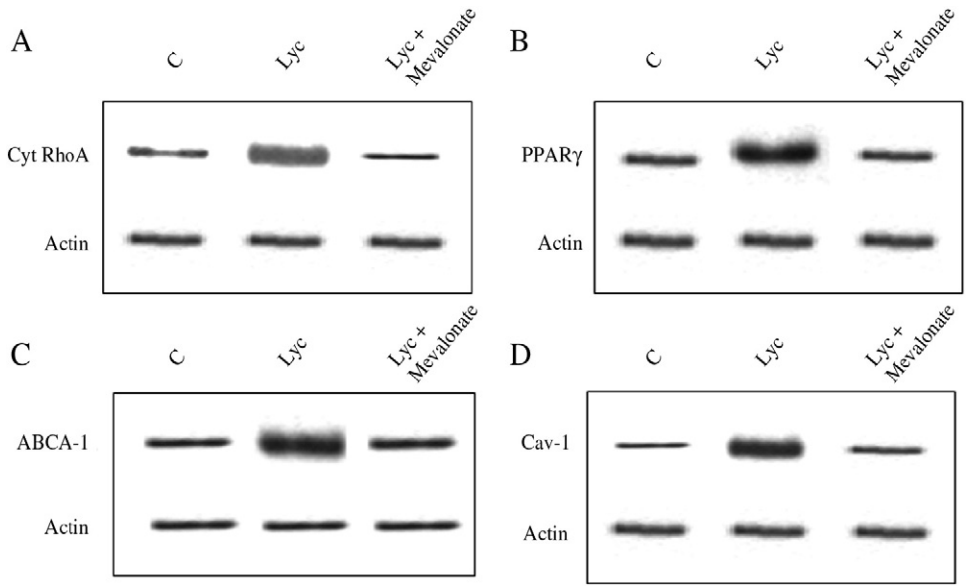

Fig. 5. Effects of combined addition of mevalonate and lycopene on cytoplasmatic RhoA (A), PPARγ (B), ABCA1 (C) and cav-1 (D) expressions in THP-1 cells. Lycopene (2 μM) was added alone and/or in combination with mevalonate (100 μM) for 24 h.

[5]. The activity of HMG-CoA reductase in animal cells has been shown to be sensitive to negative regulation by both sterols and nonsterol products of the mevalonate pathway [7,8]. Lycopene is a polyisoprenoid synthesized in plants from mevalonate via the HMG-CoA reductase pathway. In plants, as well as in animal cells, HMG-CoA reductase is regulated by an end-product repression [27]. Our results show that lycopene was able to reduce the expression of HMG-CoA reductase in a dose- and time-dependent manner in THP-1 cells. Changes in HMG-CoA reductase were already observed at 3 h of lycopene treatment. Such a control probably involves a posttranscriptional mechanism, as suggested for isoprenoids and other carotenoids, including β-carotene [28]. The inhibition of HMG-CoA reductase by lycopene was also accompanied by a reduction in intracellular cholesterol levels. These data are in agreement with other observations showing that lycopene is able to reduce cholesterol levels by inhibiting HMG-CoA reductase activity in cultured macrophages [23]. It has been recently shown that membrane association appears to be an important function in mevalonate derivative modifications of several important proteins, such as many low-molecular GTPases. Members of the small GTPase family, including RhoA, are major substrates for posttranslational modification by geranylgeranylation [5,9], a process essential for their proper membrane localization and activation [10]. GTPases function as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state, thereby mediating cellular responses through their association with numerous effector molecules, including kinases [11]. Since it has been reported that one determinant of RhoA activity is its translocation from the cytosol to cell membranes, we measured the relative amount of RhoA protein in isolated cell membranes and in the cytosol. Our results show that lycopene impaired the localization of RhoA protein in cell membranes in THP-1 cells, which led to cytoplasmatic accumulation and subsequent inactivation of this protein. Such an effect was dependent on dose and time, and it was clearly evidenced at 3 h of treatment. It has been recently demonstrated that HMG-CoA reductase inhibition, through reduced RhoA activation, resulted in decreased phosphorylation and enhanced the activity of nuclear hormone receptor PPARγ [5]. Because PPARγ is also negatively regulated through phosphorylation [12], it has been reported that RhoA could also modulate PPARγ activation and the expression of PPARγ target genes. Therefore, we measured the effects of lycopene on PPARγ expression in THP-1 cells. We observed that lycopene was able to strongly increase this transcription factor at protein and mRNA levels in a dose-dependent manner. Interestingly, such an effect occurred only at 6 h, after the reduction of HMG-CoA reductase expression and RhoA translocation into the cytosol. These data suggest a possible modulatory role of lycopene on PPARγ levels in human macrophages, confirming previous findings in rat tissues [29] and in murine fibroblasts [30]. Sharoni *et al.* [31] have proposed that some carotenoids, such as lycopene, phytoene, phytofluene and β-carotene, cause the transactivation of peroxisome proliferator response element in cells co-transfected with PPARγ. Moreover, it has been recently reported that β-carotene is able to increase PPARγ levels in MCF-7 cancer cells [32]. Several lines of evidence suggest a modulatory role of the nonsterol mevalonate intermediate GGPP and RhoA on LXR activity and on the expression of LXR-responsive genes [13,14]. Moreover, LXR is of great interest as target for the prevention and treatment of cardiovascular diseases, because several relevant genes (*i.e.*, cholesteryl ester transfer protein, cytochrome *P*450-dependent 7α-hydroxylase, ABCA1 and ABCG5/G8) are LXR regulated [33]. Therefore, we measured the levels of LXRα in THP-1 following treatment with lycopene. The carotenoid increased the expression of LXR in a dose-dependent manner. Similarly to PPARγ, such an effect started at 6 h of lycopene treatment. Key targets of PPARγ and LXRα activation are the ABC proteins, including ABCA1, and the caveolin family proteins, including cav-1. ABCA1 controls the rate-limiting step in cellular cholesterol and phospholipid efflux to apoA1 [15]. On the other hand, some studies show that cav-1 expression is associated with an enhancement of cholesterol efflux [16,34,35]. In particular, cav-1 may play a role by increasing cholesterol association with lipid rafts, from which it can move laterally to nonraft domains for efflux [16]. In this study, we demonstrated that lycopene, at concentrations within the range that activated the PPARγ receptor and LXRα receptor, dose-dependently induced the expression of both ABCA1 and cav-1 in THP-1 macrophages. The increase in ABCA1 by lycopene is interesting since ABCA1 is also suggested to be a carotenoid transporter [36]. Several studies suggest that PPARγ ligands are able to induce an up-regulation of these two proteins

[37–39]. It has been recently shown that β-cryptoxanthin induces ABCA1 and ABCG1 mRNAs and ABCA1 protein in macrophages by a mechanism involving retinoic acid receptors [40]. Such observation could be particularly interesting in view of reports showing that PPARs are ligand-activated transcription factors that mainly heterodimerize with the retinoic acid receptors. Experiments are in progress in our laboratory to evaluate the role of lycopene and other carotenoids in modulating retinoic acid receptors in human macrophages.

Ligand activation of the PPARγ has been shown to promote the differentiation of monocytic cells [41], and both ABCA1 and cav-1 expressions have been reported to be induced in THP-1 cells upon differentiation [42]. Therefore, we wanted to test whether the increase in ABCA1 and cav-1 expressions induced by lycopene in our cell model was a direct consequence of a differentiation-promoting effect of PPARγ activation. For this reason, we treated already PMA-differentiated THP-1 cells with PMA at 150 nM for another 24 h — experimental conditions reported to strongly promote macrophage differentiation and PPARγ activation [25,39], and we compared the effects of such a treatment with those obtained by lycopene addition on the expressions of ABCA1, cav-1 and a marker of cell differentiation, such as CD11b [43]. Our results show that treatment with PMA caused an increase in CD11b expression (data not shown), but it did not cause an induction of ABCA1 and cav-1 expressions. On the contrary, lycopene was able to induce both ABCA1 and cav-1 levels, without any change in CD11b levels. These results suggest that the addition of lycopene did not promote further macrophage maturation and that lycopene induction of ABCA1 and cav-1 expressions is independent of cellular differentiation, confirming previous results in cancer cells [38].

The important role of mevalonate pathway inhibition and the subsequent RhoA inactivation in the regulation of cholesterol efflux by lycopene is clearly demonstrated by our observation that the carotenoid was ineffective in inhibiting RhoA inactivation and in increasing PPARγ, ABCA1 and cav-1 expressions in the presence of mevalonate.

In conclusion, these results imply a potential role of lycopene, at concentrations that can be reached *in vivo* after supplementation [44], in attenuating foam cell formation and in preventing atherosclerosis. These occur through a cascade mechanism involving HMG-CoA reductase inhibition, RhoA inactivation and subsequent increase in PPARγ and LXRα activation and enhancement in ABCA1 and cav-1 expressions.

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