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Biochimica et Biophysica Acta 1359 (1997) 13–24

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Lovastatin induces apoptosis by inhibiting mitotic and post-mitotic events in cultured mesangial cells

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Received 29 May 1997; accepted 16 June 1997

Abstract

Lovastatin, an inhibitor of protein prenylation, was reported to inhibit DNA synthesis and induce apoptosis in cultured cells. This report describes the morphological consequences of lovastatin treatment. Lovastatin (50 μM) induced mesangial cell rounding and disassembly of actin stress fibers within 24 to 48 h. After 48 to 72 h of lovastatin treatment, the cells detached from the substratum and underwent apoptotic cell death as evidenced by condensed nuclear chromatin, nuclear fragmentation, cell blebbing and decrease in cell size. Time lapse cinematography revealed that lovastatin caused cell rounding by either inhibiting cytokinesis or cell spreading following cytokinesis. Lovastatin-induced cell rounding, detachment, and apoptosis were dependent upon cell proliferation. These effects were prevented by serum deprivation to inhibit cell proliferation or by plating cells at densities which resulted in contact inhibition of cell growth. Lovastatin-induced mesangial cell rounding and apoptosis were also prevented by the inclusion of the isoprenoids all-*trans*-farnesol or all-*trans*-geranylgeraniol in the incubation medium. These results indicate that the effects of lovastatin were mediated by inhibition of protein isoprenylation because exogenous all-*trans*-geranylgeraniol can be used only in protein prenylation. The small GTP-binding protein RhoA, which may be important for cell spreading and cytokinesis, accumulated in the cytosol following treatment with lovastatin, suggestive of its inactivation. This effect was also prevented by the inclusion of either farnesol or geranylgeraniol in the incubation medium. Thus, lovastatin-induced apoptosis in mesangial cells occurs by interfering with prenylation dependent mitotic and post-mitotic events. © 1997 Elsevier Science B.V.

Keywords: Mesangial cell; Protein prenylation; RhoA; Geranylgeraniol; Farnesol; Cytokinesis

1. Introduction

Cells grown in lovastatin, a potent inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG–CoA) reductase, develop a round, refractile morphology [1], show inhibition of cell growth [2] and undergo apoptosis [3]. Such cells display loss of actin stress fibers without apparent changes in the microtubular lattice or intermediate filament structure [1]. The main function of lovastatin is to prevent the reduction of HMG–CoA to mevalonate [4]. Mevalonate is converted into 5-carbon units (C5), called isopentenyl pyrophosphate (IPP) [5]. Two C5 units combine to form a C10 compound, geranyl pyrophosphate, which then combines with IPP to form farnesol pyrophosphate (FPP), a C15 compound. FPP is the common intermediate for the biosynthesis of choles-

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PII S0167-4889(97)00091-8

terol, dolichol and ubiquinone as well as protein isoprenylation [5]. In addition, FPP combines with another C5 unit (IPP) to form a C20 compound, all-*trans*-geranylgeranyl pyrophosphate (GGPP). These conversions are prevented in the presence of lovastatin due to the absence of the IPP units. Unbound all-*trans*-GGPP appears to be uniquely destined for the posttranslational modification of different groups of proteins [5]. The isoprenoids FPP and GGPP are transferred by farnesyl and geranylgeranyl protein transferases respectively to various proteins, such as small GTP-binding proteins, for targeting and activation.

Small GTP-binding proteins of the Ras superfamily, including Rho, Rab, Raf, Rac, Rap, etc. are involved in such diverse cellular functions as cytokinesis [6], cell motility [7], cell adhesion [8] and cell proliferation [9]. All members of this family, except Rab, have a C*AAX* sequence at their C-terminal, where *A* is any aliphatic amino acid and *X* is a COOH-terminal amino acid that specifies which prenyltransferase will act. Although recent experiments suggest more complex relationships [10], it is generally believed that C*AAX* farnesyltransferase preferentially recognizes methionine or serine at the *X* position and C*AAX* geranylgeranyl transferase prefers leucine at the *X* position [11]. During posttranslational modification, an isoprenyl group (for example, farnesyl or geranylgeranyl) is first attached to the cysteine group, then the *AAX* sequence is cleaved and the isoprenylated cysteine is carboxymethylated [12]. The Ras proteins are farnesylated while most other small GTP-binding proteins are geranylgeranylated [13]. RhoB is both geranylgeranylated as well as farnesylated [10].

Once prenylated, the small GTP-binding proteins can cycle between a GTP-bound active state and a GDP-bound inactive state [14]. RhoA, for example, forms a complex with the GDP-dissociation inhibitor (Rho–GDI) and remains GDP-bound in the cytoplasm [15]. The conversion from the GDP bound inactive form to the GTP-bound active form is regulated by guanine nucleotide exchange proteins such as the GDP-dissociation stimulator (Rho–GDS) [16]. RhoA in its GTP-bound form is then translocated to the membrane where it binds its target protein [17]. Only prenylated RhoA can form complexes with other proteins [13]. In the membrane, RhoA acts as a GTPase and exchanges its bound GTP for GDP. In the GDP-bound state, it is translocated to the cytoplasm by Rho–GDI [18].

Most of the other members of the Ras superfamily are presumed to go through similar cycles, although the exact mechanisms may vary. In the presence of lovastatin, these proteins are not able to cycle because of the absence of prenyl modification and, therefore, an inability to form complexes with other proteins. Because the small GTP-binding proteins are involved in important cellular functions, this will limit a cell's ability to function properly. While Ras is involved in cell proliferation [9], members of the Rho subfamily, RhoA, Rac1 and Cdc42 are associated with actin stress fiber formation, and the formation of lamellipodia and filopodia [19].

Lovastatin causes cell rounding and inhibits DNA synthesis in renal mesangial cells [2]. In addition, cells treated with lovastatin are blocked at G1 during cell cycle progression [20,21]. In an effort to identify a morphological basis for cell cycle arrest, we studied the cells by time lapse cinematography. These studies revealed that lovastatin prevented either cytokinesis or cell spreading following cytokinesis. Because RhoA plays an important role in actin stress fiber assembly and cell shape in cultured cells [22], we proceeded to study RhoA prenylation during lovastatin treatment of mesangial cells. In dividing cells RhoA localizes to cleavage furrows [23], and thus may play a role in cytokinesis. Our results show that lovastatin caused RhoA to accumulate in the cytosol. The effects of lovastatin on cell function and RhoA inactivation were prevented by the addition of farnesol or geranylgeraniol to the medium. Further, our findings suggest that lovastatin-induced mesangial cell apoptosis is caused by prolonged exposure to conditions of reduced cellular adherence following inhibition of post-mitotic events, a condition termed 'anoikis' [24].

2. Experimental

2.1. Materials

Lovastatin was kindly provided by Merck, Rahway, NJ and was prepared as described by Kita et al. [25] except at pH 7.4. All-*trans*-farnesol and all-*trans*-geranylgeraniol (Sigma, St. Louis, MO) were

dissolved in ethanol (1 M) and suspended in 45% w/v 2-hydroxypropyl-β-cyclodextran (β-CD) (Pharmatech, Alachua, FL) to a final stock concentration of 10 mM in an ultrasonic water bath for 20 min. β-CD prevents reprecipitation of the isoprenoids upon addition to tissue culture medium [26]. The purity of farnesol and geranylgeraniol was > 95% as assessed by gas liquid chromatography (15 m DB-23 column, temperature programmed 105–210°C, J&W Scientific, Folsom, CA). Monoclonal anti RhoA antibody was obtained from Santa Cruz Biotechnology, Santa Cruz, CA.

2.2. Cell culture

Rat glomerular mesangial cells were isolated as described elsewhere [27] and cultured in RPMI 1640 with 20% fetal calf serum and 1% antibiotic/antimycotic (all from Irvine Scientific, Santa Ana, CA).

Table 1
Cell rounding observed in mesangial cells plated at 10^4 cells cm^{-2} after 48 h of lovastatin treatment

Lovastatin concentration in μM	Degree cell rounding [a]
0	0
10	1+
20	2+
30	3+
40	3+
50	4+

[a] 1+, < 25% of the cells rounding; 2+, 25–40% of the cells rounding; 3+, 40–65% of the cells rounding; 4+, > 65% of the cells rounding.

2.3. Pharmacological treatments

Mesangial cells were plated at densities of 1, 2, or 3×10^4 cells/cm^2 in complete medium with or without lovastatin. In some experiments, the lovastatin

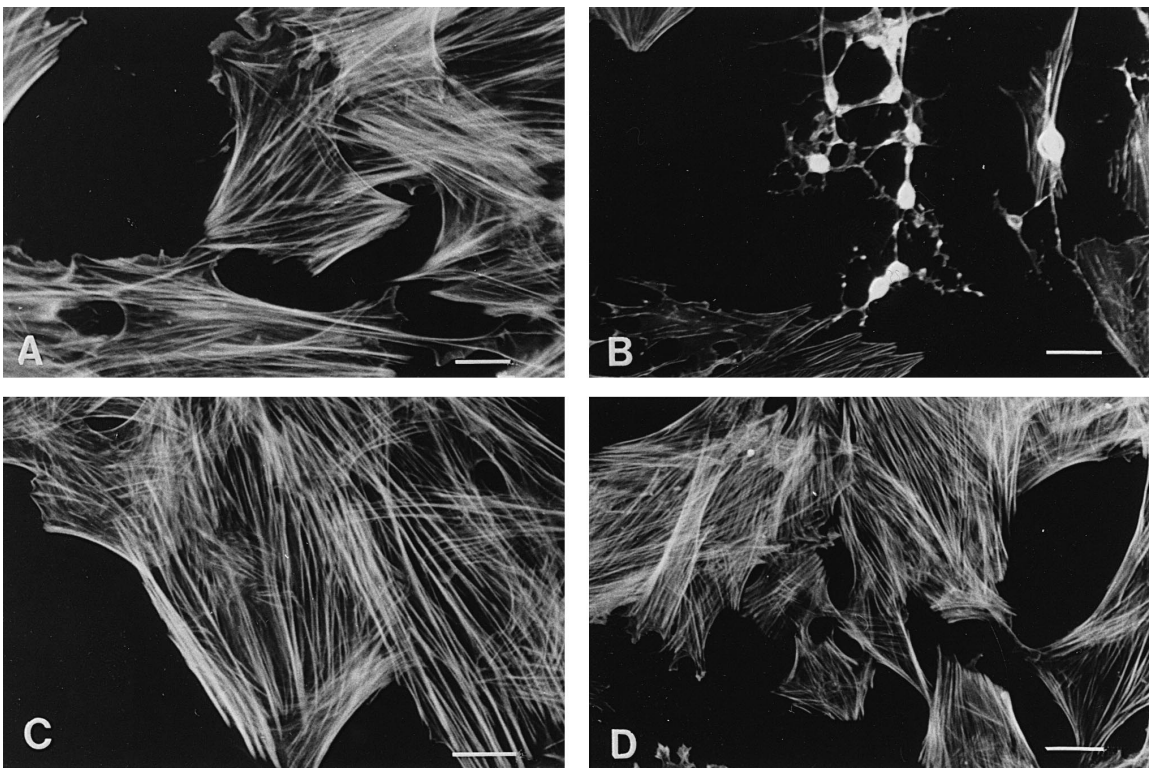


Fig. 1. Mesangial cells stained with rhodamine phalloidin to demonstrate actin stress fibers. (A) untreated mesangial cells. Note that the cells are flat and well spread and display numerous arrays of actin stress fibers. (B) 48 h of lovastatin treatment. Note that the cells are round and no longer display actin stress fibers. (C) 48 h after lovastatin treatment in the presence of geranylgeraniol (10 μM). These cells appear similar to untreated cells with numerous bundles of actin stress fibers. (D) 48 h of lovastatin treatment with farnesol (10 μM). These cells also appear similar to untreated cells. Bars = 50 μm

treated cells were also treated with farnesol or geranylgeraniol in β-CD.

2.4. Western blots

Mesangial cells were grown in 100 mm tissue culture dishes and were treated as required for predetermined lengths of time, at the end of which the cells were washed two times in cold PBS, scraped in TBS containing proteinase inhibitors and phased into detergent depleted (cytoplasmic) and detergent-enriched (membrane) fractions using Triton X-114 [28]. Proteins were quantitated and fractionated on 12% SDS–PAGE run as previously described [27]. After electroblotting onto nitrocellulose membranes, the blots was stained with a monoclonal antibody specific to RhoA. RhoA was detected by enhanced chemiluminescence after incubation with a peroxidase labelled secondary antibody, (Pierce, Rockford, IL).

2.5. Examination of apoptotic cells

Apoptotic cells were identified according to the following criteria [29,30]: condensed and fragmented nuclei, cell blebbing and decrease in cell size.

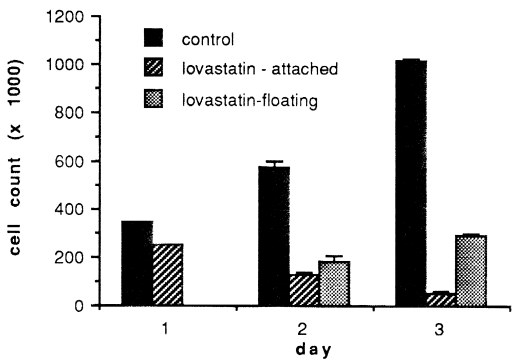


Fig. 2. The effect of lovastatin on the total number of mesangial cells. The untreated cells are in a state of exponential growth. Notice the decrease in the number of attached cells and the increase in the number of floating cells after 48 and 72 h of lovastatin (50 μM) treatment. After one day of lovastatin treatment there are no floating cells. This experiment represents the average of two dishes per condition. This was repeated 4 times with similar results. Error bars represent standard deviation from the mean.

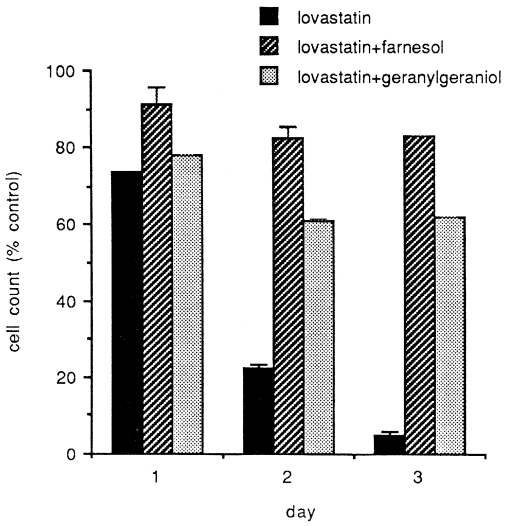


Fig. 3. The effect of lovastatin (50 μM) without and with farnesol (10 μM) or geranylgeraniol (10 μM) on mesangial cell number expressed as % of control. Farnesol and geranylgeraniol were replenished daily at the same concentrations. Notice that farnesol was more effective than geranylgeraniol in maintaining cell number. The number of counts represents the average of two dishes. This experiment was repeated 4 times with similar results. Error bars represent standard deviation from the mean.

2.5.1. Fluorescent staining

Cells were stained with the DNA binding fluorochrome Hoechst 33258 stain [30] and nuclear morphology examined under a Zeiss epifluorescence photomicroscope (Zeiss, Thornwood, NJ).

2.5.2. Transmission electron microscopy

Cells were fixed in 4% paraformaldehyde/1% glutaraldehyde for 30 min, scraped, and washed off the plastic surface with 1 mg ml^{-1} bovine serum albumin in PBS. They were then pelleted and processed for thick and thin sectioning.

2.6. Fluorescence microscopy

Mesangial cells were fixed with 3.7% paraformaldehyde in PBS for 20 min and permeabilized with 0.4% Triton X-100 in PBS for 2 min at room temperature. The cells were then incubated in rhodamine–phalloidin (diluted 1:100), rinsed and observed under standard epifluorescence optics for the visualization of actin stress fibers.

2.7. Time lapse microcinematography

Mesangial cells were plated in 25 cm^2 Corning flasks at a concentration of 2.5×10^5 cells per flask

with or without 50 μM lovastatin. They were viewed under a phase contrast microscope (Nikon model ELWD 0.3) equipped with a time lapse video camera (Panasonic model AG-6030).

2.8. Cell proliferation assay

The rate of cell proliferation was quantitated using a Cell Proliferation kit (Amersham, UK). Mesangial cells were grown on glass coverslips and pulsed for 2 h with 5-bromo-2′-deoxyuridine (BrdU). The cells were then treated with 50 μM lovastatin or left untreated for different time periods, at the end of which they were fixed in acid–ethanol. The fixed cells were processed as per manufacturer's instructions using an antibody to BrdU followed by a peroxidase-conjugated secondary antibody. The number of stained nuclei per high power field were counted and

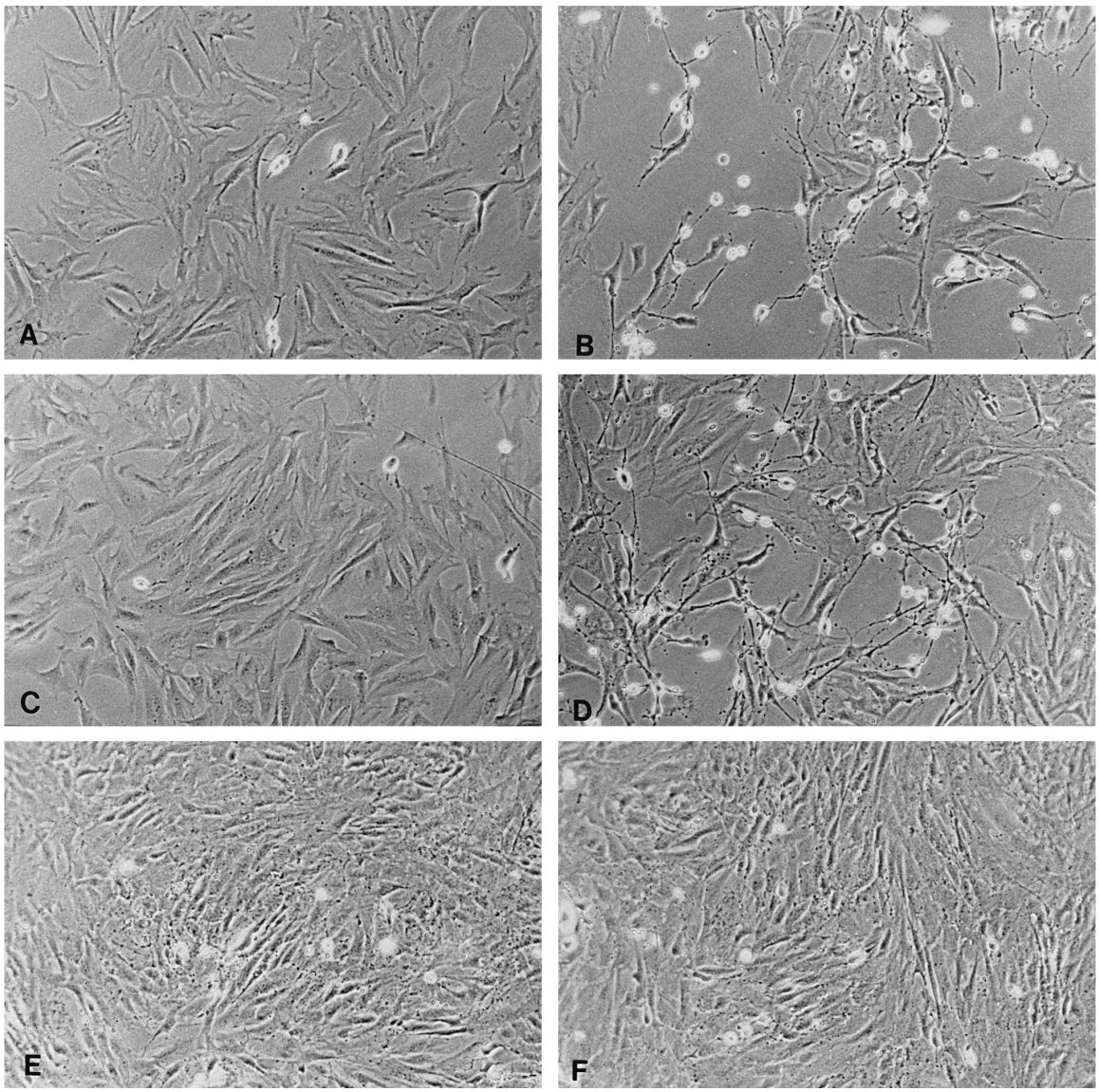


Fig. 4. Phase contrast photomicrographs of the effect of cell density on lovastatin (50 μM)-induced cell rounding. A, C, and E are untreated cells plated at 1, 2, and 3×10^4 cells cm^{-2}. B, D, and F are cells at the same platings as A, C, and E, respectively but treated with lovastatin for 48 h. Greater than 65% of the cells rounded in response to lovastatin when plated at 1×10^4. When plated at densities which approached confluence, and, hence, exhibited contact inhibition of growth (3×10^4), $< 25\%$ of the cells rounded in response to lovastatin. $200 \times$.

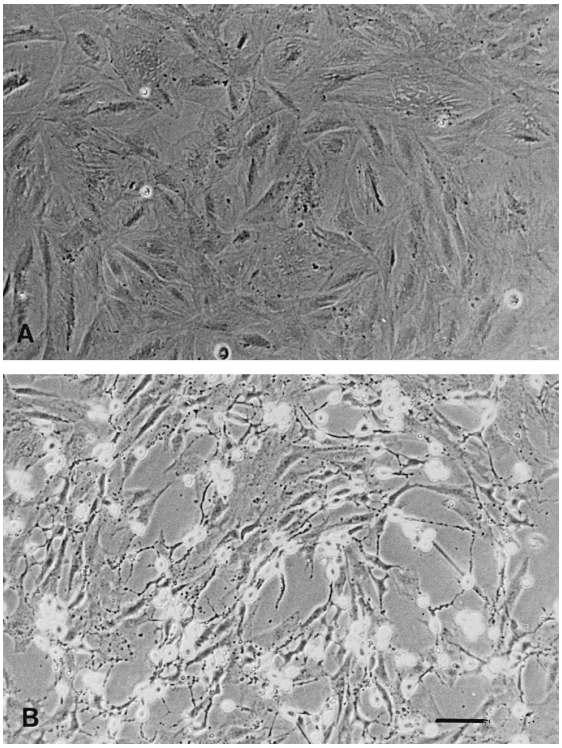


Fig. 5. Phase contrast photomicrographs of the effect of serum deprivation on lovastatin (50 μM)-induced cell rounding. Mesangial cells were serum-deprived for 24 h and then treated with lovastatin for 36 h in the absence of serum (A) while other cells were serum-deprived for 24 h and then treated with lovastatin for 36 h in the presence of serum (B). Bar = 50 μm.

were expressed as the percentage of stained vs. unstained cells.

3. Results

3.1. Effect of lovastatin on mesangial cell morphology

Lovastatin induced a dose-dependent effect on mesangial cell morphology (Table 1). Greater than 65% of the cells plated in the presence of 50 or 100 μM lovastatin changed shape from flat cells with actin stress fibers (Fig. 1A) to round cells without actin stress fibers (Fig. 1B). By 48 h, ca. 50% of the

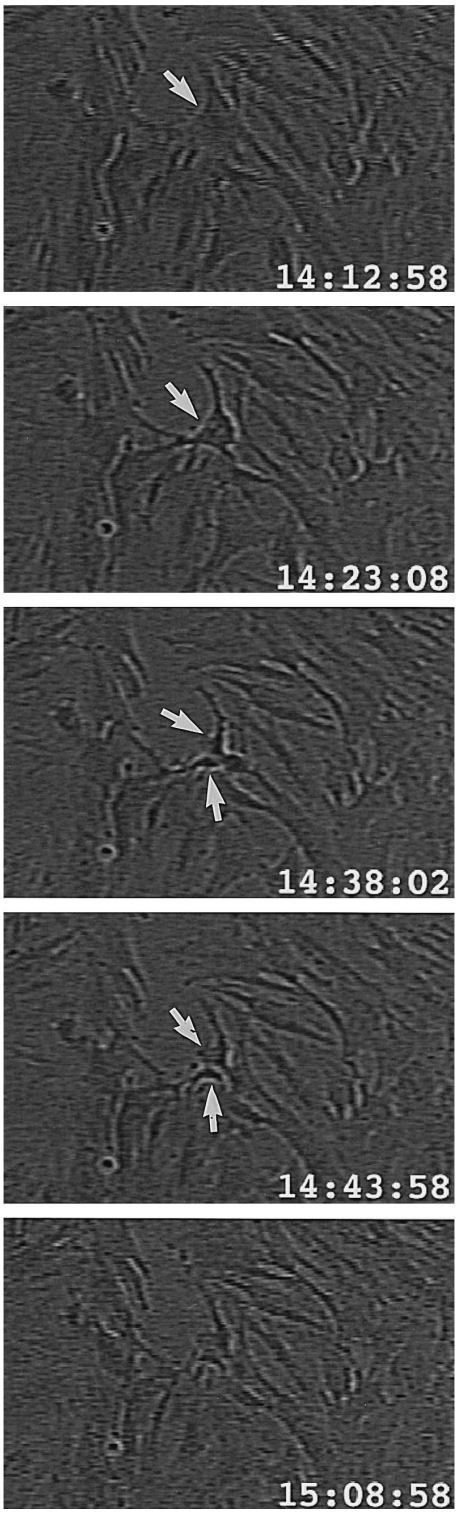
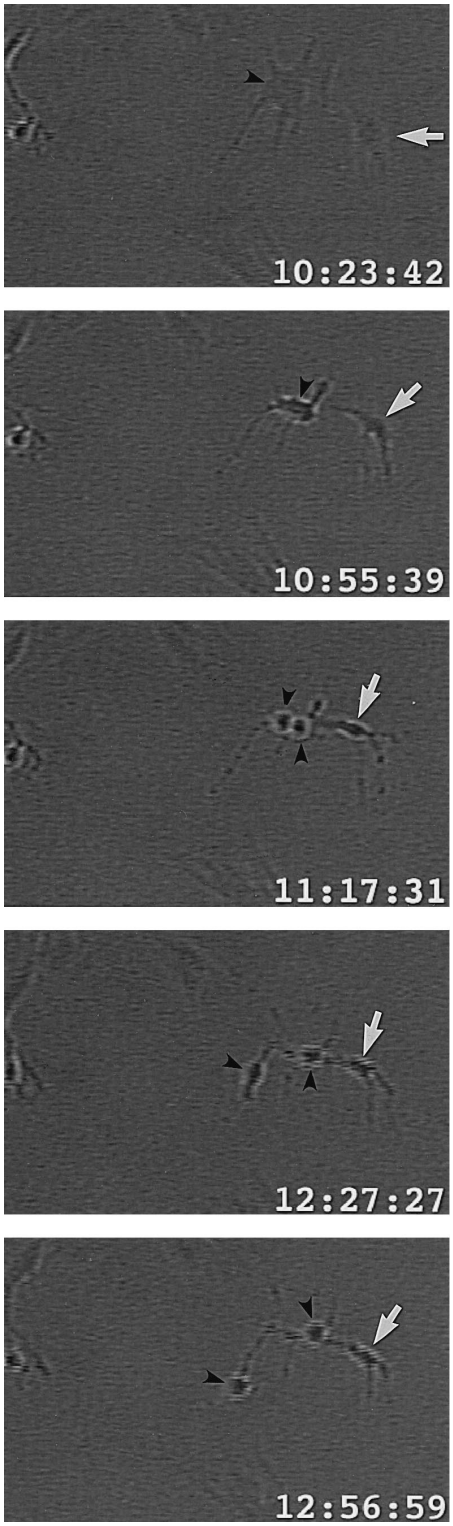


Fig. 6. Images from time lapse microcinematography of untreated mesangial cells. Cell division was first apparent 18 h after plating. The arrow depicts a cell which is undergoing division. Approximately 45 min elapse from the time cytokinesis is first evident (top image, cell has one edge lifted off surface) until the two daughter cells have respread over the surface (bottom panel).

cells were detached from the substratum (Fig. 2); for the most part, the remaining cells still attached to the substratum were rounded (Fig. 1B). By 72 h, ca. 90%



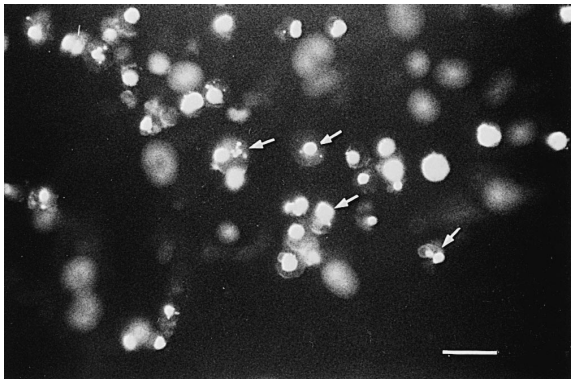


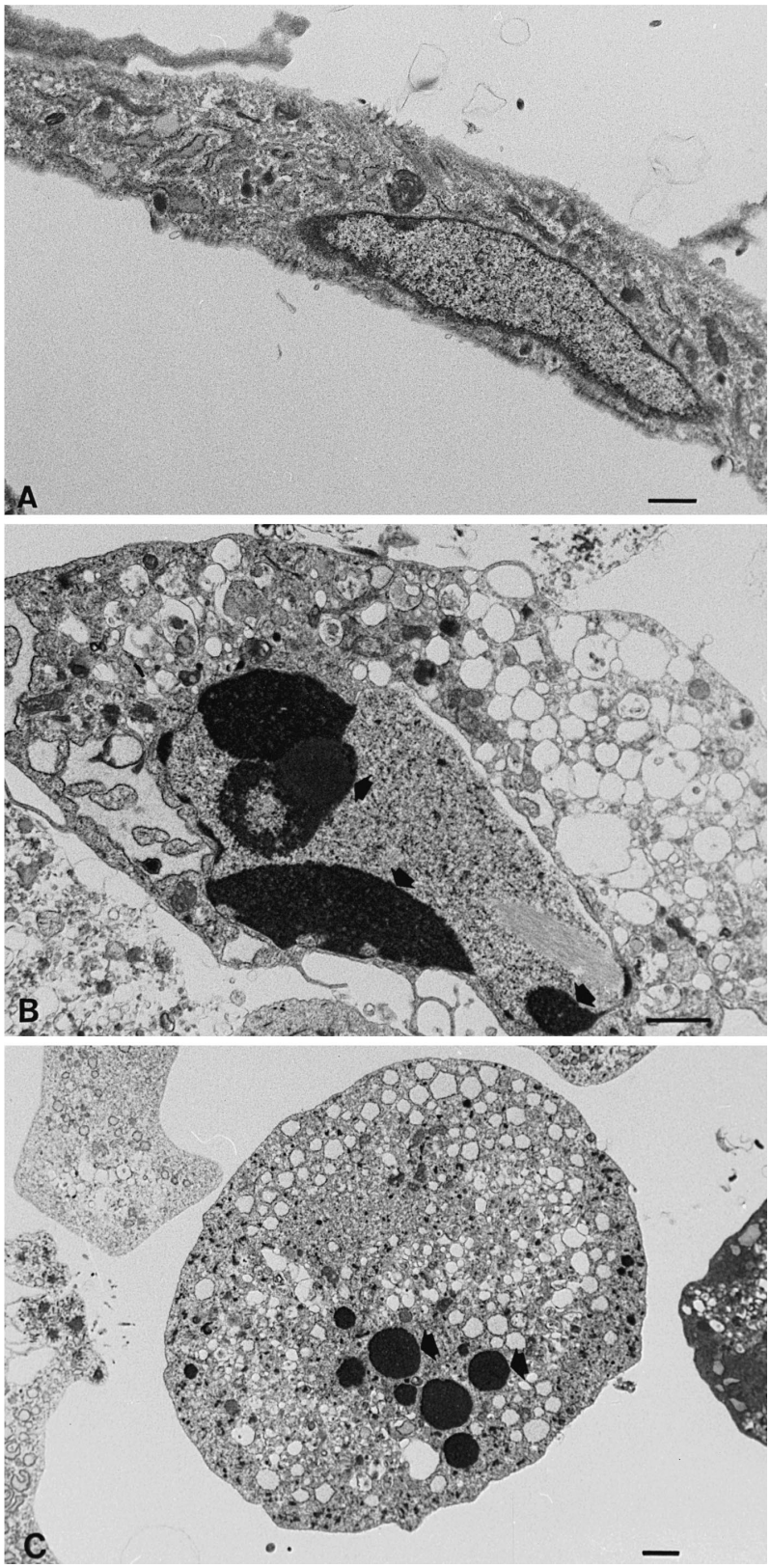
Fig. 8. Morphological characteristics of mesangial cells cultured in 50 μM lovastatin for 48 h. Cells were stained with the DNA binding fluorochrome Hoechst 33258 and examined under a epifluorescence photomicroscope with a water immersible lens. All the stained cells floated in the medium (arrows). Notice the nuclear condensation, fragmented cell nucleus and decreased cell size, features of apoptotic cells. The attached cells did not stain with Hoechst. Bar = 50 μm.

of the cells were floating in the medium (Fig. 2). Lovastatin-induced cell rounding was reversible by replacement with lovastatin-free medium; however, floating cells could not be stimulated to readhere in the absence of lovastatin. Inclusion of geranylgeraniol or farnesol dissolved in β-CD in the incubation medium together with lovastatin largely prevented cell rounding and loss of actin stress fibers (Fig. 1C and D) as well as cell detachment and inhibition of cell proliferation (Fig. 3). β-CD alone had no effect on mesangial cell morphology or adhesion.

3.2. Dependence of cell rounding on cell growth

Mesangial cells plated at low plating densities were affected most by lovastatin with $> 65\%$ of the cells rounded (Fig. 4B). However, plating cells at densities which resulted in confluent monolayers, where proliferation was inhibited by cell–cell con-

Fig. 7. Images from time lapse microcinematography of cells plated in the presence of 50 μM lovastatin. The top image shows two cells beginning to undergo mitosis. As is evident from the remaining images, one cell, (depicted with an arrowhead), undergoes cytokinesis but the daughter cells do not spread over the surface even after 2.5 h; the other cell, (depicted with an arrow) fails to undergo cytokinesis and remains round. Also, note the rounded cell along the left hand margin which had divided at some time previously but failed to spread.


A
B
C

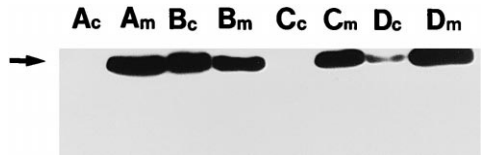


Fig. 10. Western blot analysis of the effect of lovastatin on RhoA localization. Mesangial cells were incubated with lovastatin (50 μM) for 48 h, harvested and extracted with Triton X-114 into cytosolic (c) and membrane (m) cellular fractions. PAGE was performed and proteins electroblotted and stained with an antibody to RhoA. Untreated mesangial cells (A) displayed immunoreactive RhoA in the membrane fraction. Treatment with lovastatin alone (B) caused RhoA to accumulate in the cytosolic fraction. Incubation in geranylgeraniol with lovastatin (C) resulted in a complete return of RhoA to the membrane cellular fraction while incubation in farnesol with lovastatin (D) resulted in only partial restoration. The arrow represents a 21 kDa protein marker.

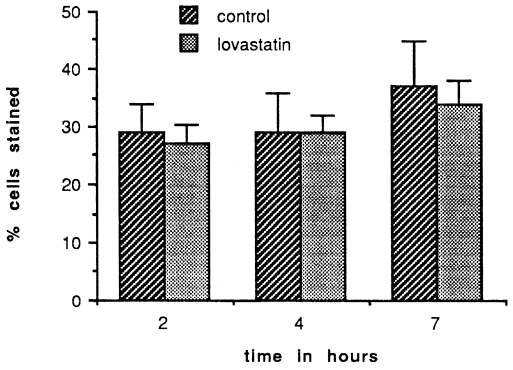


Fig. 11. Mesangial cells were pulsed with BrdU for 2 h and exposed to lovastatin (50 μM) for 2, 4, and 7 h. Cells were fixed and stained with an antibody to BrdU followed by a peroxidase-conjugated secondary antibody. Cell proliferation index was determined by calculating the percentage of cells exhibiting nuclear staining for BrdU.

tact, largely prevented cell rounding in the presence of the same concentration of lovastatin (Fig. 4D and F). Likewise, prevention of cell growth by serum deprivation for 48 h inhibited cell rounding induced by lovastatin, whereas the same cells exhibited cell rounding in the presence of lovastatin when serum was added back to the culture medium and cell division was stimulated (Fig. 5).

3.3. Sequence of events leading to G1 arrest in lovastatin

Mesangial cells were observed under time-lapse cinematography for a period of 48 h with and without lovastatin. Without lovastatin, mesangial cells were quiescent for a period of ca. 18 h after plating before evidence of cytokinesis was first observed. Immediately prior to cell division, cells detached from one edge and rounded up. Cell division followed within 45 min with the daughter cells moving apart from one another, and subsequently spreading out again (Fig. 6).

Plating cells into medium containing 50 μM lovastatin did not affect plating efficiency or initial cell spreading. Lovastatin's effect on the cells was not obvious until the cells were in mitosis. At this stage, the cells were observed to behave in one of the following manners in the presence of lovastatin: (1) After completion of cell division, the cells remained rounded and attached to the substratum; they did not respread, or (2) cytokinesis was inhibited and the cells remained in a rounded morphology. Both examples are shown in Fig. 7. Review of the entire video tape revealed that ca. 80% of the cells underwent cell division but did not respread while the remaining cells did not undergo cytokinesis.

3.4. Isoprenoid induced recovery of mesangial cells

Time lapse studies revealed that the addition of geranylgeraniol or farnesol to the rounded cells within 48 h of lovastatin treatment, caused the cells to respread and/or undergo cytokinesis similar to the untreated cells. Cells which were treated with lovastatin for longer periods of time could not be induced to respread. Similar results were obtained with ger-

Fig. 9. Ultrastructural appearance of mesangial cells either untreated (A) or treated with 50 μM lovastatin for 48 h (B, C). The untreated cells were attached to the substratum. Notice the characteristic spindle shape of mesangial cells with elliptical-shaped nucleus. B and C represent cells treated with lovastatin which were floating in the medium. Notice in B the nuclear chromatin condensation (arrowheads) and in C the fragmented nucleus (arrowheads), both characteristics of apoptotic cells. The attached cells incubated with lovastatin were round but displayed no evidence of apoptosis (not shown). Bars = 2 μm.

anylgeranyl pyrophosphate and farnesyl pyrophosphate (data not shown).

3.5. Induction of apoptosis

Mesangial cells were plated at a density of 2×10^4 cells cm^{-2} and treated with 50 μM lovastatin for 24, 48 and 72 h. Floating and attached cells were stained with Hoechst stain and nuclear morphology examined. All of the floating cells observed after 48 and 72 h of lovastatin treatment stained with Hoechst 33258 (see Fig. 8 for appearance of cells after 48 h of lovastatin treatment). This staining revealed diagnostic features of apoptosis; namely, nuclear condensation and fragmentation, and decrease in cell size (Fig. 8). Cell blebbing was also observed. None of the rounded, attached cells stained with Hoechst 33258.

In other experiments, cells were plated at 1.3×10^4 cells cm^{-2}, and treated with 50 μM lovastatin for 48 h. Floating and attached cells were examined by electron microscopy. Normal mesangial cells were flat spindle-shaped cells with elliptical shaped nuclei (Fig. 9A). After lovastatin treatment the attached cells were rounded with intact nuclei, and no evidence of apoptosis was apparent (data not shown). However, all floating cells were apoptotic, characterized by condensed nuclear chromatin (Fig. 9B) and fragmented nuclei (Fig. 9C).

3.6. Cellular localization of RhoA

To detect the cellular localization of RhoA, glomerular mesangial cells were treated with or without lovastatin in the presence or absence of farnesol or geranylgeraniol, and extracted with Triton X-114. After separation into cytosolic and membrane fractions, proteins were fractionated by SDS–PAGE, electroblotted onto nitrocellulose and stained with an antibody to RhoA. As shown in Fig. 10, in untreated mesangial cells, the immunoreactive RhoA was found in the membrane fraction (lane A_m). However, lovastatin treatment for 48 h caused RhoA to accumulate in the cytosolic fraction (lane B_C), suggestive of inactivation of RhoA (13). Mesangial cells treated with lovastatin in the presence of geranylgeraniol displayed a similar location of RhoA as untreated cells, that is, in the membrane fraction (lane C_m). Farnesol treatment resulted in partial restoration of RhoA to the membrane fraction (lane D_m).

3.7. Measure of cell proliferation

Mesangial cells were pulsed for 2 h with BrdU after exposure to lovastatin for 2, 4 or 7 h. As shown in Fig. 11, nuclear staining for BrdU did not change in the presence of lovastatin, confirming our observations that cell growth was necessary for lovastatin to affect cell morphology. Lovastatin decreased mesangial cell proliferation as evidenced by decreased nuclear staining for BrdU subsequent to cell rounding (data not shown).

4. Discussion

The HMG–CoA reductase inhibitor lovastatin has been reported to inhibit DNA synthesis [2,31,32], and cell proliferation [2,33,34] and to cause apoptosis in cultured cells [3,32]. It has been shown that these effects of lovastatin could be reversed by the addition of mevalonic acid [21] or by farnesol [2] or geranylgeraniol [37,38]. It is well established that this is due to prevention of isoprenylation of small GTP-binding proteins that are involved in these processes [1,2]. Although lovastatin is known to block cell cycle progression at G1 [20,21], the morphological basis for this is unknown. Our time lapse studies demonstrate that lovastatin blocks cell cycle progression at early G1 by preventing cell spreading and/or cytokinesis. Lovastatin is thus able to affect proliferating cells much more readily than non-proliferating cells.

Time lapse cinematography showed that the mitotic process in glomerular mesangial cells normally takes less than 1 h to complete; however, in the presence of lovastatin, some cells that had completed mitosis did not respread while other cells failed to undergo cytokinesis. This is contrary to the common belief that lovastatin inhibits DNA synthesis [2,32]. Also, our data shows that in mesangial cells lovastatin does not cause cell-rounding; rather, lovastatin prevents cells, which had already rounded up to undergo mitosis, from spreading back again. The fact that lovastatin-induced cell rounding is dependent on cell proliferation is further supported by the observation that BrdU uptake was not prevented by lovastatin in actively dividing cells, and that inhibition of cell growth, either by serum starvation or by contact inhibition, prevented this effect. Most studies report-

ing inhibition of DNA synthesis in the presence of lovastatin [2,32] measure DNA synthesis at a much later time, usually 42 h after the addition of lovastatin, when cell rounding has already occurred.

The cell rounding and loss of actin stress fibers was followed by cell detachment and apoptosis. Adherent cells maintained in suspension for long periods undergo apoptosis due to a failure to produce $p125^{FAK}$ [24,35]. This subtype of apoptosis was termed 'anoikis' or loneliness. Our data demonstrated that lovastatin-treated cells underwent 'anoikis' due to a loss of cell adhesion. This was supported by the observation that lovastatin-treated round cells still attached to the substratum did not undergo apoptosis, while the detached and floating cells did. The effects of lovastatin could be prevented by the addition of farnesol or geranylgeraniol to the medium specifying the action of lovastatin to its inhibition of isoprenoid synthesis.

We chose to study RhoA because (a) RhoA has been implicated in the formation of actin stress fibers and focal adhesion [22] and would therefore be involved in cell spreading, (b) RhoA localizes to cleavage furrows [23] and therefore may be involved in cytokinesis and (c) RhoA acts upstream of $p125^{FAK}$ [36] and is probably involved with the prevention of anoikis. Prevention of geranylgeranylation would inhibit targeting of RhoA to its active site on the membrane as well as its interaction with other proteins and therefore its function as a GTPase. Indeed, mesangial cells treated with lovastatin accumulate RhoA in the cytosolic fraction, indicative of inactive RhoA. Although both farnesol and geranygeraniol are capable of inhibiting lovastatin's action on cell rounding and stress fiber disassembly, only geranylgeraniol returned RhoA distribution to the membrane fraction completely. This implies that other small GTP-binding proteins in addition to RhoA participate in cell spreading and actin stress fiber assembly, especially ones that are farnesylated. One candidate is RhoB which can be either geranylgeranylated or farnesylated, and whose function is not clearly defined [10].

Addition of farnesol or geranylgeraniol also prevented inhibition of cell proliferation and anoikis induced by lovastatin treatment, but farnesol appeared to be more effective than geranylgeraniol (Fig. 3). These results in mesangial cells are contrary to those found in other cell lines in which farnesol was ineffective in preventing the effects of lovastatin [37,38]. Our results on mesangial cells imply the involvement of both farnesylated and geranylgeranylated proteins in cell proliferation.

Acknowledgements

This material is based upon work supported by a VA Merit Review, the Office of Research and Development, Medical Research Service, Department of Veterans Affairs. Dr. Kreisberg is a Career Scientist for the Department of Veterans Affairs. We would like to thank Ms. Sue Aguilar for her assistance in preparing the manuscript for publication, and Drs. Eugene Sprague and Robert Klebe for the use of the time lapse videomicroscope.

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