

Lovastatin Augments Apoptosis Induced by Chemotherapeutic Agents in Colon Cancer Cells

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ABSTRACT

β -Hydroxy- β -methylglutaryl coA reductase inhibitors (HRI) inhibit isoprenylation of several members of the Ras superfamily of proteins and therefore have important cellular effects, including the reduction of proliferation and increasing apoptosis. Significant toxicity at high doses has precluded the use of HRI as a monotherapy for cancers. We therefore studied whether combinations of the HRI lovastatin with standard chemotherapeutic agents would augment apoptosis in colon cancer cells. In the colon cancer cell lines SW480, HCT116, LoVo, and HT29, lovastatin induced apoptosis with differing sensitivity. Pretreatment with lovastatin significantly increased apoptosis induced by 5-fluorouracil (5-FU) or cisplatin in all four cell lines. Lovastatin treatment resulted in decreased expression of the antiapoptotic protein bcl-2 and increased the expression of the proapoptotic protein bax. The addition of geranylgeranylpyrophosphate (10 μ M) prevented lovastatin-induced augmentation of 5-FU and cisplatin-induced apoptosis; mevalonate (100 μ M) was partially effective, whereas cotreatment with farnesyl pyrophosphate (100 μ M) had no effect. These data imply that lovastatin acts by inhibiting geranylgeranylation and not farnesylation of target protein(s). Our data suggest that lovastatin may potentially be combined with 5-FU or cisplatin as chemotherapy for colon cancers.

INTRODUCTION

Lovastatin is a HRI² that reduces cholesterol synthesis by preventing the conversion of β -hydroxy- β -methylglutaryl coA

to mevalonate. The formation of farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which are downstream of mevalonate in the cholesterol synthetic pathway (1), is also inhibited. Farnesyl pyrophosphate and geranylgeranyl pyrophosphate belong to a class of molecules called isoprenoids that attach to several cellular proteins including G proteins by a posttranslational modification termed isoprenylation. The isoprenylation of G proteins is crucial for membrane attachment and normal functioning (1–4). The low molecular weight G proteins including ras, rho, and rab play crucial roles in signal transduction and therefore influence important cellular functions such as proliferation, apoptosis, and differentiation. By altering the function of these proteins, lovastatin has major effects on cells.

HRI inhibit cellular proliferation and induce apoptosis (5–9), making them potential anticancer agents. However, the use of HRI in the treatment of cancers, particularly solid tumors, has not been feasible because the doses calculated as required to produce a clinically desirable inhibition of proliferation and increase in apoptosis may be associated with significant toxicity. We postulated that HRI might augment the apoptosis induced by standard chemotherapeutic agents; thus, they could potentially be added to cancer chemotherapy regimens to improve outcomes.

MATERIALS AND METHODS

Cell Culture. SW480, HCT116, LoVo, and HT29 cells were obtained from American Type Culture Collection and maintained in DMEM or HAM solution (Life Technologies, Inc., Grand Island, NY) with 10% fetal bovine serum in an atmosphere of 95% O₂, 5% CO₂ at 37°C without antibiotics. All studies were performed with cells at 50% confluence. Lovastatin was a generous gift from Merck Laboratories (Rahway, NJ). 5-FU and cisplatin were purchased from Sigma (St. Louis, MO).

Lovastatin (10 and 30 μ M) was used to study the relative sensitivity to lovastatin-induced apoptosis in the four cell lines. Because of markedly different sensitivity to lovastatin-induced apoptosis in the cell lines, the concentrations of lovastatin used in experiments designed to study the effect of combining lovastatin with chemotherapeutic agents were then adjusted to achieve roughly similar levels of apoptosis without 5-FU or cisplatin (SW480 and HCT116 cells, 0, 5, and 10 μ M; LoVo and HT29 cells, 0, 10, and 30 μ M). Because the SW480 and HCT116 cells were more sensitive to 5-FU and cisplatin than LoVo and HT29 cells, we used 50 μ g/ml 5-FU and 20 μ g/ml cisplatin in studies with SW480 and HCT116 cells and 75 μ g/ml 5-FU and 30 μ g/ml cisplatin in studies with LoVo and HT29 cells.

To determine the relative sensitivity to apoptosis, lovastatin was added to cells at 50% confluence after changing the medium. Apoptosis was quantified after a 48-h incubation by flow cytometry. To study the effect of combinations of lovastatin and 5-FU or cisplatin on apoptosis, lovastatin was added after

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² The abbreviations used are: HRI, β -hydroxy- β -methylglutaryl coA inhibitor; 5-FU, 5-fluorouracil; TUNEL, terminal deoxynucleotidyl transferase nick end labeling; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; BrdUrd, bromodeoxyuridine.

changing the medium. After a 48-h incubation, the medium was changed again, lovastatin \pm 5-FU or cisplatin were added, and the cells were incubated for another 48 h. Apoptosis then was quantified with flow cytometry or MTT assays. For add-back experiments, mevalonate (100 μ M), farnesyl pyrophosphate (100 μ M), or geranylgeranylpyrophosphate (10 μ M; Sigma) were added with lovastatin at the beginning of the experiment and added again at 48 h when the cells were incubated with lovastatin \pm 5-FU or cisplatin. A higher dose of lovastatin (30 μ M) was used in these experiments to clarify the potential for prevention of the effect of lovastatin.

Electron Microscopy. For electron microscopy, adherent and nonadherent (floating) cells were fixed in 1% glutaraldehyde and 4% paraformaldehyde in phosphate buffer, post-fixed in 1% osmium tetroxide in phosphate buffer, dehydrated, and embedded in epoxy resin. Ultrathin (80-nm) sections were stained with uranyl and lead acetates and examined with a Zeiss M900 electron microscope at 80 kV.

Flow Cytometry. Flow cytometry was used to quantitate apoptotic cells by two methods, DNA histogram and TUNEL staining, which were performed simultaneously on the same samples. TUNEL staining was used to confirm the results observed by measurement of subdiploid cells. TUNEL staining detects cells earlier during apoptosis than measurement of subdiploid cells, so that more cells are determined as apoptotic. Cells were analyzed on a FACSORT flow cytometer (Becton Dickinson, San Jose, CA) after staining using a commercially available Apo-BrdUrd kit (Phoenix Flow Systems, San Diego, CA).

TUNEL Staining. DNA strand breaks in apoptotic cells were detected by incorporation of fluorescein-labeled deoxyuridine triphosphate into fragmented DNA by terminal deoxynucleotidyl transferase using the Apo-BrdUrd kit (10). The cells were collected and stained as described by the protocol provided by the manufacturer. The data were plotted on a dot plot, FL2-A versus FL2-W, and a singlet gate was applied. These gated cells were then plotted on dot plot FL1-H(log) versus FL2-A(lin), and cells stained with BrdUrd were counted as apoptotic. The data were also plotted on FL2-H histograms, and the number of subdiploid cells was counted as apoptotic. All flow cytometric studies were performed in triplicate and repeated three times. The data are presented as the mean \pm SD of three readings from each experiment. Similar data were obtained when the experiments were repeated. Student's *t* test was done to calculate the statistical significance between the controls (no lovastatin) and the two dose levels of lovastatin used. $P < 0.05$ was considered significant.

MTT Assay. Cells were grown in 96-well plates and treated with lovastatin for 48 h. The medium was then changed, and lovastatin and 5-FU or cisplatin were added. After 48 h, 50 μ g of MTT (Sigma) were added to each well, and the plates were incubated at 37°C for 2 h. MTT solubilization solution (10% Triton X-100 and 0.1 N HCl in anhydrous isopropanol; 100 μ) was then added, and the plates were agitated on a mechanical shaker to dissolve the crystals. Absorbance was measured spectrophotometrically at a dual wavelength of 570 and 405 nm, and the mean of six readings was used for calculations. The data are presented as the absorbance of treated cells as a percentage of the absorbance of untreated samples. Stu-

dent's *t* test was done to calculate the statistical significance between the controls (no lovastatin) and the two doses of lovastatin used. $P < 0.01$ was considered significant.

Western Blotting. Exponentially growing cells were collected by scraping, washed three times in ice-cold PBS, and resuspended in lysis buffer that contained 20 mM Tris-HCl (pH 7.4), 2 mM EDTA, 2 mM EGTA, 6 mM mercaptoethanol, 1% NP40, 0.1% SDS, and 10 mM NaF plus the protease inhibitors leupeptin (10 μ g/ml) and aprotinin (10 μ g/ml), and 0.1 mM phenylethylsulfonyl fluoride (all purchased from Sigma). After lysis with sonification, the resulting insoluble material was removed by centrifugation at 15,000 rpm for 15 min at 4°C and stored at -80°C . Protein concentrations were measured by the Bradford method, and 50- μ g samples were mixed with 2 \times Laemmli buffer, boiled for 5 min, electrophoresed in 10% SDS-PAGE, and transferred to Immobilon membranes (Millipore, Bedford, MA). Western blot analyses were then performed as described previously (11) using specific polyclonal antibodies to Bcl-2 and Bax that were raised to sequence-specific peptides at a concentration of 1:1500 (v/v). Antibody binding was detected by enhanced chemiluminescence (Amersham, Arlington Heights, IL). The images of films were obtained with a digital camera and used for densitometry measurements (KDS 120; Kodak, Rochester, NY).

RESULTS

Lovastatin Increases Apoptosis in Colon Cancer Cells.

To determine the relative sensitivities of HCT116, SW480, LoVo, and HT29 cells to lovastatin-induced apoptosis, cells at 50% confluence were incubated for 48 h with lovastatin (10 or 30 μ M). Apoptosis then was quantified by flow cytometry and measured as the percentage of subdiploid cells on DNA histogram. Lovastatin caused a dose-dependent increase in apoptosis in all four cell lines used (Fig. 1). There were marked differences in the levels of apoptosis induced by lovastatin in these cell lines. HT29 cells were most resistant to lovastatin-induced apoptosis but did undergo apoptosis at high concentrations (17.3 \pm 3.5% subdiploid cells with 100 μ M lovastatin). Apoptosis was confirmed in lovastatin-treated cells by electron microscopy, which revealed the characteristic nuclear fragmentation and chromatin condensation.

Effect of Lovastatin Pretreatment on 5-FU and Cisplatin-induced Apoptosis.

To determine whether lovastatin enhances apoptosis induced by chemotherapeutic agents, cells were pretreated with lovastatin for 48 h, followed by treatment with lovastatin \pm 5-FU or cisplatin for 48 h. Because of the marked differences in sensitivity to lovastatin-induced apoptosis, we used different doses of lovastatin in these experiments to achieve roughly similar levels of apoptosis with lovastatin alone (SW480 and HCT116 cells, 0, 5, and 10 μ M; LoVo and HT29 cells, 0, 10, and 30 μ M). Similarly, due to differences in sensitivity, 50 μ g/ml 5-FU or 20 μ g/ml cisplatin was used in SW480 and HCT116 cells, and 75 μ g/ml 5-FU or 30 μ g/ml cisplatin was used in LoVo and HT29 cells. As shown in Fig. 2 using two methods, apoptosis induced by 5-FU and cisplatin was augmented by lovastatin pretreatment in all four of the cell lines. 5-FU-induced apoptosis increased from 44.5 \pm 4.8% to 61.5 \pm 3.2% in HCT116 cells, from 21.7 \pm 3.2% to 34.8 \pm 3.1% in

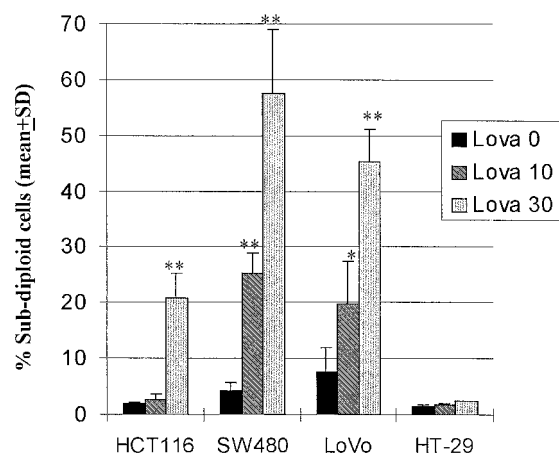


Fig. 1 Lovastatin induces apoptosis in colon cancer cells. HCT116, SW480, LoVo, and HT29 colon cancer cells were grown to 50% confluence, incubated with lovastatin (10 and 30 μM) for 48 h, and harvested for quantification of apoptosis by flow cytometry. Apoptosis was determined as the percentage of subdiploid cells. Data are presented as the mean \pm SD. $P < 0.05$ was considered statistically significant (versus no lovastatin). Similar data were obtained in three separate experiments. *, $P < 0.05$; **, $P < 0.01$.

SW480 cells, from $28.4 \pm 14.6\%$ to $74.9 \pm 6.9\%$ in LoVo cells, and from $16.6 \pm 0.8\%$ to $61.9 \pm 8.5\%$ in HT29 cells. In HT29 cells, 400 $\mu\text{g/ml}$ 5-FU induced $60.4 \pm 6.1\%$ apoptosis as compared to $61.9 \pm 8.5\%$ apoptosis induced by 75 $\mu\text{g/ml}$ 5-FU and 30 μM lovastatin.

Similarly, cisplatin-induced apoptosis increased from $8.7 \pm 2.3\%$ to $36.8 \pm 5.4\%$ in HCT116 cells, from $15 \pm 1.5\%$ to $22.3 \pm 4.9\%$ in SW480 cells, from $35.5 \pm 10.3\%$ to $81.2 \pm 7.5\%$ in HT29 cells, and from $44.1 \pm 12.1\%$ to $86.05 \pm 9.6\%$ in LoVo cells. We compared the effect of pretreatment with lovastatin on cisplatin-induced apoptosis with that of cisplatin alone in HCT116 colon cancer cells (Fig. 3). As shown in Fig. 3A, 20 μM lovastatin increased the apoptosis induced by 20 $\mu\text{g/ml}$ cisplatin from $8.7 \pm 2.3\%$ to $48.7 \pm 4.2\%$, whereas an increase in the concentration of cisplatin from 20 $\mu\text{g/ml}$ to 160 $\mu\text{g/ml}$ only increased apoptosis from $8.7 \pm 2.3\%$ to $30.8 \pm 3.1\%$ (Fig. 3B).

Lovastatin pretreatment further decreased the number of viable cells after treatment with 5-FU and cisplatin in colon cancer cells, as illustrated in Fig. 4.

Geranylgeranylpyrophosphate Prevents Lovastatin-induced Augmentation of Apoptosis Induced by 5-FU or Cisplatin. To evaluate the cellular mechanisms involved in lovastatin enhancement of 5-FU- or cisplatin-induced apoptosis, add-back experiments were performed using intermediates in the cholesterol synthetic pathway that might be involved in the lovastatin effect. Mevalonate (100 μM), farnesyl pyrophosphate (100 μM), or geranylgeranyl pyrophosphate (up to 10 μM) was added with 30 μM lovastatin, and the cells were incubated for 48 h. The medium was then changed, and cells were incubated with lovastatin \pm isoprenoids \pm 5-FU (50 $\mu\text{g/ml}$) or cisplatin (20 $\mu\text{g/ml}$) for another 48 h. Apoptosis was then quantified by flow cytometry. Fig. 5A summarizes the results of these experiments in SW480 cells. By itself, lovastatin induced apoptosis,

and it also augmented apoptosis induced by 5-FU and cisplatin. Mevalonate (100 μM) and farnesyl pyrophosphate (100 μM) failed to prevent this lovastatin effect. In contrast, geranylgeranylpyrophosphate (10 μM) prevented lovastatin-induced apoptosis and also prevented the augmentation by lovastatin of 5-FU- or cisplatin-induced apoptosis. Similar results were observed in HCT116 cells (Fig. 5B). Mevalonate (100 μM), farnesyl pyrophosphate (up to 100 μM), and geranylgeranylpyrophosphate (10 μM) did not alter apoptosis in these cells in the absence of lovastatin (data not shown).

Lovastatin Alters Bcl-2 and Bax Expression. To determine whether apoptosis accompanying lovastatin exposure was associated with changes in the expression of the Bcl-2 family of genes, Western blotting was performed on cells after exposure to lovastatin (Fig. 6). In HT29 cells, apoptosis induced by lovastatin was accompanied by an 8-fold decrease in the expression of the antiapoptotic protein Bcl-2 and a 3-fold increase in the expression of the proapoptotic protein Bax.

DISCUSSION

Evidence is provided that shows that a HRI, lovastatin, induces apoptosis in HCT116, SW480, LoVo, and HT29 colon cancer cell lines. Furthermore, pretreatment of these colon cancer cells with lovastatin results in an augmentation of apoptosis that is induced by the standard chemotherapeutic drugs 5-FU and cisplatin. The augmentation of apoptosis is prevented by geranylgeranylpyrophosphate but not by mevalonate or farnesol pyrophosphate. Lovastatin treatment results in decreased expression of the antiapoptotic protein bcl-2 and increased expression of the proapoptotic protein bax.

Lovastatin has been shown to induce apoptosis in prostate cancer cells (6), lymphocytes (7), leukemia cells (8), and hepatocytes (9) in culture. The amount of apoptosis varies from a slight increase (from 0.16% to 1.48% in hepatocytes; Ref. 9) to a massive increase (50% in HL-60 cells; Ref. 8). The present study using four colon cancer cell lines showed a wide variation in sensitivity to lovastatin-induced apoptosis. HT29 cells were the least susceptible of the four cell lines studied. These data suggest that the sensitivity to lovastatin relies not only on the tissue source of the cancer cells but also on the type of oncogenic mutations that are present. HT29 cells, which are the least sensitive to lovastatin-induced apoptosis, have normal ras, whereas SW480, HCT116, and LoVo cells, which are highly sensitive to lovastatin-induced apoptosis, all have oncogenic *k-ras* mutations. The presence of *ras* mutations may enhance lovastatin-induced apoptosis. Because HT29 cells do undergo apoptosis, despite the presence of normal ras, this suggests that apoptosis induced by lovastatin is not entirely dependent on ras transformation. Furthermore, both HT29 and SW480 cells have mutated nonfunctional p53, yet they have very different sensitivities to lovastatin-induced apoptosis. We have demonstrated previously (12) that lovastatin induces p53-sensitive apoptosis in intestinal IEC-18 cells using clones that have mutated and wild-type p53. These results show that although lovastatin causes p53-sensitive apoptosis, other factors such as *ras* mutation may be more important in altering the susceptibility to lovastatin-induced apoptosis.

Pretreatment of colon cancer cells with lovastatin aug-

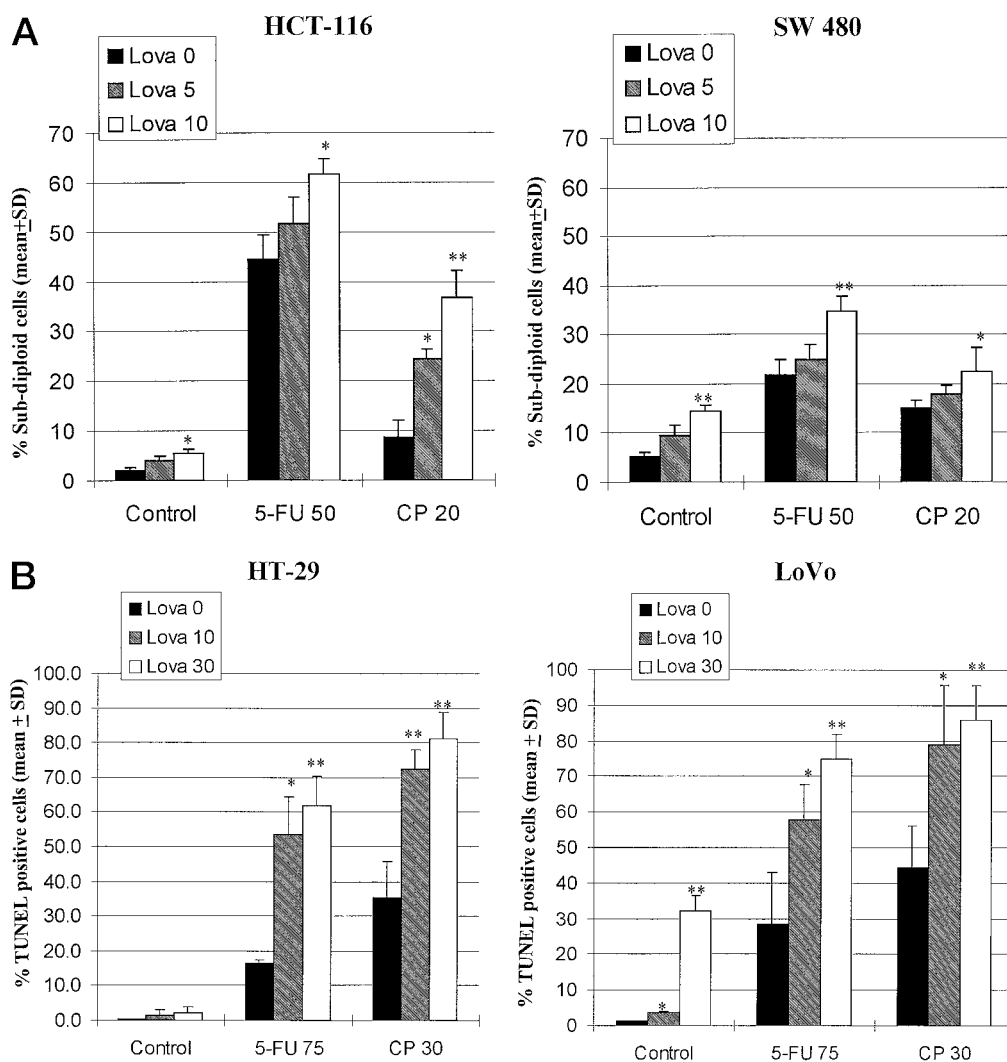


Fig. 2 Lovastatin pretreatment augments apoptosis induced by 5-FU or cisplatin. HCT116, SW480, LoVo, and HT29 colon cancer cells were grown to 50% confluence and treated with lovastatin for 48 h. Cells were then incubated for 48 h with lovastatin (HCT116 and SW480 were treated with 5 and 10 μM and HT29 and LoVo were treated with 10 and 30 μM) \pm 5-FU (50 or 75 $\mu\text{g/ml}$) or cisplatin (CP; 20 or 30 $\mu\text{g/ml}$) and harvested for quantification of apoptosis by flow cytometry. Control cells were treated with lovastatin but not with 5-FU or cisplatin. Lovastatin pretreatment resulted in a dose-dependent augmentation of apoptosis. **A**, apoptosis quantified as the percentage of subdiploid cells in SW-480 and HCT-116 cells. **B**, apoptosis quantified as the percentage of TUNEL-positive cells in HT-29 and LoVo cells. Similar data were obtained in three separate experiments. *, $P < 0.05$; **, $P < 0.01$.

mented the apoptosis induced by 5-FU and cisplatin. Indeed, lovastatin induced levels of apoptosis that could be achieved only by a severalfold increase in the doses of 5-FU or cisplatin. Whether lovastatin would also sensitize normal cells to apoptosis induced by 5-FU and cisplatin and thereby increase the incidence of adverse effects requires further investigation. There is preliminary evidence that cancer cells may be more sensitive to lovastatin-induced apoptosis than normal cells because leukemia cells and HL-60 cell lines are much more susceptible to lovastatin-induced apoptosis than the normal bone marrow progenitors (13, 14). The presence of oncogenic *ras* mutations appears to increase the sensitivity to lovastatin-induced apoptosis. Thus, it is possible that lovastatin may be combined with

chemotherapeutic agents to increase the tumor cell kill or to reduce the side effects of chemotherapeutic agents that are required to induce a remission. The addition of lovastatin to chemotherapy regimens might selectively increase apoptosis in cancer cells while sparing normal cells.

The degree of lovastatin-induced augmentation of apoptosis induced by different chemotherapeutic agents varied. In HCT116 cells, lovastatin augmented cisplatin-induced apoptosis more than 5-FU-induced apoptosis (Figs. 2A and 4). The reason for these differences is not clear. However, such data might provide a rationale for chemotherapy designed specifically for each tumor, based on the sensitivity patterns. Alternately, combinations of chemotherapeutic agents rather than single chemo-

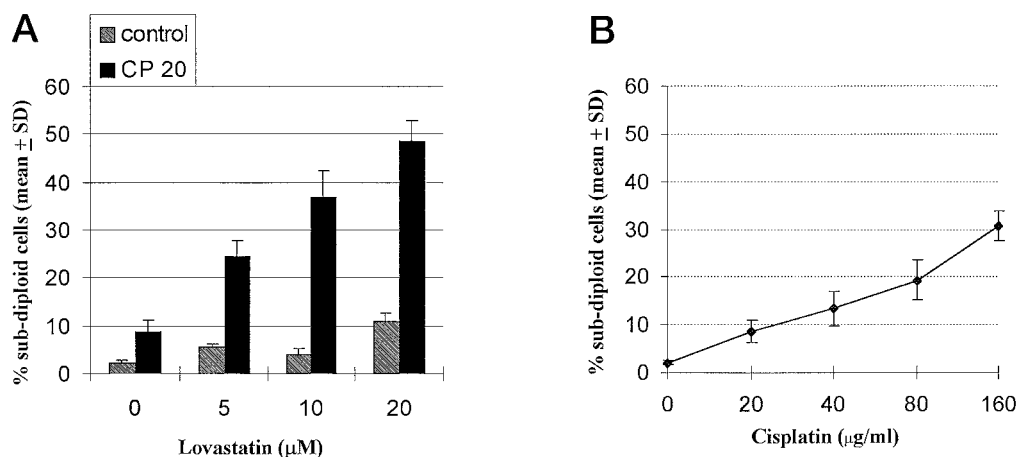


Fig. 3 Comparison of lovastatin pretreatment with cisplatin alone upon apoptosis. **A**, effect of lovastatin ± cisplatin upon apoptosis. HCT-116 cells at 50% confluence were exposed to lovastatin (0, 5, 10, and 20 μM) for 48 h. Cells were then incubated with lovastatin and without cisplatin (*control*) or with cisplatin (20 μg/ml; *CP 20*) for 48 h, and apoptosis was quantified as the percentage of subdiploid cells. **B**, dose-response curve for cisplatin-induced apoptosis. HCT116 cells at 50% confluence were treated with cisplatin (20, 40, 80, and 160 μg/ml) alone for 48 h, and apoptosis was quantified as the percentage of subdiploid cells. Note: pretreatment with lovastatin (10 or 20 μM) before cisplatin (20 μg/ml) treatment induced more apoptosis than increasing the cisplatin concentration to 160 μg/ml.

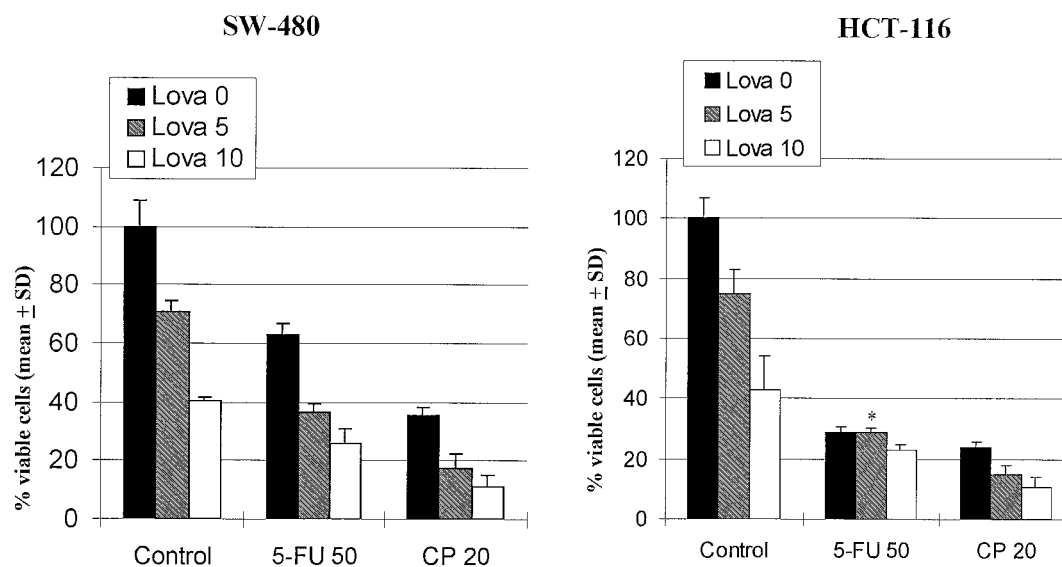


Fig. 4 Lovastatin pretreatment reduces viable cells after treatment with 5-FU or cisplatin. SW480 and HCT116 cells were grown in 96-well plates and incubated with lovastatin (0, 5, and 10 μM) for 48 h. Lovastatin ± 5-FU (50 μg/ml) or cisplatin (20 μg/ml) were added, and cells were incubated for an additional 48 h. Controls were treated with lovastatin and without 5-FU or cisplatin. A MTT assay was then performed as described in "Materials and Methods." The data are presented as the fraction of the optical density of control wells and plotted as a percentage. $P < 0.01$ was considered significant. *, not significant.

therapeutic agents might be useful when combined with lovastatin in the chemotherapy of colon cancer.

Soma *et al.* (15, 16) have reported that the HRI simvastatin has a synergistic effect with the alkylating agent carmustine in inhibiting cellular proliferation in gliomas *in vitro* (15) and *in vivo* (16). However, the present report is the first in which HRIs have augmented apoptosis induced by various chemotherapeutic agents in a concentration-dependent manner. Lovastatin might therefore be used in cycles with chemotherapeutic agents to

increase tumor cell kill, rather than being used over prolonged periods to inhibit tumor proliferation. This would minimize the adverse effects of high-dose lovastatin administered over prolonged periods and would increase its utility in cancer chemotherapy.

Lovastatin augmentation of 5-FU- or cisplatin-induced apoptosis was prevented by adding geranylgeranylpyrophosphate but not by mevalonate or farnesyl pyrophosphate. These data imply that lovastatin induces apoptosis by inhibiting the gera-

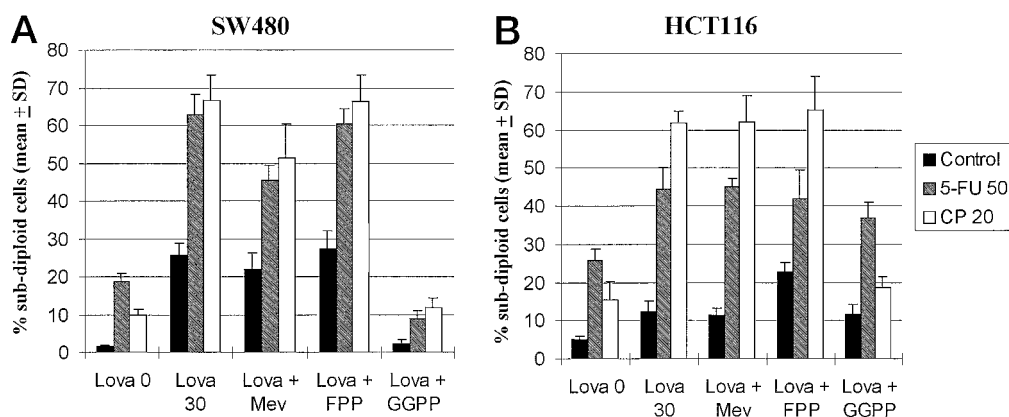


Fig. 5 Geranylgeranylpyrophosphate but not farnesyl pyrophosphate prevents augmentation of 5-FU- or cisplatin-induced apoptosis by lovastatin. SW480 and HCT116 cells were grown to 50% confluence, and then lovastatin (30 μ M) was added with mevalonate (100 μ M) farnesyl pyrophosphate (FPP; 100 μ M) or geranylgeranylpyrophosphate (GGPP; 10 μ M) and incubated for 48 h. After a change of medium, 5-FU (50 μ g/ml) or cisplatin (20 μ g/ml) was added with lovastatin and the isoprenoids, and the cells were incubated for another 48 h and collected for flow cytometry. Control cells were treated with lovastatin and isoprenoids but not with 5-FU or cisplatin. Apoptosis was quantified as the percentage of subdiploid cells. Similar results were obtained in three separate experiments. Lovastatin-induced augmentation of 5-FU- or cisplatin-induced apoptosis is abolished by the addition of geranylgeranylpyrophosphate but not mevalonate or farnesyl pyrophosphate.

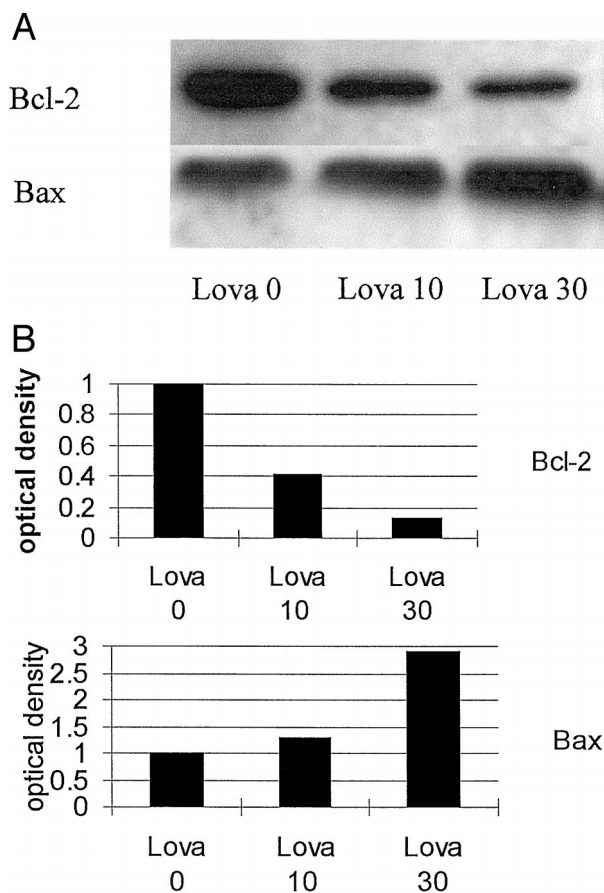


Fig. 6 Western blotting for Bcl-2 and Bax expression in lovastatin-treated cells. HT29 cells were treated with lovastatin for 48 h and then collected and handled as described in "Materials and Methods." *Lova 0*, untreated HT-29 cells; *Lova 10*, lovastatin (10 μ M); *Lova 30*, lovastatin (30 μ M). Lovastatin increased the expression of bax and decreased the expression of bcl-2. A, Western blots; B, densitometry readings of the blots.

nylgeranylation and not the farnesylation of intracellular proteins. Because ras is primarily farnesylated, inhibition of ras action is unlikely to be the predominant mechanism by which lovastatin augments apoptosis. Members of the rho family, including rho, rac, and cdc-42, which are predominantly geranylgeranylated, would appear to be more likely targets. We are currently investigating the possible role of inhibition of geranylgeranylation of rho in lovastatin-induced augmentation of 5-FU- and cisplatin-induced apoptosis.

Lovastatin treatment was accompanied by decreased expression of the antiapoptotic protein bcl-2 and increased expression of the proapoptotic protein bax. These changes may explain the increased susceptibility of these cells to undergo apoptosis. In another recent report, lovastatin induced increased levels of caspase-7 and up-regulated its mRNA (17). These data suggest that lovastatin treatment may alter the expression of several cellular proteins that may modulate apoptosis induced by chemotherapeutic agents.

To summarize, the HRI lovastatin augments apoptosis induced by standard chemotherapeutic agents such as 5-FU and cisplatin in colon cancer cells. This augmentation is due to inhibition of geranylgeranylation and is associated with decreased expression of bcl-2 and increased expression of bax. Our data suggest that lovastatin may have a potential use in the chemotherapy of colon cancer.

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