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Lovastatin Induces Apoptotic Cell Death by Activation of Intracellular Ca^{2+} Signal in HepG2 Human Hepatoma Cells

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Abstract – Although lovastatin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, has been shown to have anti-cancer actions, the effect on human hepatoma cells was not investigated. Moreover, the exact mechanism of this action is not fully understood. In this study we investigated the mechanism by which lovastatin induces apoptosis using HepG2 human hepatoblastoma cells. Lovastatin induced apoptotic cell death in a dose-dependent manner in the cells, assessed by the flow cytometric analysis. Treatment with mevalonic acid, a precursor of cholesterol, did not significantly suppress the lovastatin-induced apoptosis. Lovastatin induced a rapid and sustained increase in intracellular Ca^{2+} concentration. Treatment with EGTA, an extracellular Ca^{2+} chelator did not significantly alter the lovastatin-induced intracellular Ca^{2+} increase and apoptosis, whereas intracellular Ca^{2+} reduction with BAPTA/AM and intracellular Ca^{2+} release blockers (dantrolene and TMB-8) completely blocked these actions of lovastatin. In addition, the lovastatin-induced apoptosis was significantly reduced by a calpain inhibitor, a broad spectrum caspase inhibitor z-VAD-fmk and inhibitors specific for caspase-9 and caspase-3 (z-LEHD-fmk and z-DEVD-fmk, respectively), but not by an inhibitor specific for caspase-8 (z-IETD-fmk). Collectively, these results suggest that lovastatin induced apoptosis of HepG2 hepatoma cells through intracellular Ca^{2+} release and calpain activation, leading to triggering mitochondrial apoptotic pathway. These results further suggest that lovastatin may be valuable for the therapeutic management of human hepatoma.

Keywords ☐ Lovastatin, apoptosis, Ca^{2+} signal, calpain, mitochondrial pathway, HepG2 cells

INTRODUCTION

Lovastatin is a competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis, and is generally regarded as a safe and effective compound used extensively for the treatment of hypercholesterolemia (Goldstein and Brown, 1990). In addition, lovastatin has been shown to inhibit tumor growth and metastasis *in vivo* in several different implantable rodent tumorigenesis models, e.g. in mammary carcinoma (Alonso *et al.*, 1998; Farina *et al.*, 2002; Inano *et al.*, 1997; Shibata *et al.*, 2003), fibrosarcoma (Matar *et al.*, 1998), colon adenocarcinoma (Broitman *et al.*, 1996), melanoma (Jani *et al.*, 1993) and pancreatic neoplasias (Kusama *et al.*, 2002).

In breast cancer cell cultures lovastatin induces two cell cycle inhibitors, p21Waf1 and p27Kip1, and leads to cell cycle arrest in the G1 phase (Gray-Bablin *et al.*, 1997; Keyomarsi *et al.*, 1991; Shibata *et al.*, 2003; Rao *et al.*, 1998). In other *in vitro* studies apoptosis has been induced by lovastatin in cancer cells derived from various organs, including the breast (Alonso *et al.*, 1998; Borner *et al.*, 1995; Farina *et al.*, 2002; Shibata *et al.*, 2003). Apoptosis in response to stimulation by lovastatin appears to be caspase-3-dependent (Shellman *et al.*, 2005) and it induces cytochrome c release (Shibata *et al.*, 2004) and PARP cleavage (Marcelli *et al.*, 1998). However, the molecular mechanism activated by lovastatin in enhancing apoptosis remains unclear.

Apoptosis is a highly organized cell death process characterized by ultrastructural modification (cytoskeletal disruption, cell shrinkage, and membrane blebbing), nuclear alteration (chromatin condensation and internucleosomal DNA cleavage), and biochemical changes (activation of proteases) (Kidd, 1998). Genetic changes resulting in loss of apoptosis are likely to be critical components of carcinogenesis (Schulte-Hermann *et al.*, 1997). Many studies have demonstrated that carcinogenic processes are significantly linked to derangement of apo-

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ptosis-signaling pathways (Adams and Cory, 1998; Kastan *et al.*, 1995). Accumulating evidence suggests that apoptosis induction of cancer cells is useful for cancer treatment (Kornblau, 1998). Known anti-cancer agents induce apoptosis in cancer cells (Kamesaki, 1998). Radiation therapy for cancer is also based on apoptosis induction of cancer cells (Crompton, 1998). Although apoptotic signaling pathways are not completely known, intracellular Ca^{2+} seems to be regarded as an important regulator of apoptosis (McConkey and Orrenius, 1996).

Thus, the main purposes of this study were to investigate whether lovastatin induces apoptotic cell death in HepG2 human hepatoma cells and to elucidate the mechanism of the lovastatin-induced apoptosis specifically focused on intracellular Ca^{2+} signal.

MATERIALS AND METHODS

Materials

The HepG2 human hepatoblastoma cell line was purchased from American Type Culture Collection (Rockville, MA). Powdered Eagle's minimum essential medium (MEM) and Earle's basal salt solution (EBSS), 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide (MTT), dantrolene, 3,4,5-trimethoxybenzoic acid-8-(diethylamino)-octyl ester (TMB-8), ethylene glycol-bis-(aminoethyl ether)N,N,N',N'-tetraacetic acid (EGTA), mevalonic acid (MA), trypsin solution, sodium pyruvate, probenecid, propidium iodide (PI), ribonuclease A and all salt powders were obtained from Sigma Chemical CO. (St. Louis, MO). Calpain inhibitor I (N-acetyl-Leu-Leu-Nle-CHO) was obtained from Calbiochem (San Diego, CA). 1-(2, 5-Carboxyoxazol-2-yl-6-aminobenzfuran-5-oxyl)-2-(2'-amino-methylphenoxy)-ethane-N,N,N'N'-tetraacetoxylmethyl ester (Fura-2/AM) and bis-(o-aminophenoxy)-ethane-N,N,N,N-tetraacetic acid/acetoxymethyl ester (BAPTA/AM) were from Molecular Probes, Inc. (Eugene, OR). Lovastatin was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The inactive lactone form of lovastatin was converted to the active dihydroxy open acid form by dissolution in ethanol, followed by incubation in 0.4 M NaOH in ethanol at 50°C for 2 hr and adjusting the pH to 7.2 with 1 M HCl. Aliquots of this stock solution (10 mM) were stored at -20°C. Caspase inhibitors, pan-caspase inhibitor z-VAD-fmk (fluoromethyl ketone), caspase-8-specific z-IETD-fmk, caspase-9-specific z-LEHD-fmk and caspase-3-specific z-DEVD-fmk were obtained from MBL Inc. (Nagoya, Japan). Fetal bovine serum (FBS) and antibiotics (penicillin and streptomycin mixture) were purchased from GIBCO (Grand Island, NY). Caspase inhibitors, BAPTA/AM and Fura-2/AM were prepared as stock solutions in dimethyl sulfoxide (DMSO), then diluted with aqueous medium to the final desired concentrations. The stock solution of drugs was sterilized by filtration through 0.2 μm disc filters (Gelman Sciences: Ann Arbor, MI).

Cell culture

HepG2 cells were grown at 37°C in a humidified incubator under 5% CO_2/95% air in a MEM supplemented with 10% FBS, 200,000 IU/L penicillin, 200 mg/L of streptomycin and 1 mM sodium pyruvate. Culture medium was replaced every other day. After attaining confluence the cells were subcultured following trypsinization.

Cell viability assay (MTT staining)

Cell viability was assessed by the MTT staining method (van de Loosdrecht *et al.*, 1991). Cells from 4- to 5-day-old cultures were seeded in 24-well plates at the density of 5×10^4 cells/well. The volume of the medium in the wells was 1 mL. In control experiments cells were grown in the same media containing drug-free vehicle. After incubation with drug for 48 hr, 100 μL of MTT (5 mg MTT/mL in H_2O) were added and cells incubated for a further 4 hr. Two hundred microliters of DMSO were added to each culture and mixed by pipetting to dissolve the reduced MTT crystals. Relative cell viability was obtained by scanning with an ELISA reader (Molecular Devices, Menlo Park, CA) with a 540 nm filter.

Flow cytometry assay

For flow cytometry analysis, HepG2 cells were collected and washed twice with PBS buffer, pH 7.4. After fixing in 80% ethanol for 30 min, cells were washed twice, and resuspended in PBS buffer (pH 7.4) containing 0.1% Triton X-100, 5 mg/L PI and 50 mg/L ribonuclease A for DNA staining. Cells were then analyzed by a FACScan (BIO-RAD, Hercules, CA). At least 20,000 events were evaluated. All histograms were analyzed using WinBryte software (BIO-RAD, Hercules, CA) to determine percentage of nuclei with hypodiploid content indicative of apoptosis (Bombeli *et al.*, 1997).

Measurement of intracellular Ca^{2+} concentrations

Aliquots of the HepG2 cells were washed in EBSS. Then, 5 μM Fura-2/AM was added, and the cells were incubated for 30 min at 37°C. Unloaded Fura-2/AM was removed by centrifugation at 150×*g* for 3 min. Cells were resuspended at a density of

2×10^9 cells/L in Krebs-Ringer buffer (KRB) containing 125 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 5 mM $NaHCO_3$, 25 mM HEPES, 6 mM glucose and 2.5 mM probenecid (pH 7.4). Fura-2-loaded cells were maintained at 25°C for 90 min before fluorescence measurement. For each experiment, 0.5 mL aliquot of Fura-2-loaded cells was equilibrated to 37°C in a stirred quartz cuvette. Fluorescence emission (510 nm) was monitored with the excitation wavelength cycling between 340 and 380 nm using a Hitachi F4500 fluorescence spectrophotometer. In the data intracellular free Ca^{2+} concentrations were presented by the 340:380 nm fluorescence ratios.

Data analysis

All experiments were performed four times. Data were expressed as mean ± standard error of the mean (SEM) and were analyzed using one way analysis of variance (ANOVA) and Student-Newman-Keul's test for individual comparisons. *P* values less than 0.05 are considered statistically significant.

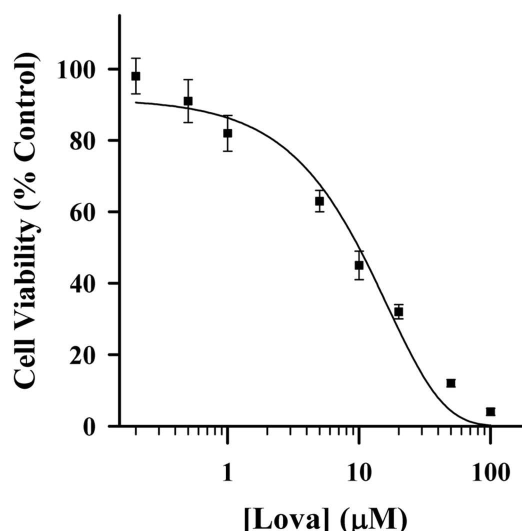

Fig. 1. Effects of lovastatin on cell viability in HepG2 human hepatoma cells. Cells were incubated with or without each concentration of lovastatin (Lova) for 48 hr. Cell viability assay was done by the MTT staining method. Results are expressed as percent change of control condition in which cells were grown in medium without drugs. Data points represent the mean values of four replications with bars indicating SEM.

RESULTS

Induction of apoptotic cell death by lovastatin

The effect of lovastatin on cell viability of the HepG2 cells were examined using the MTT staining method. Lovastatin decreased cell viability in a dose-dependent manner as depicted in Fig. 1. A significant cytotoxicity by lovastatin was started at the concentration of 5 μM. Lovastatin reduced cell viability to 50% at around 9 μM. To determine whether the reduced cell viability by lovastatin is due to apoptotic cell death, its effect on apoptosis of the HepG2 cells was studied. Flow cytometry assay was used for detection of apoptosis (Bombeli *et al.*, 1997). As shown in Fig 2, lovastatin increased the number of cells with hypodiploid nuclei, indicative of apoptosis, in a dose-

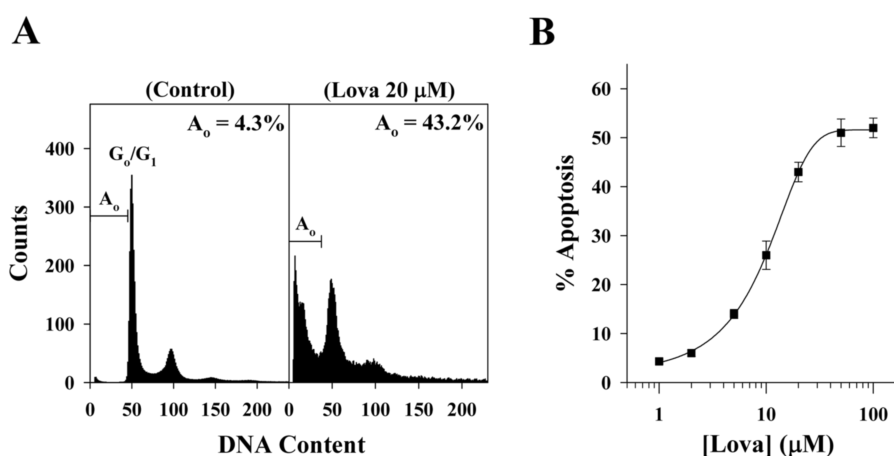

Fig. 2. Lovastatin induces apoptotic cell death of HepG2 human hepatoma cells. In figure A, after cells were treated with or without lovastatin (Lova, 20 μM) for 48 hr, the cells were stained with PI and analyzed by flow cytometry. The number of apoptotic cells was measured by flow cytometry as described in text. The region to the left of the G_o/G_1 peak, designated A_o, was defined as cells undergoing apoptosis-associated DNA degradation. In figure B, the cells were incubated with Lova for 48 hr at each designated concentration. The data represent the mean values of four replicates with bars indicating SEM.

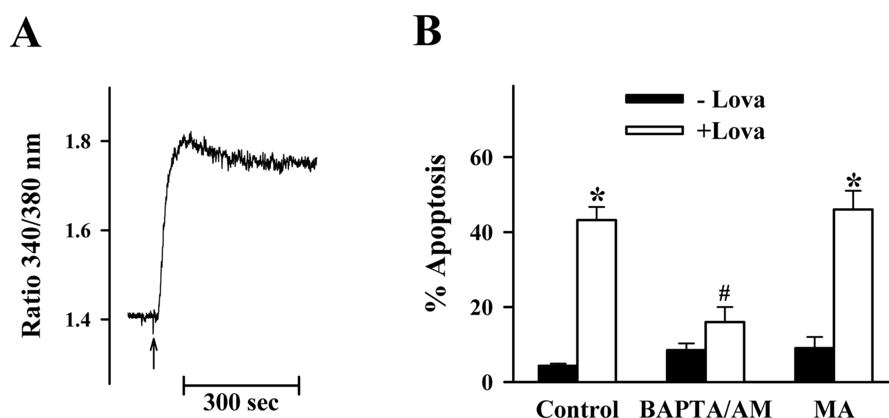

Fig. 3. Lovastatin-induced apoptosis is dependent on intra-cellular Ca^{2+} in HepG2 human hepatoblastoma cells. In figure A, intracellular Ca^{2+} concentration was assessed by Fura-2 fluorescence technique, and the data represent intracellular Ca^{2+} change with time. The arrow shows the time point for addition of lovastatin (20 μM). In figure B, the cells were incubated with or without lovastatin (Lova, 20 μM) for 48 hr. BAPTA/AM (1 μM) and mevalonic acid (MA, 1 mM) were added to the cells 30 min before treatment with Lova (20 μM). The number of apoptotic cells was measured by flow cytometry. The data represent the mean values of four replications with bars indicating SEM. *$p<0.05$ compared to control. #$p<0.05$ compared to Lova alone.

dependent manner. These results clearly showed that lovastatin induced apoptotic cell death in the HepG2 cells.

Role of intracellular Ca^{2+} signal in the lovastatin-induced apoptosis

Since lovastatin is a cholesterol-lowering drug that exerts pleiotropic functions by preventing the synthesis of mevalonic acid (MA), the precursor of non-steroidal isoprenoid compounds that play a key role in a number of cellular processes, such as post-translational modification of proteins (McTaggart, 2006), cell differentiation (Buhaescu and Izzedine, 2007) and cell proliferation (Alegret and Silvestre, 2006), we investigated whether administration of MA can inhibit the lovastatin-induced apoptosis. As shown in Fig. 3B, treatment with MA did not abrogate the lovastatin-induced apoptosis. These results suggest that lovastatin may induce apoptotic cell death through a mevalonate-independent mechanism.

Intracellular Ca^{2+} signal appears to be commonly involved in the mechanism of apoptosis (McConkey and Orrenius, 1997). Thus we examined whether lovastatin alters intracellular Ca^{2+} concentration using Fura-2 fluorescence technique (Grynkiewicz *et al.*, 1985). Lovastatin at the concentration inducing apoptosis (20 μM) rapidly increased intracellular Ca^{2+} concentration, as shown in Fig. 3A. Treatment with BAPTA/AM, an intracellular Ca^{2+} chelator, significantly suppressed the lovastatin-induced apoptosis as shown in Fig. 3B. These results suggest that lovastatin-induced apoptosis may be due to increased intracellular Ca^{2+}.

To determine the source of the lovastatin-induced intracellular Ca^{2+} increase, we measured intracellular Ca^{2+} concentration using a nominal Ca^{2+}-free medium containing 1 mM EGTA. This experimental protocol can effectively reduce extracellular free Ca^{2+} concentration, and thus, blunt available Ca^{2+} influx. Under these conditions cellular Ca^{2+} response to lovastatin was not significantly altered as illustrated in Fig. 4A. Treatment with dantrolene and TMB-8, specific inhibitors for ryanodine receptor and IP_3 receptor, respectively (Ehrlich *et al.*, 1994), almost completely inhibited the Ca^{2+}-increasing effect of lovastatin. These results indicate that the increased intracellular Ca^{2+} concentration by lovastatin may be due to Ca^{2+} release from the internal Ca^{2+} stores. Moreover, treatment with these inhibitors (dantrolene, TMB-8) also significantly prevented the apoptosis induced by lovastatin, as depicted in Fig. 4B. These results strongly support that intracellular Ca^{2+} release results in the lovastatin-induced apoptosis.

Involvement of mitochondrial pathway in the lovastatin-induced apoptosis

There are two pathways currently proposed to play major roles in regulating apoptosis in mammalian cells: an extrinsic pathway mediated by one or more death receptors and an intrinsic pathway mediated by mitochondria (Hengartner, 2000). Thus in this study we examined which pathway is involved in the lovastatin-induced apoptosis using various caspase inhibitors. As shown in Fig. 5, the results show that the lovastatin-induced apoptosis was significantly reduced by both

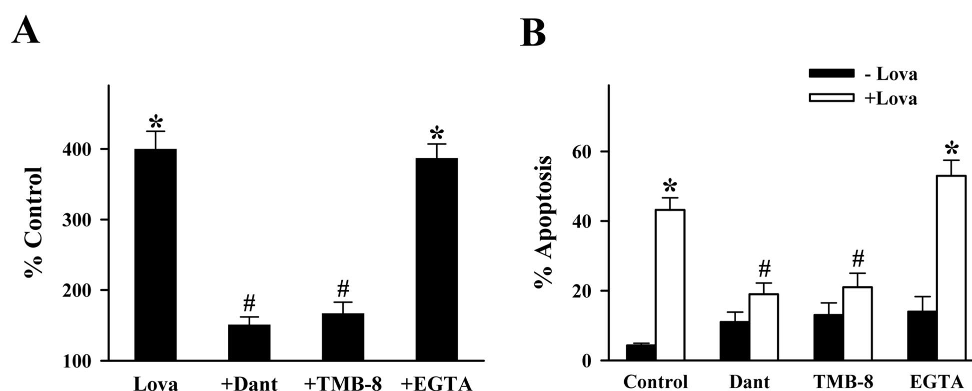

Fig. 4. Activation of Ca^{2+} release mechanism mediates the lovastatin-induced intracellular Ca^{2+} increase and apoptosis in HepG2 human hepatoblastoma cells. Intracellular Ca^{2+} concen-tration and apoptosis were assessed by Fura-2 fluorescence technique and flow cytometry, respectively. In figure A, dantrolene (50 μM) and TMB-8 (20 μM) were added 5 min before lovastatin (Lova, 20 μM) application. In the EGTA group, a nominal Ca^{2+}-free medium containing 100 μM EGTA was used. The data are expressed as percent changes of intracellular Ca^{2+} levels compared to the control condition in which the cells were incubated with Lova-free medium. In figure B, Lova (20 μM) was treated for 48 hr. Dantrolene (50 μM), TMB-8 (20 μM) and EGTA (1 mM) were added 30 min before Lova treatment. The data (A and B) represent the mean values of four replications with bars indicating SEM. *$p<0.05$ compared to control. #$p<0.05$ compared to Lova alone.

the broad spectrum caspase inhibitor z-VAD-fmk and inhibitors specific for caspase-9 and caspase-3 (z-LEHD-fmk and z-DEVD-fmk, respectively), but that apoptosis was not reduced by exposure to z-IETD-fmk, an inhibitor specific for caspase-8. Considering that caspase-8 appears to play a role in triggering only the extrinsic pathway of apoptosis (Gogvadze and Orrenius, 2006), these indirect evidence suggest that the intrinsic mitochondrial pathway may be, at least in part, engaged in the lovastatin-induced apoptosis.

Since the Ca^{2+}-dependent protease calpain seems to be involved in the mechanism of Ca^{2+}-associated apoptosis (Goll *et al.*, 2003), we investigated whether a cell permeable calpain inhibitor suppresses the lovastatin-induced apoptosis. Indeed, the calpain inhibitor significantly abrogated the apoptosis, as depicted in Fig. 5. These results further indicate that intracellular Ca^{2+} increase by lovastatin may trigger the activation of calpain, in turn, resulting in apoptosis.

DISCUSSION

HMG-CoA reductase inhibitors, statins, reduce the proliferation of a wide variety of cell types *in vitro* by inducing cell-cycle arrest at G1 phase (Kozar *et al.*, 2004; Koyuturk *et al.*, 2004; Mo and Elson, 2004; Muck *et al.*, 2004). Evidence is accumulating that statins may inhibit carcinogenesis because of their central action on important cellular functions (Hindler *et al.*, 2006). It has been suggested that the anticancer effect of statins can be potentially exploited for the cancer therapy. Retrospective studies concluded that the long-term use of statins reduces the risk of pancreatic, prostate, and colorectal cancers (Poynter *et al.*, 2005). In addition, several cancer trials have shown evidence supporting the use of statins as therapeutic anticancer agents (Brower, 2003; Chan *et al.*, 2003). The mech-

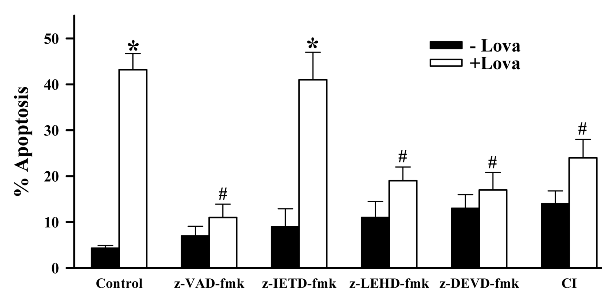

Fig. 5. Effects of inhibitors of calpain and caspases on the lovastatin-induced apoptosis in HepG2 human hepatoma cells. After cells were incubated with Lova (20 μM) for 48 h, the cells were stained with PI and analyzed by flow cytometry. The number of apoptotic cells was measured by flow cytometry as described in text. In these experiments, z-VAD-fmk, z-IETD-fmk, z-LEHD-fmk and z-DEVD-fmk were used as inhibitors of the broad spectrum caspase, the caspase-8, the caspase-9 and the caspase-3, respectively. These caspase inhibitors (100 μM) and the calpain inhibitor (CI, 10 μM) were given 30 min before Lova application. The data represent the mean values of four replicates with bars indicating SEM. *$P<0.05$ compared to control in which the cells were incubated with drug-free medium. #$P<0.05$ compared to Lova alone.

anism responsible for action of statins is considered to be the inhibition of cholesterol biosynthesis through inhibition of HMG-CoA reductase. This results in depletion of mevalonate, which is a precursor of cholesterol (Goldstein and Brown, 1990). Interestingly, elevated mevalonate synthesis has been reported in malignant breast (Dalenc *et al.*, 2005), leukemia and lymphoma (Fernndez *et al.*, 2004; van de Donk *et al.*, 2006), and prostate carcinoma cells (Mo and Elson, 2004). In this study we clearly showed, for the first time, that lovastatin induces apoptotic cell death in human hepatoma cells (Fig. 2). Treatment with mevalonic acid did not abrogate the lovastatin-induced apoptosis (Fig. 3B), suggsting that apoptosis of the hepatoma cells by lovastatin may be induced through a mevalonate-independent mechanism. Although this discrepancy between this study and others is not clearly understood, the mechanism of the lovastatin-induced apoptosis may be cell type specific.

Accumulating evidence implies that intracellular Ca^{2+} is commonly involved in the mechanism of apoptosis (McConkey and Orrenius, 1997). Consistently, in this study we also showed that lovastatin appeared to induce apoptosis through intracellular Ca^{2+} rise, since lovastatin induced a rapid and sustained increase in intracellular Ca^{2+} concentration (Fig 3A), and intracellular Ca^{2+} chelation with BAPTA/AM effectively prevented the lovastatin-induced apoptosis (Fig. 3B). In addition to apoptosis, cell proliferation and differentiation have been linked to the stimulation of intracellular Ca^{2+} signal (Munaron *et al.*, 2004). Interestingly, Ca^{2+} channel antagonists have decreased cell proliferation in a variety of cancer cells *in vitro* (Yoshida *et al.*, 2003) and *in vivo* (Taylor *et al.*, 1992).

Lovastatin appeared to elevate intracellular Ca^{2+} level through internal Ca^{2+} release, since this action of lovastatin was not altered by reducing extracellular Ca^{2+} concentration with a nominal Ca^{2+}-free medium containing 1 mM EGTA, but completely inhibited by intracellular Ca^{2+} release blockers, dantrolene and TMB-8 (Fig. 4A). Our results are in line with the previous report that simvastatin, another inhibitor of HMG-CoA reductase, induced intracellular Ca^{2+} release from IP_3-sensitive pools mediated by activation of phospholipase C-γ1 (Mutoh *et al.*, 1999). Significant inhibition of the lovastatin-induced apoptosis by these Ca^{2+} release blockers (Fig. 4B) implies that Ca^{2+} release from internal stores may act as a major trigger for the lovastatin-induced apoptotic cell death in the HepG2 cells.

There are two pathways currently proposed to play major roles in regulating apoptosis in mammalian cells: an extrinsic pathway mediated by one or more death receptors and an intrinsic pathway mediated by mitochondria (Hengartner, 2000). In the extrinsic death receptor/ligands pathway, caspase activation occurs as a direct consequence of death receptor ligation, with upstream caspase-8 cleaving and activating downstream proteases such as caspase-9 and caspase-3. In the intrinsic mitochondrial pathway, Bax, a member of the Bcl-2 family, plays the leading role. Bax normally resides in the cytosol in a quiescent state. After an apoptotic stimulus, Bax is translocated into the mitochondria and promotes the release of cytochrome c (Jurgensmeier *et al.*, 1998), possibly by forming a pore (Saito *et al.*, 2000) or a voltage-dependent anion channel (Shimizu *et al.*, 1999) in the outer mitochondrial membrane. Both the death receptor and the mitochondrial pathways are linked by cleavage of Bid by caspase-8. It is known that cleaved Bid also translocates to mitochondria and induces Bax-dependent release of cytochrome c (Luo *et al.*, 1998). Once in the cytosol, cytochrome c activates Apaf-1, which then activates procaspase-9, which, in turn, activates caspase-3, triggering apoptosis. Lovastatin-induced apoptosis was significantly reduced by both the broad spectrum caspase inhibitor z-VAD-fmk and inhibitors specific for caspase-9 and caspase-3 (z-LEHD-fmk and z-DEVD-fmk, respectively), but not by exposure to z-IETD-fmk, an inhibitor specific for caspase-8 (Fig. 5), which strongly suggests that the intrinsic mitochondrial pathway is engaged in the lovastatin-induced apoptosis.

Calpains, a family of Ca^{2+}-dependent cytoplasmic cysteine proteases are activated through an increase in intracellular Ca^{2+} (Molinari and Carafoli, 1997). The two major calpain isoforms, m- and μ-, differ in the amount of Ca^{2+} required for activation. Calpain is considered to be primarily responsible for both apoptosis (Nath *et al.*, 1996) and necrosis (Wang *et al.*, 1996), probably as a consequence of a loss of Ca^{2+} homeostasis (Squier *et al.*, 1994). Calpain has been shown to act upstream (McCollum *et al.*, 2002), or downstream of caspase activation and to contribute to the degradation phase of apoptotic cell death (Wood and Newcomb, 1999 and Wood *et al.*, 1998). Lovastatin rapidly mobilized Ca^{2+} from intracellular stores (Fig. 2A) and that the lovastatin-induced apoptosis was significantly prevented by a calpain inhibitor (Fig. 5), suggesting that Ca^{2+}-dependent activation of calpain(s) may be involved in the lovastatin-induced apoptosis in the hepatoma cells.

In conclusion, lovastatin induced apoptosis of HepG2 human hepatoma cells through intracellular Ca^{2+} release and calpain activation, leading to triggering intrinsic mitochondrial pathway. These results suggest that lovastatin may be a good

candidate for the therapeutic management of human hepatoma.

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