



ORIGINAL ARTICLE

Inhibitory Effects of Statins on Cytomegalovirus Production in Human Cells: Comprehensive Analysis of Gene Expression Profiles

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Background/Purpose: Inhibitory effects of statins on human immunodeficiency virus-1 or poliovirus replication were reported. Our aim was to clarify whether statins could inhibit the replication of cytomegalovirus (CMV) in human cells and to determine the changes in gene expression profiles in host cells treated with statins using a DNA microarray.

Methods: Human embryonic lung (HEL) fibroblast cells were infected with CMV (Towne strain) at a multiplicity of infection of 1 and were simultaneously treated with mevastatin, simvastatin, lovastatin, or pravastatin (0.001–10 μ M). HEL cells were incubated for 6 days, and progeny viral titers were quantified by plaque assay. Time-dependent effects of mevastatin or simvastatin (1 μ M) on CMV replication were also examined. We determined the effects of mevastatin or simvastatin at concentrations ranging from 0.1 μ M to 10 μ M on the expressions of CMV immediate-early-1 (IE-1) and late proteins using Western blotting. Comprehensive analysis of gene expression profiles in HEL cells treated with mevastatin (1 μ M) was performed with a DNA microarray 1 day after infection.

Results: The 50% effective concentration values for the inhibition of CMV titers by mevastatin, simvastatin, lovastatin, and pravastatin were 0.0006 μ M, 0.0055 μ M, 0.04 μ M, and 2.55 μ M, respectively. Inhibition of viral replication by mevastatin was observed when added 24 hours after infection, whereas that by simvastatin was observed when added 48 hours after infection. Mevastatin decreased the expression of the IE-1 protein, and simvastatin inhibited the expression of the late protein. We observed significant changes of cellular growth/differentiation-associated gene expressions (e.g., downregulated *cdk2* mRNA) in HEL cells treated with mevastatin.

Conclusion: Our data suggest that treatment with mevastatin could inhibit CMV replication at IE phase through altered expressions of cellular growth/differentiation-associated genes.

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1. Introduction

Endothelial and smooth muscle cells infected with human cytomegalovirus (CMV) may cause chronic inflammation in vessels, suggesting an association between arteriosclerosis and CMV infection. However, latently infected CMV is frequently activated in immunosuppressed patients, such as those with AIDS or organ

transplants, thereby causing severe morbidity and eventual mortality.¹ Symptomatic CMV infection has been treated successfully with ganciclovir (GCV), but the emergence of GCV-resistant strains is a current issue in the treatment of immunocompromised subjects with CMV infection. Although either foscarnet or cidofovir has been used as an alternative therapy against GCV-resistant strains, these treatments are not always successful.² There is a need for the development of new or alternative anti-CMV agents.³

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, statins, are used as lipid-lowering agents in clinical settings. It is demonstrated that statins play a multifactorial role in the maintenance of transplanted organs.⁴ Nie et al⁵ report that statins exert immunosuppressive effects in rats undergoing heterotopic limb

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allografts by inhibiting activation and proliferation of T cells. In addition to these immunomodulatory actions, statins are shown to have antiviral effects. Fluvastatin is described to inhibit CMV replication through a decrease of nuclear factor- κ B binding activity in human endothelial cells.⁶ However, there is very limited information regarding the inhibitory actions of various statins against CMV replication in other human cell lines. We aimed to clarify whether the activities of various agents can suppress CMV production in human embryonic lung (HEL) cells and to determine significant alterations of genetic profiles in cells treated with statins using a DNA microarray.

2. Methods

2.1. Virus, cells, and reagents

CMV Towne strain was applied throughout our investigation.⁷ CMV was propagated in HEL cells, and the clarified supernatant was stored in liquid nitrogen until use. Viral infectivity was titrated using a plaque assay method as previously reported.⁸ Four statins, mevastatin, simvastatin, lovastatin, and pravastatin, were commercially available (Calbiochem Inc., San Diego, CA, USA). These reagents were dissolved in a cell culture medium.

2.2. Cell culture

HEL cells⁹ were cultured in Dulbecco's modified Eagle's minimal essential medium (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan), supplemented with 10% heat-inactivated fetal calf serum (Z.L. Bocknek Laboratory, Ontario, Canada), L-glutamine (0.3 mg/mL), gentamicin (50 mg/mL), and amphotericin B (2.5 mg/mL). All cell cultures were maintained in a humidified incubator at 37°C in 5% CO₂/95% air.

2.3. Viral production

When HEL cells in 24-well plates (IWAKI Microplate; IWAKI Glass Co., Funahashi, Japan) reached confluency, the cells were inoculated with CMV at a multiplicity of infection of 1. After adsorption for 1 hour, the cells were supplemented with 1 mL of Dulbecco's modified Eagle's minimal essential medium containing 2% fetal calf serum in the presence or absence of various concentrations of statins for indicated time intervals. The infectious virus produced in culture supernatants was titrated using a plaque assay.⁸

2.4. Inhibitory effects of statins on cytomegalovirus production

The CMV-infected cells were treated with four statins (mevastatin, simvastatin, lovastatin, or pravastatin) at different concentrations ranging from 0.001 μ M to 10 μ M. All cells were incubated for 6 days after viral inoculation, and then, the CMV titers were measured. Based on the curves showing relationships between relative virus titers (a percentage of the control titers) and statin concentrations used, 50% effective concentration (EC₅₀) was calculated for each statin.

2.5. Time-dependent effects of statins on cytomegalovirus production

The cells were inoculated with CMV, and treatment with mevastatin (at a concentration of 1 μ M) was started at indicated times (1, 8, 24, and 48 hours) after infection. All cells were incubated for 5 or 7 days after viral inoculation, and the viral titers were quantified. Similarly, simvastatin (1 μ M) was added at various time points

(1, 24, 48, and 72 hours) after infection, and the CMV titers were determined at Day 7 after infection.

2.6. Cytotoxic activity of statins

Subconfluent monolayers of the uninfected cells were cultured on 24-well plates in the absence or presence of different concentrations ranging from 0.01 μ M to 10 μ M of mevastatin or simvastatin. They were incubated under conditions of 37°C and 5% CO₂ for 3 or 6 days. The number of viable cells was determined using trypan-blue exclusion dye staining, and the ratio of the viable cells under exposure to the statins to viable negative control cells was calculated for each concentration.

2.7. Detection of viral proteins

The CMV-infected cells were treated with the indicated concentrations ranging from 0.1 μ M to 10 μ M of mevastatin or simvastatin until 1 day after viral inoculation and were harvested for Western blot analysis to detect the expression of the immediate-early-1 (IE-1) protein. In another trial, the cells were treated with the same concentrations of the same statins until 3 or 6 days after infection and were harvested for Western blotting analysis with the antibody against the late protein. Western blot analysis for the detection of the structural IE-1 and late proteins was performed as previously described.³ The β -actin protein was applied as an internal control.

2.8. Detection of viral mRNA

The infected cells were incubated with different concentrations ranging from 0.1 μ M to 10 μ M of mevastatin until 24 hours after infection. Total RNA was extracted using a standard method and was reversely transcribed after eliminating residual genomic DNA. The cDNA products were amplified for IE-1/-2 and β -actin gene expressions through real-time reverse-transcription polymerase chain reaction (PCR) with each specific primer set and iQ SYBR Green Supermix (Bio-Rad Laboratories Inc., Tokyo, Japan) for 34 cycles (10 seconds at 95°C, 20 seconds at 55°C, and 20 seconds at 72°C) by a Mini Opticon real-time PCR using Gene Expression Macro software (Bio-Rad Laboratories Inc.). The PCR primers for the amplification of IE-1/-2 genes were used as previously reported.¹⁰ The β -actin gene was used as an internal control. The comparative expression levels of the IE-1/-2 mRNAs were calculated according to the expression levels of the β -actin mRNA.

2.9. Determination of gene expression profiles in cells by DNA microarray analysis

Total RNA was isolated from the cells using a commercially available kit (RNeasy Mini Kit; QIAGEN GmbH, Hilden, Germany). The extracted RNA was quantified by photometry at 260/280 nm, and the quality of RNA was determined by the ratio of 18S/28S ribosomal band intensities in an ethidium bromide containing 1% agarose gel through electrophoresis. Comprehensive analysis of gene expression profiles in the infected cells was performed using a Human U133 Plus 2.0 GeneChip microarray system (Affymetrix Inc., Santa Clara, CA, USA) as previously reported.¹¹ The amounts of probe-specific transcripts were determined based on the average of the differences between the perfect-match and mismatch intensities. Because replicate assays were not performed, the signal intensity of selected genes that were up- or downregulated by at least twofold changes compared with that of a control group were extracted by the GeneSpring GX software package version 7.3.1 (Agilent Technologies Inc., Santa Clara, CA, USA). Ingenuity Pathway Analysis (Ingenuity Systems Inc., Redwood City, CA, USA) was used

as an additional method for evaluating the functional significance of the induced gene expression profiles.

2.10. Validation of data by DNA microarray analysis

Real-time reverse-transcription PCR analysis was performed as described regarding the detection of viral mRNA, using prepared RNA samples. Four target genes (*cdk2*, *arf1*, *xrcc2*, and *psmb4*) in the infected cells treated with mevastatin or simvastatin at different concentrations ranging from 0.1 μ M to 10 μ M until 24 hours after infection were selected among those having significantly up- or downregulated changes based on the gene expression profiles. The oligonucleotide sequences of the forward/reverse primer set for each gene amplification were described previously.^{12–15} Comparative expression levels of the selected genes were calculated according to the expression levels of the β -actin mRNA. The expression levels of the four mRNAs are expressed as fold changes relative to the baseline expression levels in the untreated control group. Together with the validation of data by DNA microarray analysis, we also confirmed the changes of viral loads (IE-1 mRNA expression).

2.11. Data analyses

Data were analyzed using statistical analysis methods and graphing software (KaleidaGraphTM; Synergy Software, Tokyo, Japan). All data were expressed as mean $\pm$ standard deviation. The significance of the differences among values was determined by Dunnett's multiple tests after one-way analysis of variance in comparison with control cultures, and *p* values less than 0.05 were considered to indicate significance. Furthermore, Fisher's exact test was used to determine the probability that biological function assigned to each network could be explained by chance alone.

3. Results

3.1. Inhibitory effects of statins on cytomegalovirus production

The EC₅₀ values for inhibition of CMV production by mevastatin, simvastatin, lovastatin, and pravastatin were 0.0006 μ M, 0.0055 μ M, 0.04 μ M, and 2.55 μ M, respectively. Mevastatin showed the most pronounced suppressive effect.

3.2. Time-dependent effects of statins on cytomegalovirus production

Based on the EC₅₀ data, mevastatin and simvastatin were selected for the evaluation of time-dependent actions against CMV production. Mevastatin (1 μ M) exhibited significantly inhibitory effects on viral replication over a period of 5 or 7 days when started at 1, 8, and 24 hours after CMV infection but not when started at 48 hours after viral inoculation (Figure 1A). On the other hand, simvastatin (1 μ M) achieved a significant suppression of CMV production over a period of 7 days when started 48 hours as well as 1 and 24 hours after the infection (Figure 1B).

3.3. Cytotoxic activity of statins

We determined the cytotoxic activity of mevastatin or simvastatin without CMV infection according to the EC₅₀ data. Ratio of viable cells under exposure to the statins (0.01–10 μ M) to viable negative control cells for 3 days was more than 90%, and those under exposure to mevastatin and simvastatin (10 μ M) for 6 days were approximately 73% and 89%, respectively. These observations suggest that decreased viral titers by these statins simply reflect inhibitory actions of CMV replication without direct statin cytotoxicity.

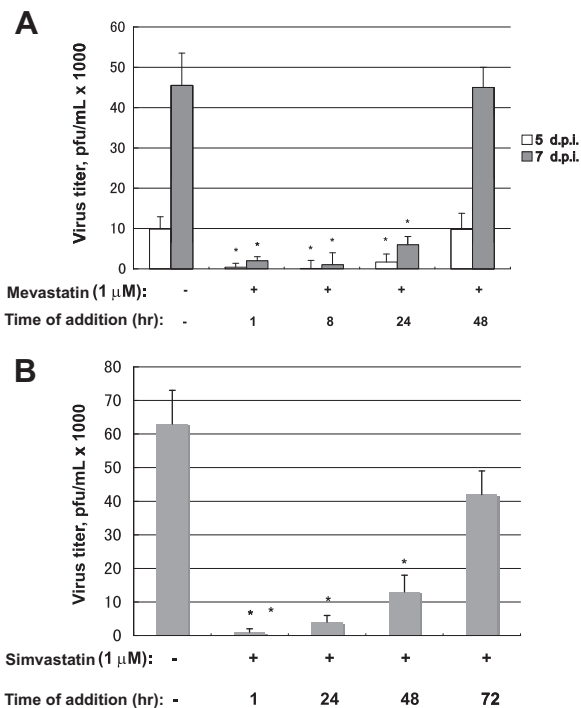


Figure 1 Time-dependent effects of mevastatin (A) or simvastatin (B) at 1 μ M on human cytomegalovirus replication. *Significantly different levels compared with those in the controls. Meva = mevastatin; Simva = simvastatin; d.p.i = days postinfection.

3.4. Viral protein

Because mevastatin and simvastatin showed different time-dependent effects, we selected these reagents for examination of variations in the production of early and late CMV proteins during statin exposure (0.1–10 μ M). Mevastatin inhibited the expression of the IE-1 protein at 1 day after viral inoculation, whereas simvastatin inhibited the expressions of the late protein at 3 and 6 days after infection (Figure 2).

3.5. Viral mRNA

We determined the ability of mevastatin to inhibit expressions of the IE-1/-2 mRNAs when incubated until 24 hours after infection, based on the protein expression data. At a concentration of 0.1 μ M, mevastatin significantly inhibited the expression of only IE-1 mRNA, whereas at 1 μ M, it significantly inhibited the expressions of both IE-1 and IE-2 mRNAs (Figure 3).

3.6. Gene expression profiles in cells by DNA microarray analysis

We performed a comprehensive analysis of gene expression profiles in the infected cells treated with mevastatin (1 μ M) until 24 hours after infection. Table 1 shows mRNAs that were up- or downregulated by at least twofold compared with the control. According to Ingenuity Pathway Analysis categories, we observed significant changes of cellular growth/differentiation-associated gene expressions in the infected cells treated with mevastatin.

3.7. Validation of data by DNA microarray analysis

Based on the results of gene expression profiles in the cells, we selected four up- or downregulated genes (i.e., *cdk2*, *arf1*, *xrcc2*, and

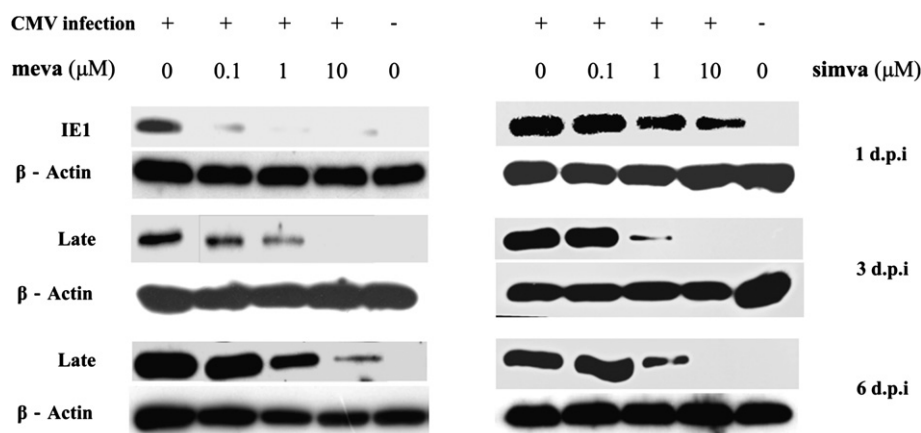


Figure 2 Expressions of viral proteins, including immediate-early-1 (IE-1) and late proteins, in human cytomegalovirus-infected cells treated with mevastatin (left) or simvastatin (right) at 0.1 μ M, 1 μ M, and 10 μ M. β -Actin protein was applied as an internal control. CMV = human cytomegalovirus; d.p.i = days postinfection; meva = mevastatin; simva = simvastatin.

psmb4) in the infected cells treated with mevastatin or simvastatin (0.1–10 μ M) until 24 hours after infection. The reasons why these four genes were selected for validation of DNA microarray data were significant levels of fold changes and estimated functions of genes. Mevastatin dose dependently induced elevations in expressions of PSMB4 and XRCC2 mRNAs and decreased expressions of CDK2, ARF1, and IE-1 mRNAs (Figure 4A and 4B). Similarly, simvastatin dose dependently increased or decreased expressions of three of the four genes except for ARF1 mRNA, together with the decreased expression of IE-1 mRNA (Figure 4C and 4D).

4. Discussion

Our observations suggest that treatment with mevastatin, simvastatin, or lovastatin at various EC_{50} concentrations of 0.0006 μ M, 0.0055 μ M, or 0.04 μ M could inhibit CMV production. Clinical trial confirmed achievable plasma concentration (0.10–3.92 μ M) of lovastatin administered at a dose of 25 mg/kg/d, which corresponds to the dose range that could trigger apoptosis of sensitive tumor types *in vitro*.¹⁶ Based on the findings of our experiment, the statin interventions should be further developed as novel or alternative strategies for treatment of CMV infection.

Statins are reported to play a crucial role in the management of sepsis in a clinical setting. Administration of statins in patients with atherosclerosis is shown to be associated with a decreased risk of subsequent sepsis, and randomized clinical trials of statins for

prevention of sepsis are warranted.¹⁷ In addition to the management of sepsis, there are several descriptions concerning the inhibitory effects of reagents on viral replication, including poliovirus,¹⁸ HIV-1,¹⁹ and hepatitis C virus.²⁰ Furthermore, combined treatment with a statin and caffeine effectively ameliorates lung damage, inhibits viral replication, and is at least as effective as the therapy with oseltamivir and ribavirin in H5N1-, H3N2-, and H1N1-infected mice.²¹ This combination seems to be more effective when administered preventatively rather than as a treatment. Therefore, it will also be important to clarify the antiviral actions of statins against influenza viruses in future investigations.

A potential mechanism of how the viral replication in CMV-infected cells could be inhibited by mevastatin seems to be cellular arrest, similar to statin effects on malignant cells in previous report.¹⁶ Comprehensive analysis of gene expression profiles in CMV-infected cells revealed that mevastatin induced significant changes of cellular growth/differentiation-associated gene expressions. In particular, this statin dose dependently induced both reduced expressions of CDK2/ARF1 mRNAs and increased expressions of PSMB4/XRCC2 mRNAs, leading to decreased expression of CMV IE-1 mRNA. CDK2 is a member of Ser/Thr protein kinase families, shows activity restricted to G1-S phase, and is essential for cell cycle G1/S phase transition. Transcriptional activation of the *cdc2* is associated with Fas-induced apoptosis of human hematopoietic cells.¹² Interestingly, mevastatin is said to inhibit cellular growth of a prostate cancer cell line through inhibition of CDK2.²² Moreover, inhibition of CDK2 activity by roscovitine inhibits CMV DNA synthesis, production of infectious progeny, and viral antigen expression in infected cells in a dose-dependent manner.²³ ARF1,

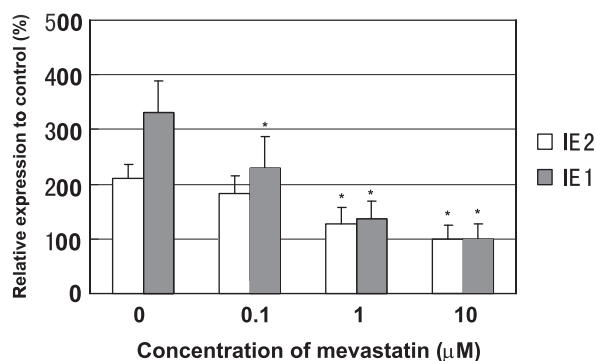


Figure 3 Expressions of viral mRNAs, including immediate-early-1 and -2 (IE-1 and IE-2) mRNAs, in human cytomegalovirus-infected cells treated with mevastatin (0.1 μ M, 1 μ M, or 10 μ M) until 24 hr after infection. *Significantly different levels compared with those in the controls. Relative expression levels of IE-1 and IE-2 mRNAs were calculated according to the expression levels of β -actin mRNA.

Table 1 Gene expression profiles in virally infected cells treated with mevastatin by DNA microarray analysis

Upregulated mRNA	Fold change	Downregulated mRNA	Fold change
<i>PSMB4</i>	4.76	<i>RPS2</i>	-7.46
<i>SLC25A37</i>	4.472	<i>CDK2</i>	-6.308
<i>XRCC2</i>	4.461	<i>C7ORF11</i>	-5.224
<i>ZNF652</i>	4.322	<i>HLA-B</i>	-5.066
<i>NCRNA00094</i>	3.972	<i>DUT</i> (includes EG:1854)	-5.002
<i>HELLS</i>	3.971	<i>ARF1</i>	-4.912
<i>UBE2B</i>	3.456	<i>GADD45GIP1</i>	-4.835
<i>RBBP6</i> (includes EG:5930)	3.427	<i>IFI16</i>	-4.705
<i>ATP6V0D2</i>	3.408	<i>ID1</i>	-4.697
<i>FARP1</i>	3.277	<i>HSPB1</i>	-4.664

To validate the data generated by DNA microarray analysis, the italicized mRNAs were selected as four target genes for real-time reverse-transcription polymerase chain reaction assays.

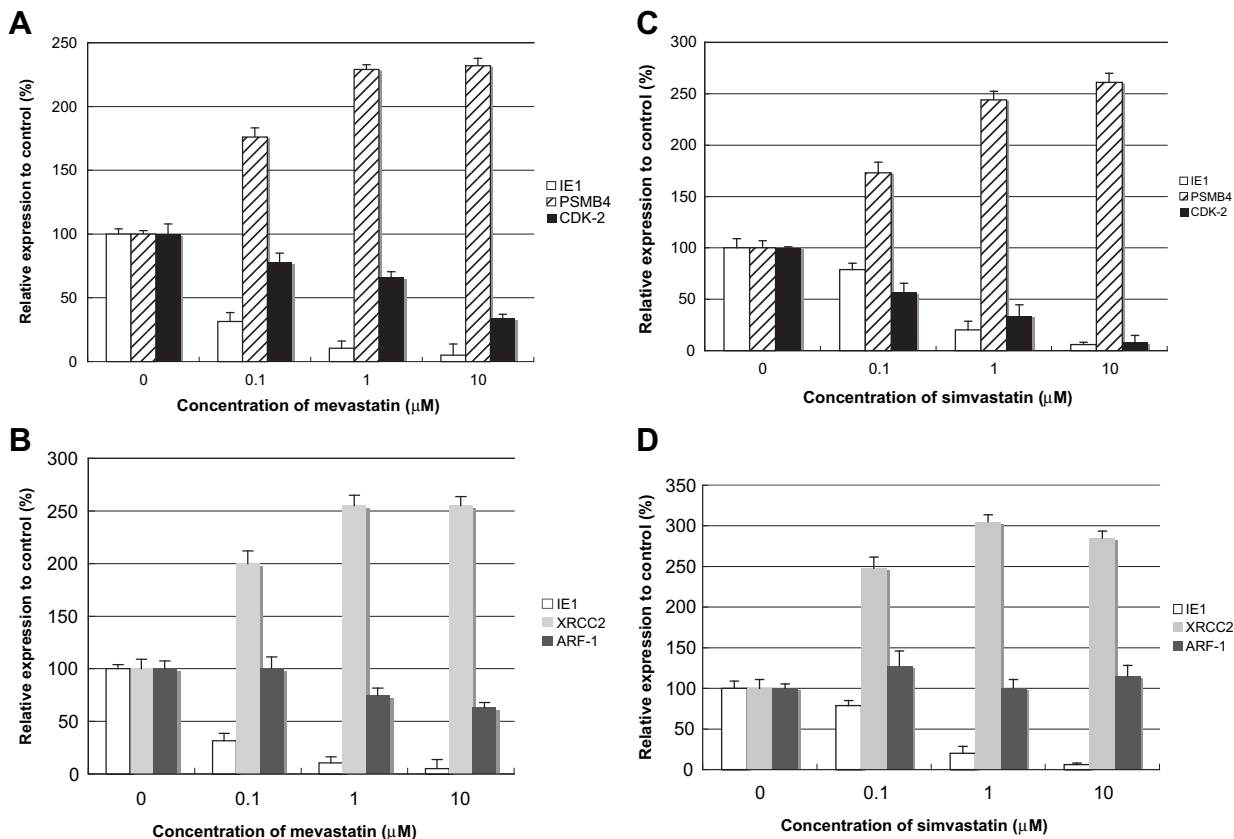


Figure 4 Dose-dependently altered expressions of four selected genes, that is, *cdk-2*, *arf1*, *xrcc2*, and *psmb4*, in human cytomegalovirus-infected cells treated with mevastatin (A and B) or simvastatin (C and D) at 0.1 μM, 1 μM, and 10 μM until 24 hr after infection. The expression levels of the four mRNAs are expressed as fold changes relative to the baseline expression levels in the untreated control group. Together with the validation of data by DNA microarray analysis, we also confirmed the changes of viral loads. IE-1 = immediate-early-1 mRNA.

a member of RAS superfamily, is localized to Golgi apparatus, and has a central role in intra-Golgi transport. This molecule regulates epidermal growth factor-dependent growth and migration of breast cancer cells through activation of phosphatidylinositol 3-kinase pathway.¹³ PSMB4, a member of proteasome B-type family, is distributed throughout the eukaryotic cells at a high concentration and cleaves peptides in an ATP/ubiquitin-dependent process in a nonlysosomal pathway. An expression survey of a panel of glioma cell lines demonstrated the expression of the PSMB4 and validity of the proteasome complex as a target for survival inhibition, which was confirmed in a series of glioma and nonglioma cell lines.²⁴ XRCC2, a member of RecA/Rad51-related protein family, participates in homologous recombination to maintain chromosome stability and to repair DNA damage. This molecule is important to preserve or restore replication forks during rapid clonal expansion of developing lymphocytes.²⁵ There are no investigations of associations between mevastatin and ARF1, PSMB4, or XRCC2. Therefore, we need to determine the detailed up- and downstream networks of CDK2, ARF1, PSMB4, XRCC2, and other molecules involved in cellular growth/differentiation in CMV-infected cells under intervention of mevastatin.

Effects of the four statins on blood cholesterol and lipoprotein levels are quantitatively similar when administered at equivalent doses. We found that mevastatin, simvastatin, and lovastatin, but not pravastatin, were able to efficiently inhibit CMV production in HEL cells. It is unclear why pravastatin could exhibit less inhibitory effects on viral replication. Pravastatin only has hydrophilic property and no β -hydroxy- δ -lactone moiety that the other three statins commonly include as chemical structures. In future basic

researches, it will be necessary to confirm significant differences in the chemical structures and biochemical activities among the statin reagents in detail. Moreover, we should examine the antiviral effects of mevastatin and simvastatin *in vivo*, using an animal model of murine CMV infection to provide experimental evidence for our hypotheses.

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References

1. Sissons JG, Carmichael AJ. Clinical aspects and management of cytomegalovirus infection. *J Infect* 2002;**44**:78–83.
2. Freitas VR, Fraser-Smith EB, Matthews TR. Increased efficacy of ganciclovir in combination with foscarnet against cytomegalovirus and herpes simplex virus type 2 in vitro and in vivo. *Antiviral Res* 1989;**12**:205–12.
3. Murayama T, Yamaguchi N, Matsuno H, Eizuru Y. *In vitro* anti-cytomegalovirus activity of Kampo (Japanese herbal) medicine. *Evid Based Complement Altern Med* 2004;**1**:285–9.
4. Suzuki J, Koga N, Kosuge H, Ogawa M, Haraguchi G, Maejima Y, Saiki H, et al. Pitavastatin suppresses acute and chronic rejection in murine cardiac allografts. *Transplantation* 2007;**83**:1093–7.
5. Nie C, Yang D, Liu G, Dong D, Ma Z, Fu H, Zhao Z, et al. Statins induce immunosuppressive effect on heterotopic limb allografts in rat through inhibiting T cell activation and proliferation. *Eur J Pharmacol* 2009;**602**:168–75.
6. Potena L, Frascaroli G, Grigioni F, Lazzarotto T, Magnani G, Tomasi L, Cocco F, et al. Hydroxymethyl-glutaryl coenzyme A reductase inhibition limits cytomegalovirus infection in human endothelial cells. *Circulation* 2004;**109**:532–6.

7. Furukawa T, Fioretti A, Plotkin S. Growth characteristics of cytomegalovirus in human fibroblasts with demonstration of protein synthesis early in viral replication. *J Virol* 1973;**11**:991–7.
8. Wentworth BB, French L. Plaque assay of cytomegalovirus strain of human origin. *Proc Soc Exp Biol Med* 1970;**135**:253–8.
9. Jacobs JP, Jones CM, Baille JP. Characteristics of a diploid cell designated MRC-5. *Nature* 1970;**227**:168–73.
10. Nishiwaki M, Fujimuro M, Teishikata Y, Inoue H, Sasajima H, Nakaso K, Nakashima K, et al. Epidemiology of Epstein-Barr virus, cytomegalovirus, and Kaposi's sarcoma-associated herpes virus infections in peripheral blood leukocytes revealed by a multiplex PCR assay. *J Med Virol* 2006;**78**:1635–42.
11. Yamakawa J, Ishigaki Y, Takano F, Takahashi T, Yoshida J, Moriya J, Takata T, et al. The Kampo medicines Orengedokuto, Bofutsushosan and Boiogito have different activities to regulate gene expressions in differentiated rat white adipocytes: comprehensive analysis of genetic profiles. *Biol Pharm Bull* 2008;**31**:2083–9.
12. Furukawa Y, Iwase S, Terui Y, Kikuchi J, Sakai T, Nakamura M, Kitagawa S, et al. Transcriptional activation of the *cdc2* gene is associated with Fas-induced apoptosis of human hematopoietic cells. *J Biol Chem* 1996;**271**:28469–77.
13. Boulay PL, Cotton M, Melançon P, Claing A. ADP-ribosylation factor 1 controls the activation of the phosphatidylinositol 3-kinase pathway to regulate epidermal growth factor-dependent growth and migration of breast cancer cells. *J Biol Chem* 2008;**283**:36425–34.
14. Mohindra A, Hays LE, Phillips EN, Preston BD, Helleday T, Meuth M. Defects in homologous recombination repair in mismatch-repair-deficient tumour cell lines. *Hum Mol Genet* 2002;**11**:2189–200.
15. Wang J, Shiels C, Sasieni P, Wu PJ, Islam SA, Freemont PS, Sheer D. Promyelocytic leukemia nuclear bodies associate with transcriptionally active genomic regions. *J Cell Biol* 2004;**164**:515–26.
16. Wong WW, Dimitroulakos J, Minden MD, Penn LZ. HMG-CoA reductase inhibitors and the malignant cell: the statin family of drugs as triggers of tumor-specific apoptosis. *Leukemia* 2002;**16**:508–19.
17. Hackam DG, Mamdani M, Li P, Redelmeier DA. Statins and sepsis in patients with cardiovascular disease: a population-based cohort analysis. *Lancet* 2006;**367**:413–8.
18. Liu S, Rodriguez AV, Tosteson MT. Role of simvastatin and methyl-beta-cyclodextrin on inhibition of poliovirus infection. *Biochem Biophys Res Commun* 2006;**347**:51–9.
19. del Real G, Jiménez-Baranda S, Mira E, Lacalle RA, Lucas P, Gómez-Moutón C, Alegret M, et al. Statins inhibit HIV-1 infection by down-regulating Rho activity. *J Exp Med* 2004;**200**:541–7.
20. Bader T, Fazili J, Madhoun M, Aston C, Hughes D, Rizvi S, Seres K, et al. Fluvastatin inhibits hepatitis C replication in humans. *Am J Gastroenterol* 2008;**103**:1383–9.
21. Liu Z, Guo Z, Wang G, Zhang D, He H, Li G, Liu Y, et al. Evaluation of the efficacy and safety of a statin/cafeine combination against H5N1, H3N2 and H1N1 virus infection in BALB/c mice. *Eur J Pharm Sci* 2009;**38**:215–23.
22. Ukomadu C, Dutta A. Inhibition of cdk2 activating phosphorylation by mevastatin. *J Biol Chem* 2003;**278**:4840–6.
23. Bresnahan WA, Boldogh I, Chi P, Thompson EA, Albrecht T. Inhibition of cellular Cdk2 activity blocks human cytomegalovirus replication. *Virology* 1997;**231**:239–47.
24. Thaker NG, Zhang F, McDonald PR, Shun TY, Lewen MD, Pollack IF, Lazo JS. Identification of survival genes in human glioblastoma cells by small interfering RNA screening. *Mol Pharmacol* 2009;**76**:1246–55.
25. Caddle LB, Hasham MG, Schott WH, Shirley BJ, Mills KD. Homologous recombination is necessary for normal lymphocyte development. *Mol Cell Biol* 2008;**28**:2295–303.