SIMVASTATIN REDUCES INSULIN-LIKE GROWTH FACTOR-1 SIGNALING IN DIFFERENTIATING C2C12 MOUSE MYOBLAST CELLS IN AN HMG-CoA REDUCTASE INHIBITION-INDEPENDENT MANNER

Takeharu OGURA, Yoshiyuki TANAKA, Tetsushi NAKATA, Tomoko NAMIKAWA, Hirofumi KATAOKA and Yoshikazu OHTSUBO

Biological Research Department, Sawai Pharmaceutical Co., Ltd., 5-2-30 Miyahara, Yodogawa-Ku, Osaka 532-0003, Japan

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ABSTRACT — Inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase occasionally cause myopathy characterized by weakness, pain, and elevated serum creatine phosphokinase (CK).

In this study, we investigated the effects of simvastatin, an HMG-CoA reductase inhibitor, on the viability and insulin-like growth factor-1 (IGF-1) signaling in differentiating C2C12 mouse myoblast cells. Simvastatin decreased cell viability and CK activity, a marker of myogenesis, in differentiating cells in a dose-dependent manner. Although the simvastatin-induced decrease in viability in proliferating and differentiating cells was completely abolished by mevalonate or geranylgeranyl-pyrophosphate, the inhibitory effects of simvastatin in differentiating cells were not abolished by mevalonate or isoprenoid derivatives of mevalonate. Moreover, the sensitivity of differentiating cells to simvastatin regarding cell viability was about 7 times higher than that of proliferating cells. After induction of differentiation in the presence of 1 μM simvastatin for 2 days, IGF-1-induced activation of ERK1/2 and Akt was significantly decreased. Although mRNA expression of the IGF-1 receptor β-chain (IGF-1Rβ) did not change, protein level of the 200 kDa IGF-1Rβ precursor was significantly increased by simvastatin in a dose-dependent manner. Mevalonate did not abolish the effect of simvastatin on IGF-1Rβ expression. These results suggest that simvastatin decreases IGF-1 signaling via a regulation of the post-translational modification of IGF-1Rβ in an HMG-CoA reductase inhibition-independent manner.

KEY WORDS: Simvastatin, Myoblasts, IGF-1 signaling, Differentiation

INTRODUCTION

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is a key enzyme in the synthesis of cholesterol and its inhibitor (statin) is widely used to treat hypercholesterolemia. Skeletal muscle myopathy, which is characterized by pain, weakness, elevated serum creatine kinase and rhabdomyolysis, is a potential adverse clinical side-effect of treatment with statins (Corsini et al., 2005; Hansen et al., 2005; Shek and Ferrill, 2001; Silva et al., 2006; Thompson et al., 2003). Statins-induced myopathy is particularly aggravated when these are combined with a fibrate (Corsini et al., 2005; Shek and Ferrill, 2001; Thompson et al., 2003), which decreases serum triglycerides by reducing hepatic production and increasing triglycerides clearance by peripheral tissues (Staels et al., 1998). In previous in vitro studies, statins reduced cell proliferation and induced apoptosis in a variety of cell lines, including skeletal muscle cells, and statin-induced cell death was abolished by mevalonate, an HMG-CoA reductase product (Denoyelle et al., 2001; Guijarro et al., 1998; Ikeuchi et al., 2004; Lee et al., 2006; Mutoh et al., 1999; Rombouts et al., 2003; Seeger et al., 2003; Yamakawa et al., 2003). Statins-induced cell death is also prevented by the isoprenoid derivatives geranylgeranyl-pyrophosphate (GGPP) and farnesyl-pyrophosphate (FPP). It was demonstrated that FPP and GGPP are essential compounds for survival and proliferation of cells through the activation of small
GTPases, such as Ras and Rho (Allal et al., 2000; Prendergast and Oliff, 2000; van de Donk et al., 2005). Numerous functions of small GTPases have been identified, including regulation of gene expression, cell growth, apoptosis, cell polarity, and membrane trafficking (Mackay and Hall, 1998; Ridley, 2001a, 2001b). Statins inhibit the geranylgeranylation of low-molecular weight proteins and reduce small GTPases activity (Cordle et al., 2005; Flint et al., 1997). These studies suggested that the depletion of GGPP is the root cause of cell death induced by statins. In rat skeletal myoblasts, however, statins induce cell death and inhibit differentiation at low doses, which do not affect the synthesis of cholesterol, and the statin-induced effects are not abolished by mevalonate (Veerkamp et al., 1996). The mechanism of the effect of statins at low doses is still unclear.

Insulin-like growth factor-1 (IGF-1) has been implicated in many anabolic pathways in skeletal muscle and plays a central role during muscle regeneration. IGF-1 stimulates myogenic differentiation and generates a pronounced hypertrophy of the muscle cells in vivo and in vitro (Bark et al., 1998; Barton et al., 2002; Machida and Booth, 2004), suggesting that IGF-1 can regulate both proliferative and differentiative responses in muscle cells. Previous reports suggest that the effects of IGF-1 on proliferation are mediated by the mitogen-activated protein kinase (MAPK) pathway, whereas the pathway leading to differentiation involves the phosphoinositide 3-kinase (PI3K) (Rommel et al., 1999).

Although it is well known that statins inhibit the proliferation and differentiation of myoblast cells, their effects on IGF-1signaling are little known. We investigated the effects of simvastatin on cell viability at different stages of differentiation and on IGF-1 signaling during differentiation in mouse myoblasts.

MATERIALS AND METHODS

Materials

Simvastatin was purchased from Lek (Vienna, Austria), GGPP and FPP were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mevalonolactone was purchased from Wako Pure Chemicals (Osaka, Japan) and was converted to mevalonate by incubation with 1N NaOH at 37°C for 1 hr in our laboratory. Recombinant mouse IGF-1 was purchased from R&D Systems (Minneapolis, MN, USA). LY294002, U0126 and SB-203580 were purchased from Biomol (Plymouth Meeting, PA, USA).

Cell culture and treatment

C2C12 mouse myoblasts, which differentiate into myotube upon the depletion of growth factors from the culture medium (Bennett and Tonks, 1997; Yaffe and Saxel, 1977), were purchased from Dainippon Sumitomo Pharma (Osaka, Japan) and cultured in growth medium (GM) composed of Dulbecco’s modified Eagle medium (DMEM, Sigma-Aldrich) supplemented with 50 IU/ml penicillin, 50 mg/ml streptomycin (Gibco-BRL, Bethesda, MD, USA) and 10% fetal calf serum (FCS, Biowhittaker, Walkersville, MD, USA). To induce differentiation, cells were plated at 10⁴ cells/cm² and cultured in GM for 24 hr. The GM was replaced by differentiation medium (DM), which contained DMEM, 0.5% FCS and antibiotics. For preparation of differentiated myoblasts, cells were cultured in DM for 4 days and differentiation checked by formation of myotubes and multi-nucleated cells by microscopic observation. All cells were maintained in a humidified 95% air 5% CO₂ atmosphere at 37°C.

MTS assay for cell viability

We used the [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS)] (CellTiter 96 AQueous One Solution, Promega, Madison, WI, USA) to investigate the viability of C2C12 cells. Cells were seeded into 96-well culture plates and incubated as indicated for each experiment. The MTS reagent was added to each well and the colorimetric reaction measured 2 hr later using Wallac 1420 ARVO Multi-label counter (Perkin-Elmer Life Sciences, Wellesley, MA, USA) at 490 nm. Results were expressed as a percentage of controls.

Creatine phosphokinase assay

Myogenic differentiation was assessed biochemically through determination of muscle creatine phosphokinase (CK) activity. Cells cultured in 24-well culture plates were washed with ice-cold phosphate-buff ered saline (PBS) and lysed in PBS containing 0.5% Triton X-100. The lysates were centrifuged at 10,000 rpm at 4°C for 10 min to remove insoluble material. The supernatant was collected and CK activity determined with CK test WAKO (Wako Pure Chemicals) as described in the manufacturer’s protocol. CK activity was calculated after correction for total protein concentration measured with Bradford protein assay (Bio-Rad protein assay, Hercules, CA, USA).

Enzyme-linked immunosorbent assay (ELISA)

Cells were washed with ice-cold PBS and then
scraped into cold lysis buffer (20 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF; 20 mM Na_2P_2O_7, 2 mM Na_3VO_4, 1% Triton-X100, 0.1% SDS, 0.5% deoxycholate, 1 mM phenylmethylsulfonyl fluoride, and 2.5 μl/ml protease inhibitor cocktail; all purchased from Sigma-Aldrich). The lysates were then separated by centrifugation at 13,000 rpm at 4°C for 10 min. Total and phosphorylated forms of extracellular signal-regulated kinase (ERK) 1/2 and Akt (protein kinase B) in the soluble extracts were determined by ELISA kits purchased from BioSource International, Inc. (Camarillo, CA, USA) and soluble proteins were quantified by Bradford protein assay (Bio-Rad Laboratories).

**Western blotting analysis**

Cells were washed with ice-cold PBS and then scraped into Western lysis buffer (1% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 0.1 mg/mL phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate in PBS). After 60 min on ice with occasional vortexing, the lysates were separated by centrifugation at 10,000 rpm at 4°C for 10 min. Soluble proteins were quantified by Bradford protein assay. Cell extracts were diluted in Laemmli sample buffer followed by boiling for 3 min. Ten μg of protein was loaded per lane and separated on 4-15% gradient polyacrylamide gels (Bio-Rad Laboratories), followed by transfer to a nitrocellulose membrane by semidry electroblotting. The blots were blocked overnight with 5% nonfat milk and incubated with polyclonal antibody against IGF-1 receptor β-chain (IGF-1R β) purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Unbound antibody was removed by washing with PBS containing 0.05% Tween-20 and the blots incubated with anti-rabbit immunoglobulin conjugated horseradish peroxidase (Santa Cruz Biotechnology). The blots were developed with ECL plus (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and quantitated using a cooled CCD camera system (FluorChem, Alpha Inotech, San Leandro, CA, USA).

**Reverse transcription-polymerase chain reaction (RT-PCR) assay**

Total RNA was isolated from C2C12 cells using ISOGEN (Nippon Gene, Tokyo, Japan) according to the manufacturer’s instructions. One microgram of total RNA was used for the RT-PCR reaction. cDNA was produced at 42°C for 30 min, and the following PCR reaction during 25 cycles, unless stated otherwise, of 30 sec at 94°C, 30 sec at 60°C, and 1 min at 72°C. PCR products were separated by electrophoresis using a 2.5% agarose gel and visualized by ethidium bromide. The ethidium bromide-stained images were recorded by a cooled CCD camera system. Primers for PCR amplification were as follows: IGF-1Rβ: 5’-GTA ACA ATCTATTCA A AGGCCTC C-3’ / 5’-GAAGGCAGAGGAGAGAAAAGG-3’, glyceraldehyde-3-phosphate dehydrogenase (GAPDH): 5’-AATGCATCCTGCACCACCA-3’ / 5’-GTAGCCCATATCATTGTCAT-3’.

**Statistical analysis**

Statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test (Dunnett, 1955). IC₅₀ values were estimated by probit analysis.

**RESULTS**

**Effects of simvastatin on the viability of proliferating, differentiating and differentiated mouse myoblasts**

We first examined the sensitivity toward simvastatin by assessing the viability of proliferating, differentiating and differentiated C2C12 cells using the MTS assay. In every case, simvastatin decreased cell viability in a dose-dependent manner (Fig. 1). Interestingly, the sensitivity of differentiating cells to simvastatin was significantly higher than that of proliferating and differentiated cells. The average IC₅₀ values in proliferating, differentiating and differentiated cells were 10.4 ± 1.2 μM, 1.42 ± 0.35 μM, and 35.3 ± 10.2 μM, respectively.

**Effects of isoprenoid derivatives of mevalonate on simvastatin-induced decrease of cell viability**

To determine if simvastatin depended on the inhibition of HMG-CoA reductase, we examined the effects of mevalonate and isoprenoid derivatives of mevalonate. C2C12 cells were treated with simvastatin in the presence of mevalonate, FPP or GGPP for 48 hr, and then cell viability was determined by MTS assay. The viability of proliferating and differentiated C2C12 cells, treated with a high concentration (10 μM) of simvastatin, was 56.3 ± 4.4% and 76.0 ± 7.1% of the control, respectively. In both cases, mevalonate (50 μM) or GGPP (10 μM) completely abolished the simvastatin-induced decrease in cell viability (Fig. 2A, 2C). In differentiating C2C12 cells, a low concentration (1 μM) of simvastatin also decreased cell viability to 66.8 ±
Fig. 1. Effects of simvastatin on cell viability. C2C12 cells were seeded in 96-well culture plates at the density of $2 \times 10^4$ cells/cm$^2$ and then incubated with various concentrations of simvastatin for 48 hr (proliferating condition). C2C12 cells were seeded in 96-well culture plates at $10^4$ cells/cm$^2$ and incubated in GM for 24 hr. Culture medium was changed to DM containing simvastatin followed by 48 hr incubation (differentiating condition). C2C12 cells were seeded in 96-well culture plates at $10^4$ cells/cm$^2$ and incubated in GM for 24 hr. Cells were treated with simvastatin for 48 hr after induction of differentiation by culturing them in DM for 4 days (differentiated condition). The viability of C2C12 cells was measured by MTS assay after treatment with simvastatin. Results are expressed as the mean ± SD ($n=6$).

Fig. 2. Effects of isoprenoid derivatives of mevalonate on simvastatin-induced decrease of cell viability. Proliferating (A), differentiating (B) and differentiated (C) C2C12 cells were treated with simvastatin for 48 hr at 10 μM, 1 μM and 10 μM, respectively, together with 50 μM mevalonate (Mev), 10 μM FPP or 10 μM GGPP. The viability of C2C12 cells was determined by MTS assay after treatment with simvastatin. Results are expressed as the mean ± SD ($n=6$). Statistical analysis was performed using Dunnett’s test. *p<0.05 versus no addition.
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7.6% of the control, but mevalonate and its isoprenoid derivatives had no effect on the decrease in cell viability in differentiating C2C12 cells (Fig. 2B). These data show that at a high concentration the effects on the viability of proliferating and differentiated C2C12 cells depend on the inhibition of HMG-CoA reductase resulting in GGPP depletion, but that another mechanism would play a role in differentiating C2C12 cells treated with a low concentration of simvastatin.

**Effects of simvastatin on myoblasts differentiation**

We assessed the effects of simvastatin on the myogenic differentiation of C2C12 cells by measuring CK activity. As shown in Fig. 3A, simvastatin decreased CK activity while inducing C2C12 cells differentiation in a dose-dependent manner. Additionally, mevalonate and its isoprenoid derivatives did not abolish simvastatin-induced inhibition in differentiating cells (Fig. 3B), the same as in the study on cell viability.

**Involvement of the MAPK/ERK and PI3K/Akt pathways in the viability of differentiating myoblasts**

To determine if MAPK/ERK and PI3K/Akt signaling are required for the survival of differentiating C2C12 cells, these cells were cultured in the presence of U0126 (MEK1/2 inhibitor), SB-203580 (p38 MAPK inhibitor) or LY294002 (PI3K inhibitor). As shown in Fig. 4, U0126, LY294002 and SB-203580 decreased the viability of differentiating C2C12 cells in a dose-dependent manner (IC₅₀=0.53 μM, 1.25 μM, 2.39 μM, respectively). Similar data were obtained when the cells were treated with AG490, a janus kinase (JAK) 2 inhibitor (IC₅₀=9.01 μM, data not shown). These results indicate that the MAPK and PI3K pathways are involved in the survival of differentiating C2C12 cells.

IGF-1 is known to be critical for myogenic progression via MAPK and PI3K pathways. Therefore, we determined whether simvastatin affected IGF-1-

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**Fig. 3. Effects of simvastatin on C2C12 cells differentiation.**

C2C12 cells were seeded in 24-well culture plates at 10⁴ cells/cm² and preincubated in GM for 24 hr. Culture medium was changed to DM and exposed to 0.1 - 1 μM simvastatin (A) or 1 μM simvastatin together with 50 μM Mev, 10 μM FPP or 10 μM GGPP (B) for 48 hr. CK activity and protein concentration in the cell lysate were then measured. Results are expressed as the mean ± SD (n=4). Statistical analysis was performed using Dunnett’s test; a p value of <0.05 versus control (A) or no addition (B) was considered significant.
induced ERK1/2 and Akt activation. C2C12 cells were treated with simvastatin in DM for 2 days and then stimulated with 50 ng/ml IGF-1. Basal levels of phosphorylated ERK and Akt were slightly but not significantly decreased by treatment of simvastatin at 1 μM (data not shown). IGF-1-induced phosphorylation of ERK1/2 and Akt was significantly decreased by simvastatin at 1 μM (Fig. 5A, 5B). Moreover, the simvastatin-induced decrease in IGF-1 signaling was not abolished by mevalonate, FPP or GGPP (Fig. 5C, 5D).

**Effects of simvastatin on IGF-1 receptor expression**

To determine if the effects of simvastatin on IGF-1-induced ERK1/2 and Akt activation were due to a reduced expression of IGF-1 receptors, we measured the expression of IGF-1Rβ after treatment with simvastatin in differentiating C2C12 cells by Western blotting and RT-PCR. IGF-1Rβ mRNA expression did not change under any condition (Fig. 6A). However, the precursor to IGF-1Rβ protein (200 kDa) and its cleaved form (95 kDa) were increased by treatment with simvastatin in a dose-dependent manner (Fig. 6B). Interestingly, although the precursor to IGF-1Rβ drastically increased to 350% of the control after treatment with 1 μM simvastatin, the increase of the cleaved form was slight (180%) by comparison. The increased expression of IGF-1Rβ proteins did not change after the addition of mevalonate.

**DISCUSSION**

The present study demonstrates that simvastatin decreased the viability of C2C12 mouse myoblasts cultured under proliferating conditions. The inhibitory effect of simvastatin in proliferating cells was abolished by either mevalonate or GGPP. Similar results were reported in studies using fibroblasts, vascular smooth muscle cells, breast cancer cells and myoblasts (Denoyelle et al., 2001; Guijarro et al., 1998; Ikeuchi et al., 2004; Lee et al., 2006; Mutoh et al., 1999; Rombouts et al., 2003; Seeger et al., 2003; Yamakawa et al., 2003). Mevalonate is not only a precursor of cholesterol but also delivers isoprenoid groups for the synthesis of FPP and GGPP. These findings indicate that the effect of simvastatin on cell viability is caused by a depletion of isoprenoid derivatives through HMG-CoA reductase inhibition. As for differentiating C2C12 cells, which were cultured under serum-starving condi-

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**Fig. 4.** Effects of inhibitors of p38 MAPK, MEK and PI3K on differentiating C2C12 cells viability. C2C12 cells were seeded in 96-well culture plates at 10⁴ cells/cm² and preincubated in GM for 24 hr. Culture medium was changed to DM and cells were exposed to 0.1 - 30 μM SB-203580, LY 294002 and U0126 for 48 hr. Then C2C12 cells viability was determined by MTS assay. Results are expressed as the mean ± SD (n=6).
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Comprehensive investigations, viability and myogenic differentiation were significantly decreased by simvastatin in a dose-dependent manner. However, the sensitivity of differentiating cells to simvastatin (IC$_{50}$=1.42 μM) was significantly higher than that of proliferating cells (IC$_{50}$=10.4 μM). Moreover, the effects of simvastatin at a low concentration were not abolished by mevalonate or isoprenoid derivatives of mevalonate. When C2C12 cells were treated with a high concentration (IC$_{50}$=35.3 μM) of simvastatin after induction of differentiation, viability decreased and the decrease was completely abolished by mevalonate or GGPP. These results suggest that other mechanisms, besides inhibition of HMG-CoA reductase, play a role in the repressive effects during differentiation of myoblasts. Elevation of serum CK activity in statin-associated myopathy is responsible for acute muscle fiber necrosis with leakage of muscle constituents into blood (Carvalho et al., 2004). Our

![Fig. 5. Effects of simvastatin on ERK1/2 and Akt activation induced by IGF-1 in differentiating C2C12 cells.](image-url)

C2C12 cells were seeded in 6-well culture plates at 10$^5$ cells/cm$^2$ and preincubated in GM for 24 hr. Culture medium was changed to DM and cells were treated with various concentrations of simvastatin (A, B) or 1 μM simvastatin together with 50 μM Mev, 10 μM FPP or 10 μM GGPP (C, D) for 48 hr. Then C2C12 cells were incubated with 50 nM IGF-1 for 10 min and lysed. The supernatant was analyzed by an ELISA specific for phospho-ERK1/2 or phospho-Akt. Results are expressed as the mean ± SD (n=4). Statistical analysis was performed using Dunnett’s test; a p value of <0.05 versus control (A, B) or no addition (C, D) was considered significant.
results in CK activity in differentiating myoblasts conflict with the elevation of serum CK level in myopathy. Concomitant cell death induced by simvastatin may lead to elevation of serum CK level as a consequence of release of muscle components.

Myoblasts differentiation is a fundamental and multistep process that occurs during muscle development and repair from muscle injury (Charge and Rudnicki, 2004; Chen and Goldhamer, 2003; Schultz and McCormick, 1994; Seale et al., 2001). During myoblasts differentiation, myoblasts fuse together to form myotube (Berendsse et al., 2003) and contractile gene expression, such as β-actin, is up-regulated (Gunning et al., 1987; Spangenburg et al., 2004). Survival, growth and differentiation of myoblasts are systemically regulated by distinct growth factor-regulated signaling pathways. IGF-1 stimulates proliferation and differentiation of myoblasts through the MEK/ERK and PI3K/Akt pathways (Galvin et al., 2003). Bennet and Tonks (1997) demonstrated that down-regulation of MAPK phosphatase-1, which inactivates ERK2 by dephosphorylation, was functionally required for the

![Fig. 6. Effects of simvastatin on IGF-1 receptor expression in differentiating C2C12 cells.](image)

C2C12 cells were seeded in 6-well culture plates at 10⁶ cells/cm² and preincubated in GM for 24 hr. Culture medium was changed to DM and exposed to simvastatin with or without 50 μM Mev for 48 hr. Expression of IGF-1 receptor β-chain was measured by RT-PCR (A) and Western blotting (B). Results from Western blotting are expressed as the mean ± SD (n=4) and statistical analysis was performed using Dunnett’s test; a p value of <0.05 versus control was considered significant.
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process of myotube formation. Basic fibroblast growth factor (bFGF) suppresses IGF-1-induced differentiation and induces myoblasts proliferation through the activation of MAPK/ERK (Tortorella et al., 2001). Sarker and Lee (2004) showed that p38 MAPK and Akt activity were upregulated but ERK1/2 was downregulated in differentiating myoblasts cultured under serum starvation. However MPDK/ERK signaling is required during the G1 phase of the cell cycle for commitment of myoblasts to DNA synthesis (Jones et al., 2001), and protects myoblasts from apoptosis caused by prolonged serum starvation (Ajenjo et al., 2004). Here we showed that both MAPK/ERK (blocked by U0126) and PI3K/Akt (blocked by LY294002) were required for survival and 1 μM simvastatin and significantly suppressed IGF-1-induced activation of ERK and Akt in differentiating C2C12 cells. Zong et al. (2000) showed that the JAK/signal transducer and activator of transcription (STAT) signaling pathway activated by IGF-1. JAK2 inhibition by AG490 significantly reduced the viability of differentiating C2C12 cells (data not shown), suggesting that IGF-1-induced activation of the JAK/STAT pathway plays an important role in myoblast differentiation. In view of the potent and anabolic effects of IGF-1 via the MAPK/ERK and PI3K/Akt pathways, our results suggest that even at a low-dose simvastatin downregulates IGF-1 signaling, resulting in a disorder or loss of skeletal muscle.

In differentiating C2C12 cells treated with 1 μM simvastatin, RT-PCR analysis showed no change of IGF-1Rβ mRNA expression, but a significant increase of IGF-1Rβ protein was measured by Western blotting analysis. Although the precursor to IGF-1Rβ drastically increased, the 95 kDa cleavage product was only slightly but still significantly increased. Additionally, treatment with mevalonate did not result in an increase of IGF-1R protein. Carlberg et al. (1996) showed that mevalonate depletion caused by lovastatin decreased N-linked glycosylation of IGF-1R and decreased IGF-1-stimulated cell growth and IGF-1 binding to IGF-1R in melanoma cells. The effects of lovastatin on IGF-1 function, however, were abolished by mevalonate. These findings show that statins reduce IGF-1R functions in an HMG-CoA reductase inhibition-dependent and -independent manner at post-translational level. In summary, the present study demonstrated that simvastatin decreases cell viability in differentiating myoblasts in an HMG-CoA reductase inhibition-independent manner and suppressed IGF-1-induced activation of ERK and Akt activation without affecting the IGF-1R mRNA levels.

REFERENCES


potential of muscle stem cells. Dev. Cell, 1, 333-342.