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Suppression of lipoprotein lipase expression in 3T3-L1 cells by inhibition of adipogenic differentiation through activation of the renin-angiotensin system

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Abstract

The renin-angiotensin system (RAS) may inhibit adipogenic differentiation by down-regulating peroxisome proliferator–activated receptor γ gene expression in adipocytes, and adipocytes express all components of the RAS, including angiotensinogen. Expression of lipoprotein lipase (LPL), which is expressed mainly in adipocytes, is considered to be affected by adipogenic differentiation. We studied whether LPL expression in mouse 3T3-L1 cells is suppressed by inhibition of adipogenic differentiation through activation of RAS by the cells. The mean 3T3-L1 cell size increased and peroxisome proliferator–activated receptor γ messenger RNA (mRNA) expression in the cells measured by reverse transcriptase polymerase chain reaction (RT-PCR) was enhanced with increase in incubation time. The LPL activity, LPL protein expression (Western blot), and mRNA expression (RT-PCR) in 3T3-L1 cells increased transiently followed by a decline during long-term incubation. Angiotensin II suppressed adipogenic differentiation, LPL activity, protein expression, and mRNA expression in 3T3-L1 cells. On the other hand, the selective angiotensin type 1 receptor blocker valsartan enhanced adipogenic differentiation and LPL activity in 3T3-L1 cells. Angiotensinogen mRNA expression in 3T3-L1 cells measured by RT-PCR was enhanced with increase in incubation time. These results suggest that LPL expression may be suppressed by inhibition of adipogenic differentiation through activation of endogenous RAS in 3T3-L1 cells angiotensin type 1 receptor.

1. Introduction

Lipoprotein lipase (LPL) that catalyzes the hydrolysis of triglycerides in the blood is produced mainly in adipocytes and skeletal muscle cells [1,2], and is believed to be transported to the surface of endothelial cells [3,4]. Recently, analysis of LPL in clinical studies was conducted by measuring LPL mass in preheparin serum [5-10]. Shirai et al [5] reported that preheparin serum LPL mass may reflect total LPL production in the whole body. Several studies reported that preheparin serum LPL mass reflects insulin resistance [5-7]. Kobayashi et al [8] reported that LPL mass in preheparin serum correlates inversely with body mass index and intraabdominal visceral fat area evaluated by computed tomography. We previously reported that the mean preheparin serum LPL mass tends to decrease with increase in number of symptoms of metabolic syndrome [9]. These reports indicate that LPL produced mainly in adipocytes may affect insulin resistance and lipid metabolism.

Adipocytes are known to release cytokines, chemokines, and many other biologically active molecules including LPL, commonly called *adipokines*. Obesity is characterized by an increased fat mass mainly due to enlarged adipocytes [11,12]. Previous studies have demonstrated that enlarged adipocytes are associated with substantial changes in adipokines [13,14]. Enlarged adipocytes are the result of the process of adipogenic differentiation mainly through activation of peroxisome proliferator–activated receptor (PPAR) γ [15,16]. However, whether LPL expression and

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production vary according to the level of adipogenic differentiation remains controversial.

Recent clinical trials [17-19] suggest that blockade of the renin-angiotensin system (RAS) may lower the risk for the development of type 2 diabetes mellitus. In the Valsartan Antihypertensive Long-term Use Evaluation trial [20], new onset of diabetes was 23% lower when treated with the selective angiotensin type 1 (AT_1) [19] receptor blocker valsartan compared with the calcium antagonist amlodipine. On the other hand, the Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication study showed that, among persons with impaired fasting glucose levels or impaired glucose tolerance, the use of ramipril for 3 years significantly increases regression to normoglycemia, whereas it does not significantly reduce the incidence of diabetes or death [21]. Although the association between RAS and glucose metabolism is slightly unclear, these reports indicate that blockade of the RAS has favorable effects on glucose metabolism.

We demonstrated that administration of valsartan increased LPL mass in preheparin serum, accompanied by enlarged low-density lipoprotein particle size as well as decreased triglyceride and hemoglobin A_{1c} in diabetic patients with hypertension [10]. Furthermore, the beneficial effects of valsartan on blood pressure and preheparin serum LPL mass were more marked in obese subjects and subjects with low LPL mass. These findings suggest that blockade of the RAS may increase LPL production relating to adipocytes. Adipocytes also express AT_1 and angiotensin type 2 receptor subtypes [22]. Janke et al [23] reported that inhibition of adipogenic differentiation by angiotensin II (Ang II) decreases, whereas stimulation of adipogenic differentiation by AT_1 receptor blockade increases, the gene expression of PPARγ and fatty acid synthase in human preadipocytes. Mature adipocytes express all components of the RAS, including angiotensinogen (the sole precursor of Ang II) as well as the angiotensin peptide–forming enzymes renin, angiotensin-converting enzyme, and chymase [23]. Therefore, we hypothesize that LPL expression and production in adipocytes are suppressed by inhibition of adipogenic differentiation through activation of endogenous RAS by the adipocytes.

In this study, we first confirmed adipogenic differentiation (indicated by cell size and PPARγ expression) of 3T3-L1 cells, and LPL activity and expression in the cells during long-term incubation. Next, we studied the effect of Ang II and/or AT_1 receptor blocker on adipogenic differentiation as well as LPL activity and expression in 3T3-L1 cells. Last, we studied angiotensinogen expression in the cells during long-term incubation.

2. Materials and methods

2.1. 3T3-L1 cells culture

Mouse 3T3-L1 cells were cultured in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum incubated at 37°C under 5% CO_2. The cells (2.5×10^4 per well) were dispensed in a 12-well plate and incubated for 48 hours. Afterward (day 0), the cells were incubated in the above-mentioned medium supplemented with dexamethasone (0.25 μmol/L), 3-isobutyl-1-methyxanthine (0.5 mmol/L), and insulin (10 μg/mL) for another 48 hours. Subsequently, the medium was replaced with Dulbecco modified Eagle medium containing 10% fetal calf serum with or without Ang II (endowed by Sigma Chemical, St Louis, MO) at a final concentration of 0.1 or 1 μmol/L, and with or without valsartan (endowed by Novartis Pharma KK, Tokyo, Japan) dissolved in dimethyl sulphoxide at a final concentration of 0.01, 0.1, and 1 μmol/L for 15 days. Valsartan was used to confirm whether AT_1 receptor blocker might affect LPL activity in 3T3-L1 cells via adipogenic differentiation. The medium was replaced once after 48 hours. Furthermore, pioglitazone (endowed by Takeda Pharmaceutical, Osaka, Japan) was used to enhance differentiation of the 3T3-L1 cells as PPARγ agonist. Pioglitazone, which dissolved in dimethyl sulphoxide at a final concentration of 10 μmol/L, was supplemented in the above-mentioned medium for the same period with or without Ang II. At days 0, 5, 10, and 15, the cells were harvested for subsequent experiments.

2.2. Measurements of 3T3-L1 cell size and oil red O staining

The 3T3-L1 cell size was measured as an indicator of adipogenic differentiation. The mean 3T3-L1 cell size was determined using particle analyzer Sysmex CDA-500 (Sysmex, Kobe, Japan) after trypsinization.

To determine the accumulated triglycerides in 3T3-L1 cells, oil red O staining was performed. Phase contrast photomicrographs (20× magnification) were recorded on digital camera.

2.3. Measurements of LPL activity

For LPL activity assay, 3T3-L1 cells were harvested, dissolved in 0.1 mol/L Tris buffer (pH 7.4), and sonicated on ice for 30 seconds using an Ultrasonic Generator model US-50 (NISSEI, Tokyo, Japan); and the homogenates were centrifuged at 2000*g* for 10 minutes (Himac CF 15D2; Hitachi, Tokyo, Japan). The supernatant was used for enzyme assay. The LPL activity was measured using triolein (Sigma) as substrate. The substrate solution was prepared as follows: 100 mg of triolein in a final volume of 7.5 mL of 1 mol/L Tris-HCl (pH 8.0) containing 0.2% Triton X-100 was sonicated on ice for 10 minutes. For measurement of LPL activity, the reaction mixture contained 50 μL of substrate solution, 25 μL of 20% fatty acid–free bovine serum albumin (Sigma) (pH 8.0), 5 μL of high-density lipoprotein (HDL) (3 mg protein per milliliter) as apolipoprotein C-II, and an appropriate amount of sample (170 μL). After incubation for 60 minutes at 37°C, the enzyme reaction was terminated by addition of 10 μL of di-isopropylfluorophosphate at 4°C. Free fatty acids released in the mixture were measured by enzymatic method (Nescauto NEFA V2; Azwell, Osaka, Japan).

2.4. Western blot analysis of LPL protein in 3T3-L1 cells

The LPL protein was detected by Western blot analysis. Cells were suspended in a lysis buffer containing 10 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 0.5% Triton X-100, 0.5 mmol/L phenylmethylsulfonyl fluoride, and 1 mmol/L EDTA for 4 hours at 4°C. After centrifugation at 12 000*g*, the concentration of proteins in supernatant was measured using the Bio-Rad protein assay (Hercules, CA). Samples were diluted 1:1 with electrophoresis sample buffer containing 100 mmol/L Tris (pH 6.8), 10% sodium dodecyl sulfate, 10% glycerol, 0.1% bromophenol blue, and 5% *b*-mercaptoethanol, then boiled for 5 minutes and electrophoresed on 10% sodium dodecyl sulfate polyacrylamide gel. The proteins were transferred into Hybond-ECL nitrocellulose (Amersham Biosciences, Piscataway, NJ). After blotting, the membrane was washed with Tris buffer saline (TBS; 100 mmol/L Tris, pH 7.5, 0.9% NaCl) and blocked with 5% bovine serum albumin in TBS, followed by a brief wash in TBS containing 0.1% Tween-20 (TTBS) and incubation with a chicken antiserum against mouse LPL (kindly provided by Masuno H, Department of Medical Laboratory Technology, Ehime College of Health Science, Japan, and Olivecrona T, Division of Nephrology, Department of Internal Medicine, Umea University, Sweden) at a dilution of 1:500 for 2 hours at room temperature. After washing with TTBS, the blot was incubated with peroxidase-labeled affinity purified antibody to chicken immunoglobulin G (H + L) (KPL, Inc, Gaithersburg, MD) at a dilution of 1:1500 for 1 hour at room temperature, washed with TTBS, and incubated with 1:3000 diluted biotinylated horseradish peroxidase streptavidin complex for 1 hour at room temperature. The antigen-antibody complex was visualized by photodetection. The bands were quantified with a digital scanning and Scion Image software (Scion, Frederick MD).

2.5. Reverse transcriptase polymerase chain reaction of LPL, PPARγ, or angiotensinogen messenger RNA in 3T3-L1 cells

Total RNA was isolated from 3T3-L1 cells grown in 6-well plate using RNeasy Mini Kit (QIAGEN, Hilden, Germany). The amount of RNA was measured as absorbance at 260 nm. Synthesis of complementary DNA (cDNA) and polymerase chain reaction (PCR) were performed using the RNA PCR Kit (AMV) Ver.3.0 (Takara, Ohtsu, Japan). A pair of gene-specific PCR primers each was designed for LPL and angiotensinogen. The following primer sets were used: LPL forward primer, 5′-CTCgATCCAgCTggACCTAA-3′; LPL reverse primer, 5′-TCCAAgTCCTCTCTCTgCAA-3′; PPARγ forward primer, 5′-gCTCTAgACgTgACAATCTgTCTgAggTCTgT-CAT-3′; PPARγ reverse primer, 5′-CgggATCCgTTgTCgg-TTTCAgAAATgCCTTgCAgTg-3′; angiotensinogen forward primer, 5′-gTACAgACAgCACCCTACTT-3′; angiotensinogen reverse primer, 5′-AACAACTTCTCCgT-gACgTg-3′. First-strand cDNA was synthesized from 1 μg of total RNA. Total RNA was mixed with 0.25 U of reverse transcriptase (RT), 1 mmol/L of deoxynucleoside triphosphate (dNTP) mixture, 2.5 μmol/L of first-strand cDNA primer (Random 9 mers; Takara, Ohtsu, Japan), 0.25 U of RNase inhibitor, and 6 μL of $MgCl_2$ reaction buffer in a final volume of 10 μL and incubated at 30°C for 10 minutes, followed by 15 minutes at 50°C, 5 minutes at 99°C, and 5 minutes at 4°C. The PCR mixture contained 0.4 μmol/L of each primer, 0.25 μL of Takara Ex Taq HS as DNA polymerase, and 5.0 mmol/L of $MgCl_2$ reaction buffer in a final volume of 40 μL for synthesizing the second-strand cDNA. Amplification was performed for 30 cycles under the following conditions: denaturation at 94°C for 5 minutes for the first cycle and 1 minute for subsequent cycles, annealing at 50°C for 1 minute, and extension at 72°C for 1 minute. The PCR products were separated by electrophoresis on a 3.0% agarose gel and then visualized by staining with 1 mg/mL ethidium bromide. The quantities of reaction products were determined using digital scanning and Scion Image software.

2.6. Statistical analysis

Stat View-J 5.0 software (SAS Institute, Cary, NC) was used for all statistical analysis. Paired *t* test was performed to determine if the differences between groups were statistically significant. A $P < .05$ was considered to be significant.

3. Results

3.1. Adipogenic differentiation of 3T3-L1 cells and changes of LPL activity and expression in cells during long-term incubation

First, we confirmed the changes of adipogenic differentiation of 3T3-L1 cells as well as LPL activity and expression in the cells during long-term incubation. The 3T3-L1 cell size, oil red O staining, and PPARγ messenger RNA (mRNA) expression in the cells were used as markers of adipogenic differentiation.

The mean 3T3-L1 cell size was 18.78 ± 0.61 μm on day 0, and the size increased with increase in incubation time. It then increased to 23.22 ± 0.76 μm on day 15 (Fig. 1A). Expression of PPARγ mRNA in 3T3-L1 cells was demonstrated by RT-PCR (Fig. 2). A significant increase (6.19-fold) in PPARγ mRNA expression was also observed over time during 15 days of incubation.

The LPL activity was confirmed in 3T3-L1 cell cultures during long-term incubation (Fig. 3A). The LPL activity increased dramatically on day 10 and declined on day 15, although both activities were significantly higher than those on day 5. Expression of LPL protein and mRNA in 3T3-L1 cells was demonstrated by Western blot analysis and RT-PCR, respectively. The LPL protein and mRNA expression also increased very significantly on day 10 compared with that on day 5 and decreased on day 15, similar to LPL activity. Especially, LPL mRNA was reduced significantly by 36.2% on day 15 compared with that on day 10.

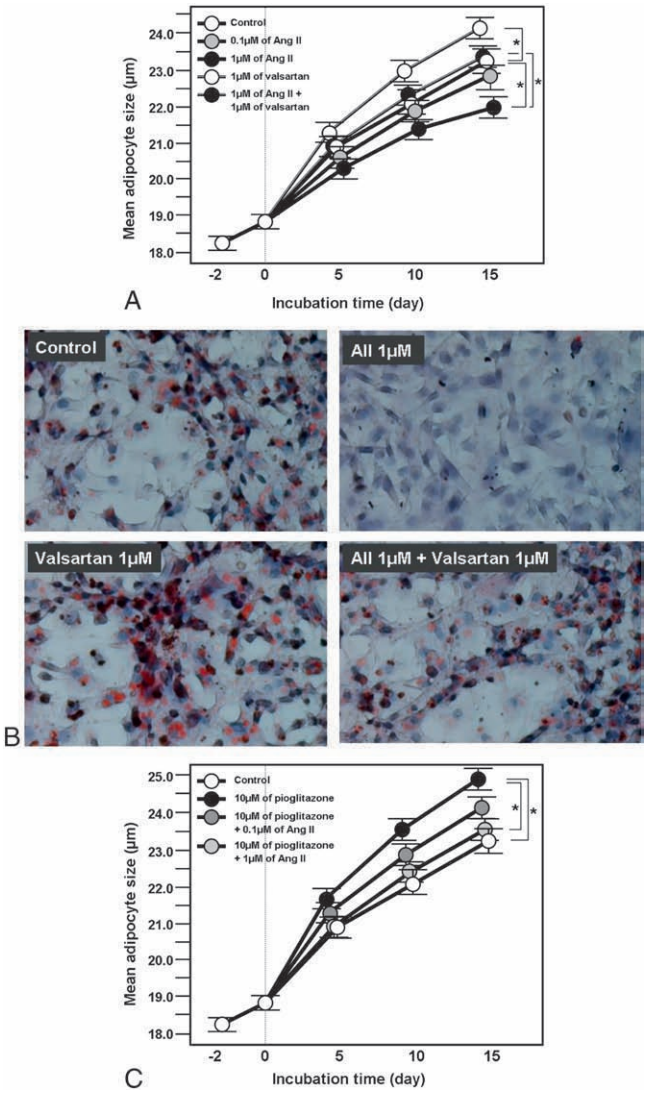

Fig. 1. The changes of 3T3-L1 cell size incubated for 15 days. At days 0, 5, 10, and 15, the mean 3T3-L1 cell size was determined using particle analyzer after trypsinization. A, The 3T3-L1 cell with or without Ang II, and with or without valsartan for 15 days after differentiation. Angiotensin II was supplemented at a final concentration of 0.1 or 1 μmol/L, and valsartan was supplemented at a final concentration of 1 μmol/L. B, Oil red O staining for triglycerides in 3T3-L1 cells. The 3T3-L1 cells were treated with or without 1 μmol/L of Ang II, and with or without 1 μmol/L of valsartan for 15 days after differentiation. Phase contrast photomicrographs (20× magnification) were recorded on digital camera. C, The 3T3-L1 cell with or without 1 μmol/L of Ang II, and with or without 10 μmol/L of pioglitazone for 15 days after differentiation. Pioglitazone was supplemented at a final concentration of 10 μmol/L. Values are expressed in mean ± SD. *$P < .05$.

3.2. Adipogenic differentiation of 3T3-L1 cells conditioned with Ang II and/or valsartan

To clarify whether Ang II affects adipogenic differentiation of 3T3-L1 cells, the cell size (Fig. 1A), oil red O staining (Fig. 1B), and PPARγ mRNA expression (Fig. 2) were examined when Ang II and/or valsartan was added to the culture for 15 days after differentiation.

Angiotensin II lowered 3T3-L1 cell size, which enlarged during long-term incubation, in a dose-dependent manner. Especially, the change of 3T3-L1 cell size was decreased significantly by 22% (the cell size on day 15 was 22.31 ± 0.70 μm) in the presence of 1 μmol/L of Ang II compared with control during 15 days of incubation. Oil red O staining showed that Ang II reduced triglyceride accumulation in 3T3-L1 cells. Similarly, 1 μmol/L of Ang II also reduced PPARγ mRNA expression, which increased during long-term incubation, in 3T3-L1 cells. On day 15, PPARγ mRNA expression in 3T3-L1 cells treated by 1 μmol/L of Ang II was suppressed significantly by 53% compared with treatment without Ang II.

Pioglitazone enlarged 3T3-L1 cell size (24.83 ± 0.70 μm) on day 15, and the change of the cell size increased significantly by 36% compared with control during 15 days of incubation (Fig. 1C). Angiotensin II lowered 3T3-L1 cell size, which was enlarged by pioglitazone, in a dose-dependent manner. The change of the cell size decreased significantly by 22% (the cell size was 23.52 ± 0.65 μm on day 15) in the presence of pioglitazone with 1 μmol/L of Ang II compared with that in the presence of pioglitazone without Ang II during 15 days of incubation.

One micromole per liter of valsartan enlarged 3T3-L1 cell size (24.17 ± 0.68 μm) on day 15, and the change of the cell increased significantly by 21% compared with treatment without valsartan during 15 days of incubation (Fig. 1A). Oil red O staining showed that valsartan promoted adipogenesis to a greater extent than control. One micromole per liter of Ang II with 1 μmol/L of valsartan enlarged 3T3-L1 cell size (23.40 ± 0.65 μm) on day 15, and the change of the cell increased significantly by 31% compared with only 1 μmol/L of Ang II. Oil red O staining showed that Ang II with valsartan promoted adipogenesis to a greater extent than only Ang II.

3.3. LPL activity and expression in 3T3-L1 cells conditioned with Ang II and/or valsartan

To clarify whether Ang II might affect LPL activity and gene expression in 3T3-L1 cells, LPL activity, LPL protein, and mRNA expression in the cells were examined in the presence of Ang II. Especially, LPL activity was examined when Ang II and/or valsartan was added to the culture for 15 days after differentiation.

Angiotensin II reduced LPL activity in a dose-dependent manner on both days 10 and 15 (Fig. 3A). Especially, LPL activity was reduced significantly by 30% and 42% on day 10 and 15, respectively, in the presence of 1 μmol/L of Ang II compared with treatment without Ang II. Furthermore, LPL activity in 3T3-L1 cells incubated with Ang II for 72 hours after 12 days after differentiation was measured on day 15 (Fig. 3B). The LPL activities were not significant between at 0, 0.1, and 1 μmol/L Ang II.

Valsartan enhanced LPL activity in a dose-dependent manner on day 15 both with and without Ang II (Fig. 4). The LPL activity was enhanced significantly by 34% in the

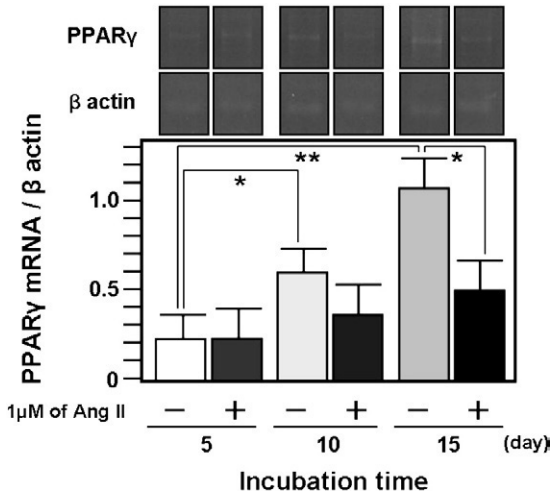

Fig. 2. The PPARγ mRNA expression in 3T3-L1 cells incubated with or without Ang II for 15 days after differentiation. The 3T3-L1 cells were treated with or without Ang II at a final concentration of 1 μmol/L. At days 5, 10, and 15, PPARγ mRNA in 3T3-L1 cells was detected by RT-PCR. The products were quantified by digital scanning and Scion Image software. Values are expressed in mean ± SD. *$P < .05$. **$P < .005$.

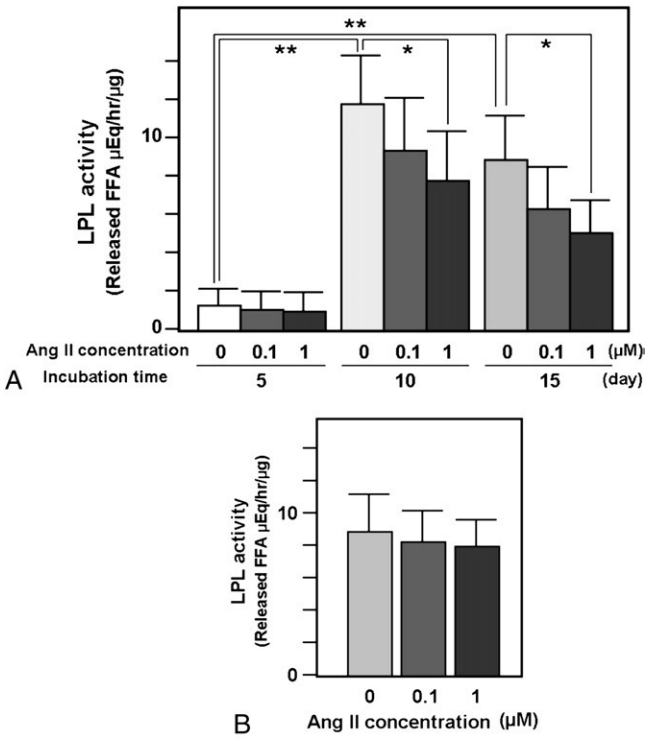

Fig. 3. The LPL activity in 3T3-L1 cells incubated with or without Ang II for 15 days after differentiation. The LPL activities were measured using triolein as substrate, and released free fatty acids in the mixture were measured by enzymatic method. A, The 3T3-L1 cells were treated with or without Ang II at a final concentration of 0.1 or 1 μmol/L for 15 days after differentiation. At days 5, 10, and 15, LPL activity in 3T3-L1 cells was measured. B, The 3T3-L1 cells were treated with or without Ang II for 72 hours after 12 days after differentiation. At day 15, LPL activity in 3T3-L1 cells was measured. Values are expressed in mean ± SD. *$P < .05$. **$P < .005$.

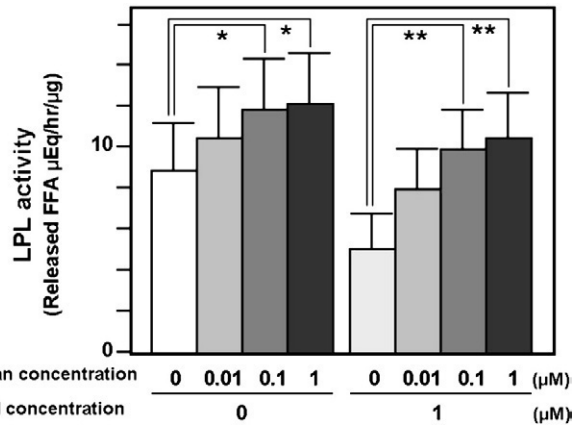

Fig. 4. The LPL activity in 3T3-L1 cells incubated with or without Ang II, and with or without valsartan for 15 days after differentiation. Angiotensin II was supplemented at a final concentration of 1 μmol/L; and valsartan was supplemented at a final concentration of 0.01, 0.1, or 1 μmol/L. The LPL activities were measured using triolein as substrate, and released free fatty acids in the mixture were measured by enzymatic method. At day 15, LPL activity in 3T3-L1 cells was measured. Values are expressed in mean ± SD. *$P < .05$. **$P < .005$.

presence of 0.1 μmol/L of valsartan and by 36% in the presence of 1 μmol/L of valsartan compared with treatment without valsartan. In the presence of Ang II, LPL activity was also enhanced significantly by 97% and 103% under treatment with 0.1 and 1 μmol/L of valsartan, respectively, compared with treatment without valsartan.

The LPL protein expression in 3T3-L1 cells treated with 1 μmol/L of Ang II was suppressed significantly by 40% and 32% on days 10 and 15, respectively, compared with treatment without Ang II (Fig. 5).

Similarly, on days 10 and 15, LPL mRNA expression in 3T3-L1 cells treated with 1 μmol/L of Ang II was also

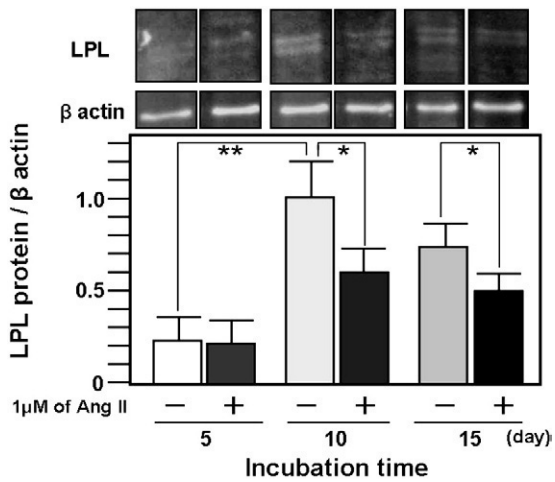

Fig. 5. The LPL protein expression in 3T3-L1 cells incubated with or without Ang II for 15 days after differentiation. The 3T3-L1 cells were treated with or without Ang II at a final concentration of 1 μmol/L. At days 5, 10, and 15, LPL protein in 3T3-L1 cells was measured by Western blot analysis. The products were quantified by digital scanning and Scion Image software. Values are expressed in mean ± SD. *$P < .05$. **$P < .005$.

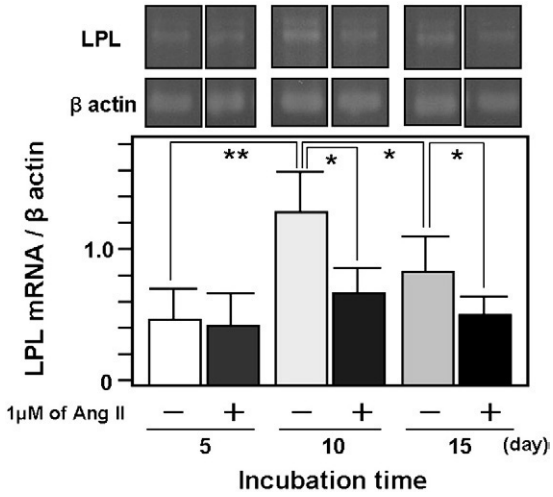

Fig. 6. The LPL mRNA expression in 3T3-L1 cells incubated with or without Ang II for 15 days after differentiation. The 3T3-L1 cells were treated with or without Ang II at a final concentration of 1 μmol/L. At days 5, 10, and 15, LPL mRNA in 3T3-L1 cells was determined by RT-PCR. The products were quantified by digital scanning and Scion Image software. Values are expressed in mean ± SD. *$P < .05$. **$P < .005$.

suppressed significantly by 43% and 31%, respectively, compared with treatment without Ang II (Fig. 6).

3.4. Angiotensinogen mRNA in 3T3-L1 cells for long-term incubation

To confirm whether activation of RAS in 3T3-L1 cells is associated with suppression of adipogenic differentiation of the cells, expression of angiotensinogen mRNA in the cells during long-term incubation was demonstrated by RT-PCR (Fig. 7). Angiotensinogen mRNA expression in 3T3-L1 cells increased significantly over time during 15 days of incubation. Angiotensinogen mRNA in 3T3-L1 cell increased with increase in incubation time. On day 15, angiotensinogen mRNA expression in 3T3-L1 cells showed a 5-fold increase compared with day 5.

4. Discussion

We hypothesized that LPL expression in adipocytes is suppressed by inhibition of adipogenic differentiation through activation of the RAS in the cells. Using 3T3-L1 cells, adipogenic differentiation was enhanced over time during 15 days of incubation; however, LPL activity and expression in the cells increased transiently. The addition of Ang II suppressed adipogenic differentiation, LPL activity, and expression in 3T3-L1 cells; in contrast, valsartan enhanced adipogenic differentiation, LPL activity, and expression in the cells. Furthermore, angiotensinogen expression in 3T3-L1 cells increased significantly over time during 15 days of incubation. Therefore, we demonstrated partially that LPL expression was suppressed by inhibition of adipogenic differentiation through activation of RAS in 3T3-L1 preadipocytes.

This is the first report that LPL activity and expression in 3T3-L1 cells decreased after a transient increase during long-term incubation. Adipocytes are known to release a variety of adipokines depending on adipocyte size [24-26]. Skurk et al [27] reported that protein secretion of leptin, interleukin (IL) 6, IL-8, tumor necrosis factor α, monocyte chemoattractant protein 1, interferon-gamma–inducible protein (IP)-10, macrophage inflammatory protein 1β, granulocyte colony-stimulating factor, IL-1ra, and adiponectin correlates positively with cell size in cultured adipocytes isolated from 30 individuals undergoing elective plastic surgery. However, the reason why adipokines including LPL behave differently depending on the level of adipogenic differentiation remains controversial. We demonstrated that both angiotensinogen expression and adipogenic differentiation increased over time in 3T3-L1 cells. Furthermore, it has been reported that adipocytes express not only angiotensinogen but also renin and angiotensin-converting enzyme [23]. These findings suggest that adipocytes may express Ang II. Therefore, the suppression of adipogenic differentiation and LPL gene expression in 3T3-L1 cells in the presence of Ang II proves our hypothesis considerably.

However, whether the Ang II–induced suppression of LPL expression in 3T3-L1 cells is mediated by adipogenic differentiation is unknown. Janke et al [23] reported that inhibition of adipogenic differentiation by Ang II decreases PPARγ gene expression in human preadipocytes. Crandall et al [28] reported that Ang II stimulated the cell cycle regulator cyclin D1, which inhibited PPARγ in adipocytes. Folli et al [29] reported that AT_1 receptor is involved in the Ang II–mediated impairment of insulin signaling, resulting in the activation of the glucose transporter and its translocation from an intracellular membrane compartment

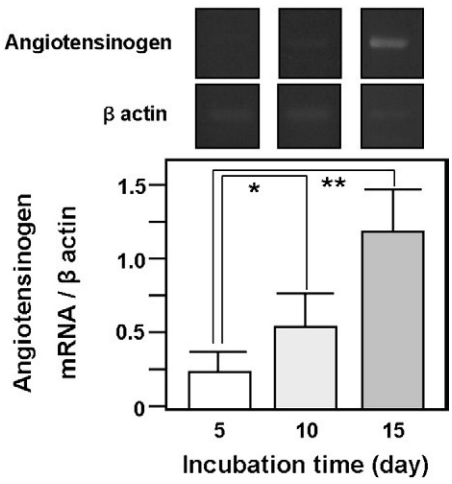

Fig. 7. Angiotensinogen mRNA expression in 3T3-L1 cells incubated for 15 days. At days 5, 10, and 15, angiotensinogen mRNA in 3T3-L1 cells was determined by RT-PCR. The products were quantified by digital scanning and Scion Image software. Values are expressed in mean ± SD. *$P < .05$. **$P < .005$.

to a plasma membrane fraction. The LPL expression is thought to be one target gene of PPARγ in adipocytes [5,30]. Therefore, the regulation of LPL expression by Ang II is suggested to be mediated by adipogenic differentiation via PPARγ expression in 3T3-L1 cells. The hypothesis was also supported by the data that Ang II tended to lower 3T3-L1 cell size enlarged by pioglitazone in a dose-dependent manner. Furthermore, LPL activity in the cells was not reduced when Ang II was incubated for 72 hours after 12 days after differentiation. In other words, with fully differentiated adipocytes, LPL activity was not suppressed by Ang II. This result supported that Ang II could have an indirect effect on LPL mediated by adipogenic differentiation in 3T3-L1 cells.

In the absence of exogenous Ang II, valsartan enlarged 3T3-L1 cell size and increased LPL activity in the cells. The result suggests that endogenous Ang II activation might play a role in LPL expression as a result of adipocyte differentiation, and AT_1 receptor might mediate these effects.

Angiotensin II has been suggested not only to stimulate adipogenic differentiation, but also to regulate lipolysis. Brink et al [31] reported that Ang II might cause weight loss in rats through a lipolytic effect. Cabassi et al [32] reported that sympathetic modulation by carvedilol and losartan reduces Ang II–mediated lipolysis in subcutaneous and visceral fat. On the other hand, Townsend [33] reported that stimulation of AT_1 receptor blocker or pressor dosages of Ang II produces no significant alteration in lipolytic activity. It is controversial whether adipocyte size might be determined by adipogenic differentiation and/or lipolysis, and there were no data with the cell size on lipolysis by the effect of Ang II in this study.

We have reported that AT_1 receptor blockade may be effective in controlling blood pressure and lipid metabolism in metabolic disorder associated with low preheparin serum LPL mass [10]. This may suggest that low preheparin serum LPL mass probably reflects activation of the RAS in enlarged adipocytes. We also have reported that preheparin serum LPL mass tends to decrease with the increase in number of symptoms of metabolic syndrome [9]. The mechanism by which LPL production in the whole body decreases in metabolic syndrome might be the activation of RAS by elevated fat mass. Therefore, LPL mass in preheparin serum may indicate insulin resistance due to activation of the RAS in enlarged adipocytes. When an adipocyte detects a limit of lipid accumulation as a result of adipogenic differentiation, the adipocyte may activate the RAS so that the cell itself may suppress LPL production.

In summary, LPL expression in 3T3-L1 cells decreases after a transient increase during long-term incubation. This phenomenon may be due to activation of the endogenous RAS together with adipogenic differentiation of 3T3-L1 cells. In other words, LPL gene expression may be suppressed by inhibition of adipogenic differentiation through activation of the endogenous RAS in 3T3-L1 cells via AT_1 receptor.

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