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Statins Induce the Gene Expression of Apolipoprotein A5 in HepG2 Cells

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Abstract：The apolipoprotein A5（apoA5）is fast gaining attention as a key regulator of plasma triglyceride concentrations. Statins are drugs that improve cholesterol levels primarily by inhibiting the rate-limiting enzyme in cholesterol synthesis. They also have a moderate ability to reduce plasma triglyceride levels. We investigated the ability of pitavastatin and pravastatin to modulate gene expression and synthesis of apoA5 in HepG2 cells. Promoter activity of the *APOA5* gene was estimated by measuring luciferase activity of plasmids with an *APOA5* promoter region transfected into human hepatoma HepG2 cells. Total RNA of HepG2 cells was extracted and analyzed by real-time quantitative PCR using *APOA5*-specific oligonucleotides. ApoA5 concentrations were measured by the ELISA. Exposure of HepG2 cells to 1–30 μM pitavastatin or 10–50 μM pravastatin resulted in significant increases in luciferase activity, and cotransfection of a peroxisome proliferator-activated receptor（PPAR）α resulted in an additional increase in *APOA5* gene expression. These effects were reversed by the addition of mevalonate or geranylgeranyl pyrophosphate, implicating HMG-CoA reductase as the relevant target of these drugs. HepG2 cells treated with pitavastatin displayed a strong induction of *APOA5* mRNA, and 5 μM pitavastatin increased the concentration of apoA5 in culture medium of HepG2 cells. Our results demonstrate that the gene expression and synthesis of apoA5 in HepG2 cells is regulated by statins in a positive manner, through suppressing the synthesis of mevalonate or its downstream products.

Key words：**Apolipoprotein A5, HepG2, luciferase activity, Pitavastatin, Statin**

INTRODUCTION

Hypertriglyceridemia is an important determinant for susceptibility to atherosclerosis [1)]. Understanding the regulation of genes that influence plasma triglyceride levels is of prime interest, and may aid in the development of therapy to reduce hypertriglyceridemia and the associated risk of atherosclerosis. Apolipoprotein A5（apoA5）is a member of the apolipoprotein family that was identified through human–mouse comparative sequence analysis [2)]. The *APOA5* gene resides ~27 kb distal to *APOA4* in the *APOA1/C3/A4/A5* gene cluster on human chromosome 11q23. The mature form of apoA5 is a 343 amino acid protein in humans. The *APOA5* gene is expressed in the liver, in which apoA5 is exported into the plasma and associates with high-density lipoprotein（HDL）and very low-density lipoprotein（VLDL）particles [3)]. It has been demonstrated that apoA5 and apoC3 independently influence plasma triglyceride concentrations in an opposite manner [4)].

Several groups have studied the mechanism underlying the effect of apoA5 on plasma triglyceride. Although apoA5 may be retained to some extent in hepatocytes, apoA5 is also secreted into the plasma. However, the total plasma concentrations of apoA5 are very low as

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compared with other apolipoproteins. It was reported that in an in-vitro setting apoA5 acted as an lipoprotein lipase (LPL) activator only if LPL was bound to heparan sulfate proteoglycans [5], and apoA5 interacted with the glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), the low density lipoprotein receptor-related protein and the mosaic type-1 receptor [6-8]. It was also offered a plausible explanation for the observations that apoA5 has a strong effect on in-vivo lipolysis of triglyceride-rich lipoproteins in mice [9, 10]. The crossbreeding of *Apoa5* knock out mice with human LPL transgenic mice demonstrated that the increased LPL activity completely compensated for apoA5 deficiency [9]. These observations underscore the crucial role of apoA5 in modulating the LPL-mediated lipolysis of triglyceride-rich lipoproteins in vivo.

Previous studies have shown that fibrates, Wy14643 or fenofibrate induce expression of the *APOA5* gene in human hepatoma cell lines [11, 12]. Fibrates are ligands for transcription factors belonging to the peroxisome proliferator-activated receptor (PPAR) subfamily of nuclear receptors, PPAR α. PPAR α is considered a major regulator of intra- and extracellular fatty acid metabolism. Statins such as pitavastatin are 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors that inhibit cholesterol synthesis. In addition to lowering LDL cholesterol, statins reduce triglyceride levels and increase HDL cholesterol. The mechanism responsible for the triglyceride-lowering effect of statins is poorly defined. In theory, statins could be related to decreased VLDL production (presumably secondary to decreased availability of hepatic free cholesterol for particle assembly), increased clearance of VLDL through the LDL receptor, increased delipidation of VLDL particles by a LPL, or a combination of these mechanisms. It was reported that atorvastatin increased LPL activity in cases of type2 diabetes with hypercholesterolemia, and simvastatin treatment caused an increase in LPL activity in rabbits [13, 14]. Recently, a combination of atorvastatin and fenofibrate increased apo A5 and decreased triglyceride through up-regulation of PPAR α [15]. In this study, we evaluated modulation of the *APOA5* gene expression by pitavastatin and pravastatin *in vitro*.

METHODS

Materials

Pitavastatin was supplied by Kowa (Nagoya, Japan). Pravastatin was purchased from Wako Pure Chemicals (Osaka, Japan). Fenofibric acid was supplied by Greran Pharmaceutical (Tokyo, Japan). Mevalonate and geranylgeranyl pyrophosphate (PP) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Cell culture experiment

Human hepatoma HepG2 cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (JRH Bioscieces, Lenexa, KS, USA) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) at 37 °C in a humidified atmosphere of 5% CO_2. Cell viability was routinely monitored by trypan blue exclusion. In all experiments, the number of dead cells never exceeded 5% of the total number.

Plasmid constructs

The pGL3/*APOA5* (–617/+18) /luc construct was created by inserting a double-stranded oligonucleotide that contained *Kpn*I and *Nhe*I restriction sites (Takara Bio, Tokyo, Japan), as reported previously[12]. Briefly, amplification of the *APOA5* promoter region was accomplished by PCR with primers, JCR45 (5'-AGTCG GTACCTCATGGGGCAAATCTTACTTTCGC-3') and JCR95 (5'-AGTCGCTAGCTCACCTGCTCACGTCTGG-3') (–617 to +18). The 651 bp fragments were subcloned into pT7Blue-T vector. The genomic subclone was digested with *Kpn*I and *Nhe*I and cloned into pGL3-Basic (Promega, Madison, WI, USA). Mutations of peroxisome proliferator response elements (PPERs) were introduced and verified by DNA sequencing [12]. The corresponding –263G>C/–262G>A/–261T>A mutated nucleotides were introduced into the wild-type pGL3-*APOA5* (–617/+18) /luc plasmid using the QuikChange site-directed mutagenesis Kit (Stratagene), with mutagenesis primers 5'-CAGGTCAGTGGGAAGGTTAAACAACATGGGGT TTGGGAG-3' and the complementary oligonucleotides (point mutation sites are underlined). Plasmid phRL-SV40 was purchased from Promega. phRL-SV40 was included in each transfection to normalize the transfection efficiency. The human PPAR α cDNA was obtained from the mRNA of the HepG2 cells by RT-PCR. The PPAR α cDNA was inserted into the pSG5 vector (Stratagene, La Jolla, CA, USA). The structures of the resulting constructs were verified by the restriction mapping and nucleotide sequencing.

Transfection and transient expression assay

HepG2 cells were seeded ($1.6x10^5$ cells/35-mm dish) for 24 h before transfection and transiently transfected in serum-free medium using Lipofectoamine reagent, according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The pGL3-*APOA5* luciferase reporter (1 μg/dish) was transfected into HepG2 cells. Effecter plasmid dosage was kept constant by the addition of appropriate amounts of the empty expression vector pSG5. To correct for variation in DNA uptake by the cells, 5 ng of the plasmid phRL-SV40 was cotransfected with each test construct. Firefly and Renilla luciferase activity was measured. pSG5-*hPPAR α* (1 μg/dish) was cotransfected with the pGL3-*APOA5* into HepG2 cells in a part of experiments. Treatment with pitavastatin, pravastatin or fenofibrate started at 4 h after transfection. Following transfection, the cells were incubated in DMEM supplemented with 5% lipoprotein-free human serum for another 24 h (3–48 h), then gently scraped into pre-chilled extraction buffer. To study the effects of mevalonic acid and geranylgeranyl pyrophosphate on statin-induced *APOA5* transactivation, each reagent was added with pitavastatin. Extracts from transfected cells were prepared by passive lysis buffer, and luciferase activity of the cell extracts was assayed by the Dual-Luciferase Reporter Assay System (Promega) and a TR717 microplate luminometer (Applied Biosystems). Independent experiments were performed at least three times in triplicate.

RNA isolation and analysis of gene expression by real-time quantitative PCR.

HepG2 cell were treated with pitavastatin for 24 h in DMEM containing 5% lipoprotein-free human serum. Total RNA was isolated with RNeasy mini kit (QIAGEN, Valencia, CA, USA). First-strand cDNA was synthesized using SuperScript VILO cDNA synthesis kit (Invitrogen), and subsequently diluted with nuclease-free water to 12.5 ng/ μl cDNA. *APOA5*, *APOA1* and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were determined by real-time quantitative PCR. Primers and probe for human *APOA1*, *APOA5* and *GAPDH* were 5'-AGACAGCGGCAGAGACTATGTGT-3' (*APOA1* forward primer), 5'-CCAGTTGTCAAGGAGCTTTAGGTT-3' (*APOA1* reverse primer), 5'-TCTGGCTCTTCTTTCAGCGTTT-3' (*APOA5* forward primer), 5'-CCGCTGGTCTGGCTGAAGT-3' (*APOA5* reverse primer), 5'-CCCATGTTCGTCATGGGTGT-3' (GAPDH forward primer) and 5'-TGGTCATGAGTCCTTCCACGATA-3' (*GAPDH* reverse primer), respectively. Briefly, 50 ng cDNA was used in duplicate per PCR run, with specific primer sets for human *APOA1*, *APOA5* and *GAPDH*. Real-time PCR was performed using a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) and measured by Syber green incorporation using the SYBR GreenER qPCR SuperMix (Invitrogen). The cycling conditions comprised 15 min polymerase activation at 95°C and 40 cycles at 94°C for 15 s and 58°C for 30 s. Data were analyzed using the Sequence Detection System program v1.3 (Applied Biosystems). Primers were verified and data were analyzed using the ΔΔCT (difference in threshold cycles) method, as described previously [16]. All data were normalized to GAPDH as an internal control, according to the manufacturer's instructions.

Preparation of recombinant apoA5 and anti-apoA5 antibody

Human *APOA5* cDNA was obtained by RT-PCR from mRNA of HepG2 cells. To facilitate the over-expression and purification of His-tagged apoA5, the *APOA5* cDNA encoding mature amino acids was cloned between the *Bam*HI and *Pst*I sites of pQE30 (QIAGEN). *Escherichia coli* JM109 cells bearing the plasmid pQE30/*APOA5* were grown in LB medium. After the absorbance at 600 nm reached 0.5, the cultures were induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside for 2 h. Purification of the His-tagged human apoA5 by Ni-nitrilotriacetic acid affinity column chromatography was performed according to the instructions of the manufacturer (QIAGEN). The recombinant apoA5 was further purified by electrophoretic elution of the respective bands obtained. Female New Zealand White rabbits were immunized three times by subcutaneous injection of 50 μg purified recombinant human apoA5 mixed with Freund's complete (first injection) or incomplete (subsequent injections) adjuvant.

Sandwich ELISA for apoA5 concentration

Each well of a microtiter plate was coated with 1.0 μg purified polyclonal antibody, and incubated at 4°C overnight. After blocking the plate with Block Ace (Dainihon Pharmacy, Osaka, Japan) for 30 min at room temperature, standard and serum samples (diluted in PBS containing 0.3% bovine serum albumin) were added to wells, and incubated for 1.5 h at room temperature. After washing with PBS containing 0.1% Tween 20, 100 μl

biotinylated polyclonal antibody (0.5 μg/ml) was added, and the plate was incubated for 1 h at room temperature. After washing the plate, avidin–horseradish peroxidase conjugate (0.2 μg/ml) in PBS containing 0.1% Tween 20 was added to each well, and incubated for 30 min at 37°C. *o*-Phenylenediamine dihydrochloride (60 μg), dissolved in 100 mM citrate buffer (pH 5.0) containing 0.012% H_2O_2, was then added to each well. After incubation for 30 min at room temperature, the reaction was terminated by addition of 20 μl of 8 N H_2SO_4, and absorbance was read at 492 nm in a microplate reader. The concentration of apoA5 was determined by reference to a standard curve constructed with purified apoA5. For intracellular concentration of apoA5, cells were collected with a rubber policeman, pelleted by centrifugation, resuspended in PBS, disrupted by sonication at 4 °C, and centrifuged at 10,000*g* for 20 min and 4 °C. The supernatants were used for analysis of intracellular apoA5. Protein concentration was determined using the BCA protein assay kit (Rockford, Illinois, USA).

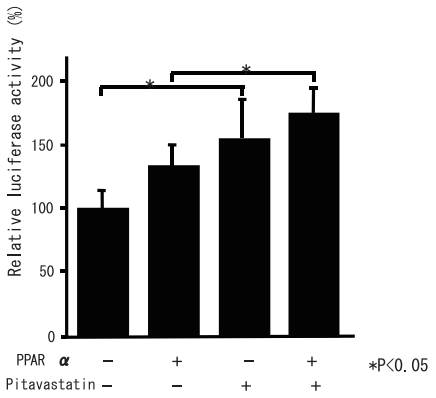

Fig. 1. Transactivation of the human *APOA5* gene promoter by pitavastatin and PPAR α. HepG2 cells were transfected with the pGL3-*APOA5* plasmid containing the −617 to +18 region of the *APOA5* promoter in the presence of cotransfected empty pSG5 or pSG5hPPAR α expression vector. Firefly luciferase activity was normalized to phRL-SV40 control activity. Values (means ± SD, n=3) are expressed relative to controls. HepG2 cells were incubated for an additional 24 h with/without pitavastatin (30 μM). Statistical differences from controls are indicated by asterisks. (*P<0.05)

Statistical analysis

Results of the reporter gene assay, the real-time quantitative PCR and the ELISA were presented as means ± SD. Comparisons of two groups were made using an unpaired t test. Statistical significance was defined as P<0.05.

RESULTS

Effect of statins on the APOA5 promoter

The 5' region of the *APOA5* gene (–617/+18 bp) was cloned into a pGL3-luciferase reporter gene basic vector. The constructed vector pGL3-*APOA5* and plasmid phRL-SV40 were simultaneously transfected into HepG2 cells, and transcriptional levels assessed by luciferase assay were compared. To determine whether pitavastatin can modulate *APOA5* gene expression, we incubated HepG2 cells in the presence or absence of pitavastain. Treatment with 30 μM pitavastatin significantly increased the activity of the firefly luciferase receptor gene driven by the human *APOA5* promoter at 24 h (Fig. 1). Furthermore, a human PPAR α expression plasmid enhanced activity of the *APOA5* promoter, and cotransfection of PPAR α with the treatment of pitavastatin resulted in an additional increase in the luciferase activity of the human apoA5 promoter (Fig. 1).

Fibrates have previously been shown to affect *APOA5* gene expression [11, 12], therefore, we compared the ability of pitavastatin, pravastatin or fenofibrate to modulate *APOA5* gene expression in HepG2 cells with a PPAR α expression vector. As shown in Fig. 2 A–C, the relative transcriptional levels of apoA5 promoter in HepG2 cells treated with pitavastatin, pravastatin and fenofibric acid increased in a dose-dependent manner. Exposure of HepG2 cells to 1–30μM pitavastatin, 10–50 μM pravastatin or 50–400 μM fenofibric acid resulted in a significant increase in luciferase activity. Pitavastatin (2 μM) and 20 μM pravastatin increased *APOA5* expression by 50 and 30%, respectively. These results indicated that pitavastatin had a greater effect than pravastatin on *APOA5* expression.

We analyzed whether the effect of pitavastatin on *APOA5* expression could be reversed by addition of mevalonate or geranylgeranyl pyrophosphate (Fig. 3A). These experiments were done without a PPAR α expression vector. As shown in Fig. 3 B and C, pitavastatin-induced *APOA5* expression and production of apoA5 in HepG2 cells were abolished in the presence of 5 mM mevalonate or 5 μM geranylgeranyl pyrophosphate.

To unequivocally characterize the PPRE requirement for statin regulation, HepG2 cells were cotransfected with/without a PPARα expression vector and an *APOA5* promoter-luciferase reporter plasmid in which the PPRE

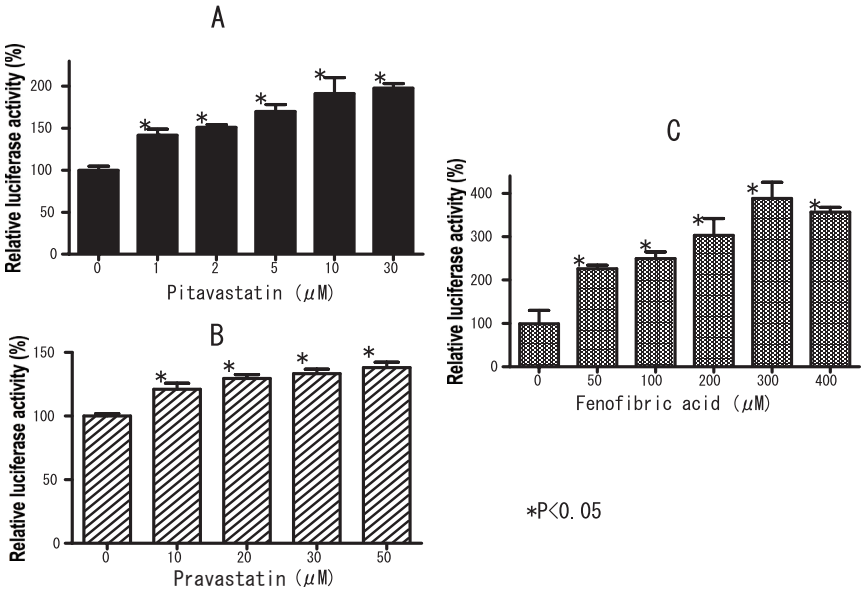

Fig. 2. Pitavastatin (A), pravastatin (B) and fenofibric acid (C) induced *APOA5* gene expression at the transcriptional level in HepG2 cells. HepG2 cells were transfected with pGL3-*APOA5* containing the −617 to +18 region of the *APOA5* promoter in the presence of cotransfected pSG5hPPAR α expression vector. HepG2 cells were incubated for an additional 24 h with various concentrations of pitavastatin, pravastatin or fenofibric acid. Firefly luciferase activity was normalized to phRL-SV40 control activity. Values (means ± SD, n=3) are expressed relative to controls. Statistical differences from controls are indicated by asterisks. (*P<0.05)

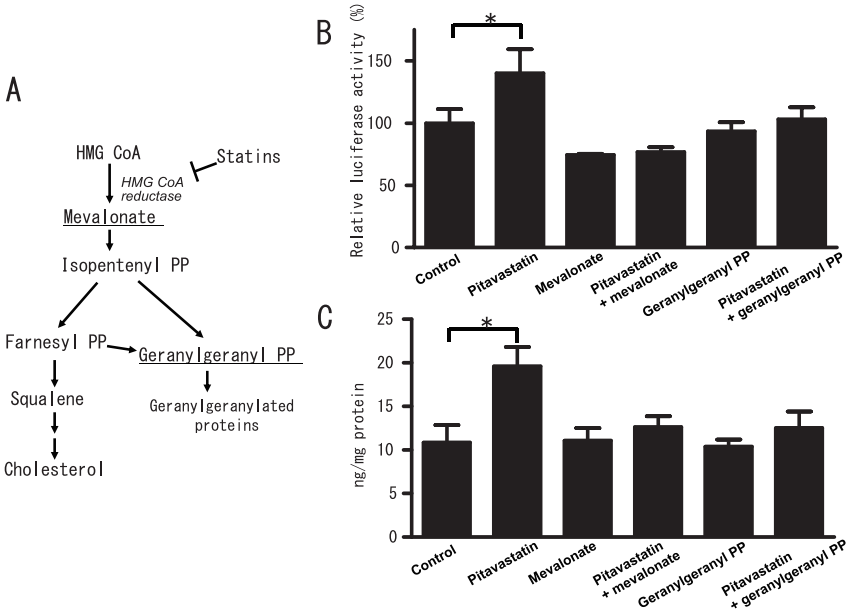

Fig. 3. Metabolic pathway for the conversion of HMG-CoA into cholesterol (A). HMG-CoA is converted to mevalonate by HMG-CoA reductase. Intermediates in the pathway are used for the synthesis of geranylgeranyl pyrophosphate (PP) and geranylgeranylated proteins. The effects of mevalonate or geranylgeranyl PP for *APOA5* gene expression at the transcriptional level in HepG2 cells (B). Pitavastatin enhanced *APOA5* gene expression by inhibition of HMG-CoA reductase activity. HepG2 cells were transfected with pGL3-*APOA5* in the absence of hPPAR α expression vector. HepG2 cells were incubated for 24 h with pitavastatin (2 μM), mevalonate (5 mM) and/or geranylgeranyl PP (5 μM), in an appropriate solvent in DMEM supplemented with 5% lipoprotein-free human serum. Firefly fuciferase activity was normalized to phRL-SV40 control activity. Pitavastatin enhanced Values (means ± SD, n=3) are expressed relative to controls. The effects of mevalonate or geranylgeranyl PP for production of apoA5 in HepG2 cells (C). HepG2 cells were incubated for 24 h with pitavastatin (2 μM), mevalonate (5 mM) and/or geranylgeranyl PP (5 μM). The relative apoA5 concentrations (ng/mg protein) in HepG2 cells were determined by ELISA (means ± SD, n=4). Statistical differences from controls are indicated by asterisks. (*P<0.05)

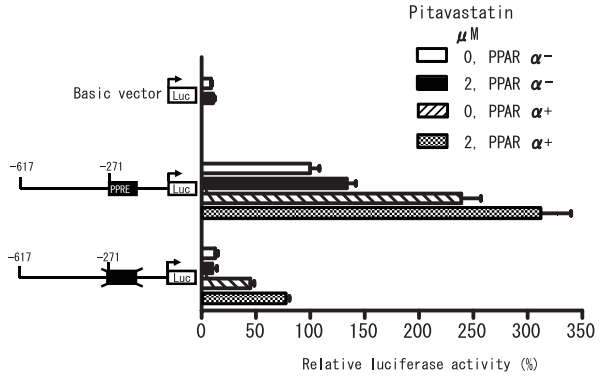

Fig. 4. PPRE requirement for statin regulation of the *APOA5* promoter. HepG2 cells were transfected with human *APOA5* promoter reporter construct (−617/+18 bp) or with a construct containing a mutation (*cross*) of the putative PPRE. Cells were cotransfected with phRL-SV40 and incubated with/ without 2μM pitavastatin in absence or presence of a PPAR α expression vector. Luciferase activity is expressed as means ± S.D. *Luc*, luciferase.

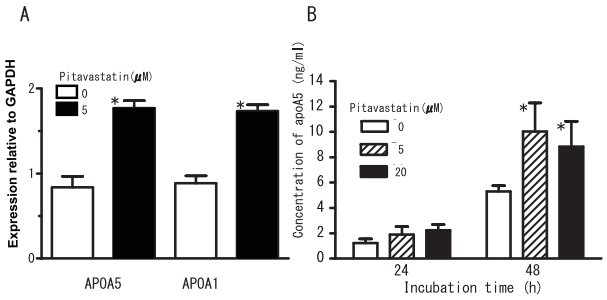

Fig. 5. Pitavastatin induced mRNA (A) and protein (B) of apoA5. (A) HepG2 cells were grown in culture medium until sub-confluent. Medium was then changed to experimental medium with or without pitavastatin (5 μM), and incubated for 24 h. *APOA5* or *APOA1* mRNA levels were determined by real-time RT-PCR. Each experimental group contained three replicates and real-time RT-PCR was run in duplicate. (B) Concentrations of apoA5 in culture medium of HepG2 after pitavastatin were determined by ELISA. Data are means ± S.D. (n=3 for each sample group). Statistical differences from controls are indicated by asterisks. ($*P$<0.05)

sequence was mutated in the 3' -hexamer. As shown in Fig. 4, luciferase activity of the reporter gene with the mutated PPRE was reduced significantly. In contrast to the native promoter construct, pitavastatin had no effect on the luciferase gene expression of the construct bearing the mutated PPRE without a PPARα expression vector.

Pitavastatin increases APOA1 and APOA5 mRNA

Total RNA was extracted and analyzed by real-time quantitative PCR using the *hAPOA1*- and *hAPOA5*-specific oligonucleotides, and GAPDH as an internal control. As shown in Fig. 5A, 5 μM pitavastatin showed a 95% increase in *APOA1*-mRNA, and a 110% increase in *APOA5*-mRNA in HepG2 cells without a PPARα expression vector. These results confirm that pitavastatin may play a role in the control of *APOA5* gene expression in human HepG2 cells.

Pitavastatin increases the concentration of the apoA5 in HepG2 culture medium

HepG2 cells were exposed to 0, 5 and 20 μM pitavastatin for 48 h. As shown in Fig. 5B, the results indicated that pitavastatin significantly increased the concentration of apoA5 in the culture medium. We showed that, in HepG2 cells, treatment with 5μM pitavastatin increased apoA5 concentration by 55 and 90 % after 24h and 48h incubation, respectively.

DISCUSSION

The primary lipid fraction targeted in the pharmacotherapy of hyperlipidemia is LDL-cholesterol. However, triglycerides in high concentrations also have atherogenic potential. We investigated *APOA5* gene regulation by statins with/without cotransfection of a PPAR α expression plasmid. We showed that pitavastatin and pravastatin modulated expression of the *APOA5* gene in HepG2 cells, and pitavastatin had a greater effect than pravastatin on *APOA5* gene expression. Statin treatment of HepG2 cells resulted dose-dependent increases in *APOA5* gene expression. In pitavastatin-treated HepG2 cells, increased levels of the *APOA1* and *APOA5* mRNA were observed by real-time PCR quantification. ApoA5 synthesis in HepG2 cells was significantly increased by the presence of pitavastatin. These data are consistent with the effects of statins on the *APOA5* gene. Cotreatment of the transfected cells with mevalonate or geranylgeranyl pyrophosphate blocked the stimulatory effects of the statin on the promoter activity of the *APOA5* gene and production of apoA5 (Fig.3 B, C). This observation associated influence of the statin on *APOA5* with its ability to inhibit HMG-CoA reductase. The cotransfection of a PPARα expression plasmid showed an additive effect. On the other hand, the mutated PPRE

caused promoter activity to revert to almost basal level, and the stimulatory effect of the pitavastatin was reduced.

Previous studies have reported that apoA5 is selectively produced by the liver and behaves as a regulator of plasma triglyceride levels [2, 17]. Since identification of the *APOA5* gene, several groups have studied its significant effects on plasma triglyceride levels. The plasma level of apoA5 is extremely low (179.2 ± 74.8 ng/ml) [18]. The plasma level of apoA5 in healthy subjects showed a negative correlation with the plasma triglyceride level and a positive correlation with HDL-cholesterol, apolipoprotein (apo) A-I and apoE levels. However, other studies in human subjects using quantitative immunoassays do not provide unambiguous support for such a relationship [19, 20]. Because plasma levels of apolipoproteins, apoC2, apoC3 or apoE can be affected by the plasma amount of lipoproteins or hypertriglyceridemia, plasma apoA5 level in patients with hypertriglyceridemia might not be related with the gene expression or secretion of apoA5 from hepatocytes.

There are a few reports describing the mechanism of the triglyceride-decreasing action of the statins. Although expression of the LDL receptor, known to be involved in LDL clearance is induced by statins, this change cannot fully explain the hypotriglyceridemic action of these drugs. Statins act by inhibiting cholesterol synthesis, which results in the activation of the transcription factor sterol responsive element-binding protein (SREBP) that controls the expression of genes involved in cholesterol homeostasis. SREBP-1c has been reported to down-regulate *APOA5* gene expression via direct binding to the functional E-box present in the human *APOA5* promoter [21]. However, *APOA5* gene expression was up-regulated by pitavastatin or pravastatin in this study. Kasim et al. reported that statin reduced plasma triglyceride levels by reducing the rate of secretion of VLDL in the hypertriglyceridemic Zucker rat model and the effect could be partially reversed by administration of mevalonate [22]. Most pleiotropic effects of statins are thought to be related to inhibition of mevalonate-dependent isoprenylation of small GTP-binding proteins. Several investigators have suggested that some of these pleiotropic effects are mediated by PPARα or PPARγ [23, 24]. It was shown that statins may exert anti-inflammatory properties involving PPARα and PPARγ via generation of PPAR ligands such as 15d-PGJ2 [25]. Such mechanism was also observed in macrophages in which statins inhibit PPARα phosphorylation by downregulating the LPS-induced calcium-dependent PKC signaling pathway [24]. It has also been reported that pitavastatin increases the mRNA levels of apoA1 and PPARα in HepG2 cells [26, 27]. The inhibition of PPARα phosphorylation by statins has been reported to be responsible for the induction of *APOA1* gene expression [28]. This regulation is accounted for by statin-dependent inhibition of geranylgeranylation of RhoA protein. Mevalonate is a key intermediate in the *de novo* synthesis of both sterol and non-sterol isoprenoids. Beneficial effects of statins against atherosclerosis can be attributed to their ability to suppress the synthesis of mevalonate or its downstream products. The present results also showed that inhibition of mevalonic acid-derived products was important for the effect of pitavastatin on *APOA5* promoter activity.

It has been reported that fibrates induce expression of the human *APOA5* gene by PPAR α agonists Wy14,643 or fenofibrate, which interact with a positive PPRE (–272/–260) located in the promoter region of *APOA5* [11]. The retinoic acid receptor-related orphan receptor- α1 (RORα1) and RORα4 also bind specially to the PPRE (–272/–260) of the promoter region and up-regulate *APOA5* gene expression in HepG2 or HuH7 cells [29]. Recently, Huang et al. reported that a combination of atorvastatin and fenofibrate increases apoA5 and decreases triglycerides through up-regulation of PPARα [15]. In this study, pitavastatin increased *APOA5* gene expression and synthesis of apoA5 in human HepG2 cells, and cotransfection of a PPARα showed an additive effect for *APOA5* gene expression.

Statins may increase *APOA5* gene transactivation and synthesis of apoA5 through inhibition of mevalonate or its downstream products. The existence of molecular cross-talk pathways between statins and PPARs offers potentially interesting perspectives for the treatment of dyslipidemia. Although the phenomenon of gene transactivation and synthesis of apoA5 by statins was shown *in vitro*, the current results provide a potential explanation for a part of statin-induced anti-hypertriglyceridemic activity.

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