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Effect of pitavastatin on transactivation of human serum paraoxonase 1 gene☆

Kikuko Ota*, Tadashi Suehiro, Kaoru Arii, Yukio Ikeda, Yoshitaka Kumon, Fumiaki Osaki, Kozo Hashimoto

Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Kochi, Japan

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Abstract

Hepatic hydroxymethyl glutary coenzyme A HMG-CoA reductase inhibitors (statins) have various anti atherosclerosis pleiotropic effects that are independent of cholesterol reduction. Human serum paraoxonase 1 (PON1) is associated with high-density lipoprotein (HDL) and inhibits the oxidative modification of low-density lipoprotein (LDL). We investigated the effects of statins on PON1 gene transcription using a reporter gene assay. Promoter activity of the PON1 gene was estimated by measuring luciferase activity of plasmids with a PON1 promoter region transfected into human hepatoma HepG2 cells and human embryonic kidney (HEK) 293 cells. Pitavastatin, simvastatin, and atorvastatin each significantly increased PON1 promoter activity, and the transactivation by pitavastatin was abrogated by mevalonic acid and farnesyl pyrophosphate (FPP), however, not by geranylgeranyl pyrophosphate. Further, PON1 promoter activity was enhanced by farnesyl transferase inhibitor (FTI), but not by geranylgeranyl transferase inhibitor (GGTI). PON1 gene transcription has been reported to be dependent on Sp1 and the transactivation by pitavastatin was completely abrogated by mithramycin, an inhibitor of Sp1. Our results suggest that pitavastatin activates transcription of the PON1 gene through the FPP pathway, which may play an important role in the anti atherosclerotic effects of statins.

1. Introduction

Human serum paraoxonase 1 (PON1) is an esterase that hydrolyses aromatic carboxylic acid esters, organophosphate, and carbamates [1], and is associated with high-density lipoprotein (HDL) [2]. Although the natural substrate of PON1 in vivo is unknown, PON1 inhibits the oxidation of not only low-density lipoprotein (LDL) but also that of HDL [3-5], and plays an important role in the suppression of development or progression of atherosclerosis [6]. Recently, it was reported that PON1-knockout mice were not protected from the progression of atherosclerosis when consuming a high-fat and high-cholesterol diet [7]. However, atherosclerotic lesion formation was decreased in PON1 transgenic mice [8].

This protein has common polymorphic sites, involving Leu-Met (L/M) at position 55 of the amino acid sequence and Gln-Arg (Q/R) at position 192 [9]. Some studies have shown that these genetic polymorphisms are involved in the development of coronary heart disease (CHD) [10-12], however, others did not find such a relationship [13,14]. Recently, we and others found a polymorphism, cytosine-thymine (C/T) at position –108 from the ATG start codon in the upstream region of the PON1 gene, which may be associated with PON1 transcriptional activity and serum concentration [15-17]. This polymorphism is present in a GC box in the PON1 gene promoter region, where a binding site of Sp1 is thought to exist [15].

We and others have also reported that PON1 activity is related to not only macroangiopathy but also microangiopathy, such as retinopathy or nephropathy, in diabetic patients [10,18,19]. Based on these results, we speculated that PON1 may have protective effects on various types of oxidation in vivo other than a lipoprotein oxidation.

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* Corresponding author. Department of Endocrinology, Metabolism and Nephrology, Kochi Medical School, Kochi University, Kohasu, Oko-cho, Nankoku, Kochi 783-8505, Japan.

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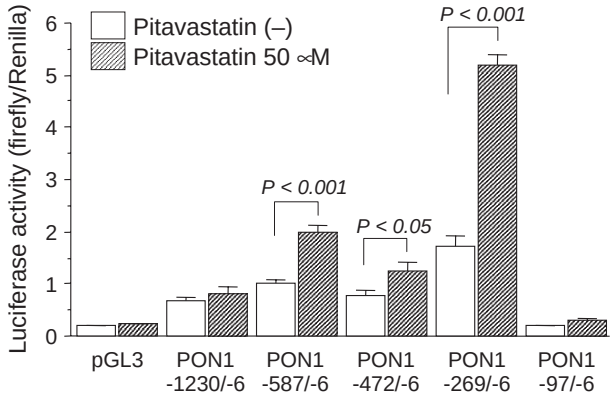

Fig. 1. The effects of pitavastatin on promoter activity of the plasmid with a different PON1 gene 5′ -flanking region length. Each plasmid was transfected into HepG2 cells, which were treated with 50 μmol/L pitavastatin or the vehicle (DMSO) at 60 minutes after transfection. Luciferase activities were measured at 24 hours after transfection. Pitavastatin significantly increased the promoter activity of every plasmid, except that plasmid with the longest fragment, PON1 (–1230/–6) and shortest fragment, PON1 (–97/–6). PON1 (–97/–6) had no GC box.

In a subanalysis of the findings of West of Scotland Coronary Prevention Study (WOSCOP), which conducted a large clinical trial using pravastatin, statins were estimated to have pleiotropic effects [20]. Further, many basic and clinical studies have shown that statins have antiatherosclerosis pleiotropic effects in addition to a cholesterol-lowering action. One of these pleiotropic effects is thought to be antioxidization [21], as it has been reported that simvastatin inhibited macrophage-dependent oxidization of LDL [22] and atorvastatin inhibited Cu-derived oxidization of LDL [23]. In addition, a clinical study reported that simvastatin normalized low levels of PON1 enzyme activity in patients with familial hypercholesterolemia [24], although the mechanism was not elucidated. This background led us to investigate whether PON1 was involved in the antioxidative effects of statins, especially transcription of the PON1 gene. We studied the effects of pitavastatin on promoter activity of the PON1 gene using a reporter gene assay method with human hepatoma HepG2 cells and human embryonic kidney (HEK) 293 cells. Our results showed that statins enhanced the promoter activity of the PON1 gene, which may have occurred primarily through a mevalonic acid-derived farnesyl pyrophosphate pathway.

2. Materials and methods

2.1. Cell cultures

HepG2 cells were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) (Sigma, St Louis, MO) supplemented with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, and 20 μg/mL streptomycin in a 90-mm plastic plate in a culture incubator with 5% CO_2 at 37°C. HEK293 cells were cultured in the same way, except for the use of high glucose DMEM (4.5 g/L).

2.2. Plasmid constructs for luciferase assay

We used plasmid constructs with the PON1 gene 5′ -flank region for a luciferase assay as reported in our previous study [25]. DNA fragments of the PON1 gene, (–1230/–6), (–587/–6), (–472/–6), (–316/–6), and (–97/–6), were introduced to pGL3 Luciferase Reporter Vectors (Promega, Madison, WI). The number of DNA fragments is shown from the ATG start codon, because the transcription site has not been identified. Further, the shortest fragment (–97/–6) did not include the GC box (–112/–103), which may be an Sp1 binding site [15].

2.3. Expression vectors

We constructed an expression vector of the Sp1 protein for mammalian cells. The insert DNA fragment was

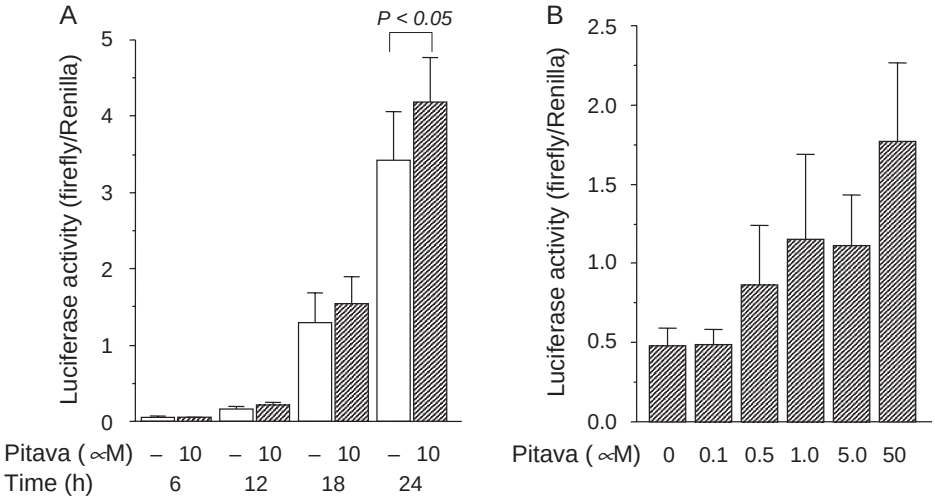

Fig. 2. (A) Time course study. PON1 (–587/–6) plasmid was transfected into HepG2 cells and treated with 10 μmol/L of pitavastatin or the vehicle (DMSO) at 60 minutes after transfection. Luciferase activities were measured every 6 hours for 24 hours. Pitavastatin significantly increased luciferase activity at 24 hours after transfection. (B) Dose-dependent study. PON1 (–587/–6) plasmid was transfected into HepG2 cells with various concentrations of pitavastatin and luciferase activities were measured at 24 hours. Pitavastatin from 0.5 to 50 μmol/L significantly increased the luciferase activity in a dose-dependent manner.

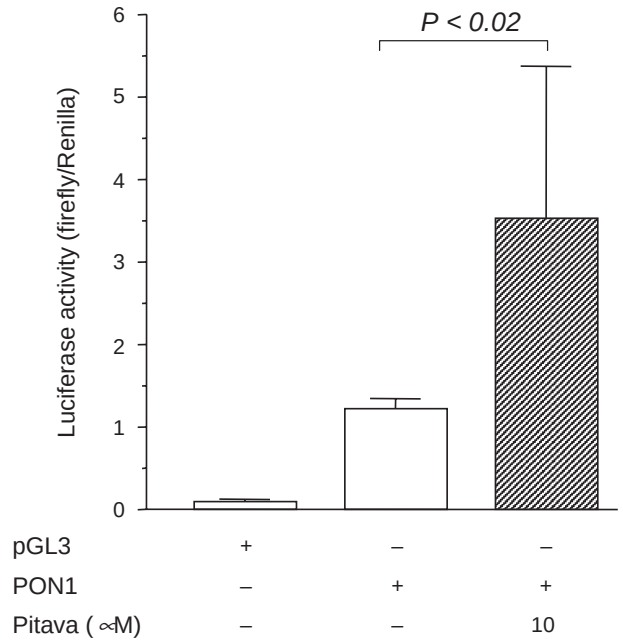

Fig. 3. Effect of pitavastatin on PON1 promoter activity in HEK293 cells. PON1 (–587/–6) plasmid was transfected into HEK293 cells and treated with 10 μmol/L pitavastatin or with the vehicle (DMSO) at 60 minutes after transfection. Luciferase activities were measured at 24 hours after transfection. Pitavastatin significantly increased the promoter activity in HEK293 cells.

amplified by a sense primer, CGGAATTCTGCCACCATGAGCGACCAAG, and an antisense primer, GCTCTAGACGGGTGCCTGATCTCAGAAG, using cDNA from mRNA obtained from the whole blood of one of the authors (T.S.) as a template. The 5′-terminal structure and ATG start codon were determined according to the report of Takahara et al, [26] and the 3′-terminal sequence was determined according to accession number J03133 in GenBank. Underlined capitals in the sense and antisense primers show the restriction sites of EcoRI and XbaI, respectively, while double-underlined capitals denote the ATG start and stop codons. The DNA fragment was introduced into a mammalian expression vector, pCL-neo Mammalian Expression Vector (Promega), and subcloned into JM109 cells. The plasmid construct was confirmed by a cycle sequencing method using a commercial kit and analyzer (BigDye Terminator Cycle Sequencing FS Ready Reaction Kit; ABI PRISM310 Genetic Analyzer, PE Applied Biosystems, Foster City, CA).

2.4. Transfection and luciferase assays

Transient transfection into HepG2 and HEK 293 cells was performed using a cationic lipid method employing Tfx-20 (Promega) and Transfast (Promega), respectively, according to the manufacturer's instructions. HepG2 cells and HEK293 cells were seeded separately into 24-well plates at 5×10^4 cells per well and grown to 80% to 90% confluence. PON1 plasmid DNA (0.4 μg/well) was then co transfected with or without Sp1 expression vectors, as well as empty vectors. Cell extracts were prepared at various times for the luciferase activity assay.

pRL-TK vectors (Promega) (0.2 μg/well), which expressed Renilla luciferase, were cotransfected as an internal control. When the Sp1 expression vector was cotransfected, we corrected it using the protein concentration of the cell

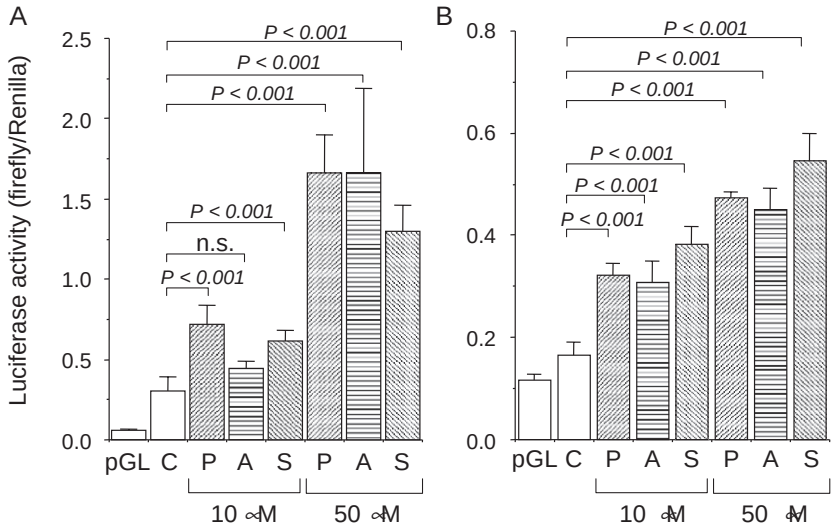

Fig. 4. Effects of 3 different statins (P, pitavastatin; A, atorvastatin; S, simvastatin) on PON1 promoter activity. PON1 (–587/–6) plasmid was transfected into both HepG2 and HEK293 cells, and then each were treated with 10 or 50 μmol/L statin, or with the vehicle (DMSO) at 60 minutes after transfection. Luciferase activities were measured at 24 hours after transfection. (A) In HepG2 cells, 10 μmol/L simvastatin and 10 μmol/L pitavastatin each significantly increased PON1 promoter activity, as did 50 μmol/L pitavastatin, atorvastatin, and simvastatin. (B) In HEK293 cells, 10 μmol/L and 50 μmol/L of each statin significantly increased promoter activity.

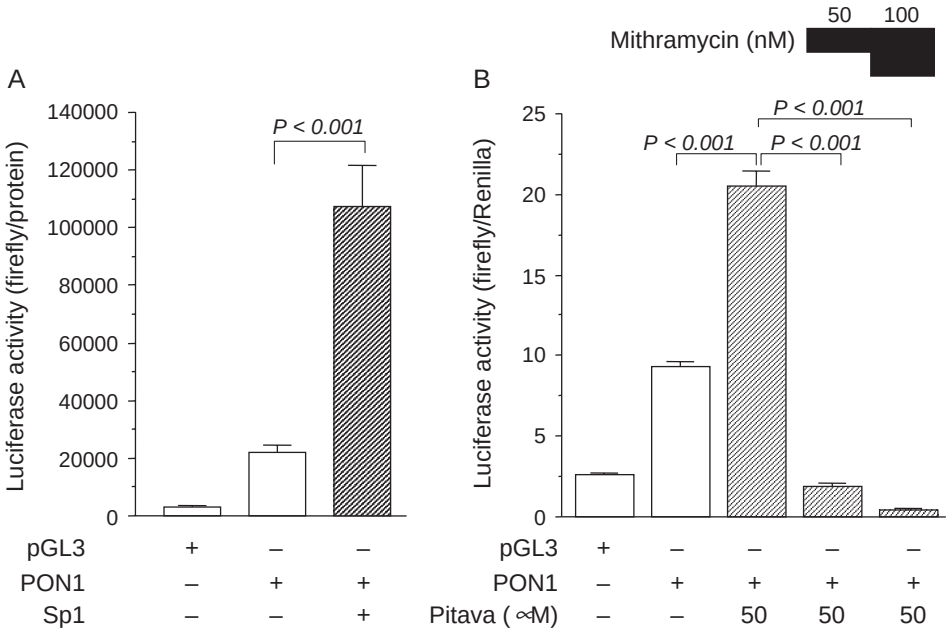

Fig. 5. Relationship of Sp1 to PON1 promoter activity. (A) PON1 (–587/–6) plasmid was transfected into HepG2 cells, and the Sp1 expression plasmid or an empty vector was simultaneously cotransfected. Luciferase activities were measured at 24 hours after transfection. Overexpression of Sp1 significantly enhanced PON1 promoter activity. (B) PON1 (–587/–6) plasmid was transfected into HepG2 cells, and treated with 50 μmol/L pitavastatin or the vehicle (DMSO) along with 10 or 50 nmol/L mithramycin at 60 minutes after transfection. Luciferase activities were measured at 24 hours after transfection. Mithramycin completely suppressed the PON1 promoter activity enhanced by 50 μmol/L pitavastatin.

lysates as an internal control, because over-expression of Sp1 activated the promoter of Renilla luciferase plasmids. Protein concentrations were measured using a commercial kit (Bio-Rad Protein Assay, Bio-Rad, Hercules, CA). Both firefly and Renilla luciferase activities in the cell lysates were measured using a Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. Promoter activities were expressed as firefly luciferase activity divided by Renilla luciferase activity or by protein concentration. Six wells were used for each

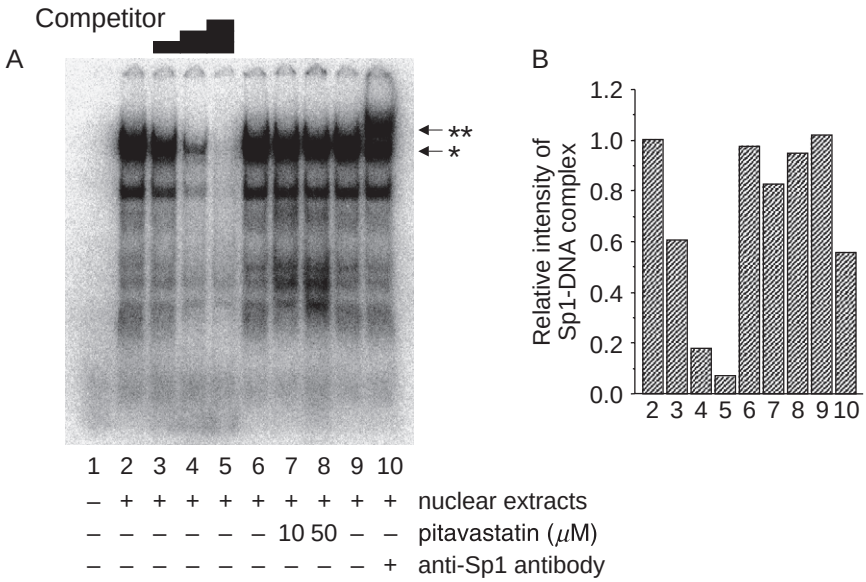

Fig. 6. EMSA results of mixtures incubated with PON1 DNA fragments and HepG2 nuclear extracts. (A) Lane 1, no nuclear extracts; lanes 2, 6, and 9, DNA fragments and nuclear extracts; lanes 3, 4, and 5, DNA fragments, nuclear extracts, and competitors (cold-DNA fragments); lanes 7 and 8, DNA fragments and nuclear extracts from HepG2 cells treated with 10 or 50 μmol/L pitavastatin, respectively; lane 10, DNA fragments, nuclear extracts, and anti-Sp1 antibody. The Sp1-DNA complex band, indicated by an asterisk, disappeared following addition of the competitors and was supershifted after addition of the anti-Sp1 antibodies (double asterisks). (B) Relative intensities of the complexes with DNA fragments and Sp1. Lane 2 was used as a control and had an intensity of 1.0. The band intensities of the nuclear extracts treated with pitavastatin (7 and 8) were the same as those of nuclear extracts without pitavastatin treatment (2, 6, and 9).

transfection condition. Each examination was repeated 3 times or more and representative results are shown.

2.5. Treatment

We used pitavastatin to study the effect of statins on PON1 promoter activity, and also compared the effect with those of atorvastatin and simvastatin. Pitavastatin and atorvastatin were obtained from Kowa (Tokyo, Japan), and simvastatin was purchased from LKT (St Paul, MN). Each statin was dissolved in dimethyl sulfoxide (DMSO), with the final DMSO concentration adjusted to 0.1%. Wells without statin were also adjusted to 0.1% by the vehicle (DMSO). Treatments with pitavastatin or other statins started at 60 minutes after transfection. For inhibition of Sp1, 50 or 100 nmol/L mithramycin (Sigma) was added 60 minutes after transfection. To study the effects of mevalonic acid, geranylgeranyl pyrophosphate (GGPP), and FPP on the statins-induced PON1 transactivation, each reagent with various concentrations was added with a statin at 60 minutes after transfection. To examine the effects of GGTI-287 (Calbochem, Darmstadt, Germany) and farnesyl transferase inhibitor (FTI)-277 (Calbochem) on PON1 promoter activity, each agent was added without a statin at 60 minutes after transfection.

2.6. HepG2 cell nuclear extraction

HepG2 cells, untreated or treated with 10 μmol/L or 50 μmol/L pitavastatin, were cultured separately in 90-mm diameter plastic plates and harvested after 24 hours. Cells on each plate were washed twice with cold phosphate-buffered saline, pH 7.4, and collected with buffer A [20 mmol/L HEPES, pH 7.9, 20% glycerol, 10 mmol/L NaCl, 1.5 mmol/L $MgCl_2$, 1 mmol/L dithiothreitol, and 0.1% NP-40, with a protease inhibitor cocktail tablet (Roche Diagnostics, Manheim, Germany)]. After incubation on ice for 5 minutes, the cells were centrifuged at 2,000 rpm at 4°C for 5 minutes. The pellets were then resuspended in buffer B, which was the same as buffer A except for 500 mmol/L NaCl instead of 10 mmol/L, and incubated on ice for 30 minutes. After centrifugation at 15,000 rpm for 15 minutes, the nuclear extract was obtained as a supernatant, after which each protein concentration was adjusted to 5.0 μg/μL, divided into plastic tubes, and preserved at –80°C.

2.7. Electrophoretic mobility shift assay

The synthetic sense and antisense strands of the oligonucleotides (–123/–93) were 5′-GACCCGGCGGGGAGGGGCGGGGCGGGCCAA-3′ and 5′-ATTGGCCCGCCCCGCCCCTCCCCGCCGGGT-3′, respectively. The 5′-ends of the oligonucleotides were labeled with [γ-^{32}P]adenosine triphosphate (ATP) using a commercial radio-labeling kit (MEGALABEL, Takara, Tokyo, Japan) with T4 polynucleotide kinase. Each HepG2 cell nuclear extract (10.0 μg) was incubated in 20 μL of solution containing 6 μL of nuclear extract buffer A, 1 μg of poly (dI-dC), 0.1% bovine serum albumin, 2 mmol/L dithiothreitol, 30 μmol/L $ZnCl_2$, and [γ-^{32}P] labeled oligonucleotides at room temperature for 20 minutes. In a competitive study, nonradiolabeled oligonucleotides were added simultaneously. For a supershift study, antibodies were incubated at room temperature for 15 minutes following the above incubation protocol. The antibody used was Sp1-specific polyclonal antibody (PEP2) (Santa Cruz Biotechnology, Santa Cruz, CA). The mixtures underwent electrophoresis on 4% polyacrylamide gels in 0.25 × TBE buffer at 200 V in a cold room. Each dried gel was analyzed by a computerized system for radioluminography (BAS2500, Fuji Photo Film, Kanagawa, Japan) and analyzing software (MacBAS version 2.3, Fuji Photo Film), and band intensities were compared using the software.

2.8. Statistical analysis

Results of the reporter gene assay are presented as mean ± S.E.M. Comparisons of 2 groups were made using an unpaired t test. Statistical significance was defined as $P < .05$.

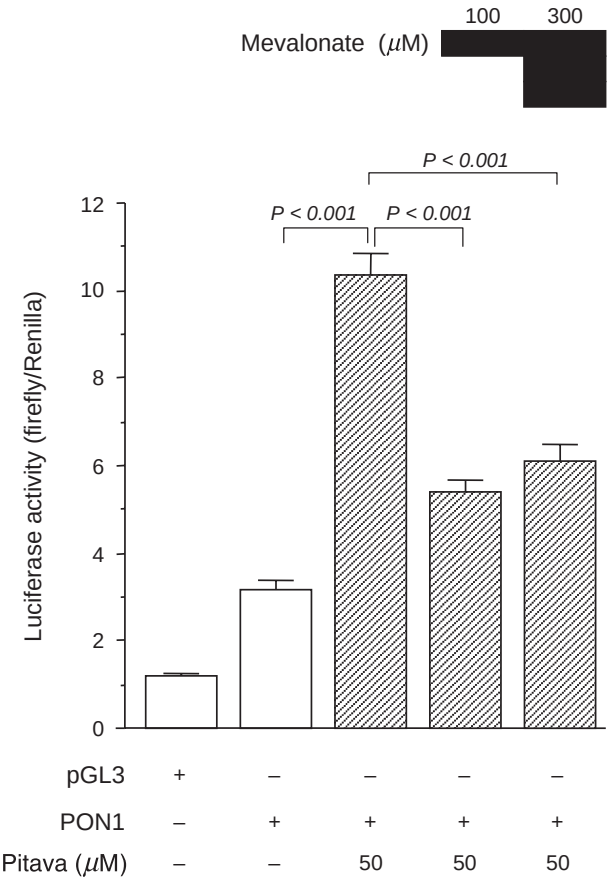

Fig. 7. Effect of mevalonic acid on PON1 promoter activity increased by pitavastatin. PON1 (–587/–6) plasmid was transfected into HepG2 cells, which were then treated with 50 μmol/L pitavastatin and 100 or 300 μmol/L mevalonic acid at 60 minutes after transfection. Luciferase activities were measured at 24 hours after transfection. Mevalonic acid at 100 or 300 μmol/L significantly abrogated the enhancement effect of pitavastatin.

3. Results

3.1. Effect of pitavastatin on PON1 promoter activity in HepG2 cells

We studied the effects of pitavastatin on promoter activity of the plasmid with different lengths of the 5′-flanking region of the PON1 gene. Each plasmid was transfected into HepG2 cells with or without 50 μmol/L pitavastatin, and luciferase activities were measured at 24 hours after transfection.

Pitavastatin significantly increased every promoter activity of the plasmid, except for plasmids with the longest PON1 (–1230/–6), and shortest PON1 (–97/–6) fragments (Fig. 1). Therefore, we used this plasmid with PON1 (–587/–6) in the subsequent experiments.

3.2. Time course and dose dependency of PON1 promoter activities

For a time course study, we transfected the plasmid with PON1 (–587/–6) into HepG2 cells with or without 10 μmol/L pitavastatin and measured luciferase activities every 6 hours for 24 hours. Pitavastatin significantly increased luciferase activity at 24 hours after transfection (Fig. 2A), and also increased in a dose-dependent manner from 0.5 to 50 μmol/L (Fig. 2B). Based on these results, we used 10 μmol/L or 50 μmol/L of statin in the following experiments and measured luciferase activity at 24 hours after transfection.

3.3. Effect of pitavastatin on PON1 promoter activity in HEK293

We confirmed the effect of pitavastatin on PON1 promoter activity in another cell line, HEK293. Ten micromoles of pitavastatin significantly increased promoter activity in HEK293 cells (Fig. 3).

3.4. Effects of different statins on PON1 promoter activity

We next examined whether statins other than pitavastatin had the same effect on PON1 promoter activity in both HepG2 and HEK293 cells. In HepG2 cells, 10 μmol/L simvastatin and pitavastatin significantly increased PON1 promoter activity, as did 50 μmol/L pitavastatin, atorvastatin, and simvastatin (Fig. 4A). Likewise, in HEK293 cells, both 10 μmol/L and 50 μmmol/L of each statin significantly increased promoter activities (Fig. 4B).

3.5. Effects of Sp1 overexpression and Sp1 inhibitor on PON1 promoter activity

We also studied the relationship of Sp1 with PON1 promoter activity. The plasmid with the PON1 promoter was transfected with or without the Sp1 expression vector. An overexpression of Sp1 significantly enhanced PON1 promoter activity (Fig. 5A), while mithramycin, an Sp1 inhibitor, nearly completely suppressed the PON1 promoter activity enhanced by pitavastatin (Fig. 5B).

3.6. Electrophoretic mobility shift assay

Electrophoretic mobility shift assay (EMSA) results of a mixture of the DNA fragment from the PON1 promoter and HepG2 nuclear extracts, are shown in Fig. 6A. The Sp1-DNA complex band, indicated by an asterisk, disappeared following addition of the competitors, unlabeled DNA fragments, and supershifted with addition of the anti-Sp1 antibodies (double asterisks). Treatment with pitavas-

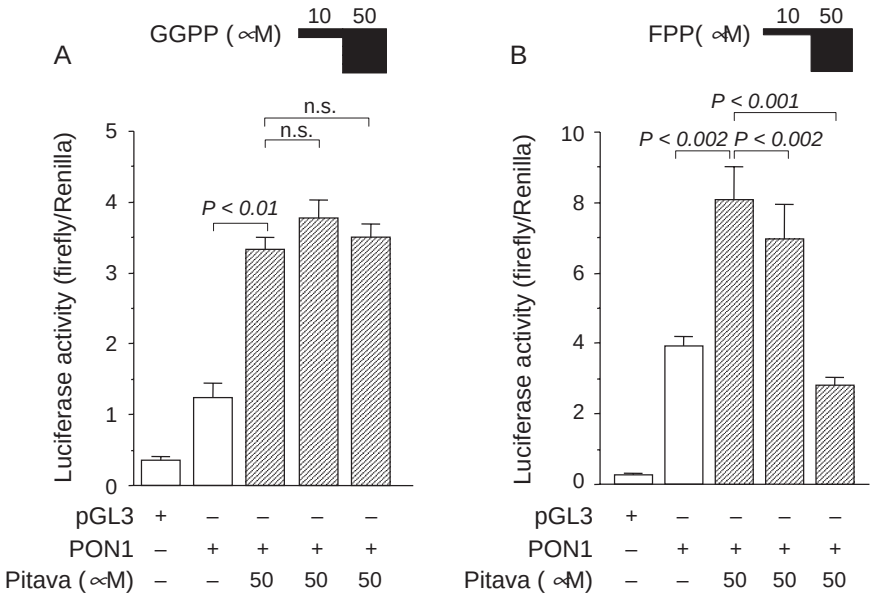

Fig. 8. Effect of GGPP and FPP on PON1 promoter activity increased by pitavastatin. PON1 (–587/–6) plasmid was transfected into HepG2 cells, which were then treated with 50 μmol/L pitavastatin and 10 or 50 μmol/L GGPP, or 10 or 50 μmol/L FPP at 60 minutes after transfection. Luciferase activities were measured at 24 hours after transfection. (A) GGPP did not abrogate the enhancement effect of pitavastatin. (B) FPP at 10 μmol/L significantly weakened the pitavastatin effect and 50 μmol/L completely canceled it.

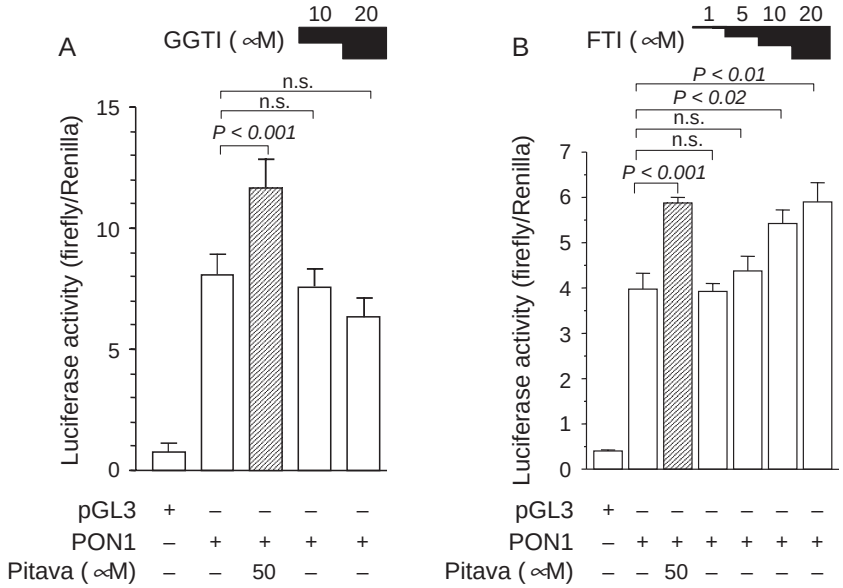

Fig. 9. Effects of GGTI = 287 and FTI = 277 on PON1 promoter activity. PON1 (–587/–6) plasmid was transfected into HepG2 cells, which were then treated with 50 μmol/L pitavastatin, 10 or 20 μmol/L GGTI = 287, or 1, 5, 10, or 20 μmol/L FTI = 277 at 60 minutes after transfection. Luciferase activities were measured at 24 hours after transfection. (A) GGTI = 287 did not enhance the activity. (B) FTI = 277 increased PON1 promoter activity in a dose-dependent manner, and 20 μmol/L FTI = 277 enhanced the activity to the same degree as 50 μmol/L pitavastatin.

tatin did not have an influence on the intensity of the Sp1-DNA complex band (Fig. 6B).

3.7. Effects of mevalonic acid, GGPP, and FPP on pitavastain-induced increase of PON1 promoter activity

We attempted to determine which pathway in cholesterol synthesis was related to the effect of statins on PON1 promoter activity. Mevalonic acid was added to the culture plates after transfection of the plasmid with the PON1 promoter. Mevalonic acid at concentrations of 100 and 300 μmol/L significantly abrogated the enhancement effect of pitavastatin on promoter activity (Fig. 7).

Next, GGPP and FPP were separately added to culture plates following transfection of the plasmid. GGPP did not have any effect on pitavastatin-induced PON1 promoter activity (Fig. 8A), whereas 10 μmmol/L FPP significantly weakened the pitavastatin effect and 50 μmol/L completely canceled it (Fig. 8B).

3.8. Effects of geranylgeranyl transferase and farnesyl transferase inhibitors on PON1 promoter activity

Finally, we investigated whether GGTI = 287 or FTI = 277 had any effects similar to the statins on PON1 promoter activity. GGTI = 287 and FTI = 277 were separately added to culture plates following transfection of the plasmid with the PON1 promoter. GGTI = 287 did not enhance the activity in contrast to pitavastatin (Fig. 9A), however, FTI = 277 increased the activity in a dose dependent manner and 20 μmmol/L FTI = 277 enhanced the PON1 promoter activity to the same degree as 50 μmmol/L pitavastatin (Fig. 9B).

4. Discussion

The present results show that pitavastatin increases PON1 promoter activity. However, because the PON1 gene promoter activity was increased by 2 other fat-soluble statins as well, we concluded that the transactivation was not specific to pitavastatin, but rather a general effect of statins.

Many pleiotropic effects of statins have been reported to depend on suppression of the synthesis of mevalonic acid-derived GGPP [27-29]. GGPP biologically activates several small G proteins, such as the Rho family, through isoprenylation of the proteins that translocate from cytoplasm to membrane [30]. Suppression of GGPP by statins decreases the isoprenylated Rho protein, which is involved in various types of signal transduction downstream of Rho.

FPP induced by mevalonic acid also activates other small G proteins, such as the Ras family, through isoprenylation and translocation of those proteins [31]. Inhibition of FPP by statins also suppresses the activation of Ras, and such inhibited Ras was shown to decrease MMP-2 secretion [32]. The present results also showed that inhibition of mevalonic acid-derived FPP, but not GGPP, was important for the effect of pitavastatin on PON1 promoter activity. Pitavastatin increased the transactivation of PON1, while FPP, but not GGPP, abrogated that effect. Further, FTI = 277, but not GGTI = 287, increased the transactivation as well. These results suggest that the primary effect of pitavastatin on the transactivation comes from the mevalonic acid-derived FPP pathway.

We have scant data available to explain why the inhibition of FPP by pitavastatin enhanced PON1 gene promoter activity; however, several mechanisms are spec-

ulated. As shown in our results, the PON1 gene promoter was activated by Sp1 and the increased transactivation of PON1 by pitavastatin was completely suppressed by the Sp1 inhibitor. The EMSA results showed that Sp1 bound the PON1 DNA fragments, including a GC box (–112/–103). Therefore, we considered that the effect of pitavastatin on PON1 transactivation was an action in conjunction with Sp1. Suppressed Ras activity by inhibition of FPP may upregulate some genes that activate the Sp1 gene promoter and PON1 gene transcription may be increased sequentially. Alternatively, it has been reported that mevalonic acid depletion by a statin induced an increase of Ras synthesis and decreased its degradation, which increased the total mass of Ras [33]. The increase of total RAS amount upregulates Ras related proteins regardless of the intracellular translocation of Ras. This mechanism has been suggested as a determinant of cell growth [34,35] and may also be important for Sp1 activity. Our EMSA results showed that there were no differences of Sp1-DNA complex band intensities between cells treated and untreated by pitavastatin, which suggests that pitavastatin itself did not enhance the binding activity of Sp1 to DNA, but rather had an effect on the action after binding, or was involved in other cofactors that are important for the function of Sp1. Several types of signal transduction occur downstream of Ras and each crosstalks with each other. The transduction downstream of Ras to Sp1 has not been identified, therefore, in the future we intend to study the pathway of Ras to Sp1 as well as other factors and to clarify the mechanism of increased PON1 transactivation by statins.

Recently, Deakin et al [36] reported that simvastatin upregulated the activity of the PON1 gene promoter in expression cassettes transfected into HepG2 cells, and patients treated with the statin showed a significant increase in serum concentrations and activities of PON1. They also found that coincubated mevalonic acid and FPP abolished the effect of the statin on PON1 transactivation, while GGPP was less effective than mevalonic acid or FPP. Their EMSA results also showed that the intensity of the band was significantly greater after incubation with nuclear extracts from simvastatin-treated cells than those from untreated cells. Their and our study results regarding the effect of GGPP were similar and showed that GGPP has less or no statin effect.

EMSA results were different between 2 studies. Our EMSA findings showed no difference in the intensities of DNA-nuclear extract complexes between pitavastatin-treated cells and untreated cells. Although the reason for the contrasting EMSA results is unknown, the use of different probes or different cell culture conditions with the statins may be responsible. Deakin et al [36] also revealed that sterol regulatory element-binding protein-2 (SREBP-2) was involved in transactivation of the PON1 gene. We found 4 sequences of the SREBP-1 binding site in the PON1 promoter region (–149/–66) using TRANSFAC (http://www.gene-regulation.com), which showed low core and matrix matches. In the future we hope to reconfirm the effect of SREBPs on PON1 transactivation.

Although the phenomenon of PON1 transactivation by statins was shown in vitro, there may be other mechanisms involved with the increase of PON1 activity or concentration by statins. The ability of PON1 to protect LDL against oxidation is accompanied by inactivation of the enzyme [37], and a statin has been reported to act as an antioxidant with lipoprotein particles [38]. Together, these findings suggest that the reduction of LDL oxidation by statins enhances PON1 activity. Therefore, other possible mechanisms should also be investigated in the future.

In conclusion, pitavastatin and other statins increased PON1 gene transactivation through an inhibition of mevalonic acid-derived FPP. We speculated that this effect may be one of the in vivo mechanisms of stain-induced antioxidization.

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