J Pharmacol Sci **103**, 383 – 390 (2007)

Journal of Pharmacological Sciences

Full Paper

Stearoyl-CoA Desaturase Activity Is Elevated by the Suppression of Its Degradation by Clofibric Acid in the Liver of Rats

Tomoaki Toyama[1], Naomi Kudo[1,*], Atsushi Mitsumoto[2], Yasuhide Hibino[1], Tadashi Tsuda[1], and Yoichi Kawashima[1]

[1]*Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan*
[2]*Faculty of Pharmaceutical Sciences, Josai International University, 1 Gumyo, Togane, Chiba 283-8555, Japan*

Received September 20, 2006; Accepted February 8, 2007

Abstract. A mechanism by which fibrates control stearoyl-CoA desaturase (SCD) in the liver was studied. Treatment of rats with 2-(4-chlorophenoxy)-2-methylpropionic acid (clofibric acid) or feeding of a fat-free diet markedly elevated hepatic activity of SCD. Both the treatment with clofibric acid and the feeding of the fat-free diet caused an increase in the steady-state level of SCD1 mRNA and enhanced transcriptional rate. The half-lives of SCD for control rats, rats treated with clofibric acid rats, and rats fed the fat-free diet were estimated to be 2.0, 3.9, and 1.9 h, respectively. Activity of palmitoyl-CoA chain elongase (PCE) was increased by both clofibric acid treatment and feeding of the fat-free diet as was observed with SCD. Steady-state level of rat fatty acid elongase 2 mRNA was increased by the treatment with clofibric acid or feeding of fat-free diet, although the transcriptional rate was not altered. Different from SCD, PCE was highly stable and its half-life was not changed by either clofibric acid or fat-free diet. These results strongly suggest that the decreased degradation of SCD is responsible for the increase in its activity in addition to increased transcription of SCD1 in the rats treated with clofibric acid.

Keywords: stearoyl-CoA desaturase, protein degradation, palmitoyl-CoA chain elongase, clofibric acid

Introduction

Fibrates are a widely used class of lipid-modifying agents and several large intervention trials have revealed the potential of fibrates to reduce cardiovascular events (1 – 3). Treatment with fibrates results in a decrease in plasma triglycerides and is usually associated with a moderate decrease in low-density lipoprotein cholesterol and an increase in high-density lipoprotein cholesterol (4). Fibrates also affect the expression of several genes involved in fibrinolysis and inflammation (5, 6). Accelerated formation of oleic acid in the liver of rodents was known as one of the pharmacological actions of fibrates.

Stearoyl-CoA desaturase (SCD) is an endoplasmic reticulum protein that catalyzes the conversion of stearoyl-CoA to oleoyl-CoA (or palmitoyl-CoA to palmitoleoyl-CoA). Recently, SCD has been shown to play an important role in lipogenesis (7). Namely, changes in the activity of SCD have been observed in a wide range of disorders including diabetes, obesity, and cancer (7 – 9). Thus, SCD is considered to be a key enzyme for developing a therapy to control these diseases.

SCD activity is elevated in the liver of insulin-resistant Zucker fa/fa rats (9) and type II diabetes ob/ob mice (7), and SCD is induced by feeding the Zucker fa/fa rats a high carbohydrate diet (10) or the administration of dehydroepiandrosterone (11) and peroxisome proliferators such as 2-(4-chlorophenoxy)-2-methylpropionic acid (clofibric acid) (12). Fibrates including clofibric acid are the ligands for peroxisome proliferator-activated receptor alpha (PPARα), which regulates expression of a number of genes responsible for fatty acid oxidation (13). At least four genes coding SCD have been cloned (14 – 17) and, among them,

*Corresponding author. naokudo@josai.ac.jp
Published online in J-STAGE: March 31, 2007
doi: 10.1254/jphs.FP0061124

SCD1 is believed to be transcriptionally regulated by PPARα (18). However, the published findings have raised the following contradictions about the action mechanism of fibrates: 1) Clofibrate (ethyl ester of clofibric acid) needs 30 hours to induce SCD1 mRNA to the maximal level in the liver when dosed to mice (18), while typical PPAR target genes are maximally induced within several hours after the treatment of Wy-14643 (a peroxisome proliferator) (19). 2) In HepG2 cells and LMH cells, induction of SCD1 mRNA is not brought about by the treatment of the cells with fibrates (20, 21). 3) Clofibric acid strongly increased SCD activity in the liver of rats (12), although SCD1 promoter weakly responds to the drug in HepG2 cells (18). These facts raise questions about whether clofibric acid induces SCD by causing transcriptional activation and whether some other regulation mechanism is operating for the induction of SCD by the drug.

Oleic acid is the fatty acid that animals are able to biosynthesize from palmitic acid. Biosynthesis of oleic acid involves sequential reactions catalyzed by SCD and palmitoyl-CoA chain elongase (PCE) (22). Under altered physiological conditions such as essential fatty acid deficiency, re-feeding after fasting, and treatment with clofibric acid, both SCD and PCE are induced, resulting in the increase in the synthesis of oleic acid in the liver of mammals (9, 10, 23 – 25), while starvation decreases the formation of oleic acid by suppressing both SCD and PCE (23 – 25). These facts made investigators speculate that there may be a common mechanism for the regulation of SCD and PCE.

The present study was undertaken to study the response of SCD to clofibric acid with regard to the degradation of its protein in vivo in comparison with the response of SCD to fat-free diet feeding and in comparison with the response of PCE to clofibric acid. This study showed that both transcriptional activation and suppression of degradation are responsible for the induction of SCD by the treatment with clofibric acid. Fat-free diet caused transcriptional activation of SCD1 but did not affect the half-life of SCD in the liver. In contrast to SCD, the prolonged half-life of PCE was not changed by clofibric acid. These results propose a new mechanism for the action of clofibric acid by which fibrates regulate the enzymes that are involved in oleic acid formation in the liver.

Materials and Methods

Materials

[2-^{14}C]Malonyl-CoA (55 Ci/mol) was purchased from Amersham Bioscience, Inc. (Tokyo). [α-^{32}P]UTP was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA). Stearoyl-CoA, palmitoyl-CoA, malonyl-CoA, clofibric acid, and bovine serum albumin were from Sigma (St. Louis, MO, USA); NADH was from Oriental Yeast Co. (Tokyo). Oligonucleotide were from Sigma Genosys (Ishikari). All other chemicals used were of analytical grade.

Animals and treatments

Male Wistar rats (4 – 5-week-old) were obtained from SLC (Hamamatsu). Animals were maintained on a 12-h light/dark cycle (lights on 7:00). Rats were fed on a standard chow (CE-2) (Clea Japan, Tokyo) ad libitum and allowed free access to water. After acclimatization for 1 week, some rats aged 6 weeks were given subcutaneous injection of clofibric acid at a dose of 100 mg/kg or 0.9% NaCl solution twice a day for 7 days, and other rats aged 5 weeks were fed a fat-free diet (26) for 2 weeks. The fat-free diet contained 14% sucrose, 14% milk casein, 45.6% corn starch, 15.5% α-starch, 5.0% cellulose, 3.5% mineral mix (AIN-93M), 1.0% vitamin mix (AIN-93M), 0.18% L-cystine, 0.25% choline bitartrate, and 0.008% butylated hydroxytoluene. Blood was obtained from the descending vena cava under diethylether anesthesia. In some experiments, cycloheximide (CHX) dissolved in 0.9% NaCl was intraperitoneally injected as an inhibitor of protein synthesis at a dose of 2.0 mg/kg (27). Rats were killed 2, 4, and 6 h after dosing CHX and then the livers were removed for the preparation of hepatic microsomes. All animal studies complied with the institutional board for animal study, Josai University.

Preparation of microsomes

Livers were perfused with ice-cold 0.9% NaCl. Part of the livers was frozen in liquid nitrogen and stored at −80°C for preparation of total RNA while another part of the livers was homogenized with 4 volumes of 0.25 M sucrose / 1.0 mM EDTA / 10 mM Tris (pH 7.4) in a Potter glass-Teflon homogenizer. The remaining homogenates were centrifuged at 18,000 × g for 20 min, and the supernatant obtained was recentrifuged under the same conditions. The resulting supernatant was centrifuged at 105,000 × g for 60 min. The pellet was resuspended in 0.25 M sucrose / 0.1 mM EDTA / 10 mM Tris (pH 7.4) and recentrifuged under the same conditions. The resulting pellet was resuspended in a small volume of 0.25 M sucrose / 0.1 mM EDTA / 10 mM Tris (pH 7.4) and used as an enzyme source (microsomes). All the operations mentioned above were carried out at 0 – 4°C. Protein concentrations were determined by the method of Lowry et al. (28) using bovine serum albumin as a standard.

Enzyme assays

SCD activity was measured spectrophotometrically as described by Oshino et al. (29), as the rate constant for the stearoyl-CoA-stimulated re-oxidation of NADH-reduced cytochrome b_5. The rates of cytochrome b_5 oxidation were measured with absorbance between 424 and 409 nm at 30°C. The cuvette contained 0.9 mg of microsomal protein and 300 μmol of Tris (pH 7.4) in a final volume of 3.0 ml. Microsomal cytochrome b_5 was reduced by 2 nmol of NADH, and re-oxidation was recorded. When the re-oxidation was completed, 20 nmol of stearoyl-CoA was added and cytochrome b_5 was reduced again by 2 nmol of NADH. The first-order constant for the re-oxidation of NADH-reduced cytochrome b_5 was calculated as described by Oshino and Sato (30). The rate constant for the re-oxidation of NADH-reduced cytochrome b_5 was measured in the presence (k) and in the absence (k^-) of stearoyl-CoA; the rate constant for SCD was given by $k^+ = k - k^-$.

The assay for microsomal palmitoyl-CoA condensation was performed by the measurement of the incorporation of [2-^{14}C]malonyl-CoA into palmitoyl-CoA (26) with some minor modifications as follows: The assay mixture for palmitoyl-CoA condensation contained 15 nmol palmitoyl-CoA, 100 nmol [2-^{14}C]malonyl-CoA (20 nCi), 12 nmol bovine serum albumin (fatty acid-free), 0.5 μmol KCN and 200 – 250 μg microsomal protein in 0.5 ml of 100 mM Tris buffer (pH 7.4). The mixture was incubated at 37°C for 4 min under nitrogen. The incubation mixture without palmitoyl-CoA was run simultaneously. After stopping the enzymatic reaction by the addition of 1 ml of 10% KOH / 90% methanol, the mixture was heated at 80°C for 30 min under nitrogen and then acidified by adding 2 ml of 6 M HCl. Reaction products were extracted four times with 2 ml of *n*-hexane. The combined *n*-hexane extract was washed with 4 ml of acidic water, transferred to a counting vial, and taken to dryness. The residue was dissolved in toluene scintillator and the radioactivity was measured by a liquid scintillation counter (LSC-5100; Aloka, Tokyo). The control value, which was obtained from the incubation without palmitoyl-CoA, was subtracted to give the net condensation rate for the palmitoyl-CoA.

Preparation of cDNA probes

SCD1 (GenBank accession no. J02585), SCD2 (GenBank accession no. AB032243), rat fatty acid elongase 1 (rELO1) (GenBank accession no. AB071985), rELO2 (GenBank accession no. AB071986), and β-actin (GenBank accession number V01217) DNA probes were synthesized by polymerase chain reaction (PCR). Amplified cDNAs were inserted into the *Sma* I site of the pBluescript vector (Stratagene, La Jolla, CA, USA). Each plasmid was denatured by treatment with NaOH and applied to a nitrocellulose filter using a slot bolt apparatus (Bio-Rad, Hercules, CA, USA). Primer sequences for probe amplification were as follows:

SCD1: sense, 5'-AAAGTTTCTAAGGCCGCTG-3', and antisense, 5'-GTCTGAGCCAGCAATCTCAA-3'; SCD2: sense, 5'-TCACCACGTTCTTCATCGAC-3', and antisense, 5'-TTACCCACTTCGCAAGCTCT-3'; rELO1: sense, 5'-CGAGACACACGAGTCAAAGG-3', and antisense, 5'-ACGTGCAGGACTGTGATCTG-3'; rELO2: sense, 5'-AGAACACGTAGCGACTCCGAA-3', and antisense, 5'-CAAACGCGTAAGCCCAGAAT-3'; β-actin: sense, 5'-TGCAGAAGGAGATTACTGCC-3', and antisense, 5'-CGCAGCTCAGTAACAGTCC-3'.

Nuclear run-on assay

Nuclei from livers of control, clofibric acid-treated rats and rats fed a fat-free diet were prepared as described by Gorski et al. (31). Briefly, freshly dissected livers were homogenized (Teflon-glass homogenizer) in 10 mM HEPES (pH 7.6) containing 2 M sucrose, 10% glycerol, 25 mM KCl, 0.15 mM spermine, 0.15 mM spermidine, and 1 mM EDTA. The homogenates were layered over a cushion of the same buffer and centrifuged at 23,000 rpm for 30 min at −2°C in a RPS-27 rotor (Hitachi Koki, Tokyo). The nuclear pellets were resuspended in a 9:1 mixture of homogenization buffer and glycerol. The homogenates were layered over cushion as described above and centrifuged under the same conditions. The nuclear pellets obtained were resuspended in a 9:1 mixture of homogenization buffer and glycerol.

A suspension of 5×10^7 nuclei in 50 μl of homogenization buffer was mixed with 2 × reaction buffer containing 100 mM Tris (pH 8.0), 10 mM $MgCl_2$, 300 mM KCl, 5 mM dithiothreitol, 2 mM ATP, 2 mM CTP, 2 mM GTP, and 200 μCi [^{32}P]UTP (410 Ci /mmol). After incubation for 30 min at 30°C, RNase-free DNase was added to the mixture, and further incubated for 10 min at 30°C. The reaction was stopped by adding 100 μl of buffer containing 20 mM Tris (pH 8.0), 2% sodium dodecylsulfate, 10 mM EDTA, and 200 μg/ml proteinase K, and then the mixture was incubated for 30 min at 42°C. The transcripts were extracted with an equi-volume of phenol / chloroform / isoamyl alcohol (25:24:1). To the extract was added 50 μg of yeast tRNA and 5 ml of 5% trichloroacetic acid containing 60 mM sodium pyrophosphate, and the mixture was incubated on ice for 30 min followed by filtration through a nitrocellulose filter (0.45-μm pore, 25-mm diameter, Millipore HAWP02500; Millipore, Billerica, MA, USA). The filter was washed three times

with 10 ml of 5% trichloroacetic acid containing 30 mM sodium pyrophosphate and then transferred to a glass vial containing 0.9 ml of 20 mM HEPES buffer (pH 7.5), 5 mM $MgCl_2$, 1 mM $CaCl_2$, and 37.5 units RNase-free DNase I. The filter was incubated at 37°C for 30 min. By the addition of 30 μl of 0.5 M EDTA and 100 μl of 10% sodium dodecyl sulfate, the reaction was terminated and the vials were then incubated for 10 min at 65°C to release the nuclear RNA from the filter. The filter was treated two times with 0.5 mL of 10 mM Tris, pH 7.4, containing 5 mM EDTA and 1% sodium dodecyl sulfate. The eluates from the filters were pooled and digested with 45 μg proteinase K for 30 min at 37°C. Transcripts were precipitated by the addition of NaCl to a concentration of 0.1 M and three volumes of 98% ethanol at −80°C. Precipitated transcripts were collected and dissolved in 1 mM EDTA, 50 mM Tris, pH 8.0. These ^{32}P-labeled transcripts were used to hybridize with 20 μg of the linearized plasmid containing rat SCD1, SCD2, rELO1, rELO2, or rat β-actin cDNA and with the plasmid lacking an insert (negative control). After the hybridization for 48 h at 65°C, the filters were washed three times with 2 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) and 0.1% sodium dodecyl sulfate for 5 min at room temperature and then washed twice with 0.2 × SSC and 0.1% sodium dodecyl sulfate for 15 min at 65°C. The radioactivities retained on the membranes were detected with an image analyzer (FLA 3000G; Fuji Film, Tokyo). Densitometric signals of SCD1, SCD2, rELO1, and ELO2 were normalized with the β-actin signal.

RNA extraction and real-time quantitative PCR

Total RNA was prepared from the rat livers using an RNeasy kit (Qiagen, Hilden, Germany). RNA was quantified spectrophotometrically based on the absorption at 260 nm. cDNA was prepared from the total RNA with avian myeloblastosis virus reverse transcriptase (Takara, Tokyo). The specific primers for SCD1, SCD2, rELO1, rELO2, and β-actin (as an internal control), designed using Primer Express 2.0 software (Applied Biosystems, Foster City, CA, USA), were as follows:

SCD1: sense, 5'-GGATGTTCTCCCGAGATTGA-3', and antisense, 5'-TGTTCGTCAGCACCTTCTTG-3'; SCD2: sense, 5'-TGCACCCCCAGACACTTGTAA-3', and antisense, 5'-GGATGCATGGAAACGCCATA-3'; rELO1: sense, 5'-GCTTCATCCACGTCCTCATGT-3', and antisense, 5'-TCAGCACAAACTGGACCAGCT-3'; rELO2: sense, 5'-AGAACACGTAGCGACTCCGAA-3', and antisense, 5'-CAAACGCGTAAGCCCAGAAT-3'.

The specific primer set for β-actin was used as described by Zhang et al. (32). PCR amplification was carried out using QuantiTect SYBR Green PCR master mix (Qiagen) with 300 nM primers. The amplification and detection were performed with an iCycler IQ real-time detection system (Bio-Rad). PCR conditions were a 15-min denaturation step at 95°C followed by 40 cycles of 15-s denaturation at 94°C, 30-s annealing at 57°C, and 30-s extension at 72°C. Reverse transcriptase-PCR products were analyzed by electrophoresis on ethidium bromide-stained agarose to ensure that a single amplicon of the expected size was indeed obtained. The relative quantity was calculated from a standard curve generated from diluted standard cDNA samples obtained from the rat liver. The quantity of tested genes was normalized with that of β-actin gene.

Statistical analyses

ANOVA was used to test for significant differences between control rats, clofibric acid-treated rat, and rats fed the fat-free diet. Where differences were significant, the statistical significance between any two means was determined using Shèffe's multiple range test. Comparison of two regression slopes was performed according to Ichihara (33).

Results

Effects of clofibric acid and fat-free diet on SCD activity and mRNA transcription

The treatment of rats with clofibric acid markedly elevated SCD activity in the liver, and feeding rats with fat-free diet also induced the activity (Fig. 1). Next, quantitative PCR was carried out with mRNA for SCD1 and SCD2 in the liver (Fig. 2). The treatment with clofibric acid increased the steady-state level of SCD1

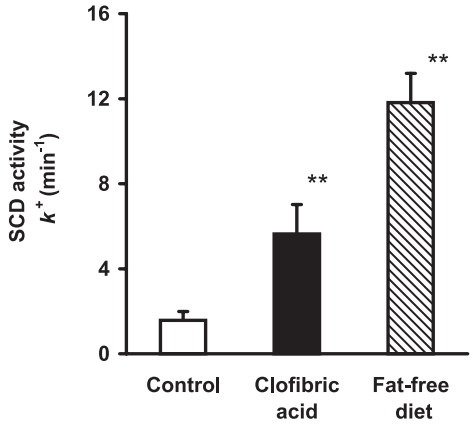

Fig. 1. Increase in activity of SCD by treatment with clofibric acid and fat-free diet feeding in the liver of rats. Rats were administered clofibric acid at a dose of 100 mg/kg, twice a day, for 7 days or fed a fat-free diet for 2 weeks. Values represent means ± S.D. for four rats. **P<0.01, compared with the control.

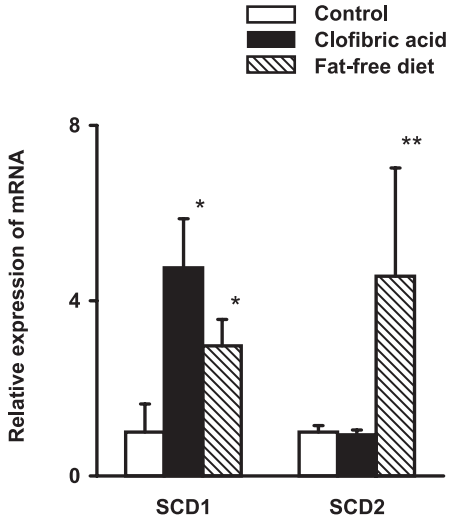

Fig. 2. Effects of treatment with clofibric acid and feeding of fat-free diet on expression of steady-state mRNA of SCD1 and SCD2 in rat liver. Rats were administered clofibric acid at a dose of 100 mg/kg, twice a day, for 7 days or fed a fat-free diet for 2 weeks. The total RNA was extracted and mRNA expression of SCD1 and SCD2 in the liver was determined by the real-time PCR method. mRNA levels of SCD1 and SCD2 were normalized to β-actin. Values represent means ± S.D. for four rats. *P<0.05, **P<0.01, compared with the control.

mRNA while that of SCD2 mRNA was not altered. Feeding of the fat-free diet enhanced steady-state levels of mRNA for both SCD1 and SCD2 in the liver. To determine whether the up-regulation of SCD1 mRNA was due to the activation of transcription, a nuclear run-on assay was carried out (Fig. 3). The transcriptional rate for SCD1 in the liver was significantly increased by the treatment with clofibric acid or feeding of fat-free diet. The transcriptional activation for SCD2 was not observed at all.

Suppression by clofibric acid of degradation of SCD in vivo

Effects of clofibric acid treatment and fat-free diet feeding on degradation of SCD in vivo were measured by the treatment of rats with CHX, an inhibitor of protein synthesis. Linear regression analyses were performed on the data sets (Fig. 4). The estimated half-lives for control rats, clofibric acid-treated rats, and the rats fed a fat-free diet were 2.0, 3.9, and 1.9 h, respectively. Comparison of the regression slope for control rats with that of clofibric acid-treated rats revealed a statistically significant difference between them (P<0.05). In contrast, the difference between control rats and the rats fed the fat-free diet was not statistically significant. To exclude the possibility that the observed difference is due to the difference in the rate of metabolic disappearance of

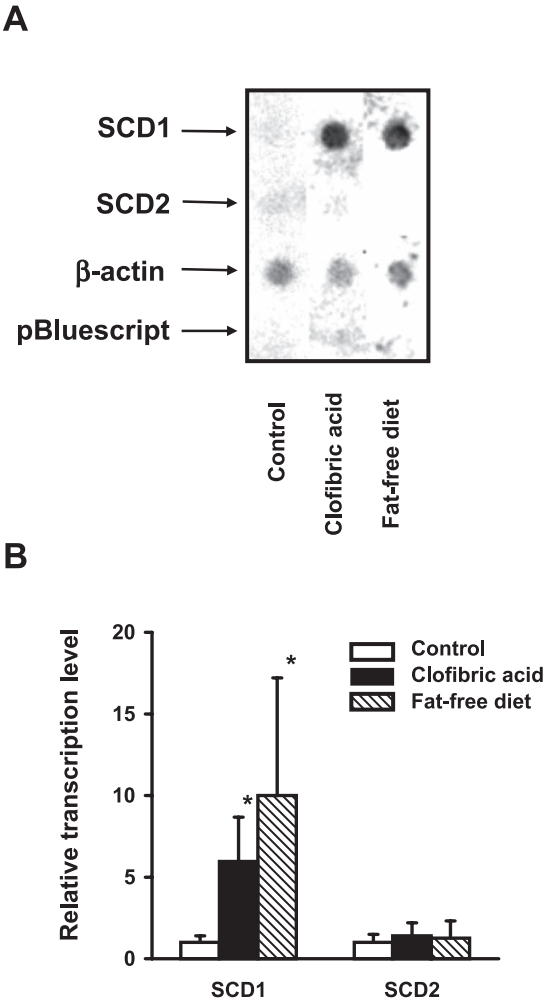

Fig. 3. Effects of treatment with clofibric acid and feeding of fat-free diet on transcriptional activity for SCD1 and SCD2 genes in rat liver. A: Nuclei were isolated from the liver of rats. ^{32}P-labelled nascent RNAs were isolated and hybridized to the nitrocellulose membrane strip dotted with 20 μg of SCD1, SCD2, β-actin cDNA, and pBluescript vector plasmid DNA. pBluescript vector plasmid DNA was used as a control for non-specific hybridization, and β-actin cDNA was used as a control. B: The intensity of hybridization spots in panel A was quantified and expressed as the amount relative to β-actin. Values represent means ± S.D. for four or eight rats. *P<0.05, compared with the control.

CHX, effects of multiple administrations of CHX on SCD half-life were examined. Rats received an additional injection of CHX at a dose of 0.8 mg/kg at 4 and 8 h after the first injection of CHX (2.0 mg/kg); and then microsomes were prepared 0, 2, 4, 8, and 12 h after the first injection of CHX. When the rats were administered CHX for multiple times, the estimated half-lives were 1.6, 3.7, and 1.8 h for the control rats, clofibric acid-treated rats, and rats fed a fat-free diet, respectively (unpublished data). Therefore, the prolonged half-life of SCD in the liver of clofibric acid-

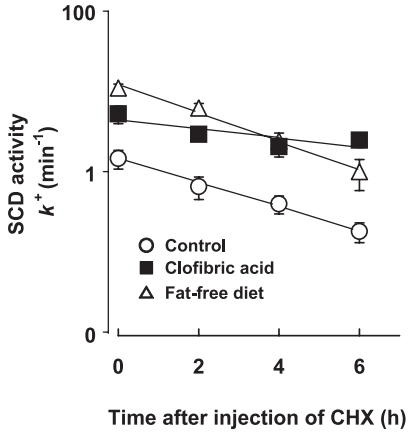

Fig. 4. Degradation of SCD in the liver of rats treated with clofibric acid or fed a fat-free diet. Some rats were administered clofibric acid at a dose of 100 mg/kg, twice a day, for 7 days. The other rats were fed a fat-free diet for 2 weeks. These rats were administered CHX (2.0 mg/kg), then hepatic microsomes were prepared at the indicated time points, and SCD activity was assayed. Values represent means ± S.D. for four rats. A single linear regression line was obtained: Control, logY = –0.337X + 1.52 (P<0.01, r^2 = 0.996); clofibric acid, logY = –0.130X + 4.72 (P = 0.178, r^2 = 0.688); fat-free diet, logY = –0.406X + 13.0 (P<0.01, r^2 = 0.990).

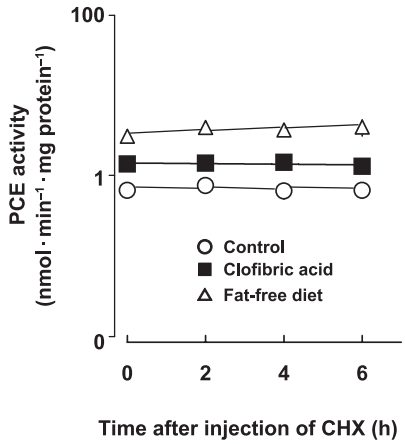

Fig. 5. Effects of CHX on PCE activity in the liver of the rats treated with clofibric acid or fed a fat-free diet. Some rats were administered with clofibric acid at a dose of 100 mg/kg twice a day for 7 days. Some rats were fed the fat-free diet for 2 weeks. Then rats were administered CHX (2.0 mg/kg). Hepatic microsomes were prepared at the specified time, and PCE activity was determined. Values represent means ± S.D. for four rats. A single linear regression line was obtained: Control, logY = –0.01X + 0.755 (P = 0.781, r^2 = 0.064); clofibric acid, logY = –0.01X + 1.55 (P = 0.544, r^2 = 0.200); fat-free diet, logY = –0.035X + 3.27 (P = 0.268, r^2 = 0.534).

treated rats is not due to altered pharmacokinetics of CHX.

Effects of clofibric acid on the production and degradation of PCE in vivo

The treatment of rats with clofibric acid markedly increased PCE activity in the liver, and the feeding of fat-free diet induced PCE activity as well (Table 1). The degradation rate of PCE was estimated by the treatment of rats with CHX in vivo. The half-life of PCE in the liver of control rats was longer than 12 h (Fig. 5). Either the treatment of rats with clofibric acid or the feeding of rats with fat-free diet did not affect the half-life of PCE at all. Recently, two genes for fatty acid elongase were cloned in rat liver and named rELO1 and rELO2 (34). Steady-state levels of mRNA for rELO1 and rELO2 were measured by quantitative real-time PCR (Table 1). Expressed level of rELO2, but not rELO1 was elevated in the liver of clofibric acid-treated rats. Feeding rats with the fat-free diet induced the expression of both rELO1 and rELO2 mRNA (Table 1). To determine whether the up-regulated mRNA of rELO2 was due to transcriptional activation, the nuclear run-on assay was performed (Table 1). No transcriptional activation was observed with genes of either rELO1 or rELO2 by the treatment of rats with clofibric acid or the feeding of rats with the fat-free diet.

Table 1. Effects of clofibric acid and fat-free diet on PCE generation and degradation in the liver of rats

	Control	Clofibric acid	Fat-free diet
PCE activity (nmol · min^{-1} · mg protein^{-1})	0.72 ± 0.13	1.51 ± 0.19**	3.30 ± 0.52**
Steady-state mRNA level (Relative expression)			
rELO1	1.00 ± 0.10	0.99 ± 0.01	1.73 ± 0.76*
rELO2	1.00 ± 0.61	11.58 ± 5.43**	8.50 ± 2.82*
Transcriptional activity (Relative expression)			
rELO1	1.00 ± 0.73	1.69 ± 0.79	1.28 ± 0.72
rELO2	1.00 ± 0.72	1.18 ± 0.27	1.48 ± 0.72

Rats were administered clofibric acid at a dose of 100 mg/kg, twice a day, for 7 days or fed the fat-free diet for 2 weeks. Values represent means ± S.D. for four to eight rats. *P<0.05, **P<0.01, compared with the control.

Discussion

Several studies showed that fibrates induce hepatic SCD activity in rodents (9, 12, 18). The induction is believed to be due to transcriptional activation via PPARα. However, fibrates increased transcription rate only a few fold when estimated with a reporter assay using the SCD promoter region (18). In addition, bifunctional enzyme, a typical PPARα-regulated enzyme, was quickly induced, whereas SCD induction required 30 h to reach the maximum level after the initiation of fibrate treatment (18, 19). These facts suggest that SCD activity is regulated by a mechanism other than transcriptional activation. Oshino et al. had shown that the half-life of SCD is approximately 4 h (27), which is much shorter than those of other members of the microsomal electron transfer system such as NADH cytochrome b_5 reductase, NADPH cytochrome *c* reductase, and cytochrome P450 (35). High turnover rate enables SCD to quickly respond to changes in physiological states. The present study demonstrated that SCD activity was increased by both activation of transcription and retardation in degradation of SCD in the liver of clofibric acid-treated rats. We estimated the content of SCD in terms of its activity but not protein. From these data, we can exclude the possibility that SCD activity is regulated by a modification of this protein, such as phosphorylation, in our experimental conditions, although to date, there has been no evidence that such protein modification regulates SCD activity. To our knowledge, this is the first evidence showing that protein degradation is responsible for the regulation of SCD activity in vivo. The characteristics of the SCD degradation system was reported by Heinamann et al. (36). They showed SCD degradation was not inhibited by pepstatine, leupeptin, *N*-acetyl-leucyl-leucyl-methional, phenylmethylsulphonyl fluoride, and lactastatine in vitro. Recently, plasiminogen-like protein was isolated from the liver of rats as a protein that was involved in SCD degradation (37). Involvement of such a protease in the activation of SCD in the liver of clofibric acid-treated rats remains to be clarified. In contrast to clofibric acid-treated rats, in the rats fed a fat-free diet, transcriptional activation but not increased protein stability was responsible for the retarded degradation of SCD. Therefore, protein stability does is not always involved in the activation of SCD. The results imply that retardation in degradation of SCD was not always involved in the process of SCD activation.

Biosynthesis of oleic acid from palmitic acid is catalyzed by both PCE and SCD. Activities of SCD and PCE are thought to be regulated in response to various physiological conditions (7 – 9, 23). The half-life of PCE has been estimated to be over 12 h, despite SCD having a very short 2-h half-life. This fact suggests that different from SCD, PCE activity remains high once induced. In fact, the half-life of PCE was not altered by the treatment of rats with clofibric acid. In the present study, transcriptional activation was not observed with rELO2 at all, even though the steady state level of rELO2 mRNA was increased by the treatment of clofibric acid and feeding of the fat-free diet. The reason why the mRNA level of rELO2 is elevated remains to be clarified.

In conclusion, the present study demonstrated that not only transcriptional activation but also suppression of protein degradation is responsible for the induction of SCD activity by clofibric acid in the liver of rats. Although the precise mechanism by which clofibric acid retards SCD degradation and the biological significance for the changes in the activity by retardation in protein degradation remain to be clarified, it would be very interesting to clarify whether xenobiotics can regulate enzyme activity through changing enzyme stability. The present results suggest a novel mechanism for the action of fibrates that may lead to better understanding of a wide variety of fibrate actions.

Acknowledgment

This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

References

1 Rubins HB, Robins SJ, Collins D, Fye CL, Anderson JW, Elam MB, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. N Engl J Med. 1999;341:410–418.

2 Rubins HB, Robins SJ, Collins D, Nelson DB, Elam MB, Schaefer EJ, et al. Diabetes, plasma insulin, and cardiovascular disease: subgroup analysis from the Department of Veterans Affairs high-density lipoprotein intervention trial (VA-HIT). Arch Intern Med. 2002;162:2597–2604.

3 Tenenbaum A, Motro M, Fisman EZ, Tanne D, Boyko V, Behar S. Bezafibrate for the secondary prevention of myocardial infarction in patients with metabolic syndrome. Arch Intern Med. 2005;165:1154–1160.

4 Fruchart JC, Brewer HB Jr, Leitersdorf E. Consensus for the use of fibrates in the treatment of dyslipoproteinemia and coronary heart disease. Fibrate Consensus Group. Am J Cardiol. 1998;81:912–917.

5 Kockx M, Gervois PP, Poulain P, Derudas B, Peters JM, Gonzalez FJ, et al. Fibrates suppress fibrinogen gene expression in rodents via activation of the peroxisome proliferator-activated

receptor-alpha. Blood. 1999;93:2991–2998.
6 Wang TD, Chen WJ, Lin JW, Cheng CC, Chen MF, Lee YT. Efficacy of fenofibrate and simvastatin on endothelial function and inflammatory markers in patients with combined hyperlipidemia: relations with baseline lipid profiles. Atherosclerosis. 2003;170:315–323.
7 Cohen P, Miyazaki M, Socci ND, Hagge-Greenberg A, Liedtke W, Soukas AA, et al. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. Science. 2002;297:240–243.
8 Falvella FS, Pascale RM, Gariboldi M, Manenti G, De Miglio MR, Simile MM, et al. Stearoyl-CoA desaturase 1 (Scd1) gene overexpression is associated with genetic predisposition to hepatocarcinogenesis in mice and rats. Carcinogenesis. 2002;23: 1933–1936.
9 Kudo N, Toyama T, Mitsumoto A, Kawashima Y. Regulation by carbohydrate and clofibric acid of palmitoyl-CoA chain elongation in the liver of rats. Lipids. 2003;38:531–537.
10 Toyama T, Kudo N, Mitsumoto A, Kawashima Y. Regulation of palmitoyl-CoA chain elongation by clofibric acid in the liver of Zucker fa/fa rats. Lipids. 2005;40:463–470.
11 Imai K, Kudo N, Koyama M, Kawashima Y. Effects of dehydroepiandrosterone on oleic acid accumulation in rat liver. Biochem Pharmacol. 2003;65:1583–1591.
12 Kawashima Y, Kozuka H. Increased activity of stearoyl-CoA desaturation in liver from rat fed clofibric acid. Biochim Biophys Acta. 1982;713:622–628.
13 Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev. 1999; 20:649–688.
14 Thiede MA, Ozols J, Strittmatter P. Construction and sequence of cDNA for rat liver stearyl coenzyme A desaturase. J Biol Chem. 1986;261:13230–13235.
15 Kaestner KH, Ntambi JM, Kelly TJ Jr, Lane MD. Differentiation-induced gene expression in 3T3-L1 preadipocytes. A second differentially expressed gene encoding stearoyl-CoA desaturase. J Biol Chem. 1989;264:14755–14761.
16 Zheng Y, Prouty SM, Harmon A, Sundberg JP, Stenn KS, Parimoo S. Scd3 – a novel gene of the stearoyl-CoA desaturase family with restricted expression in skin. Genomics. 2001; 71:182–191.
17 Miyazaki M, Jacobson MJ, Man WC, Cohen P, Asilmaz E, Friedman JM, et al. Identification and characterization of murine SCD4, a novel heart-specific stearoyl-CoA desaturase isoform regulated by leptin and dietary factors. J Biol Chem. 2003; 278:33904–33911.
18. Miller CW, Ntambi JM. Peroxisome proliferators induce mouse liver stearoyl-CoA desaturase 1 gene expression. Proc Natl Acad Sci U S A. 1996;93:9443–9448.
19 Sato O, Kuriki C, Fukui Y, Motojima K. Dual promoter structure of mouse and human fatty acid translocase/CD36 genes and unique transcriptional activation by peroxisome proliferator-activated receptor alpha and gamma ligands. J Biol Chem. 2002;277:15703–15711.
20 Hsu MH, Savas U, Griffin KJ, Johnson EF. Identification of peroxisome proliferator-responsive human genes by elevated expression of the peroxisome proliferator-activated receptor alpha in HepG2 cells. J Biol Chem. 2001;276:27950–27958.
21 Lefevre P, Tripon E, Plumelet C, Douaire M, Diot C. Effects of polyunsaturated fatty acids and clofibrate on chicken stearoyl-coA desaturase 1 gene expression. Biochem Biophys Res Commun. 2001;280:25–31.
22 Jeffcoat R. The biosynthesis of unsaturated fatty acids and its control in mammalian liver. Essays Biochem. 1979;15:1–36.
23 Kawashima Y, Suzuki Y, Hashimoto Y. Dietary control of the chain elongation of palmityl-CoA in rat liver microsomes. Lipids. 1977;12:434–437.
24 Cinti DL, Cook L, Nagi MN, Suneja SK. The fatty acid chain elongation system of mammalian endoplasmic reticulum. Prog Lipid Res. 1992;31:1–51.
25 Wang Y, Botolin D, Christian B, Busik J, Xu J, Jump DB. Tissue-specific, nutritional, and developmental regulation of rat fatty acid elongases. J Lipid Res. 2005;46:706–715.
26 Toyama T, Kudo N, Mitsumoto A, Kawashima Y. Effects of perfluorocarboxylic acids on the activities of acyl-CoA elongations in vivo and in vitro. Chem Biol Interact. 2004;150:189–198.
27 Oshino N, Sato R. The dietary control of the microsomal stearyl CoA desaturation enzyme system in rat liver. Arch Biochem Biophys. 1972;149:369–377.
28 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265–275.
29 Oshino N, Imai Y, Sato R. A function of cytochrome b_5 in fatty acid desaturation by rat liver microsomes. J Biochem (Tokyo). 1971;69:155–167.
30 Oshino N, Sato R. Stimulation by phenols of the reoxidation microsomal bound cytochrome b_5 and its implication to fatty acid desaturation. J Biochem (Tokyo). 1971;69:169–180.
31 Gorski K, Carneiro M, Schibler U. Tissue-specific in vitro transcription from the mouse albumin promoter. Cell. 1986;47: 767–776.
32 Zhang J, Chrysis D, Underwood LE. Reduction of hepatic insulin-like growth factor I (IGF-I) messenger ribonucleic acid (mRNA) during fasting is associated with diminished splicing of IGF-I pre-mRNA and decreased stability of cytoplasmic IGF-I mRNA. Endocrinology. 1998;139:4523–4530.
33 Ichihara K. Statistics for bioscience. Practical technique and theory. Tokyo: Nankodo Co., Ltd.; 1990.
34 Inagaki K, Aki T, Fukuda Y, Kawamoto S, Shigeta S, Ono K, et al. Identification and expression of a rat fatty acid elongase involved in the biosynthesis of C18 fatty acids. Biosci Biotechnol Biochem. 2002;66:613–621.
35 Schimke RT, Doyle D. Control of enzyme levels in animal tissues. Annu Rev Biochem. 1970;39:929–976.
36 Heinemann FS, Ozols J. Stearoyl-CoA desaturase, a short-lived protein of endoplasmic reticulum with multiple control mechanisms. Prostaglandins Leukot Essent Fatty Acids. 2003;68:123–133.
37 Heinemann FS, Korza G, Ozols J. A plasminogen-like protein selectively degrades stearoyl-CoA desaturase in liver microsomes. J Biol Chem. 2003;278:42966–42975.