

# Fibrates suppress chenodeoxycholic acid-induced RANTES expression through inhibition of NF- $\kappa$ B activation

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## Abstract

Fibrates, hypolipidemic agents, are reported to be effective in treatment of primary biliary cirrhosis. However, the mechanism involved in therapeutic benefits of fibrates in primary biliary cirrhosis remains unknown. In contrast, hepatic regulated upon activation, normal T-cell expressed and secreted (RANTES) is increased in patients with primary biliary cirrhosis and bile acids up-regulate RANTES expression in hepatocytes. The role of fibrates in bile acid-induced RANTES expression was investigated in human hepatoma cells; 100  $\mu$ M of bezafibrate and fenofibrate decreased expression of chenodeoxycholic acid-induced RANTES mRNA and protein. In addition, luciferase enzyme assay using RANTES promoter-luciferase reporter plasmid revealed that 100  $\mu$ M of bezafibrate and fenofibrate transcriptionally reduced chenodeoxycholic acid-induced RANTES gene expression. Moreover, bezafibrate clearly repressed DNA-binding activity of nuclear factor- $\kappa$ B (NF- $\kappa$ B) induced by chenodeoxycholic acid. Therefore, fibrates might be inhibitory agents of inflammatory cell migration by RANTES to the liver in patients with primary biliary cirrhosis, possibly indicating that fibrates are therapeutic agents in primary biliary cirrhosis. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** RANTES; Bile acid; Chenodeoxycholic acid; NF- $\kappa$ B (Nuclear factor- $\kappa$ B); Fibrate; PPAR $\alpha$  (peroxisome proliferator-activated receptor)

## 1. Introduction

Primary biliary cirrhosis is a chronic cholestatic liver disease characterized by gradual inflammatory destruction of intrahepatic bile ducts with later portal fibrosis and, ultimately, cirrhosis and infiltration of large numbers of T lymphocytes, into the affected portal tracts (Kaplan, 1987; Yeaman et al., 1988). Several studies have suggested that most of these infiltrating T lymphocytes to liver are a memory type of CD4<sup>+</sup> T lymphocytes (Krams et al., 1990; Li et al., 1991; Bjorkland et al., 1991; Leon et al., 1995). It is well known that regulated upon activation, normal T-cell expressed and secreted (RANTES), which is one of the CC chemokines, mainly migrates memory type of CD4<sup>+</sup> T lymphocytes to inflamed tissues (Schall et al., 1990; Ward et al., 1998). In fact, hepatic RANTES was increased in patients with primary biliary cirrhosis (Maltby et al., 1996). Thereafter, we have already shown that chenodeoxycholic acid, an endogenous hydrophobic bile acids accumulating in the liver during cholestasis such as

primary biliary cirrhosis (Sherlock, 1994; Khandelwal and Malet, 1994), transcriptionally induced RANTES expression in human hepatoma cells via nuclear factor- $\kappa$ B (NF- $\kappa$ B) activation (Hirano et al., 2001). Thus, it is possible that increased endogenous bile acids play an important role in inflammatory cell migration by RANTES to the liver in patients with primary biliary cirrhosis.

Ursodeoxycholic acid, which is a 7 $\beta$  isomer of chenodeoxycholic acid, is considered to be a nonhepatotoxic hydrophilic bile acid that may reverse potential hepatotoxicity of endogenous bile acids (Heuman et al., 1991), and is often used in patients with cholestasis to improve liver dysfunction (Lindor and Burnes, 1991; O'Brien et al., 1991; Bouchard et al., 1993). Notably, a beneficial effect of ursodeoxycholic acid in patients with primary biliary cirrhosis has been demonstrated in double-blind controlled studies (Leuschner et al., 1989; Poupon et al., 1991; Corpechot et al., 2000). Additionally, it was recently reported that bezafibrate, a hypolipidemic agent, is effective in primary biliary cirrhosis treatment (Iwasaki et al., 1999; Nakai et al., 2000; Kurihara et al., 2000). In their reports, bezafibrate is more profitable than ursodeoxycholic acid in patients with primary biliary cirrhosis. However, the mech-

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anism involved in therapeutic benefits of bezafibrate in primary biliary cirrhosis is yet unknown. In general, fibrates such as bezafibrate and fenofibrate constitute a group of hypolipidemic agents that is used in treatment of hypertriglyceridemia and combined hyperlipidemia (Schoonjans et al., 1996). These fibrates promote  $\beta$ -oxidation and suppress acetyl CoA carboxylase activity in the liver (Schoonjans et al., 1996). In addition to these pharmacological effects, fibrates activate the peroxisome proliferator-activated receptor (PPAR)  $\alpha$ , a member of the nuclear hormone receptor superfamily (Issemann and Green, 1990). These PPAR $\alpha$  are reported to be involved in cell proliferation and inflammatory response as well as lipid metabolism (Desvergne and Wahli, 1999; Delerive et al., 2001).

The present report shows for the first time that bezafibrate and fenofibrate transcriptionally reduced chenodeoxycholic acid-induced RANTES expression in human hepatoma cells, at least in part through inhibition of both DNA-binding activity and transcriptional activation of NF- $\kappa$ B. Collectively, fibrates might be inhibitory agents of migration of inflammatory cells by RANTES to the liver in patients with primary biliary cirrhosis.

## 2. Materials and methods

### 2.1. Cell culture and chemical reagents

The human hepatoma cell line HLE was provided by Japanese Cancer Research Resources Bank (JCRB) (Doi, 1976). Cells were cultured in the minimum essential medium (MEM) supplemented with 20% fetal calf serum, 100  $\mu$ g/ml penicillin, and 100 U/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> in air. The human hepatoma cell line HepG2 was cultured as described previously (Hirano et al., 2001). From Mitsubishi Pharma (Osaka, Japan), chenodeoxycholic acid was received and dissolved in ethanol. Gas chromatography demonstrated that chenodeoxycholic acid composition purity was at least 99.5%. The chenodeoxycholic acid was used at 100  $\mu$ M because total bile acid serum concentration in patients with cholestasis was reported to be around 10–100  $\mu$ M (Batta et al., 1989; Stiehl et al., 1990; Crosignani et al., 1991; Jazrawi et al., 1994). Bezafibrate and fenofibrate were received from KISSEI Pharmaceutical (Nagano, Japan) and KAKEN Pharmaceutical (Tokyo, Japan), respectively, and dissolved in dimethyl sulfoxide.

### 2.2. Reporter and expression vectors

RANTES promoter-luciferase reporter plasmid was a kind gift from Dr. A.M. Krensky (Stanford University School of Medicine) and described previously (Hirano et al., 2001). The  $\kappa$ B1 site at positions from –53 to –44 and the  $\kappa$ B2 site at positions from –39 to –30 of the RANTES promoter were respectively mutated in 5' -flanking sequence of the

RANTES gene by oligonucleotide-directed, site-specific mutagenesis according to instructions delivered with the site-directed mutagenesis kit (Promega). The mutant sequences utilized for the  $\kappa$ B1 and the  $\kappa$ B2 were GGAAACTtaC and GGtaATGCCC, respectively. Lower case letters represent mutant nucleotides. pECEp65 and pECEp50 were also described previously (Hirano et al., 1998a). The mammalian expression vector pCMX-mouse PPAR $\alpha$  was a kind gift from Dr. K. Umosono (Kyoto University) and constructed by inserting the cDNA insert of PPAR $\alpha$  into pCMX (Umesono et al., 1991; Kliewer et al., 1994).

### 2.3. Enzyme-linked immunoassay (ELISA) for RANTES

In 60-mm collagen-coated culture dishes, HLE cells were grown to confluence and treated with bile acids. Supernatants were collected and analyzed for RANTES content. Levels of RANTES were measured using a RANTES monoclonal antibody sandwich ELISA employing two anti-RANTES antibodies recognizing different, noncompeting determinants according to instructions delivered with the Quantikine Human RANTES Immunoassay (R&D systems, Minneapolis, MN).

### 2.4. RNA extraction and semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted from hepatoma cells according to the method of Chomczynski and Sacchi (1987), which includes a single step of acid guanidium thiocyanate and phenol/chloroform extraction; RNA was quantified spectrophotometrically. Synthesis of the first strand of cDNA and PCR analysis were performed according to instructions delivered with the RNA PCR Kit (AMV) Ver.2 (TaKaRa) as described previously (Maruyama et al., 2000). In brief, 500 ng of total RNA were subjected to first-strand cDNA synthesis in a 20- $\mu$ l reaction containing 10 mM Tris–HCl (pH. 8.3), 50 mM KCl, 5 mM MgCl<sub>2</sub>, 1  $\mu$ M of each dNTP, in presence of 2.5  $\mu$ M random 9 mer nucleotides, 20 U RNase inhibitor, and 5 U avian myeloblastosis virus reverse transcriptase. After completion of first-strand cDNA synthesis, the reaction was stopped by heat inactivation (5 min, 99 °C). For semiquantitative RT-PCR analysis, cDNA amounts equivalent to 500 ng of total RNA were subjected to PCR amplification in a 50- $\mu$ l reaction containing 10 mM Tris–HCl (pH. 8.3), 50 mM KCl, 5 mM MgCl<sub>2</sub>, 200  $\mu$ M of each dNTP, 20  $\mu$ M of each primer, and 2.5 U of TaKaRa Taq DNA polymerase. For RANTES mRNA, HLE cell samples were amplified at 94 °C for 5 min, at 56 °C for 90 s, and at 72 °C for 120 s, followed by 28 cycles at 94 °C for 30 s, at 56 °C for 90 s, and at 72 °C for 120 s. The following primers were used: for RANTES, sense 5'-GCTGTCATCCTCATTGCTAC-3', antisense 5'-TCCATCCTAGCTCATCTCCA-3'; for GAPDH, sense 5'-ACATCGCTCAGACACCATGG-3', antisense 5'-GTAGTTGAGGTCAATGAAGGG-3'. Samples of 10  $\mu$ l of the PCR products were electrophoresed through

1.5% agarose gels and visualized by ethidium bromide. Then, PCR was performed at different cycle numbers for each primer set to ensure that the assay was in the linear range for each molecule tested.

### 2.5. Preparation of whole cell extracts and electrophoretic mobility shift assay (EMSA)

Whole cell extracts were prepared as described previously (Hirano et al., 1998b). Briefly, cells were washed twice with phosphate buffered saline (PBS) and incubated in 20 mM HEPES (pH. 7.9), 350 mM NaCl, 1 mM MgCl<sub>2</sub>, 0.5 mM EDTA, 0.1 mM EGTA, 1% Nonidet P-40, 0.5 mM dithiothreitol, and 0.4 mM 4-(2-amino-ethyl)benzenesulfonyl fluoride hydrochloride (Boehringer Mannheim) on ice at 15 min. After centrifugation at 14,000 rpm for 20 min, the supernatant was used as a whole cell extract. Equal amounts of whole cell extracts (10 µg of protein) were incubated with 30,000 cpm of <sup>32</sup>P-labeled H<sub>2</sub>k oligonucleotide probe for binding NF-κB. Reactions were performed in 20 µl of binding buffer containing 20 mM HEPES (pH. 8.4), 60 mM KCl, 4% Ficoll, 5 mM dithiothreitol, 1 µg of bovine serum albumin, and 2 µg of poly(dI-dC) for 20 min at 30 °C. The reaction mixture was loaded on a 4% polyacrylamide gel and run in 1X tris-borate-EDTA (TBE) buffer. The gel was dried and subjected to autoradiography.

### 2.6. Transient transfection and luciferase enzyme assays

Transient transfection was performed as described previously (Hirano et al., 2001). In brief, HLE or HepG2 cells were plated in six-well plastic dishes (IWAKI Glass, Funabashi, Japan) to 30–50% confluency and were washed three times with PBS; then medium was replaced with Opti-MEM medium (Life Technologies, Grand Island, NY). The plasmid mixtures were mixed with 4 µl of Trans-IT lipofection reagent (Life Technologies) and added to the culture. After 6 h of incubation, the medium was replaced with fresh medium supplemented with 10% fetal calf serum and cells were treated by chenodeoxycholic acid and fibrates for 24 h. For p65, p50, and PPARα expression experiments, HepG2 cells were incubated with fresh medium with 10% fetal calf serum for 24 h after transfection and harvested. After normalization of transfection efficiency by β-galactosidase expression, luciferase activity was determined by Lumat LB9501 (Berthold Japan, Tokyo, Japan).

## 3. Results

### 3.1. Effect of fibrates on chenodeoxycholic acid-induced RANTES production in HLE cells

To examine effects of fibrates on chenodeoxycholic acid-induced RANTES production in HLE cells, we used two fibrates: bezafibrate and fenofibrate. For measurement of

antigenic RANTES protein, conditioned media were collected from cells treated with bezafibrate or fenofibrate in addition to chenodeoxycholic acid. As shown in Fig. 1, no remarkable changes in RANTES protein production were shown by 100 µM of bezafibrate or fenofibrate alone for 48 h (lanes 2 and 3). By contrast, 100 µM of chenodeoxycholic acid significantly increased RANTES protein production for 48 h (lane 4). Moreover, chenodeoxycholic acid-induced RANTES proteins in conditioned media were clearly decreased by 100 µM of either bezafibrate or fenofibrate (Fig. 1, lanes 5 and 6). Cell viability was intact in the medium containing indicated concentrations of chenodeoxycholic acid and fibrates (data not shown). Next, to test effects of fibrates on chenodeoxycholic acid-induced RANTES mRNA expression, semiquantitative RT-PCR was performed. As shown in Fig. 2A and B, PCR products of RANTES or GAPDH in presence or absence of chenodeoxycholic acid and/or two fibrates were gradually increased during 16–36 cycles, respectively. Densitometric quantification showed that chenodeoxycholic acid-induced RANTES mRNA expression was clearly reduced by 100 µM of bezafibrate or fenofibrate (Fig. 2C, lanes 3 and 4 contrasted with lane 2). In contrast, neither of 100 µM of two fibrates, bezafibrate and fenofibrate, influenced RANTES mRNA expression (data not shown).

### 3.2. Effect of fibrates on chenodeoxycholic acid-induced RANTES promoter activity in HLE cells

To examine chenodeoxycholic acid effects on RANTES gene expression, luciferase enzyme assay was performed

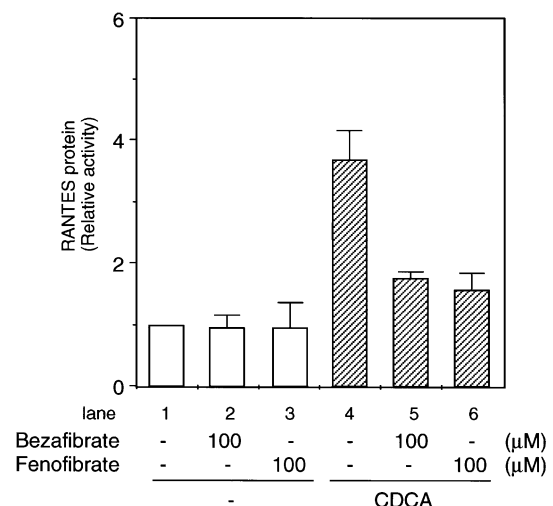


Fig. 1. Effect of fibrates on antigenic chenodeoxycholic acid-induced RANTES production in HLE cells. ELISA was performed using conditioned media as described in Materials and methods. Conditioned media were collected after treatment with 100 µM of chenodeoxycholic acid and/or fibrates for 48 h. Levels of RANTES protein in unstimulated cells were taken as 1.0. Experiments were performed quadruplicately. Results are presented as mean ± S.E. of three independent experiments. CDCA—chenodeoxycholic acid.

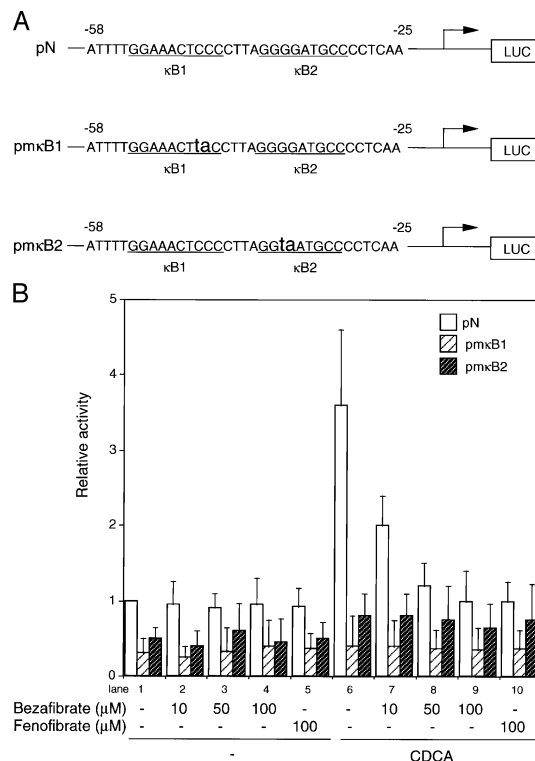
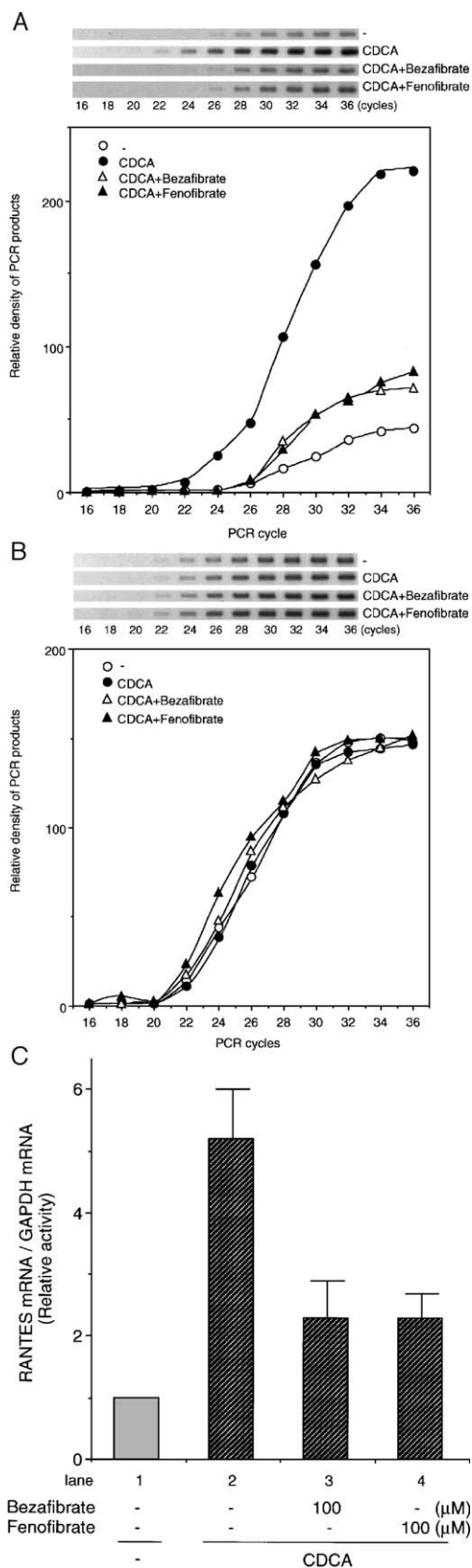


Fig. 3. Effects of fibrates on chenodeoxycholic acid-induced RANTES gene expression. (A) Schematic maps of the RANTES reporter constructions. Two NF- $\kappa$ B binding sites were shown as  $\kappa$ B1 and  $\kappa$ B2. Here, pN was wild type RANTES promoter construct plasmid. Mutation of  $\kappa$ B1 or  $\kappa$ B2 site is shown as pm $\kappa$ B1 or pm $\kappa$ B2, respectively. Lower case letters represent mutant nucleotides. (B) Transcriptional effect of bezafibrate and fenofibrate on chenodeoxycholic acid-induced RANTES gene expression. Cells were transfected by lipofection with 200 ng of a reporter plasmid containing the RANTES promoter. After transfection, cells were co-incubated with 100  $\mu$ M of chenodeoxycholic acid and/or the indicated concentration of fibrates. After 24 h, cellular extracts were prepared for luciferase enzyme assay. Experiments were performed quadruplicately. Levels of luciferase activity of a reporter plasmid alone in unstimulated cells were taken as 1.0 (lane 1). Results are mean  $\pm$  S.E. of three independent experiments. CDCA—chenodeoxycholic acid.

using the reporter plasmid. The RANTES gene upstream sequence contains the two putative NF- $\kappa$ B binding sites ( $\kappa$ B1 and  $\kappa$ B2; -44 and -30 relative to the transcription start site, respectively). Fig. 3A presents mutations of individual *cis*-acting elements of NF- $\kappa$ B binding sites. The base vector for these constructions was the wild-type pN construct (Fig. 3A, upper). Fig. 3B shows that bezafibrate or

Fig. 2. Effect of fibrates on chenodeoxycholic acid-induced RANTES mRNA expression. Semiquantitative RT-PCR analysis was performed using total RNA in HLE cells as described in Materials and methods. (A) RANTES mRNA. (B) GAPDH mRNA. Cells were treated with 100  $\mu$ M of chenodeoxycholic acid and/or fibrates for 24 h. GAPDH was used as an internal control. (Bottom) Densitometric quantification of PCR products of RANTES and GAPDH. (C) Densitometric quantification of RANTES mRNA/GAPDH mRNA ratio at 28 PCR cycles. RANTES mRNA/GAPDH mRNA ratio in unstimulated cells was taken as 1.0. Experiments were performed quadruplicately. Results are presented as mean  $\pm$  S.E. of three independent experiments. CDCA—chenodeoxycholic acid.

fenofibrate did not change basal levels of RANTES gene expression (lanes 2–5) and chenodeoxycholic acid-induced RANTES gene expression was obviously down-regulated by bezafibrate in a dose-dependent manner (lanes 6–9). Additionally, 100  $\mu$ M of fenofibrate reduced RANTES gene expression at the basal level (Fig. 3B, lane 10 contrasted to lane 6). In contrast, mutation of either  $\kappa$ B1 or  $\kappa$ B2 ablated chenodeoxycholic acid-inducibility and fibrates-mediated repression, suggesting that both sites were required for chenodeoxycholic acid induction and fibrates repression of RANTES (Fig. 3B, striped column). In HepG2 cells, also hepatoma cells, chenodeoxycholic acid-induced RANTES gene expression was completely diminished by both fibrates (data not shown). These results might indicate that fibrates down-regulated chenodeoxycholic acid-induced RANTES production at the transcriptional level.

### 3.3. Roles of PPAR $\alpha$ in inhibition of chenodeoxycholic acid-induced RANTES gene expression by fibrates in human hepatoma cells

To examine effects of fibrates on chenodeoxycholic acid-induced DNA-binding activity of NF- $\kappa$ B in HLE cells, EMSA was performed. As shown in Fig. 4A, 100  $\mu$ M of

bezafibrate inhibited DNA-binding activity of NF- $\kappa$ B induced by 100  $\mu$ M of chenodeoxycholic acid for 10 h (lanes 2, 3). In contrast, bezafibrate alone did not influence NF- $\kappa$ B activation (Fig. 4A, lane 4). Thereafter, 100  $\mu$ M of fenofibrate also repressed DNA-binding activity of NF- $\kappa$ B in HLE cells (data not shown). Next, to identify Rel proteins associated with chenodeoxycholic acid-induced NF- $\kappa$ B–DNA complexes, competition and supershift analyses were performed. Whole cell extracts from HLE cells activated by 100  $\mu$ M of chenodeoxycholic acid for 10 h were used. Successful competition was observed using unlabeled NF- $\kappa$ B probe (Fig. 4B, lane 2), whereas an unrelated oligonucleotide was ineffective (Fig. 4B, lane 3). Moreover, chenodeoxycholic acid-induced DNA-binding activity of NF- $\kappa$ B was clearly supershifted by anti-p65 and p50 antibodies (Fig. 4B, lanes 4 and 5 compared with lane 1). These results imply that fibrates repressed chenodeoxycholic acid-induced RANTES gene expression via inhibition of NF- $\kappa$ B. To further study the mechanism of inhibitory effect of fibrates in RANTES gene expression, we investigated PPAR $\alpha$  effects on NF- $\kappa$ B activation. Luciferase enzyme assay was performed using expression plasmids of p65, p50, and PPAR $\alpha$ . Then, HepG2 cells were incubated in culture medium containing mixtures of RANTES promoter-lucifer-

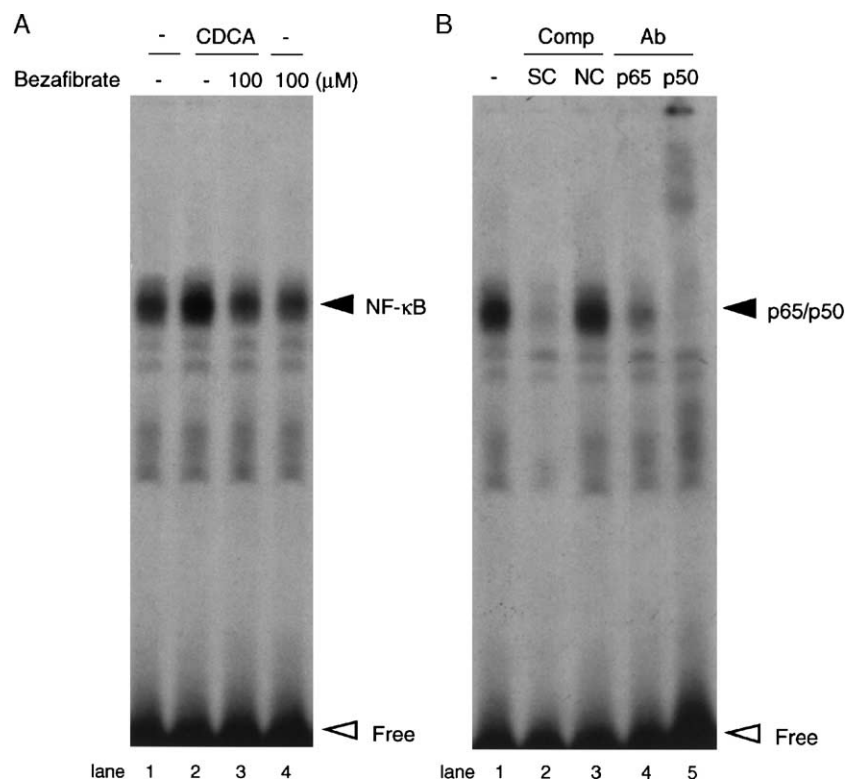


Fig. 4. Effect of bezafibrate on chenodeoxycholic acid-induced DNA-binding activity of NF- $\kappa$ B in HLE cells. EMSA was performed using H<sub>2</sub>k oligonucleotides for a probe as described in Materials and methods. (A) Effect of bezafibrate on chenodeoxycholic acid-induced NF- $\kappa$ B activation. Cells were treated with 100  $\mu$ M of chenodeoxycholic acid and bezafibrate for 10 h. Specific NF- $\kappa$ B band and free DNA are shown as closed and open triangles, respectively. Data is representative of three similar experiments. (B) Competition and supershift analysis. For competition, EMSA was performed using specific (SC) or nonspecific (NC) oligonucleotides on extracts obtained following 10 h of 100  $\mu$ M chenodeoxycholic acid. For supershift analysis, EMSA was performed using anti-p65 or p50 antibody on extracts obtained following 10 h of 100  $\mu$ M chenodeoxycholic acid. Specific NF- $\kappa$ B band (p65 and p50) and free DNA are shown as closed and open triangles, respectively. Data is representative of three similar experiments. CDCA—chenodeoxycholic acid.



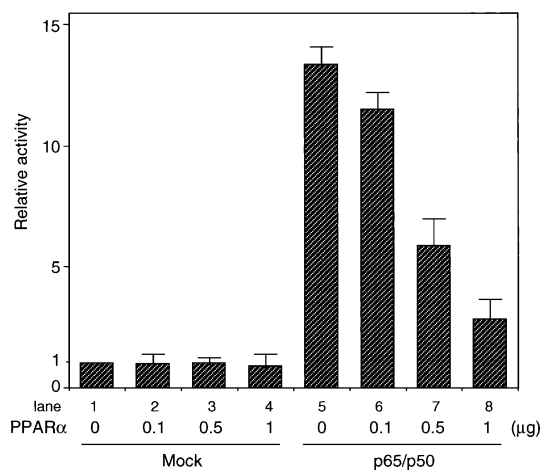


Fig. 5. PPAR $\alpha$  inhibited p65/p50-induced RANTES gene expression. HepG2 cells were transfected by lipofection with 200 ng of a reporter plasmid containing the RANTES promoter and the indicated expression plasmids. After 24 h, cellular extracts were prepared for luciferase enzyme assay. Experiments were performed quadruplicately. Levels of luciferase activity of a reporter plasmid alone in unstimulated cells were taken as 1.0 (lane 1). Results are mean  $\pm$  S.E. of three independent experiments.

ase reporter plasmid and indicated amounts of pCMX-mouse PPAR $\alpha$  with or without 1  $\mu$ g of pECEp65 and pECEp50 per well for 6 h. After transfection, cells were cultured with fresh medium for 24 h and harvested. Luciferase enzyme assay showed that PPAR $\alpha$  distinctly reduced p65-induced RANTES gene expression in a dose-dependent fashion (Fig. 5, lanes 5–8), even though RANTES gene expression was not influenced by PPAR $\alpha$  alone (Fig. 5, lanes 1–4). Given these results, we infer that fibrates inhibited chenodeoxycholic acid-induced expression of RANTES mRNA and protein, at least in part, through PPAR $\alpha$ -mediated pathway.

#### 4. Discussion

In the present study, we found that fibrates inhibited chenodeoxycholic acid-induced RANTES production in human hepatoma cells through inhibition of NF- $\kappa$ B, suggesting that this pharmacological effect of fibrates might be a therapeutic mechanism in patients with primary biliary cirrhosis. Fibrates including bezafibrate have been reported to up-regulate expression of multiple resistant gene 2 (*mdr2*) in the mouse (Chianale et al., 1996). They have demonstrated that an increase of *mdr2* expression by fibrates secreted hepatic phospholipid into the biliary tract and inactivated hydrophobic endogenous bile acids in the biliary tract by micellization (Chianale et al., 1996). Moreover, *mdr2*-knockout mice revealed that the initial pathologic lesion was chronic nonsupportive destructive cholangitis similar to the one in primary biliary cirrhosis (Smit et al., 1993). Therefore, it is possible that these findings provide a rationale for the role of fibrates in improving primary biliary

cirrhosis. In addition to them, we presented another possible mechanism for fibrates via PPAR $\alpha$ -mediated pathway in improving primary biliary cirrhosis.

Functional studies indicate that multiple *cis*-acting elements interspersed within the RANTES promoter sequence contribute to promoter activity upon cell activation (Nelson et al., 1993). The upstream sequence of the RANTES gene contains a number of putative *cis*-acting elements for transcription factors such as activator protein-1, nuclear factor-interleukin 6, and NF- $\kappa$ B (Nelson et al., 1993). Our previous study asserted that mutation on NF- $\kappa$ B binding sites in the RANTES promoter markedly reduced chenodeoxycholic acid inducibility, indicating that NF- $\kappa$ B is a potent inducer of RANTES expression in response to chenodeoxycholic acid (Hirano et al., 2001). Reduction of RANTES expression by fibrates appears at a transcriptional level because bezafibrate and fenofibrate did inhibit chenodeoxycholic acid-induced RANTES promoter activity. This effect seems to stem from inhibition of NF- $\kappa$ B activation, as suggested by reporter gene assay and EMSA. In addition, our data revealed that PPAR $\alpha$  overexpression inhibited p65-driven RANTES gene transcription, possibly suggesting that inhibition of NF- $\kappa$ B activation could result from PPAR $\alpha$ -mediated pathway. In fact, Delerive et al. (1999) have reported that PPAR $\alpha$  physically interacts with p65 via its Rel homology domain which mediates homo- and heterodimerization and interaction with inhibitor of NF- $\kappa$ B in human aortic smooth muscle cells. Alternatively, inhibitory effects might occur through competitive binding of transcriptional coactivators by PPAR $\alpha$  or by PPAR $\alpha$ -induced transcription factors. Moreover, longer exposure to fibrates was found to induce I $\kappa$ B $\alpha$  mRNA and protein expression in primary smooth muscle cells and hepatocytes (Delerive et al., 2000). Future study should investigate these inhibitory effects of fibrates via PPAR $\alpha$  on chenodeoxycholic acid-induced RANTES expression in human hepatocytes.

It was demonstrated that fibrates suppress bile acid synthesis via PPAR $\alpha$ -mediated down-regulation of cholesterol 7 $\alpha$ -hydroxylase and sterol 27-hydroxylase expression (Patel et al., 2000; Post et al., 2001). Additionally, bezafibrate treatment changed bile acid composition in bile with cholic acid being increased and chenodeoxycholic acid being decreased, suggesting PPAR $\alpha$  involvement in regulation of expression of sterol 12 $\alpha$ -hydroxylase (Hunt et al., 2000). In line with these reports, fibrates might improve cholestasis and down-regulate hepatic RANTES expression in patients with primary biliary cirrhosis since endogenous hydrophobic bile acids such as chenodeoxycholic acid were decreased in hepatocytes.

Recently, bile acids have been shown to bind and activate the orphan nuclear receptor, farnesoid X receptor (Wang et al., 1999; Makishima et al., 1999; Parks et al., 1999); farnesoid X receptor binds to DNA as a heterodimer with retinoid X receptor, recognizing an inverted hexanucleotide repeat separated by a single base (an IR-1 motif) (Forman et al., 1995). Although no IR-1 element has been identified in

the RANTES promoter, further study should examine whether interaction between farnesoid X receptor and PPAR $\alpha$  is related to inhibitory effects of fibrates on chenodeoxycholic acid-induced RANTES gene expression.

In summary, we indicate that fibrates decreased chenodeoxycholic acid-induced RANTES gene expression in human hepatoma cells, at least in part through inhibition of NF- $\kappa$ B activation, possibly indicating that fibrates are inhibitory agents of migration of inflammatory cells by RANTES to the liver in patients with primary biliary cirrhosis.

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