CYP4F3B is induced by PGA_1 in human liver cells: a regulation of the 20-HETE synthesis

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Abstract The regulation of the human liver-specific cytochrome P450 4F3B (CYP4F3B) isoform, a splice variant of the CYP4F3 gene with strong substrate specificity for long chain fatty acids, is yet an unsolved question. This report provides the first evidence that CYP4F3B is uniquely induced by prostaglandin A_1 (PGA_1) in human hepatocyte-like HepaRG cells and leads to the synthesis of 20-hydroxy-eicosatetraenoic acids (HETEs). Real time PCR, immunoblot analysis with a specific antipeptide antibody, and determination of fatty acid ω-hydroxylase activity demonstrate that PGA_1 treatment strongly increases expression of CYP4F3B. This induction drives the production of 20-HETE (19-fold increase). SiRNA-mediated-silencing of CYP4F3 suppresses both 20-HETE synthesis and PGA_1 induced 20-HETE production. Taken together, these results provide evidence that CYP4F3B is the key enzyme to produce 20-HETE by ω-hydroxylation of arachidonic acid in liver cells. Since 20-HETE is a potent activator of PPARα and an important inflammatory mediator, CYP4F3B may exert important functions in lipid homeostasis and in inflammatory diseases.—Antoun, J., S. Goulitquer, Y. Amet, Y. Dreano, J-P. Salaun, L. Corcos, and E. Plée-Gautier. **CYP4F3B is induced by PGA_1 in human liver cells: a regulation of the 20-HETE synthesis. *J. Lipid Res.* 2008.** 49: **2135–2141.**

Supplementary key words cytochrome P450 • human hepatoma cells • HepaRG • fatty acid hydroxylase • prostaglandin • inflammation

The cytochrome P450 4F (CYP4F) subfamily has over the last few years come to be recognized for its dual role in modulating the concentrations of eicosanoids as well as in the metabolism of clinically significant drugs (1). Among this family, CYP4F3 catalyses the ω-hydroxylation of leukotriene B4 (LTB4) (2), epoxyeicosatrienoic acids (EETs), hydroxy-eicosatetraenoic acids (HETEs) (3), and a large number of long chain fatty acids. Cytochrome P450 4F3B (CYP4F3B), an isoform encoded by the CYP4F3 gene, results from an alternative premRNA splicing event that occurs specifically in liver (4). It was found to catalyze the inactivation of LTB_4 with a 44-fold lower efficiency than CYP4F3A, the neutrophil-specific enzyme (3, 5); but it utilizes arachidonic acid as a substrate for omega-hydroxylation. For these reasons, it was proposed that CYP4F3B could be the key enzyme to produce 20-HETE, an important eicosanoid mediator derived from phospholipase-released arachidonic acid (4, 6). 20-HETE exerts important biological functions, including blockade of Ca^{2+}-activated K^+ channels or Na^+/K^+-ATPase activity and activation of the peroxisome proliferator-activated receptor α (PPARα) (7–9). 20-HETE derivatives can be stored esterified to cellular glycerophospholipids within cells (10), can act as autocrine and paracrine lipid signaling molecules (1), or can be excreted conjugated to glucuronide (11).

In liver, fatty acid ω-hydroxylation is a minor metabolic pathway that accounts for 5–10% of total fatty acid oxidation (12). Although the expression levels of many cytochromes P450 can be induced by a variety of endo- and xenobiotics, little is known about the regulation of CYP4F3B gene expression. In order to study the regulation of this gene in liver cells, we have chosen HepaRG as a model, a recently derived human hepatoma cell line, which is the closest cell line from human hepatocytes ever reported (13). Indeed, HepaRG cells remain capable of expressing most of the liver-specific functions, including most, if not all, liver CYPs (14).

The cyclopentenone prostaglandins, a subfamily of prostaglandins (PG), are naturally occurring derivatives of PGs

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Abbreviations: AA, arachidonic acid; CYP, cytochrome P450; EET, epoxyeicosatrienoic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HETE, hydroxy-eicosatetraenoic acids; LTB4, leukotriene B4; NICI, negative ion chemical ionization; PG, prostaglandin; PPAR, peroxisome proliferator-activated receptor; SIM, single ion monitoring; Z9(10)-EpSTA, 9,10-epoxystearic acid.

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E_1, E_2, and D_2 (15). Prostaglandin A_1 (PGA_1) is formed by dehydration within the cyclo-hydroxy-pentanone ring of PGE_1 (16). It belongs to a class of eicosanoids that display various biological activities. Among cyclopentenone prostaglandins, PGA_1 behaves as a "circulating hormone," and its levels are significantly increased in hypertension (17). It may be one of the agents released during inflammatory reactions (18). Free PGA_1 has been found in human peripheral blood (19), but it can also be bound to albumin or conjugated with glutathione (20). PGA_1 has some unique biological activities that are different from those of classic prostaglandins, such as induction of cell differentiation and antitumor and antivirus activities (15). Recently, cyclopentenone PGs have been subject of considerable interest due to their potential use as therapeutic agents. In vivo studies have shown beneficial effects of these mediators in several models of inflammation and tissue injury (21). Prostaglandins exert their actions through incompletely elucidated mechanisms. Some prostanoids can activate cell surface receptors or can also act as agonists of the PPAR nuclear receptor (22) and even induce the heat-shock response in human cell lines (23–25).

In the present report, we demonstrate that PGA_1 upregulates expression of CYP4F3B in the HepaRG cell line. This induction leads to the generation of 20-HETE, which is further exported as a glucuronide conjugate. SiRNA-mediated-knock-down of CYP4F3 suppresses production of 20-HETE glucuronide. This is the first report of the regulation of the production of this lipid mediator by an endobiotic compound in human liver cells. Furthermore, this study provides the first demonstration that CYP4F3B is the key enzyme to produce 20-HETE in hepatocyte-like cells.

MATERIALS AND METHODS

Chemicals

PGs, LPS, fatty acids, and insulin were from Sigma (Saint Quentin Fallavier, France). 20-HETE and 20-HETE-d6 were from Cayman Chemical European Platform (SPI-Bio, Montigny le Bretonneux, France). Radiolabeled [1-^{14}C]9,10 epoxystearic acid (EpSTA) was synthesized as described (3). Culture media and glutamine were from Gibco BRL (Cergy-Pontoise, Fance), sera from Perbio (Brebières, France), and hydrocortisone hemisuccinate from Upjohn Pharmacia (Guyancourt, France). Human recombinant CYP4F3B and CYP4F3A were from Gentest BD Bioscience (Le Pont de Claix, France).

Cell culture

HepaRG cells were seeded in William's E media supplemented with 5 U/ml penicillin, 5 μg/ml streptomycin, 10% fetal calf serum (Hyclone: Fetalclone II Bovine serum product), 200 mM L-Glutamine, 5 μg/ml insulin, and 5.10^{-5} M hydrocortisone. After 12 days, the medium was supplemented at first with 1% DMSO and then with 2% DMSO until the 24th day, when the different treatments were applied, according to previous reports (26).

Preparation of siRNAs

CYP4F3B siRNA were designed and purchased from Qiagen (Courtaboeuf, France). The sense and the antisense strands of siRNAs were: r(GGU CUC CUA UAC ACA CAA A)dTdT (sense), r(UUU GUG UGU AUA GGA GAC C)dTdT (antisense). The target sequence of the CYP4F3B siRNA was: AAG GTC TCC TAT ACA CAC AAA.

siRNAs transfection

siRNA were transfected within a ratio of 37.5 ng siRNA to 3 μl of cationic liposome (HiPerfect Transfection Reagent, QIAGEN) in order to obtain 5 nM as a final siRNA concentration in the culture media. Then siRNA complexes were added drop-wise onto highly confluent and differentiated hepatoma cells. The cells were incubated under normal growth conditions (37°C, 5% CO_2) during 48 h. Nonsilencing siRNA (5 nM) was used as a negative control (Qiagen). Gene silencing was monitored by Real Time RT-PCR.

RNA extraction and quality control

Total RNA was extracted using the ABI Prism™ 6100 Nucleic Acid PrepStation (Applied Biosystems, Courtaboeuf, France) as described (26). The integrity of total RNA was checked on an Agilent 2100 Bioanalyser using the RNA 6000 Nano LabChip kit (Agilent Technologies, Palo Alto, CA).

Reverse transcription and real-time PCR assays

Fifty nanograms of total RNA (10 μl) were reverse-transcribed into cDNA using the high-capacity cDNA Archive Kit (Applied Biosystem) with random hexamers according to the supplier's instructions. The reporter for the Taq Man probes, all purchased from Applied Biosystems [ref Hs001668521_m1for CYP4F3, ref Hs99999905_m1 for glyceraldehyde-3-phosphate dehydrogenase (GAPDH)] was FAM. Data were normalized to GAPDH signal. For detection with a fluorescent dye (SYBR Green), reactions were conducted in a 25 μl final volume containing 10 ng cDNA, 12,5 μl of Syber green PCR Master Mix (Applied Biosystems), and 7,5 pg of each primers. Primers (Proligo, Paris, France) sequences were as follows: CYP4F3A reverse: 5′CCA CCA GCA GCA CAT ATC AC3′ (in exon 4), CYP4F3B reverse: 5′TGA TGA CAG ACC GGA TGA TG3′ (in exon 3), and CYP4F3A/B forward: 5′ATT GGT TCT TGG GTC ACC TG3′.

Assay of monooxygenase activity in microsomes

The microsomal fractions were prepared as described previously (26). The ω-hydroxylation of 9,10-epoxystearic acid (Z9 (10)-EpSTA) was determined by HPLC as previously reported using [1-^{14}C]9,10-EpSTA at the concentration of $4K_m$ (0.15 mM; specific activity 74 kBq/ml) as substrate (3). We verified that the reaction was linear up to 20 min, which excludes detergent effects of the substrate.

Immunoquantification of CYP4F3B protein

The CYP4F3B-specific antipeptide antibody was raised against the aminoacids encoded by the CYP4F3B-specific exon (position 232-374 from sequence AF054821) (Eurogentec, Seraing, Belgium). Proteins from cell microsomal preparations (30 μg) were separated by electrophoresis on 9% SDS-polyacrylamide gels and then electrophoretically transferred to nitrocellulose sheets. These sheets were blocked with PBS containing 3% (w/v) BSA and 0.05% (v/v) Tween 20 for 30 min at room temperature before overnight incubation, at 4°C, with rabbit anti-CYP4F3B primary polyclonal antibody.

Preliminary experiments showed that this primary polyclonal antibody does not react with CYP4A11 or CYP4F3A proteins (see fig. 1B). The detection with the secondary antibody was performed as described (26).

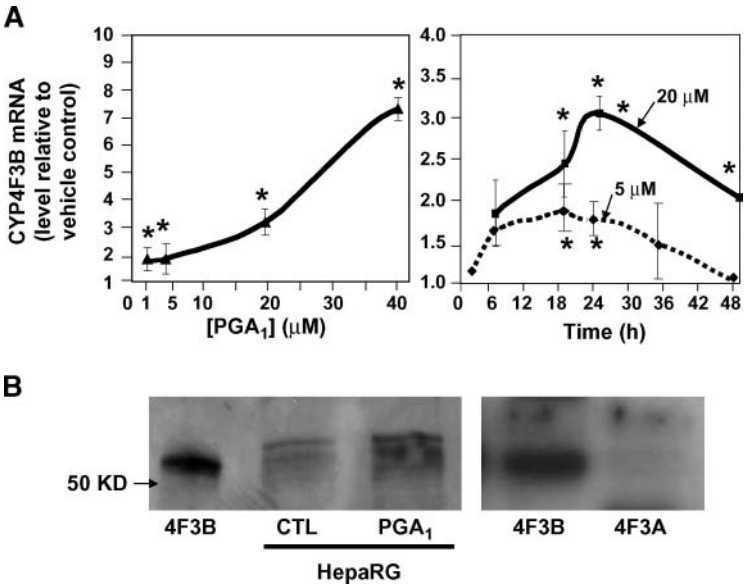

Fig. 1. Prostaglandin A_1 (PGA_1) induces cytochrome P450 4F3B (CYP4F3B) gene expression and protein content A: Dose-response regulation (left panel) and time-course induction (right panel) of CYP4F3B mRNA by PGA_1. Fully differentiated HepaRG cells were incubated during 24 h with 0, 1, 5, 20, or 40 μM PGA_1 or with 5 μM or 20 μM of PGA_1 for 2, 6, 18, 24, 36, and 48 h. CYP4F3B mRNA levels were determined by real-time RT-PCR. The results are shown as fold increase in comparison with the control sample (treated with vehicle) after normalization to the GAPDH signal. Data were expressed as mean ± SEM (n = 4) of induction vs. vehicle control. * $P < 0.05$ B: Immunoblot analysis of CYP4F3B content in microsomes (30 μg of protein per lane) from fully differentiated HepaRG cells treated or not for 24 h with 20 μM PGA_1. Relative amounts of protein loaded and transferred onto the blot were assessed by Coomassie blue staining and quantified by image processing scan analysis (Gel Doc XR, Biorad, France); the intensity of each lane varied by no more than 5%. 4F3B: 5 pmol of human recombinant CYP4F3B (Gentest) as a positive control. 4F3A: 5 pmol of human recombinant CYP4F3A (Gentest) as a negative control. This figure is representative of three independent experiments with identical results.

Quantification of 20-HETE by GC/MS/NICI

Fifty nanograms of 20-HETE-d6 internal standard was added to each sample. To detect conjugated 20-HETE, 20 ml of culture medium was first freeze-dried and hydrolyzed overnight at 37°C with 20 μl of *Helix Pomatia* Juice (Villeneuve la Garenne, BioSepra) and 3 ml of 2 M acetate buffer pH 5.2; the reaction was stopped by adding 3 ml 0.6 N perchloric acid. Extraction was carried out with 2 × 2 ml of ethyl acetate. The supernatants were combined and dried under a gentle stream of oxygen-free nitrogen at room temperature. The dried samples were suspended in 100 μl acetonitrile. Pentafluorobenzyl esters and trimethylsilyl (TMS) ethers were formed according to the method described by Nithipatikom et al. (27). Samples were analyzed with a Hewlett-Packard 5873 Mass Selective Detector interfaced to a Hewlett-Packard 6890 Series+ gas chromatograph. A 2 μl volume of sample was injected into a capillary column (HP-5MS, J and W Scientific) with 0.25 mm internal diameter, 30 m length, and 0.25 μm-film thickness. The compounds were ionized by negative ion chemical ionization (NICI) using methane as the reagent gas. The data were collected in both total ion current and single ion monitoring chromatograms. The concentrations were determined by the ratio of 20-HETE peak area (*m/z* 391) to the peak area of 20-HETE-d6 (*m/z* 397) as internal standard. Position of the hydroxyl on the ω carbon was confirmed by GC/MS with electronic impact using methyl-TMS derivatives.

Statistical analysis

Values were expressed as mean ± SD from at least three separate experiments. Treatments (kinetic and dose-response experiments) were compared by one-way ANOVA followed by Tukey's posthoc test. Experiments including only two groups were compared with nonparametric Mann and Withney test. Differences were considered significant if $P < 0.05$.

RESULTS

CYP4F3B gene expression is up-regulated by PGA_1 in a dose- and time-dependent manner

Because CYP4F3 may be transcribed as two isoforms differing by the alternative inclusion of either one of two possible exons (4), giving rise to CYP4F3A or CYP4F3B, CYP4F3B specific primers were designed to analyze mRNA expression levels. We found a threshold cycle of 27.4 ± 0.02 for CYP4F3A and 19.3 ± 0.02 for CYP4F3B in fully differentiated HepaRG cells. This result indicates that, as in human liver (5), CYP4F3A mRNA was much less expressed than CYP4F3B mRNA in HepaRG cells. We analyzed CYP4F3 mRNA gene expression in response to a panel of putative modulators in differentiated HepaRG cells. Several CYP4F3 substrates such as LTB4 (1 μM), long chain fatty acids at the concentration of 100 μM (linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid), epoxidized and hydroxylated fatty acids at the concentration of 10 μM (epoxystearic acid, coronaric acid, vernolic acid, 8-HETE, 11-HETE, 12-HETE),

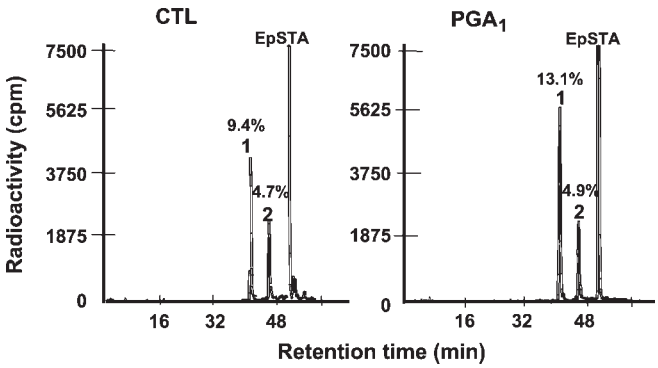

Fig. 2. PGA_1 induces CYP4F3B enzyme activity RP-HPLC profiles of metabolites generated from 150 μM [1-^{14}C] Z9(10)-EpSTA by microsomes from HepaRG cells untreated (CTL) or treated with 20 μM PGA_1 during 24 h (PGA_1). Peaks 1 and 2 were identified as 18-hydroxy Z9(10)-EpSTA and 9-10 dihydroxy-octadecanoic acid, respectively [previously characterized by mass spectrometry by our group (3)]. Percent of radioactivity in peak 1 and 2 relative to substrate are noted. Data shown are representative of three independent experiments with similar results.

PPAR ligands [15-deoxy$\Delta_{12,14}$ PGJ_2 (1μM), 2-bromo-palmitic acid (25 μM), clofibric acid (100 μM)] and prostaglandins (PGA_1, PGH_2, PGE_2, PGE_1, PGB_1) at the concentration of 1 and 5 μM were added to the culture medium of HepaRG cells during 24 h. Strikingly, the cyclopentenone PGA_1 was uniquely identified as a potent inducer of CYP4F3B. HepaRG cells were incubated during various periods of time with 5 μM or 20 μM PGA_1 or with increasing concentrations (1–40μM) of PGA_1 for 24 h. CYP4F3B gene expression was increased in a time-and dose-dependent manner (**Fig. 1A**). A significant 1.8-fold increase in CYP4F3B mRNA level was detected in response to 1 μM PGA_1 treatment. This induction reached more than 6-fold for 40 μM PGA_1 after 24 h (Fig. 1A). To determine whether the raise in CYP4F3B mRNA level was associated with an increase of CYP4F3B protein content, immunoblot analyses were performed with a anti-CYP4F3B polyclonal antibody specifically raised against the peptide sequence encoded by the liver-specific CYP4F3B exon (position 232–374 from sequence AF054821) (Fig. 1B). As expected, a signal was detected with recombinant CYP4F3B (Gentest) but not with recombinant CYP4F3A (Gentest) with the anti-CYP4F3B antibody, demonstrating that the anti-CYP4F3B was fairly specific. A 3-fold increase of CYP4F3B protein was observed following treatment of HepaRG with

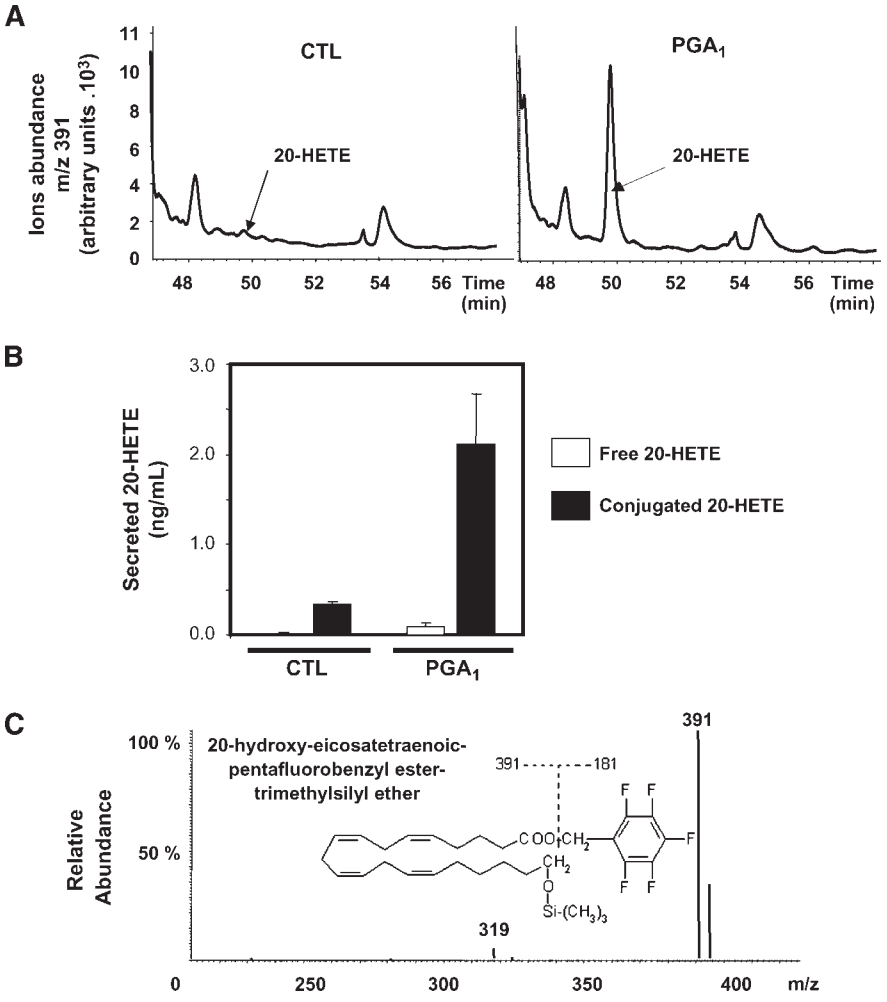

Fig. 3. Characterization and quantification of 20-hydroxy-eicosatetraenoic acid (HETE) in the culture media of HepaRG A: Representative partial gas chromatograms, acquired in single ion monitoring (SIM) mode monitoring *m/z* 391, from cell culture media of HepaRG (after hydrolysis) treated (PGA_1) or not (CTL) during 24 h with 20 μM PGA_1. The amount of 20-HETE in the samples was determined by comparing the area of 20-HETE peak to that of the internal standard. B: Free 20-HETE (without hydrolysis) and conjugated 20-HETE (after hydrolysis with Helix Pomatia Juice) was determined by gas chromatography in the HepaRG culture medium after treatment or not with 20μM PGA_1 during 24 h. The amount of 20-HETE in the samples was determined by comparing the area of 20-HETE peak to that of the internal standard. Each value represents the mean ± SEM of data obtained from three independent experiments. C: Mass spectra of 20-HETE-PFB-ester-TMS-ether in the negative ion chemical ionization mode (NICI); the mass spectra shows a major abundance for the fragment *m/z* 391 $[M\text{-}H\text{-}PFB]^-$ and a minor fragment, *m/z* 319, corresponding to the subsequent loss of [TMS-H].

20 μM PGA_1 for 24 h. The Western blot analysis shows two bands in HepaRG cell samples, which are both induced by PGA_1. Although their identity is not known, they may correspond to posttranslational modifications or, alternatively, to distinct translation initiation or termination sites. To analyze the activity of CYP4F3B, we chooe 9,10-epoxystearic acid [Z9(10)-EpSTA] as a discriminating CYP4F3B substrate (3). Z9(10)-EpSTA was incubated with microsomes from HepaRG cells treated or not with 20 μM PGA_1. The metabolite rate, based on the formation of ω-hydroxylated Z9(10)-EpSTA (peak 1), was significantly increased ($P < 0.05$) by the PGA_1 treatment (metabolic rate of 11.07 ± 0.44 nmol/min/mg protein vs. 8.16 ± 0.49 nmol/min/mg protein in untreated cells) (**Fig. 2**).

PGA_1 triggers 20-HETE production

Since 20-HETE was proposed to be a major endogenous product for CYP4F3B catalysis, we analyzed 20-HETE synthesis by HepaRG cells treated or not with PGA_1 (**Fig. 3A**). To evaluate free and conjugated 20-HETE in cell culture medium we quantified the secreted 20-HETE after hydrolysis or not with Helix Pomatia Juice (Fig. 3B). Interestingly, 20-HETE, which was under limit of detection within cells, was readily detected in the culture medium as a conjugate. As expected, hydrolysis of the metabolites with *Helix Pomatia* juice gave a peak with the same retention time as 20-HETE by GC/MS analysis (Fig. 3A). The identity of the metabolite was confirmed by mass spectrometry (Fig. 3C). The mass spectra showed a major ion fragment at *m/z* 391 $[M\text{-}H\text{-}PFB]^-$ and a minor fragment, at *m/z* 319, corresponding to the subsequent loss of $Si(CH_3)_3$ (TMS). The production of conjugated 20-HETE in the culture medium was increased by 19.3 ± 2.4-fold (from 0.021 ± 0.004 ng/ml/24 h to 0.361 ± 0.076 ng/ml/24 h) when cells were treated with PGA_1 (Fig. 3A). Production of glucuronide-conjugated EETs in the culture medium was not significantly affected by the treatment (data not shown). 20-HETE synthesis was increased after incubation of HepaRG cells with arachidonic acid (100 μM during 24 h) and with lipopolysaccharide treatment (100 ng/ml during 24 h) as a PLA2 inducer (28) (**Fig. 4**). These results suggest that 20-HETE synthesis was correlated to the level of arachidonic acid released from membranes through PLA2 activation or added to HepaRG cell medium, further supporting the proposition that HepaRG cells can produce 20-HETE from arachidonic acid.

CYP4F3B silencing blocks 20-HETE production and PGA_1-induced 20-HETE synthesis

To fully demonstrate the major role of CYP4F3 in 20-HETE production, HepaRG cells were transiently transfected with CYP4F3B siRNA. Significant suppression of CYP4F3B expression was observed (**Fig. 5**), whereas CYP4F3A, CYP4F2, or CYP4A11 gene expression was not affected (data not shown). In the CYP4F3B-silenced cells, the 20-HETE production was highly reduced (Fig. 5). In addition, PGA_1-mediated induction of 20-HETE production was also affected when CYP4F3B was silenced (Fig. 5).

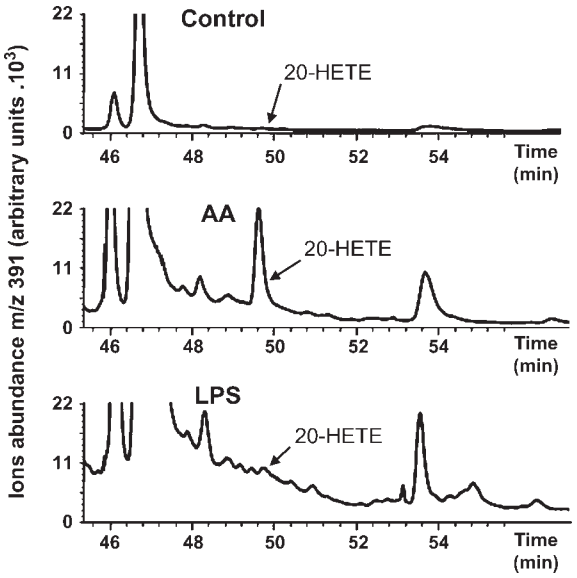

Fig. 4. Effect of arachidonic acid (AA) and LPS on 20-HETE production. Representative partial gas chromatograms acquired in SIM mode monitoring *m/z* 391, from cell culture media of HepaRG (after hydrolysis) treated or not during 24 h with 100 μM AA or after 100 ng/ml lipopolysaccharide (LPS). Peak corresponding to 20-HETE are indicated.

DISCUSSION

It was previously shown that CYP4F3B utilizes arachidonic acid as a substrate for omega-hydroxylation and generates 20-HETE (4) (29). This report provides the first evidence that PGA_1 induces CYP4F3B in human hepatocyte-

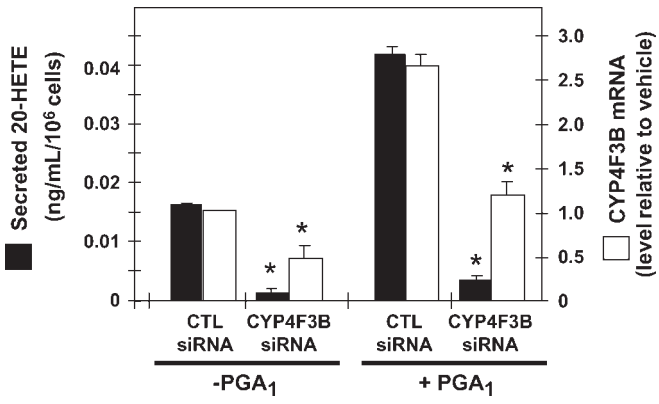

Fig. 5. Effect of CYP4F3B-blocking siRNA on 20-HETE production. Cells were transfected with siRNA negative control (CTL siRNA) or specific of CYP4F3B (CYP4F3B siRNA) as described in Materials and Methods. After 4 h, the medium was replaced with fresh medium during 20 h. Incubation continued for an additional 24 h, without (-PGA_1) or with 20 μM PGA_1 (+PGA_1). Medium and cells were collected 48 h after transfection to evaluate 20-HETE concentration (closed bar) relative to 10^6 cells and CYP4F3B mRNA content (open bar). CYP4F3B mRNA level is expressed in comparison with the CYP4F3B expressed in control cells transfected with a nonsilencing siRNA and incubated without PGA_1. * $P < 0.05$.

like cells. This effect was fairly specific as treatment of cells with several other PGs (PGE_1, PGE_2, PGH_2, PGB_1) did not influence CYP4F3B mRNA. The CYP4F3B gene-expression induction resulted in a marked increase of 20-HETE, which was found as a glucuronide conjugate in cell supernatant. CYP4F3B mRNA silencing prevented these effects, which points to a major role of CYP4F3B in 20-HETE synthesis in this human hepatoma cell model.

The cyclopentenone prostaglandins are a family of lipids that have a wide spectrum of biological actions. PGA_1 lowers arterial pressure in hypertensive patients and can inhibit cell proliferation, presumably through its ability to regulate the expression of a variety of stress-induced and cell-cycle-related genes (15). In vivo, cyclopentenone prostaglandins such as PGA_1 have antitumor effects (15). PGA_1 has also been reported to activate PPARα and PPARγ, and hence may bear potential for regulation of lipid homeostasis (30). Yet the molecular signaling pathway involved in the induction of CYP4F3B mRNA expression remains to be unraveled. To our knowledge, it is presently not possible to predict the interaction constant between PGA_1 and putative targets. However, most of biological actions involving cyclopentenone prostaglandins, including PGA_1, PGA_2, and PGJ_2, do not depend on binding to G-protein-coupled prostanoid receptors, but result from their direct interaction with other cellular target proteins. Therefore, mechanisms by which PGA_1 could act include activation of heat-shock proteins (31) or PPAR (30) or inhibition of NF-κB (32) or increased oxidative stress (33). In our study, PPARα activation is unlikely to be the basis for the PGA_1-induced CYP4F3 expression because clofibric acid, a PPARα activator, did not mimic the effects of PGA_1, while we have shown that clofibric acid could induce CYP4A11 gene expression in HepaRG cells (26). Even though the present study reveals a specific effect of PGA_1 on CYP4F3B expression, an important question is whether the induction of 20-HETE production relates to some known biological activity of PGA_1. It is difficult to predict if the CYP4F3B-induced gene expression by prostaglandin is relevant to either a biological or to a pharmacological property, or both.

High concentrations of arachidonic acid (AA) were used here in order to determine the capacity of HepaRG cell to metabolize AA. It would remain to see whether 20-HETE could still be detected with lower amounts of AA as a precursor.

Cyclopentenone prostaglandins are found in body fluids at nanomolar concentrations (15). These concentrations are low compared with what is required for their biological activity, but they may in fact represent only an underestimate of the intracellular concentration. The same dilemma was found for 15-deoxy$\Delta_{12,14}$ PGJ_2. Hence, the reported concentrations of 15-deoxy$\Delta_{12,14}$ PGJ_2 necessary to activate PPARγ (μM range) greatly exceed those found in the plural exudate (pM) (34). PGA_1 is very unstable in serum and may be preferentially found as a conjugate to glutathione, glucuronic acid, or sulfate (15, 35). Furthermore, in several pathological conditions, including hyperthermia, infection, or inflammation, local prostaglandin concentrations in the micromolar range can be detected (36). Indeed, while other eicosanoids, such as leukotrienes and lipoxins, usually evoke bioactions in the nanomolar or subnanomolar range in vivo, PGs mostly require micromolar concentrations to elicit biological effects (29, 38–41). It remains to be determined if the level of PGA_1 required to achieve a sufficiently high local concentration in vivo to induce 20-HETE production in target cells or tissues can indeed be reached.

The biological function of 20-HETE starts to be elucidated in humans. It is an important autocrine and paracrine factor that has diverse biological functions. With both pro- and antihypertensive properties, it is involved in the regulation of cardiac contractility, cellular proliferation, and inflammation (37–39). The liver expresses the highest level of CYP4F3B, but little information is available on the autocrine biological properties of 20-HETE in this tissue. 20-HETE could participate in the modulation of liver diseases and in the renal functional disturbances in patients with hepatic cirrhosis (40). 20-HETE has also been shown to promote cell growth and angiogenesis (41). As prostaglandin and leukotriene hydroxylases, CYP4F enzymes can modulate the levels of eicosanoids during inflammation (1). In rodent hepatocytes, CYP4F gene expression is down-regulated by the proinflammatory cytokine IL-1β (42). By contrast, in human hepatocyte-like cells, CYP4F3 was up-regulated by the antiinflammatory cyclopentenone PGA_1.

Our results show that, in human liver cells, 20-HETE is excreted mainly as a glucuronide conjugate. In vivo, it was previously observed that the concentration of free 20-HETE (20–40 pg/ml in human urine) was about 10-fold lower than the corresponding concentration of the 20-HETE glucuronide (11). The biological activity of glucuronidated 20-HETE is not yet known. In this study, 20-HETE glucuronide did not seem to regulate, by an autocrine mechanism, the expression of PPARα target genes such as CYP4A11 (data not shown). 20-HETE can be further metabolized by cyclooxygenases to 20-hydroxy PGG_2 and 20-hydroxy PGH_2 (43) or to the 1-20-dicarboxylic derivative of arachidonic acid by alcohol dehydrogenase (29). These products are potent activators of PPARα and PPARγ (44). Because several lines of evidence suggest that PPARγ functions to dampen inflammation and injury (45), the PGA_1-induced 20-HETE production in liver could explain the anti-inflammatory activity of this cyclopentenone prostaglandin through induction of the synthesis of 20-HETE.

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