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Up-regulation by clarithromycin of α_1-acid glycoprotein expression in liver and primary cultured hepatocytes

Takafumi Komori[a], Hirofumi Kai[a], Kazuki Shimoishi[a], Koki Kabu[a], Ayumi Nonaka[a], Toru Maruyama[a], Keiji Tamura[b], Masaki Otagiri[a,*]

[a]*Faculty of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan*
[b]*Saikin Kagaku Institute, Sendai, Miyagi 981-0917, Japan*

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Abstract

α_1-Acid glycoprotein (AGP) is the major transport protein for cationic drugs, endogenous ligands, and some anionic drugs in plasma. Hepatic synthesis and secretion of AGP are altered during acute inflammation as well as by a number of drugs. This alteration could influence the binding of drugs and its biological function. Macrolide antibiotics are widely used in the treatment of a variety of infectious diseases. The effects of macrolide antibiotics have been studied with respect to rat AGP expression *in vivo*. After the individual administration of six macrolides to rats, with the exception of oleandomycin, five increased AGP levels in serum. Of these five, clarithromycin (CAM) was the most potent inducer of AGP, which reached a maximum level between 3 to 7 days after administration. CAM increased the steady-state level of AGP mRNA in liver as well as protein level in serum in a dose-dependent manner. In addition, CAM increased AGP mRNA levels in primary cultured hepatocytes. In the luciferase promoter assay, CAM potentiated dexamethasone-increased promoter activity of the AGP gene, which contained the glucocorticoid response element, in cultured rat hepatocytes, although CAM itself had no effect on its activity. The effect of CAM and dexamethasone was diminished by glucocorticoid response element deletion or mutation or by adding the antiglucocorticoid, RU486. Further, in the mouse mammary tumor virus (MMTV) promoter containing functional glucocorticoid response element, CAM potentiated dexamethasone-increased promoter activity. In the adrenalectomized rats, CAM did not increase AGP levels in serum. These findings suggest that CAM may cause transcriptional induction of AGP, at least in part, via a glucocorticoid-mediated mechanism.

Keywords: α_1-Acid glycoprotein; Clarithromycin; Glucocorticoid-mediated mechanism; Hepatocytes

1. Introduction

AGP is the major transport protein for cationic drugs, endogenous ligands (progesterone), and some anionic drugs in plasma [1]. It contains a variable glycan chain at five glycosylation sites, which may play a role in modulating its immune responses [2,3]. The hepatic synthesis and secretion of AGP, as well as its glycosylation, are altered during acute inflammation [4]. This could influence the binding of drugs and its biological function. AGP gene expression was increased by IL-1, tumor necrosis factor-α, IL-6, IL-8, IL-11, leukemia inhibitory factor, oncostantin M, and glucocorticoids [5,6]. Cis-acting regulatory elements have been identified in the rat AGP gene; these are responsive to IL-1, IL-6, and glucocorticoids [7,8]. AGP gene expression is also increased by phenobarbital and rifampicin via mechanisms that are independent of the inflammatory pathway [9–12].

Macrolide antibiotics are widely used in the treatment of a variety of infectious diseases. It has recently been recognized that long-term treatment with macrolides appears to improve chronic inflammatory processes such as diffuse panbronchiolitis, bronchial asthma, and human T-cell leukemia virus (HTLV)-1-associated myelopathy, the effects that are not due to their antimicrobial properties. Of these macrolides, CAM, and erythromycin uniquely suppressed mRNA levels as well as the release of IL-6 at therapeutic and non-cytotoxic concentrations [13]. We recently reported that a single administration of CAM increased the serum levels of AGP in normal and diabetic rats [14].

* Corresponding author. Tel.: +81-96-371-4150; fax: +81-96-362-7690.

E-mail address: otagirim@gpo.kumamoto-u.ac.jp (M. Otagiri)

Abbreviations: AGP, α_1-acid glycoprotein; CAM, clarithromycin; DEX, dexamethasone; GR, glucocorticoid receptor; GRE, glucocorticoid response element; IL, interleukin; and PCR, polymerase chain reaction.

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However, the issue of whether CAM increases the AGP level at transcriptional level or not remains unclear, because the method employed for the quantitation of AGP in serum is affected by alterations in glycosylation. In the present study, we addressed three questions: 1. Do other macrolides increase the serum level of AGP? 2. Does CAM affect the glycosylation of AGP? 3. If not, does CAM increase AGP mRNA levels? To answer these questions, macrolides were repeatedly administered into rats once a day for 3 or 7 days, because in this way, the effects of macrolides become considerably more obvious. Moreover, we investigated the mechanism of AGP gene expression by CAM in cultured hepatocytes by promoter analysis using the 5′-flanking region of the rat AGP gene.

2. Materials and methods

2.1. Materials

CAM was a generous gift from Taisho Pharmaceutical. Erythromycin, troleandomycin, oleandomycin, spiramycin, josamycin, DEX, RU486, actinomycin D, and rat AGP were purchased from Sigma. Plasmids that contain, in its *Pst*I site, the full-length cDNA of rat AGP was a generous gift from Dr. Heinz Baumann (Roswell Park Cancer Institute, Buffalo, NY). MMTV-CAT was a kind gift from Dr. Pierre Chambon (CNRS, INSERM, Universite Louis Pasteur, France). Human β-actin cDNA (2.0 kb) was obtained from Clontech Laboratories. All other reagents used in this study were of analytical grade.

2.2. Animals

Male Wistar rats weighing 200–250 g were treated with macrolides [1–25 mg/kg in 30% dimethyl sulfoxide, subcutaneously (s.c.)] and were sacrificed 24 hr after the last injection. To produce rats with inflammation, they were treated with turpentine oil (2 mL/kg, s.c.) [15]. Adrenalectomized rats and sham-operated rats were purchased from Kyudo Farm(Japan).

2.3. AGP quantitation in serum

Antiserum to rat AGP was prepared by the immunization of rabbits using a 50/50 emulsion of antigen/Freund's complete adjuvant, which corresponds to antigen amounts of 0.75 mg. Following one booster injection at 3 weeks, two immunizations were performed over a period of 1 month. AGP concentrations in rat serum were measured by a radial immunodiffusion assay using rat AGP and rabbit anti-rat AGP antiserum. In a typical experiment, radial immunodiffusion plates consisting of 1% agarose in 7 mM Na_2HPO_4, 2 mM NaH_2PO_4, 145 mM NaCl, 5 mM EDTA, pH 7.0, were used with the antiserum. After authentic AGP and serum samples (5 μL) were applied to wells, the plates were allowed to stand for 48 hr at room temperature; at that time, a fully developed precipitation ring was observed. The ring diameter was subsequently measured and quantified.

2.4. Isolation and culture of hepatocyte

Hepatocytes were isolated from the livers of 180–230 g male Wistar rats by collagenase perfusion, and plated onto 35-mm tissue culture dishes precoated with collagen. Cultured hepatocytes were incubated in a humidified chamber maintained at 37° and 5% CO_2/95% air with Williams' Medium E supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, 50 μg/mL gentamycin, and 10 mM HEPES. The medium was changed 4 hr later to remove unattached hepatocytes. After shifting the cultures to serum-free medium, drug incubations were started at 18 hr after plating the hepatocytes and the culture medium was renewed every 24 hr. Drugs were added to the culture medium as concentrated stock solutions in dimethyl sulfoxide (for macrolides) or ethanol (for DEX and RU486). The final concentration of organic solvent in the culture medium was 0.1%.

2.5. Northern blot analysis

Total RNA was isolated from rat livers or cultured hepatocytes and analyzed by Northern blot hybridization. RNA samples (7.5 μg) were resolved on denaturing 1% agarose gels, transferred to GeneScreen Plus nylon membranes (Amersham) by blotting, and baked for 2 hr at 80°. Membranes were prehybridized for 6 hr at 42° in 5 × SSPE (0.75 M NaCl, 50 mM NaH_2PO_4, 5 mM EDTA, pH 7.4), 10 × Denhardt's solution (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin), 2% SDS, 100 μg/mL of sonicated and denatured salmon sperm DNA, and 50% formamide. Membranes were hybridized for 24 hr at 42° in the above solution, which contained full-length AGP cDNA (740 bp) and human β-actin cDNA (2.0 kb) probes radiolabeled by random priming with [α-^{32}P] dCTP (3000 Ci/mmol) (Amersham). β-actin was used as an internal control to insure consistent transfer of the mRNA. Membranes were then washed at room temperature with 2× SSC and 0.1% SDS. Radioactive signal intensity was quantified with a bio-imaging analyzer (BAS1000), and the results were expressed as the difference from control values.

2.6. Construction of reporter plasmids

A 380-bp fragment of the rat AGP gene, spanning nucleotides −360 to +20 relative to the transcription start site [16], was amplified by PCR by using rat genomic DNA as template and the following sequences: 5′ primer, 5′-TACTTCCCGAGAGTGTTCATTCATGTGGACATAGA-3′, and 3′ primer, 5′-GCCCAGGAAGAGCTAACTGGTGGCTGGAGAGTGCA-3′. The PCR product was generated through 40 cycles of the following

steps: denaturing temperature at 94° for 2 min, annealing temperature 60° for 1 min, and elongation temperature 72° for 1.5 min, and was cloned into a pCRII vector by the TA Cloning Kit (Invitrogen). Then, using this clone containing rat AGP gene as a template, and the following sequences: 5′ primer, 5′-CTCAGAGCTCCCTCAGGGGTTGATTTA-3′, and 3′ primer, 5′-AATTCTCGAGGCCCAGGAAGAGCTA 3′, PCR was performed according to the following profile: 94° for 2 min, 55° for 1 min, 72°C for 1.5 min, 40 cycles. The PCR fragment was cloned into the *Sac*I to *Xho*I site upstream of the pGL2-Basic luciferase reporter plasmid (Promega). The resulting construct is referred to as pAGP(−360)Luc. A series of deletion constructs were also prepared by PCR using 5′ primer, 5′-GCAGGAGCTCGTGTCAGGGCTGGCT-3′ and 5′-TCT GGAGCTCCTGGCGACGCCCATG-3′, and 3′ primer, 5′-AATTCTCGAGGCCCAGGAAGAGCTA-3′, and the luciferase reporter plasmid named pAGP(−172)Luc and pAGP(−60)Luc, respectively. pAGP(−360)Luc-M is the construct pAGP(−360)Luc with a mutated GRE site. It was prepared by using a Transformer Site-Directed Mutagenesis kit (Clontech) and rAGP-mGRE as a mutation primer. It contains −132 bp to −95 bp with a mutated GRE site indicated in lower case, bold letters, 5′-GCTGGCTTGAGGGAA **ag**TTTT **aa**GCAAGACATTTCCC-3′. pMMTV-luc was prepared by using pMMTV-CAT (gift from Dr. P. Chambon) and the pGL2-Basic luciferase reporter plasmid. All constructs and cloned fragments were sequenced by the dideoxy chain termination method [17].

2.7. Transfection of primary cultured rat hepatocyte

Primary cultured rat hepatocytes were plated in standard Williams' Medium E onto 35-mm collagen-coated dishes. At 6 hr after plating, the culture medium was replaced with 1 mL of Opti-MEM containing a premixed complex of 8 μg of Lipofectin reagent and 1 μg of the reporter construct in combination with 0.1 μg of pSV-βgal as a control for measuring transfection efficiency. The transfection mixture was replaced with standard Williams' Medium E 12 hr later. Hepatocytes were further harvested 24 hr after transfection. The luciferase activity in cell extracts was measured by using the Luciferase Assay system according to the manufacture's instructions (PicaGene Luminescence Kit). The β-galactosidase activity was determined by an enzymatic assay using *o*-nitrophenyl β-D-galactopyranoside as the substrate, and the absorbance was measured at 420 nm. Luciferase activity in each sample was normalized to that of β-galactosidase.

2.8. mRNA stability

Hepatocytes were incubated with 0.5 μg/mL actinomycin D for 0.5 hr prior to the addition of 4×10^{-6} M CAM at 18 hr after plating ($t = 0$). The 18-hr-old cultures were further treated for 1, 3, and 6 hr and harvested for isolation of total RNA, and AGP mRNA levels were analyzed by Northern blot hybridization.

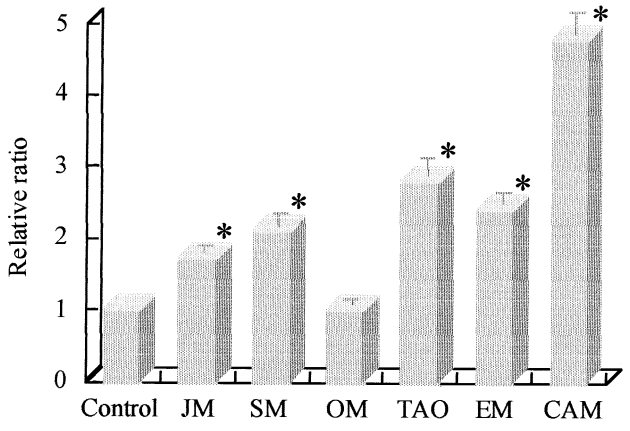

Fig. 1. Effects of macrolides on AGP protein levels in rat serum. The abbreviations used are as follows: JM, josamycin; SM, spiramycin; OM, oleandomycin; TAO, troleandomycin; EM, erythromycin; CAM, clarithromycin. Male Wistar rats (seven rats per group) were treated for 3 days s.c. with each macrolide at a dose of 25 mg/kg/day. Blood was withdrawn 24 hr after the last injection and centrifuged at 3000 *g* for 10 min. The levels of AGP protein in rat serum were measured by a radial immunodiffusion assay. All values are presented as the mean ± SD of seven different rats and as the relative ratio of control values. Data were analyzed by Duncan's multiple range test (a non-parametric test). Significantly different from the control, $*P < 0.05$.

2.9. Statistical analyses

The differences among groups were assessed by Duncan's multiple range test (a non-parametric test) or Student's *t*-tests. A $P < 0.05$ denoted the presence of a statistically significant difference.

3. Results

3.1. Effects of macrolide antibiotics on AGP levels in rat serum

For this *in vivo* study, a series of 14-membered (CAM, erythromycin, troleandomycin, and oleandomycin) and 16-membered macrolide antibiotics (spiramycin and josamycin) were chosen (Fig. 1). CAM, erythromycin, troleandomycin, spiramycin, and josamycin, but not oleandomycin, increased serum AGP levels at a dose of 25 mg/kg/day for 3 days. Of the macrolides tested, CAM was the most potent inducer of AGP. In order to further analyze the effect of CAM on AGP expression, rats were treated for 7 days with different doses of CAM (1 to 25 mg/kg/day) (Fig. 2). The effect of CAM was observed 24 hr after the administration of doses of 15 and 25 mg/kg. The maximal effect of CAM was observed after 3 days and this effect remained constant up to 7 days after administration. The AGP levels induced by CAM (25 mg/kg/day, for 7 days) was similar to the AGP

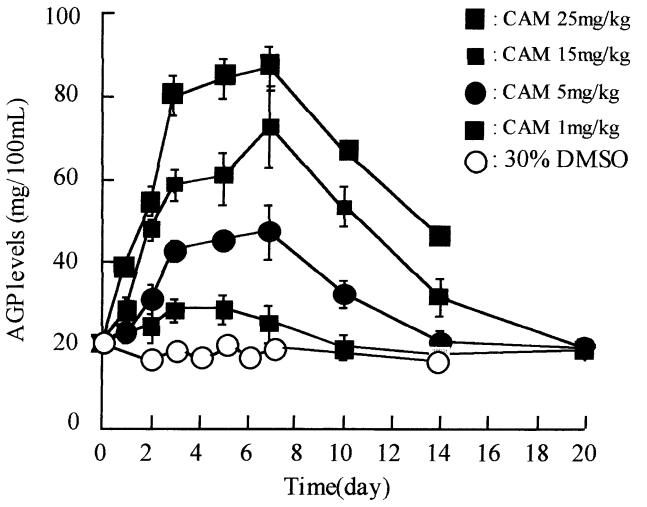

Fig. 2. Time-course and dose-response effects of CAM on AGP protein levels in rat serum. Male Wistar rats (six rats per group) were treated for 7 days s.c. with CAM at doses of 1, 5, 15, or 25 mg/kg/day. Blood was withdrawn 24 hr after the last injection and centrifuged at 3000 *g* for 10 min. The levels of AGP protein in rat serum were measured by a radial immunodiffusion assay. All values are presented as the mean ± SD of six different rats.

levels in the rats with inflammation induced by turpentine oil (2 mL/kg, s.c.) (data not shown). In addition, CAM increased other acute-phase proteins including α_2-macroglobulin, hemopexin, and haptoglobin, but not transferrin and albumin (data not shown).

The method employed here for quantitation of AGP in serum is affected by an alteration of the glycosylation of AGP. Thus, to determine whether an increase of AGP by CAM is due to the change of AGP glycan heterogeneity, the effect of CAM on the oligosaccharide structure of AGP in serum were examined by Crossed affino Con A immunoelectrophoresis [18]. However, CAM had no effect on the extent of glycosylation of AGP (data not shown).

3.2. Effect of CAM on AGP mRNA levels in liver and in primary cultured rat hepatocytes

The effect of CAM on AGP mRNA level in the liver of other rats was analyzed by Northern blot hybridization. CAM (15 mg/kg, s.c.) led to an increase in AGP mRNA level in liver 12 hr after administration and reached a plateau by 24 hr (data not shown). CAM dose-dependently increased AGP mRNA in liver 24 hr after administration (Fig. 3A). To better understand the up-regulation of AGP by CAM, the following experiments were conducted in primary cultured rat hepatocytes. Cultured hepatocytes were incubated with CAM 18 hr after plating and were harvested 48 hr later to measure AGP mRNA. CAM at more than 4×10^{-7} M significantly increased in AGP mRNA in cultured hepatocytes (Fig. 3B).

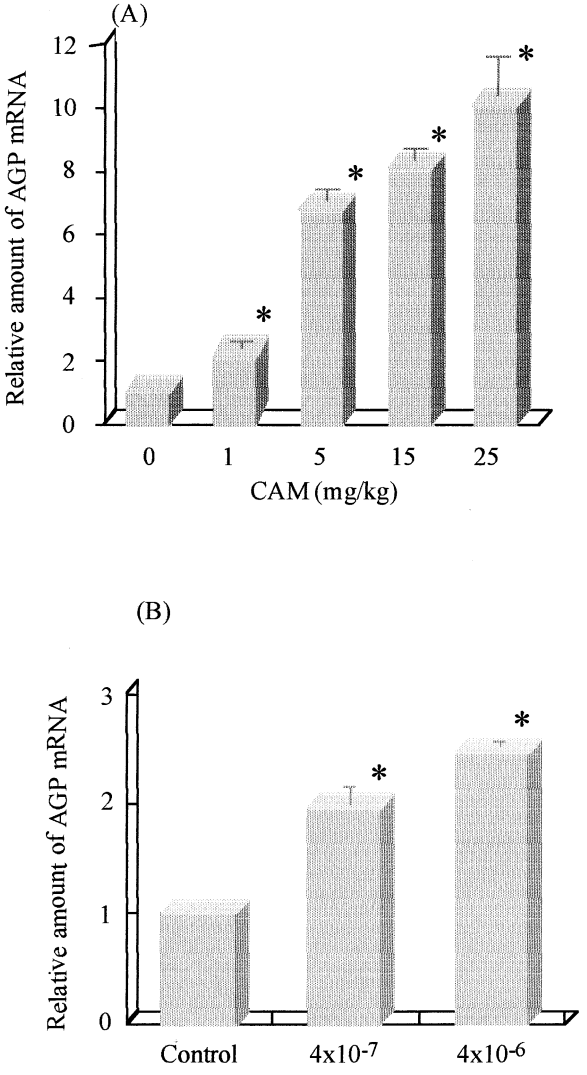

Fig. 3. (A) Dose-dependent effect of CAM administration on AGP mRNA levels in rat liver. Male Wistar rats (four rats per group) were treated for 3 days s.c. with CAM at doses of 1, 5, 15, or 25 mg/kg/day. At 24 hr after the last administration, the rats were killed, and the livers were dissected to prepare total RNA, as well as to analyze AGP mRNA levels by Northern blot hybridization. All values are expressed as the mean ± SD of four different rats and as the relative ratio of AGP mRNA/β-actin mRNA in control rats. (B) Dose-dependent effects of CAM treatment on AGP mRNA levels in primary cultured rat hepatocytes. Eighteen-hr-old hepatocytes were treated for 48 hr with medium alone or medium containing 4×10^{-7} M or 4×10^{-6} M CAM. Hepatocytes were harvested from individual dishes for isolation of total RNA, and AGP mRNA levels were analyzed by Northern blot hybridization. All values are expressed as the mean ± SD of four independent experiments and as the relative ratio of AGP mRNA/β-actin mRNA in control hepatocytes. Data are analyzed by Duncan's multiple range test (a non-parametric test). Significantly different from the control, $^*P < 0.05$.

3.3. Effect of CAM on the DEX-induced (or GR-mediated) expression through the GRE of the 5′-flanking region of rat AGP gene

To determine whether the effect of CAM on AGP mRNA was due to changes in transcriptional activation, we

performed promoter analysis in primary cultured rat hepatocytes. Cultured hepatocytes 6 hr after plating were transfected with plasmids, and 12 hr later hepatocytes were treated for 24 hr with medium alone or medium containing 10^{-5} M CAM, 10^{-8} M DEX, or 10^{-5} M CAM plus 10^{-8} M DEX with or without 10^{-6} M RU486. The reason why the time for treatment with drugs is shorter than that in the case of Fig. 3B is that primary cultured hepatocytes was easily damaged by the transfection to maintain the cells for longer time. As shown in Fig. 4A, CAM did not increase the luciferase activity of pAGP(−60)Luc, pAGP(−172)Luc, or pAGP(−360)Luc by itself, whereas it potentiated the DEX-induced promoter activity of pAGP(−172)Luc and pAGP(−360)Luc, both of which contain GRE. The promoter activity of pAGP(−60)Luc was not affected by DEX, because the GRE is located at −121 to −107 in the rat AGP 5′-flanking region. In addition, the effects of DEX and CAM were blocked by a mutation of GRE (pAGP(−360)Luc-M) as well as of a glucocorticoid antagonist, RU486 (Fig. 4A). To confirm whether the effect of CAM is specific for the AGP promoter, we used pMMTV-luc, a glucocorticoid-responsive promoter [19]. The promoter activity of pMMTV-luc increased by DEX was significantly potentiated by CAM (Fig. 4B). Taken together, the results indicated that GRE may play a role in CAM-responsive transcriptional activation in AGP.

3.4. Effect of cam on AGP levels in adrenalectomized rats

To determine whether the CAM effect *in vivo* is similarly operating through the glucocorticoid-mediated pathway, we examined the effect of CAM on AGP in adrenalectomized rats. As shown in Fig. 5, CAM did not increase serum AGP levels 4 days after administration in the adrenalectomized rats, although CAM increased serum AGP level in sham-operated rats. Next, we examined whether CAM induced the production of endogenous glucocorticoids in normal rats. However, CAM did not affect the endogenous level of corticosterone in serum (data not shown).

3.5. Effect of CAM on AGP mRNA stability

Finally, we examined the issue of whether the accumulation of AGP mRNA expression by CAM might be a result of transcript stability as well as of post-transcriptional regulation. Primary cultured rat hepatocytes were incubated with actinomycin D, as an RNA synthesis inhibitor, in the presence or absence of CAM, and the decay rates of AGP mRNA were then examined. The level of AGP mRNA 6 hr after the addition of actinomycin D with CAM was higher than that found in the absence of CAM (Fig. 6). We found that CAM may slightly increase the stability of AGP mRNA (t1/2 = 6.8 ± 0.8 hr for control, t1/2 = 14.8 ± 1.7 hr for CAM-treated).

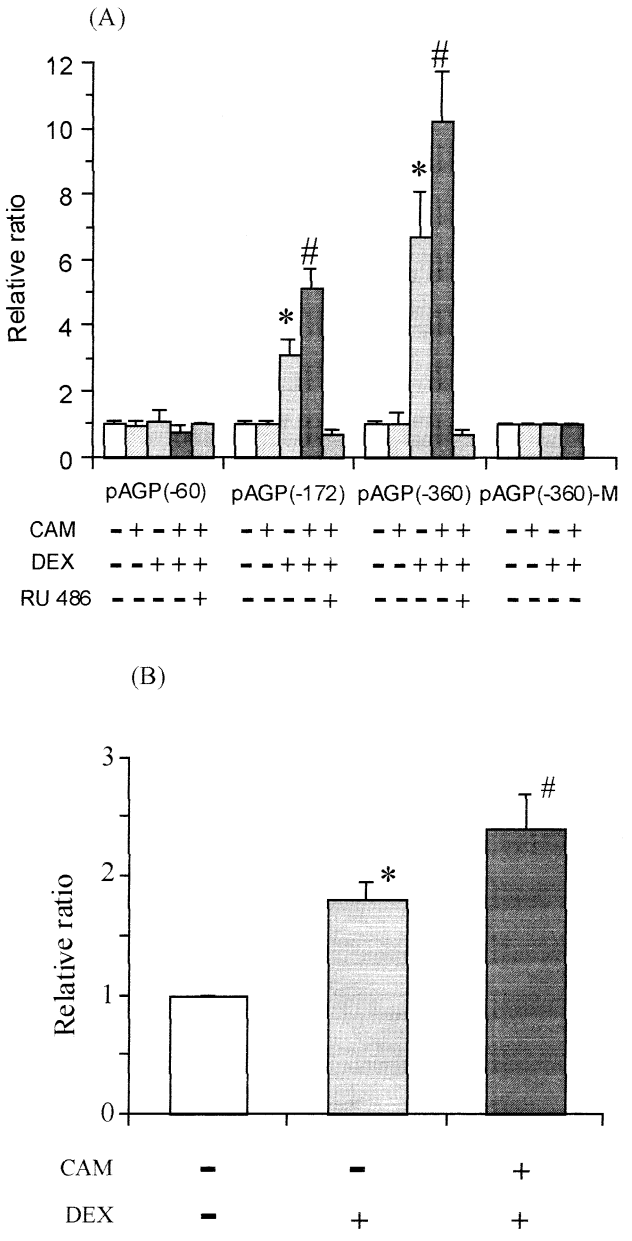

Fig. 4. (A) Effects of CAM and DEX on luciferase activity in primary cultured rat hepatocytes transiently transfected with AGP-reporter constructs. Six hours after plating, primary cultured rat hepatocytes were transiently transfected with 1 μg of luciferase reporter plasmid, pAGP(−60)Luc, pAGP(−172)Luc, pAGP(−360)Luc, or pAGP(−360)Luc-M, plus 0.1 μg of pSV-β-galactosidase. Following transfection for 12 hr, hepatocytes were treated for 24 hr with medium alone or medium containing 10^{-5} M CAM, 10^{-8} M DEX, or 10^{-5} M CAM plus 10^{-8} M DEX with or without 10^{-6} M RU486. Hepatocytes were harvested to measure luciferase and β-galactosidase activity. (B) Effect of CAM on DEX-induced expression of pMMTV-luc constructs. At 6 hr after plating, primary cultured rat hepatocytes were transiently transfected with 1 μg of luciferase reporter plasmid plus 0.1 μg of pSV-β-galactosidase. Following transfection of pMMTV-luc for 12 hr, hepatocytes were treated for 24 hr with medium containing DEX at 10^{-8} M in the presence or absence of 10^{-5} M CAM. All values are expressed as the mean ± SD of six independent experiments and as the relative ratio of normalized luciferase activity by β-galactosidase activity in corresponding control hepatocytes. Data are analyzed by Duncan's multiple range test (a non-parametric test). Significantly different from the corresponding control, *$P < 0.05$; #$P < 0.05$ vs. DEX-treated group.

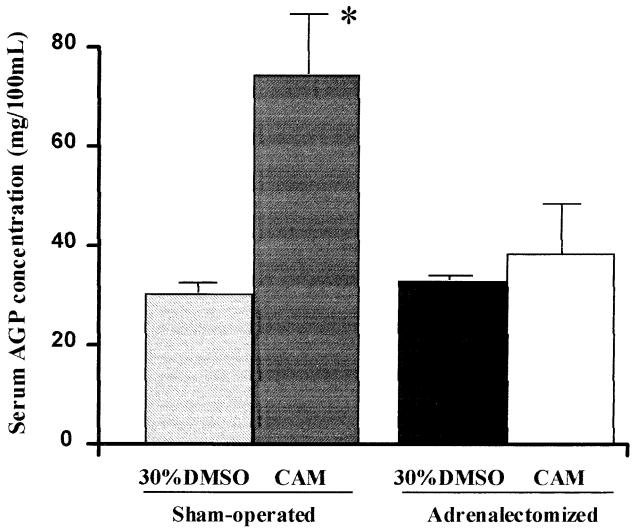

Fig. 5. Effect of CAM on AGP protein levels in rat serum. The adrenalectomized and sham-operated male Wistar rats (six rats per group) were treated for 4 days s.c with CAM at doses of 25 mg/kg/day. Blood was withdrawn 24 hr after the last injection and centrifuged at 3000 *g* for 10 min. The levels of AGP protein in rat serum were measured by a radial immunodiffusion assay. Data are analyzed by Duncan's multiple range test (a non-parametric test). Significantly different from the corresponding control, $*P < 0.05$.

4. Discussion

The findings herein show that the five macrolides examined caused an increase in serum AGP levels. It is possible that these macrolides may influence the binding of other drugs on AGP, as well as the biological function of AGP. Of these, CAM appeared to be the most potent inducer *in vivo.*

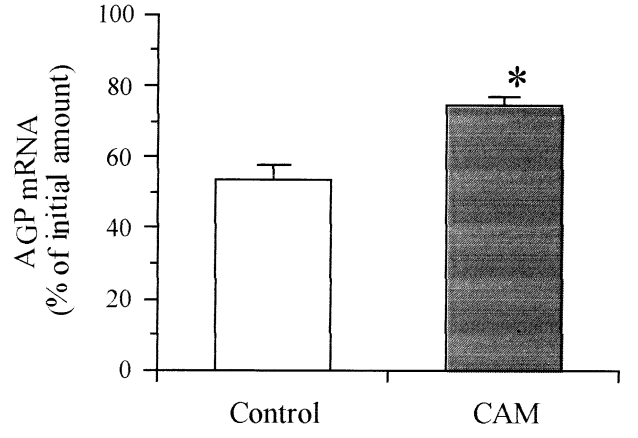

Fig. 6. Effect of CAM on the rate of degradation of AGP mRNA in primary cultured rat hepatocytes. Hepatocytes were incubated with 0.5 μg/mL actinomycin D for 0.5 hr prior to the addition of 4×10^{-6} M CAM at 18 hr after plating ($t = 0$). The 18-hr-old cultures were further treated for 6 hr and harvested for isolation of total RNA and AGP mRNA levels, which were analyzed by Northern blot hybridization. All values are expressed as the mean ± SD of four independent experiments and as the percentage of AGP mRNA/β-actin mRNA in hepatocytes at $t = 0$. Data are analyzed by Student's *t*-tests. Significantly different from the control, $*P < 0.05$.

In an *in vitro* study, the suppressive effect of CAM on IL-6 expression is equivalent to that of erythromycin in bronchial epithelial cells [13]. Therefore, it seems that the differences in the extent of AGP induction *in vivo* among these macrolides may be due to differences in hepatic uptake or the stability of the macrolide. In support of this possibility, two studies have concluded that the bioavailability of CAM is significantly higher than that of erythromycin [20], and that CAM accumulates 7-fold more than erythromycin in liver (S. Morimoto, personal communication, 1998).

For the quantitation of AGP in rat serum, a radial immunodiffusion assay was employed using rabbit anti-rat AGP antiserum. AGP has variable glycan chains at five glycosylation sites, and this glycosylation is altered by both physiological and pathological conditions. In addition, the pattern of glycosylation of AGP is responsible for the binding of drugs and its biological functions [2,3]. The antiserum used here will grossly recognize the oligosaccharide structure of AGP. Thereby, if CAM affects the AGP glycosylation, it may be necessary to reconsider the validity of our quatnitative data. We found by Crossed affino Con A immunoelectrophoresis that the increased AGP synthesis in turpentine-induced inflammatory rats was accompanied by an increase in reactivity for Con A (data not shown), suggesting altered levels of glycosylation of AGP. These alterations may be involved in the regulation of post-translational changes by cytokines such as IL-1 and IL-6 [4]. In contrast, CAM did not affect the pattern of glycosylation (data not shown). Thus, it appeares that CAM dose not qualitatively modulate the oligosaccharide structure and the roles of AGP.

An increase in AGP mRNA levels by CAM was observed in primary cultured rat hepatocytes as well as *in vivo.* Although CAM slightly increased the stability of AGP mRNA, we assumed that the main mechanism for the up-regulation of AGP mRNA is at the transcriptional activation level. To examine the mechanism of action of CAM on transcription, we carried out promoter analysis using several luciferase reporter constructs containing the rat AGP 5′-flanking region. First, although we used a rat AGP gene ranging from −360 to +20 bp in the promoter analysis, CAM itself had no effect on promoter activity. We next specifically focused on the involvement of a potential glucocorticoid-response on the effect of CAM, because this region contains GRE, which is located between positions −121 to −107. We indicated that CAM potentiated the DEX-induced promoter activity of pAGP(−172)Luc and pAGP(−360)Luc, both of which contain GRE. The potentiation of DEX-induced transcription by CAM was abolished in the absence of GRE sequences and in combination with the antiglucocorticoid, RU486. In addition, the effect of CAM was not specific for the AGP promoter, because MMTV promoter containing GRE was also more activated by CAM in combination with DEX than DEX alone. In support of this, CAM did not increase AGP levels in serum in adrenalectomized rats. In addition, because CAM did not

increase enodogenous corticosterone in serum, CAM may directly affect the glucocorticoid signaling pathway. An immunosuppressive macrolide, such as FK506 or rapamycin has been reported to potentiate GR-mediated gene expression by increasing the nuclear translocation of GR [19]. The nuclear translocation was mediated via the binding of the macrolides to hsp56, which is associated with cytosolic GR. It is possible that macrolides may up-regulate AGP gene expression by increasing the nuclear translocation of GR, although whether CAM increases the nuclear translocation of GR remains to be determined.

The data presented in this study clearly showed that CAM strongly increased AGP level in plasma and AGP mRNA levels in liver *in vivo*, and that in primary cultured rat hepatocytes, CAM induced transcriptional activation of the AGP gene, at least in part, through GR-mediated regulation.

Acknowledgments

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References

[1] Kremer JMH, Wilting J, Janssen LHM. Drug binding to human α_1-acid glycoprotein in health and disease. Pharmacol Rev 1988;40:1–47.

[2] Bennett M, Schmid K. Immunosuppression by human plasma α_1-acid glycoprotein:importance of the carbohydrate moiety. Proc Natl Acad Sci USA 1980;77:6109–13.

[3] Bories PN, Feger J, Benbernou N, Rouzeau J-D, Agneray J, Durand G. Prevalence of tri- and tetraanttenary glycans of human α_1-acid glycoprotein in release of macrophage inhibitor of interleukin-1 activity. Inflammation 1990;14:315–23.

[4] Pos O, Moshage HJ, Yap SH, Snieders JPM, Aarden LA, Gool JV, Boers W, Brugman AM, Dijk WV. Effect of monocytic products, recombinant interleukin-6 on glycosylation of α_1-acid glycoprotein: studies with primary human hepatocyte cultures and rats. Inflammation 1989;13:415–27.

[5] Richards CD, Brown TJ, Shoyab M, Baumann H, Gauldie J. Recombinant oncostatin M stimulates the production of acute phase proteins in HepG2 cells and rat primary hepatocytes in vitro. J Immunol 1992;148:1731–6.

[6] Baumann H, Gauldie J. The acute phase response. Immunol Today 1994;15:74–80.

[7] Prowse KR, Baumann H. Hepatocyte-stimulating factor, β_2 interferon, and interleukin-1 enhance expression of the rat α_1-acid glycoprotein gene via a distal upstream regulatory region. Mol Cell Biol 1988;8:42–51.

[8] Klein ES, DiLorenzo D, Posseckert G, Beato M, Ringold GM. Sequences downstream of the glucocorticoid regulatory element mediate cycloheximide inhibition of steroid induced expression from the rat α_1-acid glycoprotein promoter: evidence for a labile transcription factor. Mol Endocrinol 1988;2:1343–51.

[9] Bertaux O, Fournier T, Chauvelot–Moachon L, Porquet D, Valencia R, Durand G. Modification of hepatic α_1-acid glycoprotein and albumin gene expression in rats treated with phenobarbital. Eur J Biochem 1992;203:655–61.

[10] Fournier T, Mejdoubi N, Lapoumeroulie C, Hamelin J, Elion J, Durand G, Porquet D. Transcriptional regulation of rat α_1-acid glycoprotein gene by phenobarbital. J Biol Chem 1994;269:27175–8.

[11] Fournier T, Mejdoubi N, Monnet D, Durand G, Porquet D. Phenobarbital induction of α_1-acid glycoprotein in primary rat hepatocyte cultures. Hepatology 1994;20:1584–8.

[12] Fournier T, Vranckx R, Mejdoubi N, Durand G, Porquet D. Induction of rat α_1-acid glycoprotein by phenobarbital is independent of a general acute-phase response. Biochem Pharmacol 1994;48:1531–5.

[13] Takizawa H, Desaki M, Ohtoshi T, Kikutani T, Okazaki H, Sato M, Akiyama N, Shoji S, Hiramatsu K, Ito K. Erythromycin suppresses interleukin 6 expression by human bronchial epithelial cells: a potential mechanism of its anti-inflammatory action. Biochem Biophys Res Commun 1995;210:781–6.

[14] Komori T, Nonaka A, Maruyama T, Otagiri M. Effect of clarithromycin on α1-acid glycoprotein levels in normal and diabetic rats. Res Commun Mol Pathol Pharmacol 1998;101:233–40.

[15] Scotte M, Horon M, Masson S, Lyourni S, Banine F, Teniere P, Lebreton JP, Daveau M. Differential expression of cytokine genes in monocytes, peritoneal macrophages and liver following endotoxin- or turpentine-induced inflammation in rat. Cytokine 1996;8:115–20.

[16] Reinke R, Feigelson P. Rat α_1-acid glycoprotein. J Biol Chem 1985; 260:4397–403.

[17] Sanger J, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977;74:5463–7.

[18] Bog–Hansen TC, Prahl P, Lowenstein H. A set of analytical electrophoresis experiments to predict the results of affinity chromatographic separations: fractionation of allergens from cow's hair, and dander. J Immunol Methods 1978;22:293–307.

[19] Ning Y-M, Sanchez ER. Potentiation of glucocorticoid receptor-mediated gene expression by immunophilin ligands FK506 and rapamycin. J Biol Chem 1993;268:6073–6.

[20] Morimoto S, Adachi T, Misawa Y, Nagate T, Watanabe Y, Omura S. Chemical modification of erythromycins IV. Synthesis and biological properties of 6-*O*-methylerythromycin B. J Antibiotics 1990;43:544–9.