J. Biochem. 131, 71–78 (2002)

Adenovirus Vector-Mediated Reporter System for *In Vivo* Analyses of Human *CYP3A4* Gene Activation[1]

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Received October 4, 2001; accepted October 27, 2001

The use of cultured mammalian cells and artificial promoters for analyses of gene regulation gives results that are sometimes inconsistent with *in vivo* events and thus inconclusive. To understand the *in vivo* mechanism of chemically mediated *CYP3A4* gene activation, we have used a natural promoter of the *CYP3A4* gene and an adenovirus as a reporter vector. The adenovirus reporter vector (AdCYP3A4-362) was constructed with a proximal promoter region (–362 to +11 nt) of the *CYP3A4* gene and a luciferase-reporter gene. AdCYP3A4-362 was then infected into mice, and both the reporter and mouse CYP3A activities were measured. Clear increases in the reporter activity were observed in livers of all mice treated with chemicals. The profile of the *CYP3A4* gene activation with chemicals was in good agreement with that of endogenous mouse CYP3A-mediated testosterone 6β-hydroxylase. Introduction of nucleotide mutations in the receptor-binding region (ER-6) of the *CYP3A4* promoter resulted in diminished reporter activity. These results indicate the advantage of the adenovirus-mediated *in vivo* system over the currently available *in vitro* systems for gene transcriptional activation.

Key words: adenovirus, CYP3A4, induction, *in vivo*, PXR.

Cytochrome P450 (P450) plays an essential role in the oxidative biotransformation of endogenous compounds such as steroids, fatty acids, prostaglandins, and of exogenous chemicals including drugs, natural plant products, carcinogens, and environmental pollutants (*1, 2*). A number of CYP3A forms have been identified from experimental animals and humans. In human, four forms, CYP3A4 (*3*), CYP3A5 (*4*), CYP3A7 (*5*), and CYP3A43 (*6–8*), have been identified and characterized. The predominant P450 form, CYP3A4, is a major microsomal P450 in liver and intestine and has been reported to be involved in the metabolism of more than 60% of medically relevant drugs (*9*). The *CYP3A4* gene is transcriptionally activated by treatment with such drugs as phenobarbital, clotrimazole, troglitazone, rifampicin and nifedipine (*10–12*). A nuclear receptor, termed pregnane X receptor (PXR), has been shown to be responsible for the activation of the *CYP3A* genes (*13–16*). PXR binds to everted repeat TGA(A/G)CT separated by six nucleotides (ER-6 motif) in the promoter region of *CYP3A4* and *CYP3A7* genes as a heterodimer with retinoid X receptor α and mediates transcriptional activation of these genes with several chemicals (*17*).

Gene transcriptional activation has generally been studied *in vitro* with cultured cells. Cultivation and immortalization, however, cause the alteration of cell properties and often loss of some intrinsic function that is observed in the tissue. The expression profile of individual proteins in cell lines is thus different from that in the original tissue. In particular, the expression profiles of drug-metabolizing enzymes are dramatically changed even in the primary cultured cells compared to intact liver tissue. Therefore, the results obtained with cultured cells are not always consistent with phenomena observed in *in vivo* studies.

Several mammalian cell lines have been used for *in vitro* studies of the *CYP3A* gene regulation. HepG2 and Caco-2 cells, derived from human liver carcinoma and human colonic tumor, respectively, responded to the reporter activity mediated from a *CYP3A4* reporter plasmid construct (*18–20*), although the intrinsic CYP3A4 is not expressed in these cell lines (*21–23*). CV-1, a cell line derived from kidney of African green monkey, has also been used for studies of the *CYP3A4* gene activation (*14*), but expression of monkey CYP3A forms is obscure in this cell line. To detect a reporter activity mediated through the activation of the *CYP3A4* gene, strong promoters, TK and SV 40, have been substituted for the natural promoter, and multiple ER-6 or direct repeat-3 of TGA(A/G)CT motifs have been constructed in the 5′-upstream regions of these promoters (*18, 24–26*). As a result, high efficiencies of the gene activation were observed. A recent study has, however, reported that the *CYP3A4* gene with a natural promoter and ER-6 motif was not activated by chemicals (*18*). These results suggest that artificial promoters may alter the gene activation observed in intact tissues, and that *in vivo* study is necessary to elu-

[1]This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture (Ministry of Education, Culture, Sports, Science and Technology) and the Ministry of Health and Welfare (Ministry of Health, Labor, and Welfare), Japan.

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Abbreviations: DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethylsulfoxide; ER-6, everted repeat separated by six nucleotides; PBS, phosphate-buffered saline; PXR, pregnane X receptor; TCID, tissue culture infectious dose.

cidate precisely the activation of the *CYP3A4* gene with chemicals.

In the present study, we have established an *in vivo* analysis system for the activation of the *CYP3A4* gene by use of an adenovirus as a reporter vector.

MATERIALS AND METHODS

Materials—Rifampicin, dexamethasone and clotrimazole were obtained from Sigma Chemical (St. Louis, MO). The chemicals used for the *in vitro* study were dissolved in dimethylsulfoxide (DMSO). Restriction endonucleases and DNA modification enzymes were purchased from Takara Shuzo (Kyoto). Dulbecco's modified Eagle's medium (DMEM) and fetal calf serum were obtained from Gibco (Rockville, USA) and Roche Diagnostics (Indianapolis, USA), respectively.

Animals—Male mice (ddY strain), weighing 30–33 g and aged 6 weeks at the time of use, were obtained from Japan SLC (Shizuoka) and treated intraperitoneally with dexamethasone, rifampicin and clotrimazole as suspensions in corn oil at 100 mg/kg/day or with vehicle (control) for 3 days. The mice were infused intraperitoneally with 1×10^9 $TCID_{50}$/mouse of the adenovirus vector 4 h after the second administration of drugs and sacrificed 2 days later. Livers were removed and washed with cold phosphate-buffered saline (PBS). The livers were homogenized in 3 volumes of 25 mM Tris-HCl buffer, pH 7.4. The homogenates were centrifuged at 9,000 ×*g* for 20 min. The supernatants (S9 fractions) were used for the assay of luciferase and testosterone 6β-hydroxylase activities and protein concentration.

Cell Culture and Cell Extract—Cells were cultivated in 5% CO_2 at 37°C in DMEM supplemented with 10% fetal calf serum, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 0.25 μg/ml of amphotericin B, 3.5 g/liter of glucose, and 10 ml/liter of 100×MEM non-essential amino acid solution (Gibco).

The cells were seeded in 24-well tissue culture plates at 2×10^5 cells per well in 0.5 ml of DMEM, and on the next day the growth medium was removed and the cells were treated with the medium containing 10, 30, or 100 μM drug at a final concentration of 0.1% DMSO for 1 day. After removal of the medium, the cells were infected with 0.05 ml of AdCYP3A4-362 solution for 1 h, then the medium containing the drug was added to the wells.

Two days after infection, the cells were washed with PBS and suspended in 0.1 ml of Reporter Lysis Buffer (Promega, Madison) in a microcentrifuge tube. The cell suspension was centrifuged at 12,000 ×*g* for 2 min at 4°C, and the cell extract was used for luciferase assay and determination of protein concentration.

Construction and Generation of Adenovirus Reporter Vector (AdCYP3A4-362)—The luciferase reporter plasmid, pGL3-Basic vector lacking eukaryotic promoter and enhancer sequences, was purchased from Promega. The promoter region (–362 to +11 nt) of the human *CYP3A4* gene was inserted into *Hin*dIII and *Bgl*II sites of the pGL3-Basic vector. The mutated promoter at the ER-6 region was produced by PCR according to methods previously reported (*27*). Oligonucleotides used were 5′-GAATATGAATTCAAAGGACGTCAGT-3′ and 5′-ACTACTGACGTCCTTTGAATTCATAT-3′ where the underlined letters indicate the nucleotide substitutions. Binding of nuclear factor(s) to these double-strand oligonucleotides was not ob-served on electrophoretic mobility shift assay. The *CYP3A4* gene constructs were verified by DNA sequencing (*28*).

Adenovirus reporter vectors were generated according to the COS-TPC method (*29*) with the aid of a Adenovirus Expression Vector Kit from Takara Shuzo. The pGL3-Basic vector containing the human *CYP3A4* promoter (pGLCYP-3A4-362) was inserted into the expression cassette cosmid, pAxcw, containing a nearly full-length Ad5 genome with E1 (454–3328 nt) and E3 (28592–30470 nt) deletions without promoter (*30*). The expression cosmid and the parental adenovirus DNA terminal protein complex were co-transfected into 293 cells by calcium phosphate precipitation using a CellPhect Transfection Kit (Pharmacia, New Jersey) for homologous recombination. The recombinant adenovirus was isolated and propagated into the 293 cells according to the manual from Takara's kit. The titer of AdCYP3A4-362 and AdCYP3A4-362M stock solutions, determined by the assay of 50% tissue culture infectious dose ($TCID_{50}$) for 293 cells, was 2.5×10^9 $TCID_{50}$/ml and 2.6×10^9 $TCID_{50}$/ml, respectively. $TCID_{50}$ is approximately equivalent to the plaque forming unit (*31*). The viral stock solution was stored at –80°C and diluted with the culture medium for the *in vitro* study.

Luciferase Assay—Luciferase assay was performed according to the manufacturer's instructions for Promega using the Luciferase Assay System and Turner Designs TD-20/20 Luminometer (Promega). The activity was expressed as relative light units (RLU)/mg of protein. The effect of drugs was presented as the ratio of treatment to no treatment.

Other Assays—Testosterone 6β-hydroxylase assay was carried out according to the previous report (*32*). Protein concentration was determined according to the procedure of Bradford with bovine serum albumin as the standard (*33*).

Statistics—Statistical analysis was performed by the *t*-test or the Dunnett's test using the SAS System v8 for Windows (SAS Institute, Cary, NC, USA).

RESULTS

Construction of Adenovirus Reporter Vector (AdCYP3A4-362)—A COS-TPC method established by Saito was used to produce an adenovirus reporter vector with the promoter region of the *CYP3A4* gene (*29*). In this method, the adenovirus reporter vector was generated finally by homologous recombination in 293 cells. Vectors (pGL3-Basic and pAxcw) lack eukaryotic promoter and enhancer sequences. pAxcw is a basic vector containing adenovirus genome DNA from which ΔE1 and ΔE3 are eliminated. Consequently, a DNA fragment of about 7 kbp can be inserted into the vector. At first, a pGLCYP3A4-362 reporter vector carrying the promoter region (–362 to +11 nt) of the *CYP3A4* gene was constructed with the pGL3-Basic vector. Then, pGLCYP3A4-362 (5.1 kbp) was directly inserted into the *Swa*I site of pAxcw to produce AdCYP3A4-362 (Fig. 1). The level of the luciferase activity in cells infected with AdCYP3A4-362 depends on the function of the *CYP3A4* promoter. A recombinant adenovirus vector, AdCYP3A4-362M, in which two nucleotides were substituted in ER-6 of the *CYP3A4* promoter, was also produced to verify the activation of the *CYP3A4* gene by the binding of PXR.

Infection of Adenovirus Vector to HepG2 Cells—HepG2

cells were used to evaluate AdCYP3A4-362. The luciferase activity measured 48 h and 96 h after the AdCYP3A4-362 infection depended on the amount infected, as shown in Fig. 2. The activity was 10 to 20 times higher at 96 h than at 48 h. The level of luciferase activity increased depending on viral dosage. As shown in Fig. 3, the reporter gene expression increased linearly for 96 h and maintained a high level for 8 days after the infection. Based on the these results, the induction experiment was carried out at a dose of 8.3×10^4 $TCID_{50}$ in HepG2 cells (Fig. 4). Clotrimazole did not affect the luciferase activity 24 h after the adenoviral infection, but a 10-fold increase was observed at 48 h (1,028% of control) and at 120 h (1,161% of control).

To verify the induction efficiency, the reporter activities of adenovirus AdCYP3A4-362 and classical pGLCYP3A4-362 were compared. As shown in Fig. 5, the induction by AdCYP3A4-362 (8-fold) was four times higher than that by pGLCYP3A4-362 (2-fold) in clotimazole-treated HepG2 cells.

Infection of Adenovirus Vector to Mouse—The adenovirus vector was intravenously administered into mice (ddy). High luciferase activity was observed after intravenous administration of 1×10^9 $TCID_{50}$ at 24 h, but some mice died due to the toxicity. Therefore, the virus was administered intraperitoneally to male mice. The animals tolerated the transfection at the 1×10^9 $TCID_{50}$ dose, thus this ip dose was adopted for the infection. The luciferase activity was

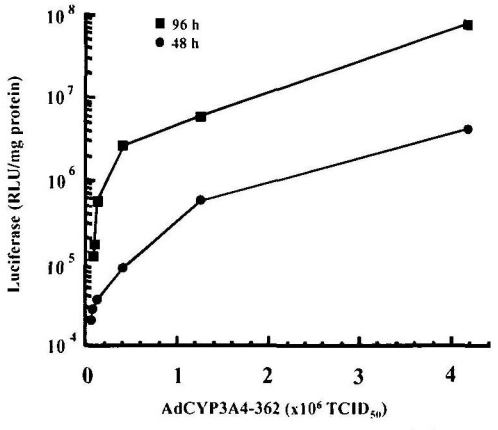

Fig. 2. **Dose-dependency of the luciferase activity expressed in HepG2 cells infected with AdCYP3A4-362.** HepG2 cells seeded at a density of 5×10^5 cells in 24-well culture plates the day before the infection were incubated with various amounts of the adenovirus for 1 h and further cultured for 48 h (circle) and 96 h (square). Enzyme preparation and assay were carried out as described in "MATERIALS AND METHODS." The luciferase activity is expressed as relative light units (RLU) to protein content and is the average from four wells.

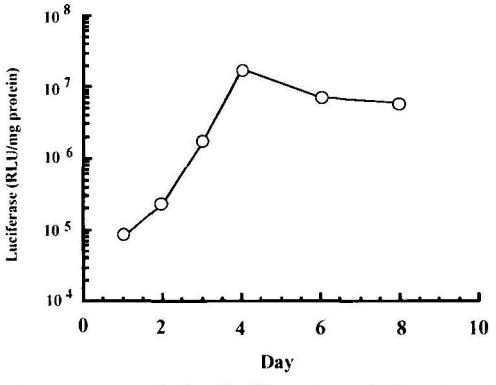

Fig. 3. **Time course of the luciferase activity expressed in HepG2 cells infected with AdCYP3A4-362.** Cells used at 5×10^5 cells per well of 24-well culture plate were cultivated for 1, 2, 3, 4, 6, and 8 days after infection of the adenovirus at 8.3×10^4 $TCID_{50}$ for 1 h. The luciferase activity in cell lysate was measured as described in "MATERIALS AND METHODS" and expressed as relative light units (RLU) per mg of protein. Values are the mean from four wells.

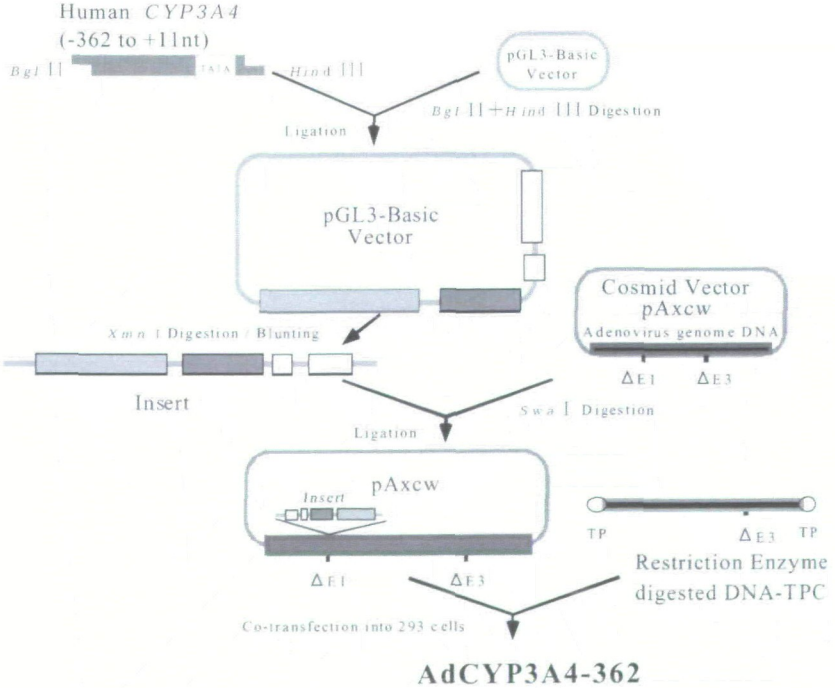

Fig. 1. **Construction of CYP3A4 adenovi reporter vector (AdCYP3A4-362).**

determined with mouse liver S9 fraction. As shown in Figs. 6 and 7, the levels of the reporter activity in livers were dependent on the amount of the adenovirus vector up to a dose of 1×10^9 $TCID_{50}$ per mouse, reached a peak 5 days after the adenovirus infection, then decreased gradually. The reporter activity was, however, detectable for at least 2 weeks.

Effect of Drugs on the Reporter Activity Expressed in Mammalian Cells—To compare the reporter activity mediated through the *CYP3A4* promoter among mammalian cells, HepG2, H-4-II-E, COS-1, and LS174T cells were infected with AdCYP3A4-362 and treated for 3 days with 10 μM dexamethasone, rifampicin or clotrimazole. The luciferase activity varied not only among the chemical treatments but also among the cell lines (Fig. 8). In HepG2 cells, the reporter activity 48 h after the transfection of AdCYP3A4-362 increased in the presence of clotrimazole (820% of control) or dexamethasone (240% of control), but not in the presence of rifampicin. In COS-1 and LS174T cells, the reporter activity was only increased by treatment with dexamethasone (151% of control) and rifampicin (172% of control), respectively. In H-4-II-E cells derived from rat hepatoma, significant activation was not observed after treatment with these drugs. The reporter activity increased up to 5 times at 100 μM rifampicin in the LS174T cell line (184 to 489% of control). Clear induction by rifampicin was not observed in HepG2, H-4-II-E, and COS-1 cell lines.

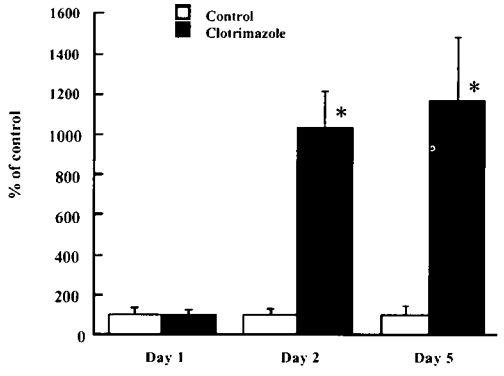

Fig. 4. **Effect of clotrimazole on the luciferase activity in HepG2 cells infected with AdCYP3A4-362.** Cells were seeded at 2×10^5 cells/well of 24-well plate (Day −2) and cultured in the presence of 10 μM clotrimazole for 24 h. After infection with AdCYP3A4-362 at 8.3×10^4 $TCID_{50}$ for 1 h (Day 0), the cells were exposed to 10 μM clotrimazole for 48 h. Luciferase expressed in cells was detected as described in "MATERIALS AND METHODS." Results are expressed as a percentage of the average for the control treated with 0.1% DMSO. Data are the means ± SD from four wells. * Significantly different from the control at $p < 0.01$ by *t*-test.

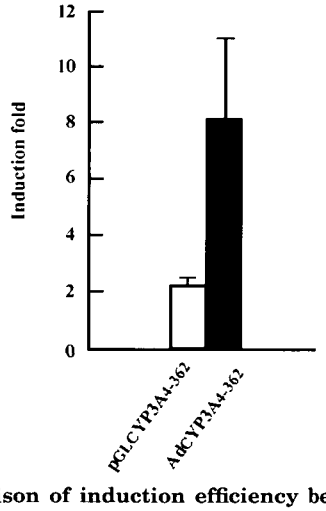

Fig. 5. **Comparison of induction efficiency between AdCYP3A4-362 and pGLCYP3A4-362.** For pGLCYP3A4-362, HepG2 cells (3×10^5 cells/well) were transfected with 3 μg of the DNA by calcium phosphate transfection according to the previous report (*40*). For AdCYP3A4-362, HepG2 cells were transfected according to the method described in Fig. 4. Both cell cultures were exposed to 10 μM clotrimazole for 48 h. Luciferase expressed in cells was detected as described in "MATERIALS AND METHODS." Results are expressed as a percentage of the average for the control treated with 0.1% DMSO. Data are the means ± SD from four wells.

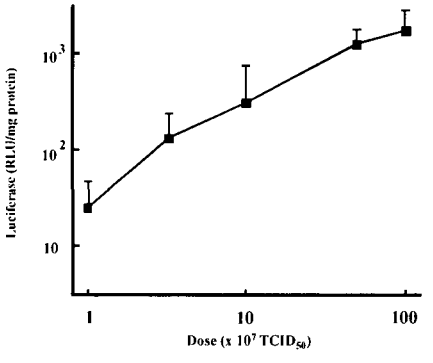

Fig. 6. **Dose-dependency of the luciferase activity in mouse livers after intraperitoneal administration of AdCYP3A4-362.** Various amounts of AdCYP3A4-362 were intraperitoneally injected into male mice. Two days thereafter, the livers were prepared for luciferase assay. The assay was performed as described in "MATERIALS AND METHODS." The luciferase activities are expressed as relative light units (RLU) to protein content of liver S9 fraction. Each point is the mean ± SD of three animals.

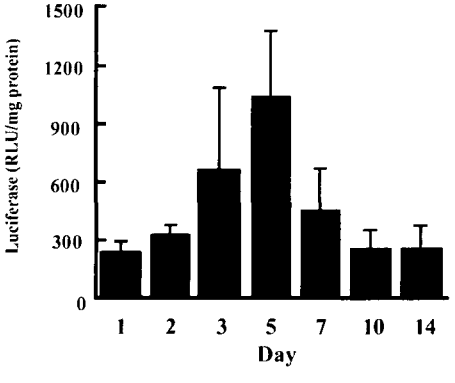

Fig. 7. **Time course of luciferase activity expressed in liver of mice after intraperitoneal administration of AdCYP3A4-362.** AdCYP3A4-362 was intraperitoneally administered to male mice at a dose of 1×10^9 $TCID_{50}$/animal. Liver samples were prepared according to the method described in "MATERIALS AND METHODS" 1, 2, 3, 5, 7, 10, and 14 days after the treatment. The luciferase activity is expressed as relative light units (RLU) per mg of protein. Results are given as means ± SD of three animals.

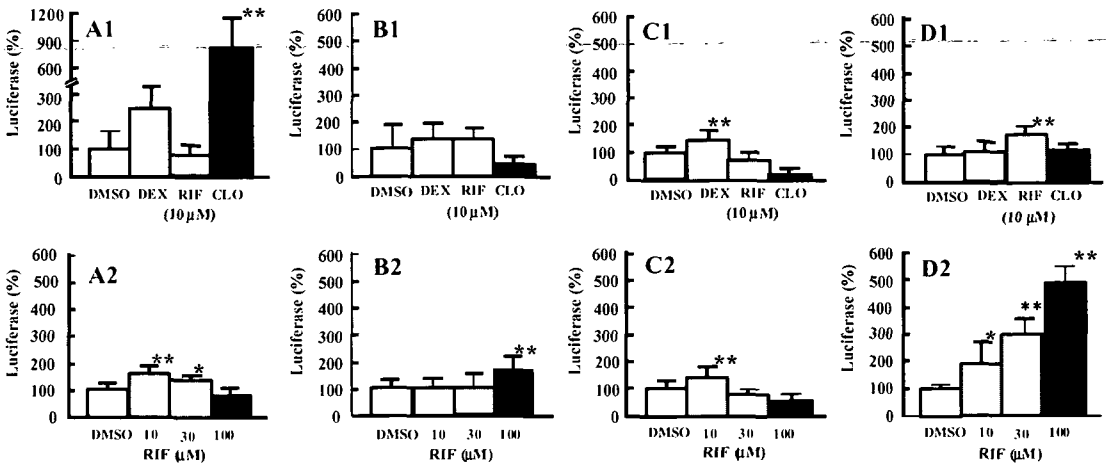

Fig. 8. **Effect of drugs on luciferase activity expressed in various cell lines infected with AdCYP3A4-362.** A1 and A2: HepG2, B1 and B2: H-4-II-E, C1 and C2: COS-1, D1 and D2: LS174T. DMSO, dimethylsulfoxide (0.1%); DEX, dexamethasone; RIF, rifampicin; CLO, clotrimazole. All transfection experiments were performed with AdCYP3A4-362 at 8.3×10^4 $TCID_{50}$ in 24-well culture plates as described "MATERIALS AND METHODS." Luciferase activities are expressed as the percentage of the average for the control treated with 0.1% DMSO. Error bars are the standard deviation ($n = 6$). * and **: Significantly different from the control with DMSO at $p < 0.05$ and $p < 0.01$ by Dunnett's test, respectively.

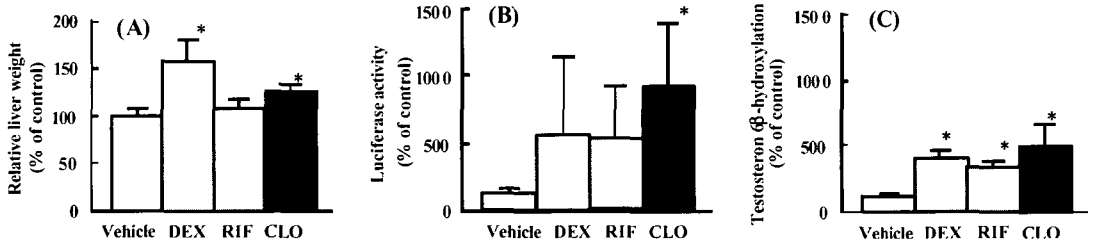

Fig. 9. **Effects of drugs on liver luciferase and testosterone 6β-hydroxylation activities in mouse infected with AdCYP3A4-362.** Vehicle, corn oil; DEX, dexamethasone; RIF, rifampicin; CLO, clotrimazole. Animals were treated intraperitoneally with drugs at a dose of 100 mg/kg/day for 3 days and infused with AdCYP3A4-362 4 h after the second dose. The amount of virus injected was 1×10^9 $TCID_{50}$/animal in a volume of 0.4 ml. Two days after the adenovirus injection, livers were weighed and homogenized to obtain the S9 fraction. The luciferase and testosterone 6β-hydroxylase activities were determined as described in "MATERIALS AND METHODS." Values were calculated based on the average from control mice. Each bar represents means ± standard deviation of five animals. * Significantly different from the vehicle control group at $p < 0.01$ (Dunnett's test).

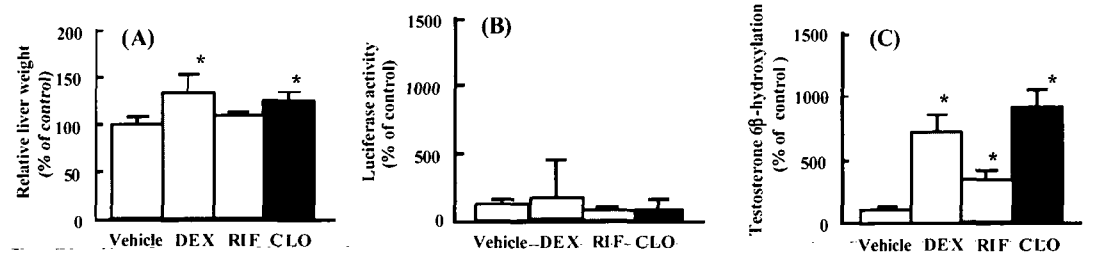

Fig. 10. **Effects of nucleotide substitution in ER-6 region on induction of the liver luciferase and testosterone 6β-hydroxylation activities in mouse.** Vehicle, corn oil; DEX, dexamethasone; RIF, rifampicin; CLO, clotrimazole. Drugs were administered intraperitoneally at a dose of 100 mg/kg/day for 3 days, and AdCYP3A4-362M was infused at 1×10^9 $TCID_{50}$/animal 4 h after the second dose. Livers were weighed and homogenized to obtain the S9 fraction 2 days after the dose of adenovirus. The luciferase and testosterone 6β-hydroxylase activities were determined as described in "MATERIALS AND METHODS." Data are shown as a percentage of control mean obtained from the vehicle group and represent means ± standard deviation of five animals. * Significantly different from the vehicle control group at $p < 0.01$ (Dunnett's test).

Effect of Drugs on the Reporter Activity Expressed in Mouse Livers—To verify *in vivo* activation of AdCYP3A4-362, the reporter activity of mouse livers was monitored after administration of drugs. The influence of AdCYP3A4-362 transfection on liver weight and endogenous testosterone 6β-hydroxylase activity of liver S9 fraction was also determined (Fig. 9). Mice were treated intraperitoneally with dexamethasone, rifampicin or clotrimazole at a dose of

100 mg/kg/day for 3 days. The chemicals used increased the reporter activity in mouse liver as compared to the control (543 to 905% of control; Fig. 9B). The reporter activity was increased 5 times by treatment with dexamethasone or rifampicin, and 9 times with clotrimazole. The liver weights of mice treated with drugs, except for rifampicin (109% of control), were significantly larger than those of the control group (126 and 158% of control; Fig. 9A). Endogenous testosterone 6β-hydroxylase activity was also increased after the treatment with these drugs (341 to 484% of control; Fig. 9C). The activities were about 3.5 to 5 times higher than the control. The change in profile of the testosterone 6β-hydroxylation is similar to that of the reporter activity expressed in mice.

To confirm that *in vivo* gene activation of the reporter is mediated through the *CYP3A4* promoter, a mutated construct of AdCYP3A4-362 at the ER-6 site (AdCYP3A4-362M) was produced. In the case of AdCYP3A4-362M, the reporter activity in liver was not affected by treatment with drugs, except for dexamethasone (Fig. 10B). The luciferase activity was increased only 1.5 times by treatment with dexamethasone. On the contrary, activity of mouse CYP3A forms was increased about 3.5 times by rifampicin, and 7 and 9 times by dexamethasone and clotrimazole, respectively (348 to 912% of control; Fig. 10C).

DISCUSSION

The present study examined the use of an adenovirus as a reporter vector of *in vivo* transcriptional activation of the *CYP3A4* gene. Initial attempts using a pGLCYP3A4-362 with the *CYP3A4* promoter (–362 to +11 nt) derived from pGL3-basic vector resulted in a minimal activation in HepG2 cells. Therefore, AdCYP3A4-362 was constructed by ligating the whole pGLCYP3A4-362 into a cosmid vector, including adenovirus gene DNA, and then by homologous recombination in 293 cells. AdCYP3A4-362 was assessed in HepG2 cells. High reporter activity was detected and depended on the amount of AdCYP3A4-362 infected (Fig. 2). The activity showed the highest level at 4 days after the infection and maintained a high level for 8 days (Fig. 3). The advantage of the adenovirus reporter vector over the classical reporter vector is in chemical induction studies. The reporter activity mediated through AdCYP3A4-362 is more than four times higher than that through pGLCYP3A4-362 in HepG2 cells. Moreover, clear induction profiles with drugs were observed on AdCYP3A4-362. The transfection method of AdCYP3A4-362 is very simple compared to that of an ordinary reporter vector. The high reporter activity was obtained only by adding the virus into the culture medium at the dose of 8.3×10^4 $TCID_{50}$. Under these conditions, the significant induction mediated through the *CYP3A4* promoter was clearly observed after treatment with drugs. Furthermore, the reporter activity was observed in mouse liver as well as cultured cells, depending on the dose.

Recently, adenovirus vectors have been used for gene therapy in cancer (*34*) and hereditary disease (*35*). Adenovirus vectors have high efficiencies for transformation of livers and display a high expression level of recombinant protein in humans as well as in experimental animals (*35–37*), although several factors limiting the application of adenovirus have been reported. Adenovirus can cause significant cytotoxicity and immunological responses in the infected animal, which limit the duration and level of target gene expression. The target gene is, however, expressed for from several weeks to several months in animals. As described in "MATERIALS AND METHODS," the period from infection to sacrifice in our experiment was at most 3 to 4 days, and a high reporter activity was maintained in livers during this period. When the virus was injected *via* the tail vein, some mice died. The reason is not clear, but seemed to be attributable to the cytotoxicity of adenovirus; thereafter, the virus was injected intraperitoneally. Consequently, all infected mice survived and supported the expression of the reporter activity for at least 2 weeks. For this *in vivo* experiment, therefore, the limitation of the adenovirus application was not a problem.

In *in vitro* gene activation studies of *CYP3A4*, artificial promoters like SV40 and TK have generally been used. These promoters can be used to study regulation of the *CYP3A4* gene in several cell lines, even though the cells do not express CYP3A4. Recently, a natural promoter has been used for regulation study of the *CYP3A4* gene. It was reported that the promoter region (–362 to +53 nt) of the *CYP3A4* gene did not work to activate the reporter gene upon treatment of HepG2 cells with rifampicin (*18*). As expected previously, the activation level and profile produced by drug treatment differed greatly among cultured cell lines. As shown in Fig. 8, the reporter gene was strongly activated by clotrimazole but not by rifampicin in HepG2 cells. On the contrary, the reporter activity was increased in LS174T cells by treatment with rifampicin, depending on the dose. HepG2 cells are derived from human liver carcinoma, but the endogenous CYP3A4 is not expressed or induced in this cell line (*11*). LS174T cells are derived from human colonic adenocarcinoma and reported to have endogenous CYP3A4 after treatment with rifampicin (*38*). Both rifampicin and clotrimazole are known to induce CYP3A4 strongly in human livers (*14, 39*). These results make it difficult to assess the true mechanism of the CYP3A4 induction using cultured cells.

In the present *in vivo* experiment, ER-6 of the CYP3A4 gene was shown to mediate the CYP3A4 induction in mouse. The activity was increased in a dose-dependent manner and was retained for at least 2 weeks. Both reporter and mouse endogenous CYP3A activities (testosterone 6β-hydroxylase) were increased in parallel by treatment of ddy mice with drugs (Fig. 9). Similar results were observed on BALB/c (data not shown). The *in vivo* activation was further confirmed by a mutation experiment of the *CYP3A4* promoter. The *CYP3A4* induction was not clearly detected in mice infected with AdCYP3A4-362M after treatment with rifampicin or clotrimazole. This experiment supported the idea that the *CYP3A4* induction was mediated through the ER-6 region of the *CYP3A4* gene in mouse livers. Recently, a *cis*-acting element (dNR1) working as a distal enhancer has been found at around –7729 nt of the *CYP3A* gene. It was reported that dNR1 worked co-operatively with ER-6 in activation of the *CYP3A4* gene (*18*). The full gene activation by drugs was observed by the existence of both elements in cultured cells. Adenovirus reporter vector, including both ER-6 and dNR1 regions, may mediate higher gene activation by chemicals in mouse livers. We have undertaken an experiment to determine whether dNR1 is essential for the *CYP3A4* induction in liver by use

of adenovirus as the gene mediator.

Recent *in vivo* experiments with gene transgenic and knock-out mice have provided a lot of novel information that was not predicted from *in vitro* experiments. Therefore, convenient methods are required for studies of the *in vivo* gene transcriptional activation. In this way, the success of our *in vivo* study of the *CYP3A4* gene activation by use of adenovirus as a reporter vector may provide a powerful tool for analysis of the *in vivo* gene regulation.

We gratefully acknowledge Dr. Katsuki of National Institute for Basic Biology, Okazaki National Research Institutes, and Dr. Saito of the Institute of Medical Science, the University of Tokyo, for their support of this project.

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