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ACCELERATED COMMUNICATION

Development of A Real-Time in Vivo Transcription Assay: Application Reveals Pregnane X Receptor-Mediated Induction of CYP3A4 by Cancer Chemotherapeutic Agents

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ABSTRACT

We report the development of a rapid real-time assay that measures the transcription of luciferase reporter genes in transduced mouse hepatic cells in vivo. Luciferase activity is noninvasively measured by whole-body optical imaging within hours of the hydrodynamic injection of as little as 1 μg of naked DNA. Transcription of genes introduced as linearized DNA can be serially assayed for weeks in each animal. Transcription was quantified by extracorporal monitoring of bioluminescence as well as or better than by traditional in vitro bioluminescence assay. Our assay allows the measurement of transcription as it occurs, under the most informative biological conditions (i.e., in a living, intact organ). Furthermore, it substantially reduces the cost, time, and number of animals required for analysis of gene expression. The utility of the method is demonstrated in the discovery that topotecan and etoposide are ligands of pregnane X receptor that induce CYP3A4 transcription.

An in vivo gene transcription assay would have broad application in studies of hepatic gene expression and provide a unique complementary tool for the toxicologic testing of drugs under development. Most current studies of mammalian gene transcription are conducted in vitro, in replicating cells transfected with plasmids that contain DNA-regulatory sequences upstream of reporter genes. However, liver-specific gene expression is extinguished in replicating hepatocyte cultures as they lose crucial transcription factors. Moreover, cell cultures cannot recapitulate the full spectrum of hepatic responses to xenobiotic agents (e.g., inflammatory response). Although transgenic mice have been useful in the investigation of gene regulation in vivo, they require considerable time and money and the breeding of large numbers of animals over several generations. Furthermore, gene expression can be affected by random integration of the transgene, and several animals must be sacrificed each time expression is analyzed.

We took advantage of a method in which naked DNA is rapidly introduced into mouse liver by hydrostatic pressure (Liu et al., 1999; Zhang et al., 1999; Chen et al., 2001; Yang et al., 2001). This technique results in expression of the transgene in 40% of hepatocytes (Liu et al., 1999). More persistent (9-month) transgene expression can be achieved by infusion of linearized plasmids that concatenate in vivo (Chen et al., 2001), and the method can be used to insert transposon DNA into the adult mouse genome (Yant et al., 2000). We used this technique in conjunction with a novel photon detection system that allows quantitative whole-body imaging of bioluminescence generated by the reporter luciferase. This imaging method has been used previously to monitor the growth and localization of luciferase-tagged bacteria or tumor cell lines injected into mice (Sweeney et al., 1999; Contag et al., 2000; Rocchetta et al., 2001). It has also been used to monitor gene expression in replicating cells implanted in mice after transfection in vitro (Iyer et al.,

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ABBREVIATIONS: PXR, pregnane X receptor; XRE, xenobiotic response element; SXR, steroid and xenobiotic receptor; bp, base pair(s); AHR, aryl hydrocarbon receptor; CAR, constitutive androstane receptor; PCN, pregnenolone-16α-carbonitrile; SV40, simian virus 40.

2001), but it has not been used to quantify expression of reporter DNA in the transduced hepatic cells of live mice. A primary objective of our study was to develop a method of measuring transcription of genes in vivo and in real time that does not require the sacrifice of the experimental animal. Finally, we applied this new technique to determine in vivo whether some anticancer agents are pregnane X receptor (PXR) ligands and activators of CYP3A4 transcription.

Materials and Methods

Reporter Plasmids

CYP3A4. CYP3A4 (+53 to −362)-LUC, CYP3A4 +53 to −362(7836/7208)-LUC, and CYP3A4 (+53 to −13,000)-LUC (hereafter referred to as CYP3A4-362-LUC, CYP3A4-7836-LUC, and CYP3A4-13-kb-LUC, respectively) were generously provided by Dr. Chris Liddle (University of Sydney, Westmead, Australia). The CYP3A4-7836-LUC plasmid was linearized with *Sac*I.

CYP1A1. Rat CYP1A1-CAT was obtained from Dr. Fujii-Kuriyama (Department of Biomolecular Science, Graduate School of Life Science, Tohoku University, Sendai, Japan) (Fujisawa-Sehara et al., 1987) and the CYP1A1 promoter fragment, containing two xenobiotic response elements (XREs) in the inverse orientation (XRE1, −1007 to −1021 bp and XRE2, −1088 to −1092 bp), was subcloned into pGL2Basic (Promega, Madison, WI) to create CYP1A-LUC.

CYP2B1. A luciferase reporter plasmid containing −2413 bp of the CYP2B1 5′-flanking region, including the phenobarbital-responsive enhancer module, was described previously (Kocarek et al., 1998), and the CYP2B1 promoter was deleted to obtain −2145-CYP2B1-LUC.

MDR1. The promoter of the human *MDR1* gene −10,612/−522 (relative to the ATG start site) or −9,912/+180 (relative to the transcription initiation site) was amplified from genomic DNA and ligated into the *Kpn*I/*Sma*I site of pGL3Basic (Promega) to produce −10-kb-MDR1-LUC. SV40-β-galactosidase (BD Biosciences Clontech, Palo Alto, CA) contains an SV40 promoter inserted upstream of the Lac*Z* cDNA encoding β-galactosidase.

Mice and Treatments

Male (8–15 weeks old) FVB and C57BL/6 mice (Charles River Laboratories, Inc., Wilmington, MA) were housed in the St. Jude Children's Research Hospital animal facility. Mice were treated intraperitoneally with dexamethasone (300 mg/kg), pregnenolone-16α-carbonitrile (PCN) (300 mg/kg), spironolactone (100 mg/kg), rifampin (5–100 mg/kg), sodium phenobarbital (75 mg/kg), transnonachlor (50 mg/kg) (Velsicol Chemical Corp., Chicago, IL), 3-methylcholanthrene (0.02 mg/kg), etoposide (100 mg/kg), tamoxifen (100 mg/kg), or topotecan (8 mg/kg). All drugs were administered in a vehicle of 90% water/10% dimethyl sulfoxide. All mice were simultaneously treated with DNA and drugs or steroids.

Hydrodynamic Infusion of DNA and In Vivo Optical Imaging

All animals within an experiment were matched for age and body weight. Mice were given a rapid (5-s) tail vein injection of DNA (25 μg total) in sterile saline in a volume equal to 10% of body weight. Seven hours later, animals were anesthetized intraperitoneally with a 0.2-ml mixture of ketamine and xylazine. Anesthetized mice were given an i.p. injection of firefly D-luciferin (30 mg/ml luciferin in sterile saline dosed with 50 μl/10 g of body weight) (Xenogen Corp., Alameda, CA). Optical images were obtained by a Xenogen imaging system (http://www.xenogen.com/demo4.html). The images were quantitatively analyzed by IGOR Pro 4.0 image analysis software (WaveMetrics, Lake Oswego, OR).

Exposure times depended on signal strength, with the usual imaging timed over the next 5 to 30 min. Results were repeated two to three times in independent animals.

Luciferase Activity In Vitro

Immediately after optical imaging, the mouse livers were removed and flash frozen. Mouse liver (200 mg) was combined with 0.1 M Tris-HCl, pH 7.8, 2 mM EDTA, and 0.1% Triton X-100, homogenized, and centrifuged at 13,000*g* for 10 min (4°C) (Liu et al., 1999). The homogenate was diluted 60-fold with HEPES buffer, 10 μl was added to 100 μl of luciferase assay reagent (Promega), and fluorescence was measured on an OptiComp luminometer (MGM Instruments, Hamden, CT). Luciferase activities were normalized to protein concentration.

Transient Transfection Studies

NIH 3T3 cells (100×10^5 cells per 2.2-cm well plated on day 1) were cotransfected on day 2 by LipofectAMINE (Invitrogen, Carlsbad, CA) with 1.5 μg of CYP3A4-7836-LUC reporter plasmid, 150 ng of HNF-4 expression plasmid, and 150 ng of either vector (pSG5) or hPXR expression plasmid and 800 ng of SV40-β-galactosidase (Promega) plasmid. Alternatively, the NIH 3T3 cells were cotransfected with 600 ng of $TK(MH100)_4$-LUC and 200 ng of vector (PM2) or GAL4-PM2-SXR plasmid (generously provided by Dr. Bruce Blumberg, University of California, Irvine, CA), and 800 ng of SV40-β-galactosidase plasmid. All cells were washed and incubated with fresh medium with and without drug 18 h after transfection. Luciferase activities were determined 24 h later using a luciferase assay system (Promega) and an OptiComp 1 luminometer and normalized to β-galactosidase activity.

Results

We chose drug metabolism genes and the multiple drug resistance gene *MDR1* (also termed *ABCB1*) for this study because they are activated by well-described mechanisms involving nuclear hormone receptors, which are the sensors of pharmacologically important xenobiotic ligands. Through these receptors, physiological ligands (such as hormones and bile acids) regulate the expression of target genes involved in bile acid and sterol homeostasis (Chawla et al., 2001). We first determined the minimum dose of plasmid required to allow measurement of expression of the reporter gene in untreated mouse liver. Mice were injected with various concentrations of plasmids in which the *CYP3A4* or the *MDR1* promoter was immediately upstream of the luciferase gene (CYP3A4-LUC or MDR1-LUC, respectively). Seven hours later, we administered luciferin and optically imaged the bioluminescence through the skin of each mouse. We observed dose-dependent expression of luciferase, and basal transcriptional activity was measurable with as little as 5 μg of plasmid (Fig. 1, A and B). (Luciferase expression was measurable with only 1 μg of CMV-LUC plasmid; data not shown.) Optical imaging of the excised livers of these mice and the anhepatic mouse demonstrated that all measurable bioluminescence was localized to the liver (data not shown).

When luciferase enzyme activity was measured in homogenates of the excised livers (Liu et al., 1999), the results corresponded closely with those of the in vivo optical bioluminescence assays in the same mice (Fig. 1, A and B).

Because transcription assays are routinely used to define regulatory regions important in gene transcription, we designed experiments to determine whether the hydrodynamic infusion method can be used to analyze the promoter activity of various deletion constructs. We found that the basal transcription of three CYP3A4-LUC constructs containing various deletions in the *CYP3A4* 5′-flanking region was readily detectable by optical imaging after infusion of 2 μg of plasmid. When the distal *CYP3A4* 5′-flanking nucleotides (−7836- to −7208-bp) were fused to the 5′ end of the promoter in the CYP3A4-LUC construct, transcription of the reporter was markedly enhanced in vivo (Fig. 1C), as it had been when the same plasmids were used in vitro (Goodwin et al., 1999).

We next examined the transcription of P450s induced by unique receptor pathways. Environmental toxins, such as dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), planar polychlorinated biphenyls, and 3-methylcholanthrene, activate the aryl hydrocarbon receptor (AHR)-AHR nuclear translocator heterodimer and induce transcription of CYP1A1. Anticonvulsant drugs, such as phenobarbital, activate the constitutive androstane receptor (CAR) and enhance transcription of CYP2B, causing some drug interactions. Induction of CYP2B expression by phenobarbital and other xenobiotics, such as the organochlorine pesticide transnonachlor, is a biomarker of hepatic tumor promotion in rodents (Lubet et al., 1989).

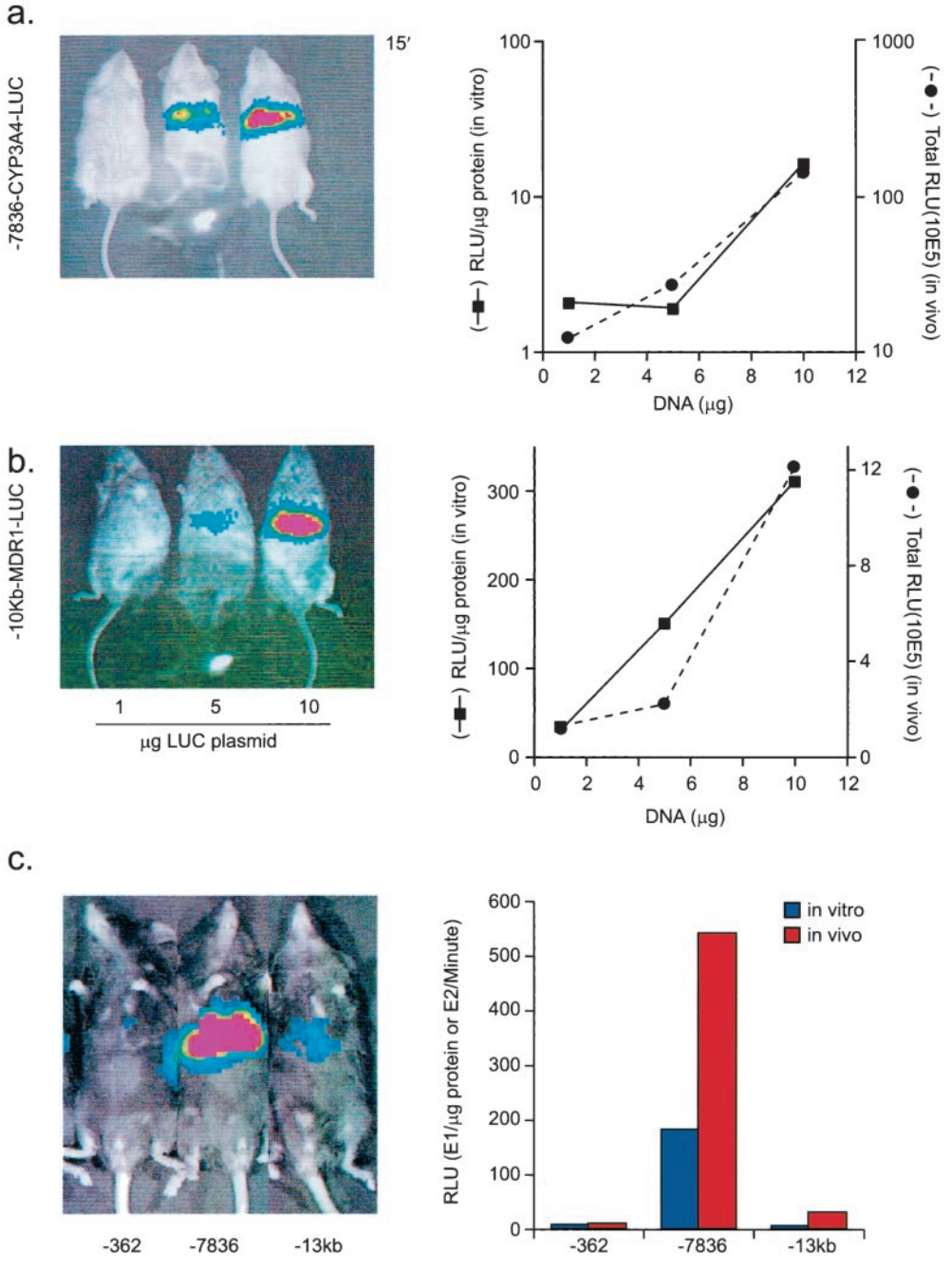

Fig. 1. External imaging of luciferase reporter activity in mouse liver. Plasmids bearing the indicated reporter constructs were combined with SV40-Lac*Z* plasmid and hydrodynamically infused. After 7 h, mice were anesthetized and injected with firefly D-luciferin and optically imaged. Emitted light is shown as a pseudocolor graphic over the black and white image of each animal. A, mice that received 1, 5, or 10 μg of −7836-CYP3A4-LUC. B, mice that received 1, 5, or 10 μg of −10-kb-MDR1-Luc. C, mice that received 2 μg of −362-CYP3A4-LUC, −7836-CYP3A4-LUC, or −13,000-CYP3A4. Graphs at right show in vivo and in vitro bioluminescence measurements obtained from the livers of the same mice, according to the quantity of DNA injected. Each graphed data point represents measurements from two to three animals.

Expression of *CYP3A* and *MDR1* is induced by endobiotic and xenobiotic molecules that ligate the nuclear PXR/steroid and xenobiotic receptor (SXR) (also termed NR1I2) (Synold et al., 2001). Because CYP3A and MDR1 participate in the detoxification and elimination of many drugs, their PXR/SXR-induced expression gives rise to many drug interactions. Infusion of the various CYP3A4-LUC and MDR1-LUC plasmids and treatment with well characterized ligands of the various receptors activated reporter transcription in each case (Fig. 2).

Chen et al. (2001) reported that the extent of expression of DNA that is linearized before hydrodynamic infusion is 10 to 100 times that of equivalent circular DNA and that the transgene is expressed for a period of at least 9 months because the linear plasmids efficiently concatenate in vivo and remain extrachromosomal in hepatic cells. In contrast, the expression of genes in circular plasmids 1 week after injection is reportedly less than 1% of the level of expression 24 h after injection (Liu et al., 1999). We therefore injected 40 μg of linear or closed circular CYP3A4-LUC plasmid into mice and investigated luciferase transcription at various time points. An inducer of CYP3A transcription (PCN) was administered to mice 7 h before imaging. Expression of luciferase from the circular plasmid declined steadily; it had declined 98% 3 weeks after injection, and after 4 weeks, little fluorescence was detected. Three and four weeks after injection, bioluminescence in the mice that had received CYP3A4-LUC–linearized plasmids was 10 times that in mice that had received closed circular plasmids (Fig. 3). Twelve weeks after DNA injection, bioluminescence was still detectable in mice with linearized but not circular CYP3A4-LUC plasmid (data not shown). Therefore, this technique allows serial real-time measurement of hepatic gene transcription in a single live mouse. Extracorporal monitoring of bioluminescence from a transcription activation assay provided results equal to or better than those obtained by traditional in vitro assays conducted with the same constructs (Figs. 1 and 2) and allowed these measurements to be made in vivo in a physiologically normal environment and in real time. Because the animal need not be sacrificed, the time course of transcription can be documented.

We next showed a practical application of this technique. We determined whether we could use the in vivo transcription assay to identify new PXR ligands. Hydrodynamic infusion of CYP3A4-LUC and treatment with a number of anticancer drugs demonstrated that etoposide, tamoxifen, and topotecan increased CYP3A4-LUC transcription in vivo (Fig. 4A). To determine whether this was a PXR-dependent mechanism, we cotransfected NIH 3T3 cells, which are deficient in PXR nuclear hormone receptor, with CYP3A4-LUC and PXR. Addition of 10 μM rifampin or 10 and 50 μM etoposide or topotecan produced a dramatic increase in *CYP3A4*-LUC reporter activity but only in those cells cotransfected with the human PXR plasmid (Fig. 4C). Tamoxifen (at 10 and 50 μM) was extremely toxic to both NIH 3T3 and other cells tried and, thus, could not be definitively established in this cellu-

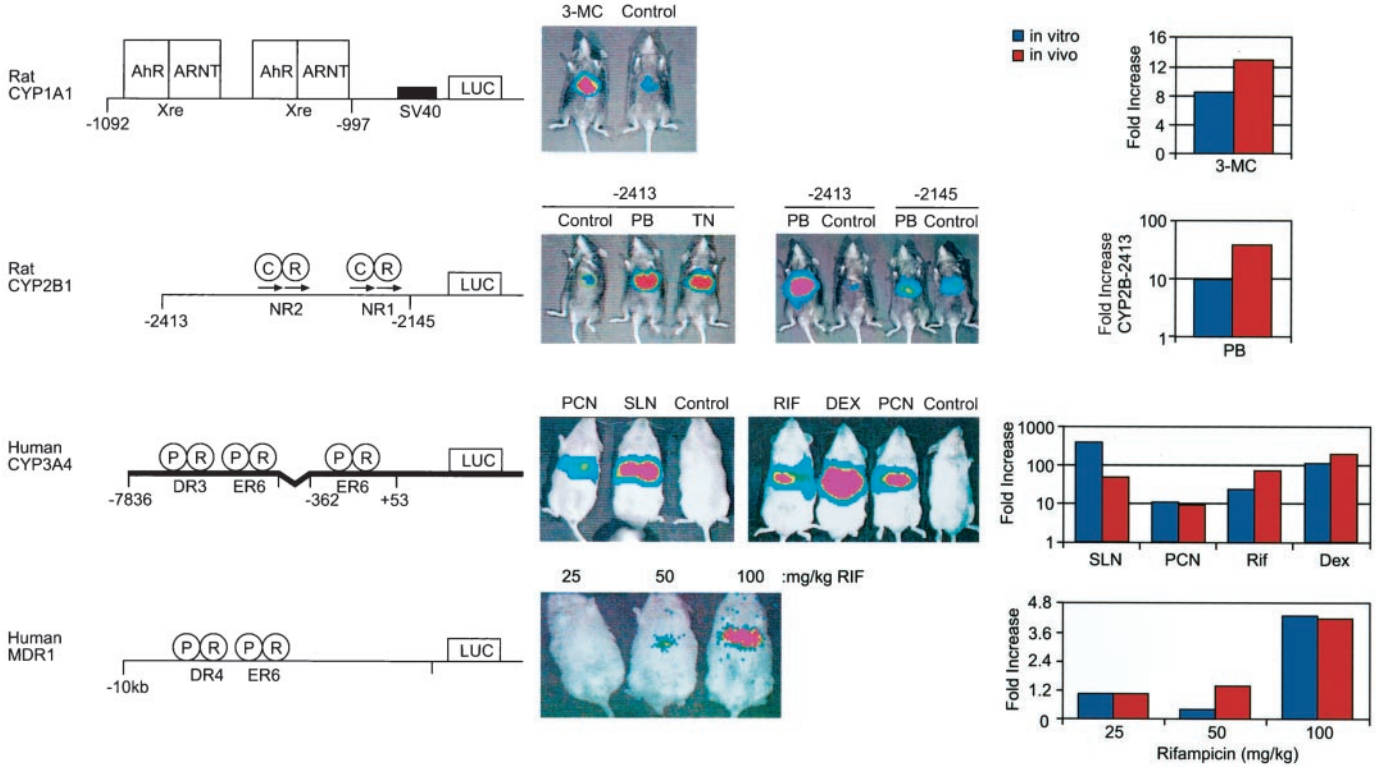

Fig. 2. In vivo monitoring of induction of the transcription of xenobiotic detoxification genes. Mice were hydrodynamically infused with plasmids encoding CYP1A1-LUC (10 μg), −2413-CYP2B1-LUC (2 μg), −2145-CYP2B1-LUC (2 μg), −7836-CYP3A4-LUC (2 μg), or −10-kb-MDR1-LUC (5 μg). Plasmids were administered with SV40-Lac*Z*. Each mouse received 25 μg of DNA. Diagrams at left show XRE binding sites for the AHR-AHR nuclear translocator heterodimer in the CYP1A1 promoter, nuclear receptor 1 and 2 (NR1 and NR2) binding sites for the CAR(C)-retinoid X receptor (R) heterodimer in the rat CYP2B1 promoter, and the direct or everted repeat (DR3, DR4, and ER6) binding elements for the PXR(P)-RXR(R) heterodimer in the CYP3A4 and MDR1 promoters. Photographs show mice after intraperitoneal administration of dexamethasone (DEX) or PCN, spironolactone (SLN), rifampin (RIF), sodium phenobarbital (PB), transnonachlor (TN), 3-methylcholanthrene (3-MC), or vehicle control (CT). Graphs show hepatic bioluminescence measured in vivo and in vitro as described in Fig. 1.

lar model as a PXR ligand. To determine whether etoposide and topotecan bind directly to PXR as ligands, we cotransfected NIH 3T3 cells with a GAL-PXR/SXR construct containing only the ligand binding domain of PXR/SXR fused to the DNA binding domain of the yeast transcription factor GAL4, along with a reporter containing the GAL4 response element fused to a luciferase reporter. Etoposide and topotecan at concentrations ranging from 10 to 50 μM readily increased PXR-GAL4 transcriptional activity in a PXR-dependent fashion (Fig. 4B).

Discussion

Although a variety of methods allow the rapid introduction of DNA into hepatocytes in vivo (e.g., direct injection of DNA into the liver and transduction with adenoviral vectors), such approaches are limited by expression of the DNA only at the site of injection and by the development of an immune response to adenovirus that specifically inhibits the expression of many drug detoxification genes (Muntane-Relat et al., 1995). Our new in vivo real-time transcription assay is free of such limitations. We used six different gene reporter constructs identical to those widely used to measure transcription activity in cell lines. Our results demonstrate that this technique is broadly applicable and requires only the technology already at hand in most laboratories. Because animals need not be sacrificed, transcription can be measured as it occurs, under the most informative biological conditions (i.e., in a living, intact organ). This technique is not limited to investigation of the transcription of P450 or other drug-metabolizing genes. If promoter constructs for genes encoding growth factors or cytokines and their receptors were introduced into animals, their transcription could be monitored in real time during physiological processes, such as organ regeneration or inflammation, or during chronic pathological processes, such as the development of cirrhosis or neoplasia. Only a small set of mice would be needed. With existing technology, such studies would require that several mice be sacrificed at each time point.

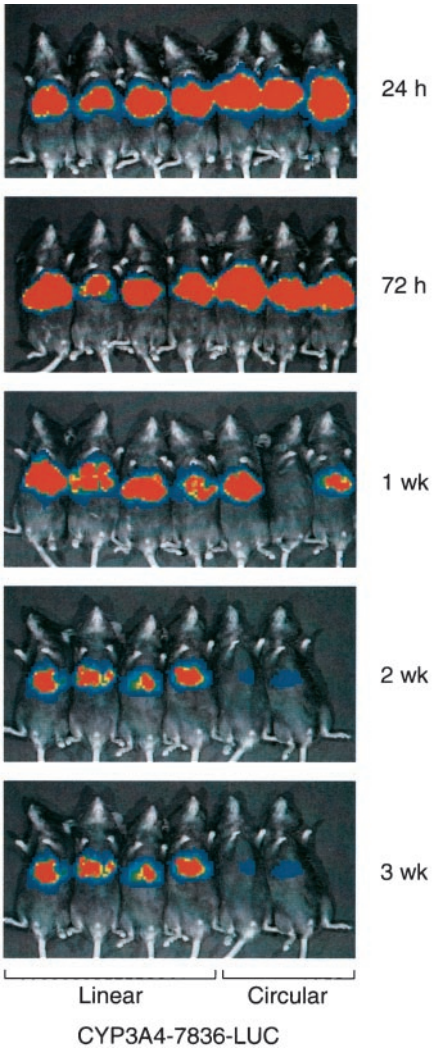

Fig. 3. Bioluminescence is more persistent after administration of linearized plasmids than after administration of circular plasmids. Mice were hydrodynamically infused with circular or linearized −7836-CYP3A4-LUC. Mice were treated with PCN 7 h before optical imaging.

The power of this technique was demonstrated in the rapid discovery in vivo of several anticancer drugs (etoposide, topotecan, and tamoxifen) as transcriptional activators of CYP3A4. Traditional in vitro PXR screening techniques further identified etoposide and topotecan as PXR ligands. This result demonstrates a further advantage of the in vivo system for testing compounds that are not toxic to quiescent hepatocytes in vivo (e.g., tamoxifen) but cannot be screened well in vitro in replicating cell models due to extensive toxicity. Moreover, this in vivo approach capitalizes on the additional pharmacokinetic parameters that can influence transcriptional activation. For example, because replicating cells lose expression of drug-metabolizing enzymes, these in vitro screening models would not detect those chemicals where the metabolite, but not parent drug or steroid, is the ligand for a nuclear hormone receptor and transcriptional activator (Gant et al., 1991). Thus, the in vivo model would complement existing in vitro screening methods. Our finding that tamoxifen, etoposide, and topotecan induce CYP3A4-LUC and are PXR ligands is important because the efficacy of anticancer drug therapy is related to systemic exposure. Indeed, it has previously been demonstrated that concurrent treatment with CYP3A4 inducers and chemotherapies metabolized by CYP3A leads to decreased therapeutic effect and long-term survival (Relling et al., 2000) and that CYP3A expression correlates with sensitivity to anticancer drugs in human cancer xenografts (Zembutsu et al., 2002).

In conjunction with the increasing development of genetically modified mice, our real-time transcription assay will permit immediate testing of the in vivo roles of *cis*-acting regulatory elements and their cognate binding factors in the transcription of reporter constructs. Many additional pharmaceutical and toxicological applications can be envisioned. Reporter constructs that carry DNA regulatory sequences of genes implicated in the hepatotoxicity of xenobiotic agents could be used with traditional toxicity screens to aid in the selection of drug candidates for development. Indeed, microarray analysis of changes in gene expression in mouse liver after exposure to various chemicals has revealed a diagnostic set of 12 RNAs that can be used to classify chemicals into five toxicologically informative subsets (Thomas et al., 2001). "Toxicogenomic" profiling of transcripts could be used to assess the potential toxicity of specific chemicals. When DNA sequences that regulate expression of genes implicated in hepatotoxicity are well defined, linearized reporter gene

constructs can be employed to monitor the expression of the genes during acute or chronic toxicity testing of drugs or chemicals.

Because many drugs act through receptors, reporter constructs could also be used in vivo to confirm mechanisms of drug action. Reporter constructs bearing DNA-regulatory se-

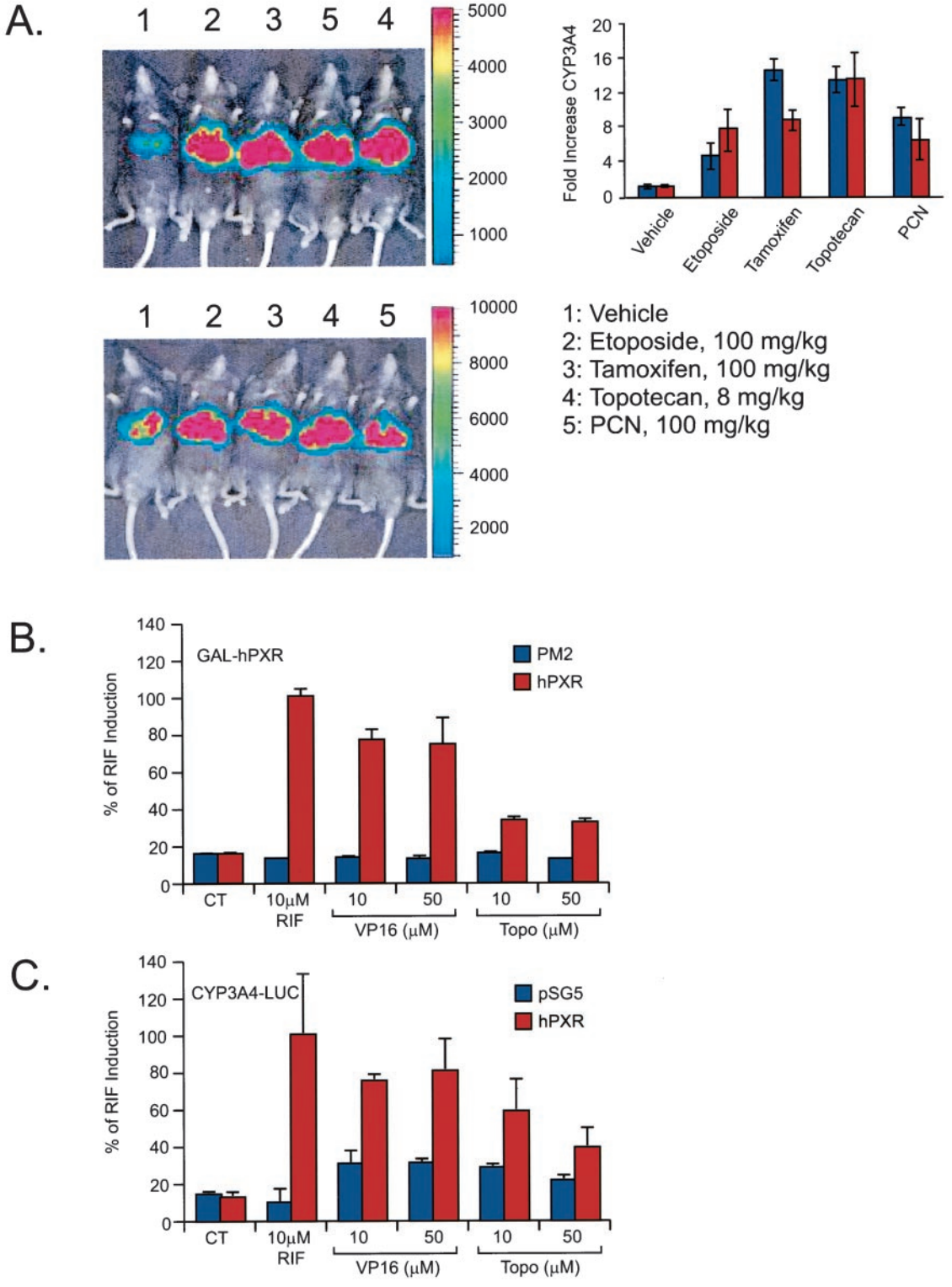

Fig. 4. Anticancer drugs induce CYP3A4 transcription. Mice were hydrodynamically infused with −7836-CYP3A4-LUC (A). Mice were treated with the indicated concentrations of drugs and optically imaged 7 h later. Red, in vivo; blue, in vitro; two separated experiments are represented. NIH 3T3 cells were transfected with TK(MH100)$_4$-LUC reporter and either vector (PM2) or GAL-hPXR/SXR plasmid (B). NIH 3T3 cells were transfected with −7836-CYP3A4-LUC with hPXR or empty vector (pSG5) (C). Cells were treated with rifampin (RIF), etoposide (VP-16), or topotecan (Topo). Luciferase activities were normalized to β-galactosidase activity, and the fold increases in treated groups over untreated controls were determined and expressed as a percentage of rifampin induction. Values represent the mean ± S.D. from triplicate determinations of a single experiment that was performed at least three times.

quences activated by steroid and nuclear hormone receptors would be powerful sensors of physiological endocrine, lipid, retinoid, fatty acid, and sterol signaling in vivo (Chawla et al., 2001). Those that carried target sequences for xenobiotic sensors, such as PXR/SXR and CAR, would be particularly useful in drug development. For example, mice that express the human SXR transgene could be infused with a plasmid in which the reporter gene is linked to the promoter of a drug-detoxifying gene, such as *CYP3A4* or *MDR1*, to evaluate the ability of human SXR receptor ligands to induce expression of these genes (Xie et al., 2000). Similarly, infusion of CYP3A4-LUC plasmid into the livers of mice with and without the MDR1 efflux pump would simultaneously identify drugs that are able to induce expression of CYP3A4 and those that will undergo MDR1-mediated efflux.

Hydrodynamic infusion of DNA can be used with genes other than reporter genes (Yant et al., 2000; Yang et al., 2001). Sequences that encode P450 gene products that participate in detoxification, growth factors, cytokines, or their receptors could be substituted for the reporter genes, and the resulting constructs could be used to create mice that carry the human transgene of interest. Transgenic "humanized" mice could be of significant value in the preclinical evaluation of new drugs. These in vivo approaches would greatly complement and validate in vitro drug screening assays that detect potential drug interactions. Moreover, the in vivo assay would faithfully recapitulate all of the pharmacokinetic variables in effect in the intact liver, such as absorption, distribution, metabolism, export, and inflammatory processes, that affect responses to xenobiotic agents. Although these variables cannot be recapitulated in cell cultures, they influence ligand availability, receptor activation, and the initiation of transcription in the entire animal.

These same imaging techniques could be used to examine protein-protein interactions in mouse liver in vivo. For example, two-hybrid assays using the ligand binding domain of nuclear receptors fused to the yeast transcription factor GAL4-DNA binding domain hydrodynamically introduced along with a reporter plasmid containing the GAL4 response element fused to a luciferase reporter could be used as ligand screening assays. Furthermore, because many cell processes require protein-protein interactions, construction of any combination of plasmids that ultimately monitor the protein-protein interaction with a luciferase reporter readout would be amenable to hydrodynamic infusion into mouse liver and noninvasive screening in vivo. An additional likely benefit of this technique, a reduction in the number of animals needed for investigation of gene expression, will benefit the entire scientific community.

Acknowledgments

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References

Chawla A, Repa JJ, Evans RM, and Mangelsdorf DJ (2001) Nuclear receptors and lipid physiology: opening the X-files. *Science (Wash DC)* **294:**1866–1870.
Chen ZY, Yant SR, He CY, Meuse L, Shen S, and Kay MA (2001) Linear DNAs concatemerize in vivo and result in sustained transgene expression in mouse liver. *Mol Ther* **3:**403–410.
Contag CH, Jenkins D, Contag PR, and Negrin RS (2000) Use of reporter genes for optical measurements of neoplastic disease in vivo. *Neoplasia* **2:**41–52.
Fujisawa-Sehara A, Sogawa K, Yamane M, and Fujii-Kuriyama Y (1987) Characterization of xenobiotic responsive elements upstream from the drug-metabolizing cytochrome P-450c gene: a similarity to glucocorticoid regulatory elements. *Nucleic Acids Res* **15:**4179–4191.
Gant TW, Silverman JA, Bisgaard HC, Burt RK, Marino PA, and Thorgeirsson SS (1991) Regulation of 2-acetylaminofluorene-and 3-methylcholanthrene-mediated induction of multidrug resistance and cytochrome P4501A gene family expression in primary hepatocyte cultures and rat liver. *Mol Carcinog* **4:**499–509.
Goodwin BJ, Hodgson E, and Liddle C (1999) The orphan human pregnane X receptor mediates the transcriptional activation of *CYP3A4* by rifampicin through a distal enhancer module. *Mol Pharmacol* **56:**1329–1339.
Iyer M, Wu L, Carey M, Wang Y, Smallwood A, and Gambhir SS (2001) Two-step transcriptional amplification as a method for imaging reporter gene expression using weak promoters. *Proc Natl Acad Sci USA* **98:**14595–14600.
Kocarek TA, Kraniak JM, and Reddy AB (1998) Regulation of rat hepatic cytochrome P450 expression by sterol biosynthesis inhibition: inhibitors of squalene synthase are potent inducers of CYP2B expression in primary cultured rat hepatocytes and rat liver. *Mol Pharmacol* **54:**474–484.
Liu F, Song YK, and Liu D (1999) Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. *Gene Ther* **6:**1258–1266.
Lubet RA, Nims RW, Ward JM, Rice JM, and Diwan BA (1989) Induction of cytochrome P450b and its relationship to liver tumor promotion. *J Am Coll Toxicol* **8:**259–268.
Muntane-Relat J, Ourlin J-C, Domergue J, and Maurel P (1995) Differential effects of cytokines on the inducible expression of CYP1A1, CYP1A2 and CYP3A4 in human hepatocytes in primary culture. *Hepatology* **22:**1143–1153.
Relling MV, Pui CH, Sandlund JT, Rivera GK, Hancock ML, Boyett JM, Schuetz EG, and Evans WE (2000) Adverse effect of anticonvulsants on efficacy of chemotherapy for acute lymphoblastic leukaemia. *Lancet* **356:**285–290.
Rocchetta HL, Boylan CJ, Foley JW, Iversen PW, Letourneau DL, McMillian CL, Contag PR, Jenkins DE, and Parr TR (2001) Validation of a noninvasive, real-time imaging technology using bioluminescent Escherichia coli in the neutropenic mouse thigh model of infection. *Antimicrob Agents Chemother* **45:**129–137.
Sweeney TJ, Mailander V, Tucker AA, Olomu AB, Zhang W, Cao Y, Negrin RS, and Contag CH (1999) Visualizing the kinetics of tumor-cell clearance in living animals. *Proc Natl Acad Sci USA* **96:**12044–12049.
Synold TW, Dussault I, and Forman BM (2001) The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat Med* **7:**584–590.
Thomas RS, Rank DR, Penn SG, Zastrow GM, Hayes KR, Pande K, Glover E, Silander T, Craven MW, Reddy JK, et al. (2001) Identification of toxicologically predictive gene sets using cDNA microarrays. *Mol Pharmacol* **60:**1189–1194.
Xie W, Barwick JL, Downes M, Blumberg B, Simon CM, Nelson MC, Neuschwander-Tetri BA, Brunt EM, Guzelian PS, and Evans RM (2000) Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature (Lond)* **406:**435–439.
Yang J, Chen S, Huang L, Michalopoulos GK, and Liu Y (2001) Sustained expression of naked plasmid DNA encoding hepatocyte growth factor in mice promotes liver and overall body growth. *Hepatology* **33:**848–859.
Yant SR, Meuse L, Chiu W, Ivics Z, Izsvak Z, and Kay MA (2000) Somatic integration and long-term transgene expression in normal and haemophilic mice using a DNA transposon system. *Nat Genet* **25:**35–41.
Zembutsu H, Ohnishi Y, Tsunoda T, Furukawa Y, Katagiri T, Ueyama Y, Tamaoki N, Nomura T, Kitahara O, Yanagawa R, et al. (2002) Genome-wide cDNA microarray screening to correlate gene expression profiles with sensitivity of 85 human cancer xenografts to anticancer drugs. *Cancer Res* **62:**518–527.
Zhang G, Budker V, and Wolff JA (1999) High levels of foreign gene expression in hepatocytes after tail vein injections of naked plasmid DNA. *Hum Gene Ther* **10:**1735–1737.

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