

Cytochrome P450 Expression in Rat Ocular Tissues and Its Induction by Phenobarbital

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Composition and inducibility of cytochrome P450 (CYP) in ocular tissues were investigated using reverse transcription-polymerase chain reaction (RT-PCR) and immunoblot techniques. Composition of ocular CYPs is more restricted than that of hepatic forms. High levels of CYP2B1/2 and reduced levels of CYP2C11 expression were detected in rat ocular tissues. Phenobarbital induced CYP2B1/2 expression in the lens but not in the rest of the eye, whereas CYP2C11 induction was observed in both regions. In addition, CYP2B1/2 proteins were found to be prominent in the retina. Our data indicate eye-specific regulation of CYP expression.

Key words — cytochrome P450, ocular tissue, lens

INTRODUCTION

The cytochrome P450 (CYP) superfamily has the predominant role in xenobiotic phase I metabolism, primarily because of its extensive heterogeneity and associated broad substrate specificity. In rats, over 40 forms of CYP are expressed, with the highest levels of expression and greatest number of forms occurring in the liver.¹⁾ However, extrahepatic CYP expression is also important because of the potential for these CYPs to regulate organ-specific effects such as toxicity and tumorigenesis. Studies in recent years have revealed that drug metabolism is associated with a variety of non-hepatic tissues.²⁾ Little is known about CYP expression in ocular tissues,^{3,4)} although this region is important as the eye is the first site of exposure for eye-drugs and air pollutants. We therefore investigated CYP expres-

sion and induction in rat ocular tissues using reverse transcription reverse transcription-polymerase chain reaction (RT-PCR) and immunoblot techniques.

MATERIALS AND METHODS

Materials — Phenobarbital (Phe), β -Naphthoflavone (BNF), dexamethasone (DEX), isoniazid and 3-methylcholanthren (3-MC) were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). Clofibrate was obtained from ICN Biochemicals Inc. Rat CYP-competitive RT-PCR Set was purchased from Takara Biochemicals (Shiga, Japan). Reagents for RT-PCR were obtained from Stratagene (California, U.S.A.). Isogen was from WAKO chemicals (Tokyo, Japan). Primary antibodies to rat CYP2B1/2 and CYP2C11 were purchased from Invitrogen Co. (Carlsbad, CA, U.S.A.).

Treatment of Animals — Male SD rats (260–290 g bodyweight) were kept at 22°C with a 12-hr on, 12-hr off light cycle and water and a standard laboratory diet *ad libitum*. For induction studies, rats were treated by intraperitoneal administration of an inducing agents for 4 days, then sacrificed by CO₂ overdose on day 5. Inducing agents and doses were: BNF, 100 mg/kg bodyweight (in corn oil); PB, 100 mg/kg (in saline); Clofibrate, 200 mg/kg (in corn oil); DEX, 50 mg/kg body weight (in corn oil); Isoniazid, 200 mg/kg (in saline); 3-MC, 60 mg/kg (in corn oil). Control groups received only corn oil or saline.

RNA Isolation and RT-PCR — Freshly enucleated rat eyes were briefly immersed in saline, and the lens and other ocular tissues was excised. Total RNA was isolated from eyes, lens and other ocular tissues, respectively, using Isogen reagent according to the manufacturer's instructions. RNA concentration and purity were determined spectrally. Reverse transcription (RT) using 10 μ g total RNA and amplification reactions were performed using standard protocols as in the kit. Reactions were performed for 30 cycles in 50 μ l with 1.0 min incubation at 94°C, 1.5 min incubation at 56°C, and 1.5 min incubation at 72°C.

Immunoblot Analysis — Eyes were dissected into parts such as retina, aqueous humor and cornea. Ocular tissues were minced on ice, followed by centrifugation at 9000 $\times g$ for 15 min. Microsomes were sedimented at 107000 $\times g$ for 60 min. Final microsomal pellets were resuspended in 250 mM sucrose containing buffer. Microsomal proteins

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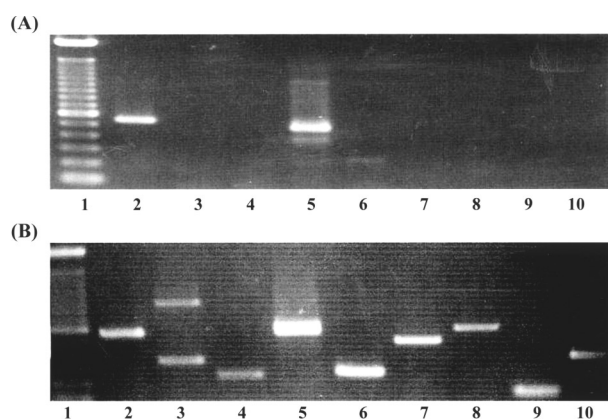


Fig. 1. RT-PCR Analysis of CYP Expression in Untreated Rat Ocular (A) and Hepatic Tissues (B)

RT-PCR was performed with total RNA from rat ocular and hepatic tissues using CYP isozyme-specific primers. Lane 1, 100 bp DNA ladder; Lane 2, β -actin; Lane 3, CYP1A1; Lane 4, CYP1A2; Lane 5, CYP2B1/2; Lane 6, CYP2C11; Lane 7, CYP2E1; Lane 8, CYP3A1; Lane 9, CYP3A2; Lane 10, CYP4A1.

(100 μ g) from each fraction were separated using SDS-polyacrylamide gel electrophoresis.⁵⁾ Proteins were electrophoretically transferred onto PVDF membranes, which were then blocked using 5% non-fat dry milk in TBS for 1 hr at room temperature. The filter was incubated with *anti*-CYP2B1/2 antibody in TBS containing 2% milk for an additional 1 hr, then washed. Cross-reacted proteins were detected by peroxidase-conjugated secondary antibody (1/3000 dilution).

RESULTS AND DISCUSSION

Expression of CYP in Rat Ocular Tissues

We measured CYP expression in rat lens and hepatic tissues by RT-PCR using isozyme-specific

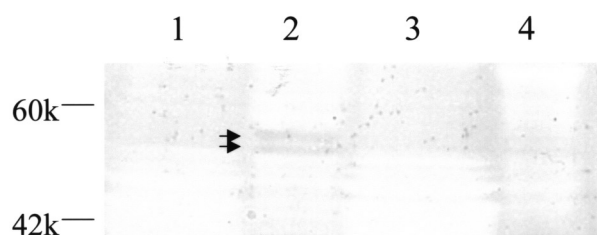


Fig. 2. Immunoblot Analysis of CYP2B1/2 Expression in Untreated Rat Ocular Tissues

Eyes were dissected into several parts and microsomal proteins of each fraction isolated in the presence of protease inhibitors. Proteins (100 μ g) from each tissue were solubilized in SDS-lysis buffer, electrophoresed in 10% SDS-polyacrylamide gel followed by electroblotting on PVDF membrane. Lane 1, total eye; Lane 2, retina; Lane 3, aqueous humor; Lane 4, cornea. Arrows indicate positions of the two positively stained bands.

primers. All CYP subfamilies examined were detected in livers (Fig. 1), whereas we detected relatively high levels of CYP2B1/2 expression and reduced levels of CYP2C11 transcripts in eyes. CYP2B1/2 expression in rat eyes was further verified by detecting CYP2B1/2 proteins using *anti*-CYP2B1/2 antibody. We detected two cross-reactive proteins, predominantly in the retina fraction with very faint bands in cornea (Fig. 2). From the sizes ranging around 55 kDa, these bands correspond to CYP2B1/2 proteins. Due to difficulties in preparing microsomes from lenses, CYP2B1/2 proteins were not measured in lenses. Further analysis must be performed to localize CYP expression in rat eyes.

Induction of CYP in Rat Ocular Tissues

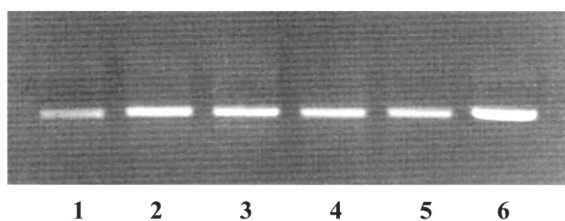
To further investigate CYP functions in the eyes, effects of 6 known inducing reagents on CYP expression in rat ocular tissues were examined. As summarized in Table 1, only Phe exhibited ability to in-

Table 1. RT-PCR Detection of CYP mRNA Expression in Rat Ocular Tissues

CYP	1A1	1A2	2B1/2	2C11	2E1	3A1	3A2	4A1
Untreated	—	—	+	±	—	—	—	—
BNF	—	—	+	±	ND	ND	ND	ND
3-MC	—	—	+	±	ND	ND	ND	ND
Phe	—	—	++*	++	—	—	—	ND
DEX	—	—	+	±	—	—	—	ND
Isoniazid	—	—	+	±	—	—	—	ND
Clofibrate	—	—	+	±	—	—	—	—

First-strand cDNA was synthesized from 10 μ g total RNA extracted from eyes of untreated rats or rats treated with BNF, 3-MC, Phe, DEX, isoniazid or clofibrate. DNA fragments were amplified by PCR as described in Material and Methods. All CYPs were detected in the case of rat hepatic RNA under the identical conditions (data not shown). PCR products were submitted to electrophoresis on 1.5% agarose gels and visualized by staining with ethidium bromide. ND, not determined. +, positive detection of predicted DNA fragments. ±, weak positive DNA fragment. —, no product was detected. ++, induction was observed. *, induction was observed in lens.

(A) 2B1/2



(B) 2C11

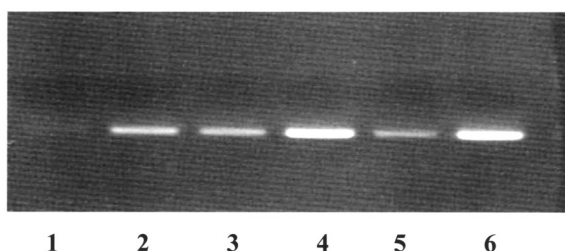


Fig. 3. RT-PCR Analysis of CYP2B1/2 and CYP2C11 Expression in Phenobarbital-Treated Rat Ocular Tissues
RT-PCR was performed with total RNA (10 μ g) from ocular tissues of corn oil- (control) or phenobarbital-treated (Phe+) rats using CYP2B1/2 (A) or CYP2C11 (B) specific primers. Lane 1, lens (control); Lane 2, lens (Phe+); Lane 3, retina and cornea (control); Lane 4, retina and cornea (Phe+); Lane 5, liver (control); Lane 6, liver (Phe+).

duce CYP2B1/2 and CYP2C11 expressions in ocular tissues, although DEX is known to induce CYP2B1/2 expression in rat livers.⁶⁾ Phe induced CYP2C11 expression in both lens and other ocular tissues whereas CYP2B1/2 induction by Phe was observed only in the lens (Fig. 3). The difference in CYP2B1/2 induction between lens and the rest of the eye suggests lens-specific expression of Phe-responsive nuclear factors such as RXR and NF-1.⁷⁾ CYP2C11 induction in the lens is striking because of low expression in the untreated state. We speculate that CYP induction may influence ocular functions. The other inducers did not demonstrate any effects on CYP expression in ocular tissues, whereas in livers expected induction was observed (data not shown).

Recently, primary congenital glaucoma has been associated with CYP1B1 gene mutation in humans.⁸⁾ Furthermore, it has been reported that cytotoxic metabolites of acetaminophen could produce cataract in mice.^{9,10)} Taken together, we assume CYP functions might play important roles in maintaining normal eye functions, not only by detoxifying xenobiotics such as drugs, air pollutants and their metabolites, but also by metabolizing endogenous molecules which are harmful to ocular tissues, especially the lens, because clarity of the lens must be

maintained over a long life time. Further studies must be performed to clarify the functions of CYP and phase II enzymes in ocular tissues.

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