Changes in the Gene Expression of Drug Metabolizing Enzymes during Cataractogenesis in Shumiya Cataract Rats

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The Shumiya cataract rat (SCR) is a hereditary cataract model in which lens opacity appears spontaneously in both the nuclear and perinuclear regions of 10–11 week old animals. The expression of drug metabolizing enzymes, including cytochrome P450 (CYP) and conjugation enzymes, was investigated in the ocular tissues of SCRs during cataractogenesis. Significantly high levels of gene expression were detected for *CYP1A1*, *CYP2B2*, *CYP2C11* and *CYP2E1* in normal lenses, whereas *CYP1A1* and *CYP2C11* were at lower levels in the cataractous lenses. The gene expression profiles of conjugation enzymes were also compared between normal and cataractous ocular tissues in which a moderate reduction in *ST1A1* and *ST1B1* expression, and an extensive loss of *UGT1A1* expression, were observed in the SCR animals that had developed cataracts. In contrast, the elevated expression of the GSTA family of genes was evident in cataractous lenses. The reduced expression of several drug metabolizing enzymes in the cataractous ocular tissues might therefore be related to cataractogenesis *via* the accumulation of endogenous or exogenous toxic substances.

Key words ——— cataract, conjugation, P450, ocular tissues, Shumiya cataract rat

INTRODUCTION

The Shumiya cataract rat (SCR) was developed as a hereditary cataract model and is derived from the congenic fatty hypertensive rat (SHR-fa).[1] Approximately 67% of SCR animals develop spontaneous nuclear and perinuclear cataracts, regardless of sex, at 10–11 weeks of age. However, the genes responsible for cataractogenesis remain to be elucidated.[2,3] Recently, primary congenital glaucoma has been shown to be associated with a *CYP1B1* gene mutation in humans.[4] Furthermore, it has been reported that cytotoxic metabolites of acetaminophen can induce the formation of cataracts in mice.[5,6] These observations suggested that the CYPs and other drug metabolizing enzymes play important roles in maintaining normal eye functions.

Previously, we have reported the characterization of drug metabolizing enzymes in rat ocular tissues. Our previous studies have demonstrated phenobarbital-induced *CYP2B1/2* and *CYP2C11* expression in the rat lens,[7] and both age- and gender-related differences in the expression levels of these CYP genes.[8] To further elucidate the underlying mechanisms of cataractogenesis, we have now investigated the expression profiles of drug metabolizing enzymes in the ocular tissues in a strain of hereditary cataract rats.

MATERIALS AND METHODS

Materials ——— RNA isolation reagents were purchased from Wako chemicals (Tokyo, Japan). PCR primers were purchased from Takara Biochemicals (Shiga, Japan) and from Sigma (St. Louis, MO, U.S.A.). Reagents for RT-PCR were obtained from Stratagene (La Jolla, CA, U.S.A.).

Animals ——— Shumiya cataract rats were bred and maintained at the Kyoritsu University of Pharmacy. SCRs were kept at 22°C with a 12 hr light/dark cycle, allowed free access to water and fed a standard laboratory diet. These animals were then divided into normal and cataractous lens groups when they had reached 14 weeks of age. For ocular tissue preparations, 5 female rats were used. The protocols that were adopted in these animal experiments were approved by the Committee of the Ethics of Animal Experiments at the Kyoritsu University of Pharmacy.

RNA Isolation and RT-PCR ——— Freshly enucleated rat eyes were briefly immersed in saline, and both the lens and extralenticular regions were excised. Total RNA was isolated from both these tissues and from liver controls with Isogen reagent, (Wako Chemicals, Tokyo, Japan) according to the manufacturer's instructions. Following RNase-free

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DNase treatment at 37°C for 30 min, RNA was precipitated with ethanol and dissolved in H_2O. The concentrations of the RNA isolates were determined by spectrophotometric scanning at 210–320 nm. Reverse transcription (RT), using 5–10 μg total RNA, and standard amplification was then performed using standard protocols. PCR reactions were performed in 50 μl volumes for 1 min at 94°C, 1.5 min at 56°C and 1.5 min at 72°C. Cycle numbers were varied from 30 to 35, depending on the amount of the target mRNA. The amplified products were then separated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and visualized by UV illumination. The intensities of the PCR fragments were determined using image analysis software (Eastman Kodak Co., NY, U.S.A.). The quantities of cDNA used in the PCR reactions were normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels in each preparation and measured using a quantitative PCR analyzer (ABI7700, Perkin Elmer, California, U.S.A.). Primers for amplification of CYPs were obtained from Takara Biochemicals, and for amplifying sulfotransferases (SULTs), UDP-glucuronosyl transferases (UGTs) and glutathione S-transferases (GSTs) were designed from published cDNA sequences.[8)]

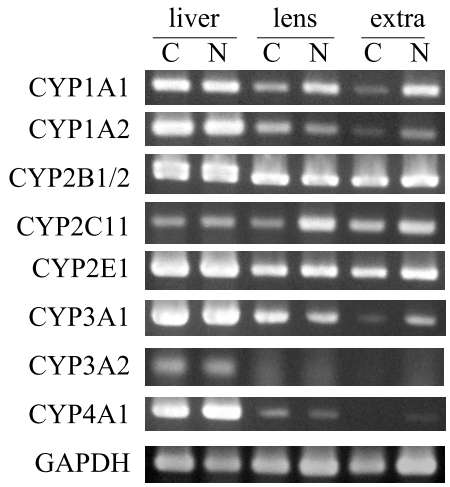

Fig. 1. CYP Expression in the Ocular Tissues of SCR with Cataractous Lenses (C) or Normal Lenses (N)

Total RNAs were isolated from the livers (liver), lenses (lens) and ocular tissues without lens (extra), and subjected to isoform-specific RT-PCR analyses. The primer set used for *CYP2B1/2* recognizes both *CYP2B1* (upper band) and *CYP2B2* (lower band). The product sizes are 332 (*CYP1A1*), 237 (*CYP1A2*), 550 (*CYP2B2*), 249 (*CYP2C11*), 474 (*CYP2E1*), 581 (*CYP3A1*), 117 (*CYP3A2*) and 345 (*CYP4A1*) and 345 (*GAPDH*) in base pairs, respectively.

RESULTS AND DISCUSSION

Expression Profiling of CYPs in the Ocular Tissues of Shumiya Cataract Rats

We determined the expression profiles of CYPs in the ocular tissues of normal and cataractous SCRs by RT-PCR analysis. We also measured the expression levels of these genes in the livers of these animals as a control. The results are shown in Fig. 1. In normal eyes, a relatively high level of expression for *CYP1A1*, *CYP2B2*, *CYP2C11* and *CYP2E1* was detected in both the lenses and extralenticular tissues (designated as extra). In contrast, a weak signal or lack of expression was observed for *CYP3A2* and *CYP4A1* in normal tissue. In the cataractous eyes, however, the expression levels of *CYP1A1* and *CYP2C11* were lower than normal and subsequent densitometric analyses of the PCR products revealed a 5–10 fold (*CYP1A1*) and 2–3 fold (*CYP2C11*) reduction. Although the endogenous substrates of *CYP1A1* and *CYP2C11* are not yet known, these CYPs may play a role in the metabolism of xenobiotics and other substances in systemic circulation or in the external environment. They would thus constitute a metabolic barrier that limits the entry of these compounds. The decreased expression of these CYPs may therefore increase the toxicity of substances, such as environmental chemicals and food constituents, which we speculate may enhance or modulate cataractogenesis in SCRs.

It has been well established that several isozyme-specific transcription factors are involved in the regulation of CYP gene expression. Our preliminary data have indicated the presence of aryl hydrocarbon receptor (AhR) and retinoid X receptor (RXR) mRNAs, but the absence of contitutive androstane receptor (CAR) and pregnane X receptor (PXR) transcripts, in rat ocular tissues (unpublished observations). It is thus possible that genetic variation in the SCRs affects the expression of transcription factors, thereby modulating CYP gene expression.

Expression Profiling of Conjugation Enzymes in the Ocular Tissues of Shumiya Cataract Rats

We determined the expression levels of three known families of conjugation enzymes (SULTs, UGTs and GSTs) in the ocular tissues of SCRs. Our data are shown in Fig. 2. Significant levels of the phenol-sulfating SULTs, *ST1A1* and *ST1B1*, and the hydroxysteroid-sulfating SULTs, *ST2A1* and *ST2A5*, were detectable in both the lenses and extralenticular tissues. However, no *ST1C1* or *ST2A2* transcripts

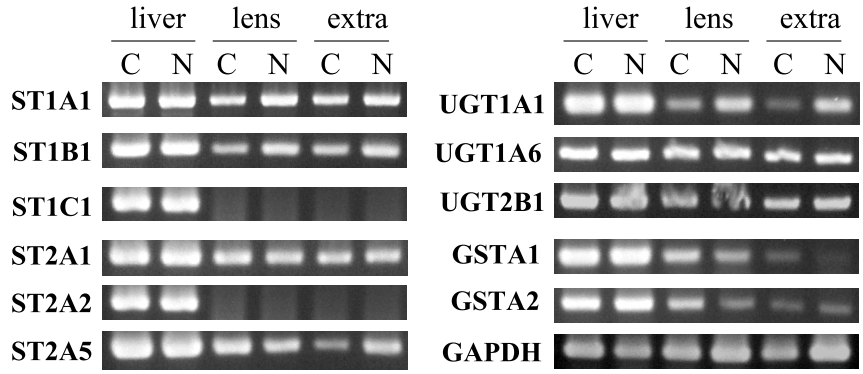

Fig. 2. Comparison of the Expression Levels of Conjugation Enzymes in the Ocular Tissues of SCR with Caratactous Lenses (C) or Normal Lenses (N)

Total RNAs were isolated from the livers (liver), lenses (lens) and ocular tissues without lens (extra), and subjected to isoform-specific RT-PCR analyses. The product sizes are 858 (*ST1A1*), 335 (*ST1B1*), 915 (*ST1C1*), 377 (*ST2A1*), 377 (*ST2A2*), 377 (*ST2A5*), 240 (*UGT1A1*), 508 (*UGT1A6*), 580 (*UGT2B1*), 213 (*GSTA1*), 360 (*GSTA2*) and 345 (*GAPDH*) in base pairs, respectively.

were evident throughout the ocular tissues, despite the fact that these sulfotransferases are the principal phenol- and hydroxysteroid-sulfating enzymes in the liver.[9] Furthermore, a moderate reduction (~20%) in both *ST1A1* and *ST1B1* expression levels was observed in both eye regions under study in cataractous rats. The expression of the UGT genes *UGT1A1*, *UGT1A6* and *UGT2B1* was found to be abundant in both the ocular tissue and liver of these rats. However, a significant reduction (20–40%) in the levels of *UGT1A1* expression was observed in cataractous eyes. Moreover, the expression levels of *GSTA1* and *GSTA2* were low in normal eyes but were increased by 2–3 fold in cataractous lenses.

In conclusion, the expression levels of the three groups of conjugation enzymes analyzed in our present study were higher in normal eyes, with exception of GSTA family, which has been known as selenium-independent glutathione peroxidase in the livers.[10] Conjugation reactions involving xenobiotics have been reported as a major protective pathway in the exclusion of specific substances *via* the transporters of sulfo- and glucuronide conjugates. Moreover, cataracts induced by the administration of acetoaminophen and naphthalene in experimental animals have been attributed to oxidative stress in the ocular tissues caused by xenobiotics.[11–13] We hypothesize that these effects may be due to the toxic metabolites of CYPs, accumulated as a result of insufficient conjugation enzyme activity. Lower CYP expression levels and reduced conjugation activities in the ocular tissues of cataractous SCRs might therefore cause lens opacity due to damage to the lens proteins by the accumulation of reactive compounds.

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