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). 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. Recently, primary congenital glaucoma has been shown to be associated with a CYP1B1 gene mutation in humans. Furthermore, it has been reported that cytotoxic metabolites of acetaminophen can induce the formation of cataracts in mice. 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, and both age- and gender-related differences in the expression levels of these CYP genes. 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
DNase treatment at 37°C for 30 min, RNA was precipitated with ethanol and dissolved in H₂O. 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)

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 constitutive 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 ST1CI or ST2A2 transcripts
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 liver.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 acetaminophen 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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REFERENCES


