Changes in the Gene Expression Patterns of the Cytochrome P450s in Selenite-induced Cataracts in the Rat

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A single injection of an overdose of selenite is known to induce cataracts in young rat eyes. In our current study, we have investigated the gene expression profiles of the cytochrome P450s (CYPs) in the ocular tissues of rats developing such selenite-induced cataracts. Seven days after the injection of selenite, the expression levels of the CYP1A1 and CYP2E1 genes in the lenses of these animals increased and a slight reduction in the expression of the CYP3A gene family could be observed in the extralenticular tissues. When pantethine, an antioxidant, was administered 30 min before selenite injection, the extent of the CYP gene expression changes diminished. These results suggest that selenite-induced oxidative stress may play a role in both the induction and downregulation of CYP genes. In addition, the elevated expression of CYP1A1 and CYP2E1 in the lens may underlie the enhancement of cataract development induced by selenite.

Key words —— cataract, cytochrome P450, ocular, selenite

INTRODUCTION

Selenite-overdose induced cataracts in young rats are thought to be caused by augmented calpain activation,[1] but the exact mechanisms leading to the development of cataracts in this manner are still not fully known. Previous studies have reported differential gene expression in lens epithelial cells from selenite injected rats,[2,3] and two of these genes have further been reported to be involved in apoptosis. Other studies have reported the involvement of cytochrome P450 (CYP) expression in the chemical induction of cataracts in rats.[4,5] CYP is a major family of enzymes involved in the metabolism of xenobiotics[6] and the CYP family members also catalyze the bioactivation and inactivation of a wide variety of endogenous compounds, including steroid hormones and eicosanoids.[7] Most of the CYPs are localized in the liver but some are expressed in extrahepatic tissues, such as the small intestine, lung and kidney.

Previously, we have reported the characterization of CYP expression in rat ocular tissues, and shown that phenobarbital induces CYP2B1/2 and CYP2C11 expression in rat lenses.[8] In additional reports, we have also demonstrated the age- and gender-related expression of the CYPs and phase II conjugation enzymes[9] and the changes in the gene expression of drug metabolizing enzymes in Shumiya cataract rats (SCR).[10] The aim of the current study was to determine whether the activities of drug metabolizing enzymes are related to the induction of cataracts in rats by selenite. To this end, we have analyzed the CYP gene expression profiles in the ocular tissues of selenite-induced cataract rats.

MATERIALS AND METHODS

Materials —— Sodium selenite and was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). D-pantethine was purchased from Sigma (St. Louis, MO, U.S.A.). CYP primers were purchased from Takara Biochemicals (Shiga, Japan) and primers for conjugating enzymes were designed as described and purchased from Sigma Genosys (Hokkaido, Japan). Reagents for reverse transcription (RT)-PCR and RNA preparation were obtained from Stratagene (La Jolla, LA, U.S.A.) and Wako Pure Chemical Industries, LTD., respectively.

Cataract Induction —— A single subcutaneous injection of sodium selenite (30 μmol/kg body weight) was administered to 13-day-old Sprague

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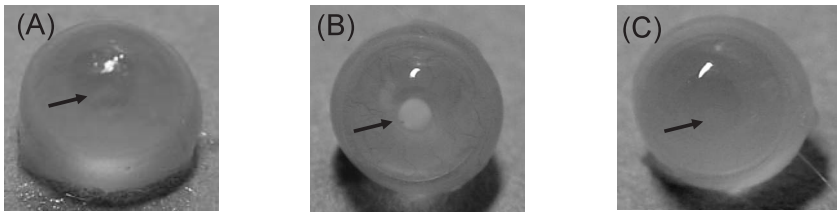

Fig. 1. Induction of Cataracts in the Rat by Selenite

13 day old SD rats were treated with saline (A), selenite (B) and D-pantethine prior to selenite (C). The eyeballs were harvested and enucleated 7 days after these treatments. The lenses are indicated by arrows.

Dawley (SD) rats (Sankyo Laboratory, Shizuoka, Japan). For pretreatment by D-pantethine, this agent was administered 30 min prior to selenite injection at 1.5 mmol/kg body weight.

RNA Isolation and RT-PCR —— Freshly enucleated rat eyes were briefly immersed in saline, and the lens and the extralenticular tissues were excised. Total RNA was then isolated from these tissues using Isogen reagent according to the manufacturer's protocol. RT using 10 μg total RNA and amplification reactions were performed using standard protocols. Reactions were performed for 30 (liver) or 35 (ocular) cycles, in a final volume of 25 μl, for 1.0 min at 94°C, 1.5 min at 56°C, and 1.5 min at 72°C. The quantities of cDNA used in these PCR amplifications were normalized with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels in each preparation, and measured using a quantitative PCR analyzer (ABI7700, Perkin-Elmer, Yokahama, Japan). The band intensities of the PCR products on agarose gels were determined using an image analyzer and associated software (Kodak, Rochester, NY, U.S.A.).

Statistics —— Data were analyzed using Student's *t*-test and *p*-values below 0.05 were considered to be statistically significant.

RESULTS

A single subcutaneous injection of sodium selenite (30 μmol/kg body weight) into 13 day old SD rats resulted in the formation of nuclear cataracts in 100% of the cases by day seven after this treatment, as described previously (Fig. 1).[2] The expression of the CYP genes in the ocular tissues of the cataract-induced and normal control rats was then analyzed by semi-quantitative PCR. In the controls, we detected relatively high levels of CYP2B2 and CYP3A1 in the lens, and strong expression also of the CYP1A1, CYP2B2 and CYP2E1 genes in the extralenticular tissues (Fig. 2). Only weak expression of the CYP1A2 and CYP3A2 genes was detected in the control ocular tissues.

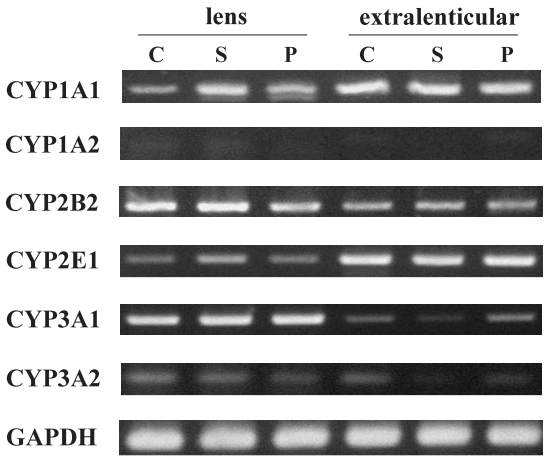

Fig. 2. CYP Gene Expression Analyses in the Ocular Tissues of a Selenite-induced Cataract Rat Model

Total RNAs were isolated from the lenses and extralenticular tissues of enucleated rat eyes, and subjected to isoform-specific RT-PCR. Selenite-induced cataract rats (S), control rats (C), and rats pretreated with D-pantethine prior to selenite injection (P) were analyzed. The PCR product base pair sizes are 332 (CYP1A1), 237 (CYP1A2), 249 (CYP2B2), 474 (CYP2E1), 581 (CYP3A1), 117 (CYP3A2) and 345 (GAPDH).

Selenite treatment was found to induce the expression of CYP1A1 and CYP2E1 in the lens, but to slightly downregulate the expression of CYP3A1 and CYP3A2 genes in the extralenticular tissues (Figs. 2 and 3). The induction and suppression of the CYP genes by selenite were observed to be partly diminished in both cases by pretreatment with D-pantethine (an antioxidant pantothenic acid analogue) prior to selenite injection (Figs. 2 and 3).

DISCUSSION

In the present report, we demonstrate that changes occur in the CYP gene expression profiles

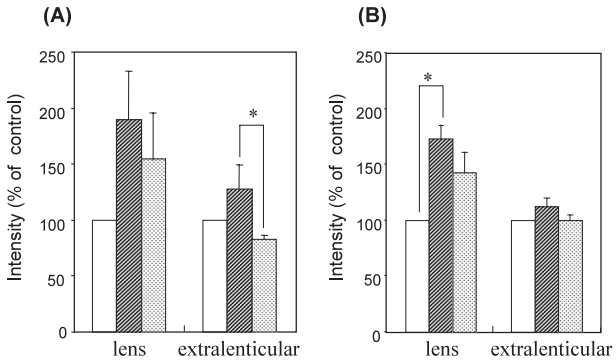

Fig. 3. Changes in the Expression Levels of the CYP1A1 (A) and CYP2E1 Genes in the Cataract Rat Model

The band intensities of the PCR products were measured and calculated using an image analyzer. The control measurements were assigned a value of 100%. Each bar represents the mean of three independent experiments including standard errors. Open bars, control (saline injected); hatched bars, selenite injected; dotted bars, D-pantethine pretreatments prior to selenite injection. $^{*}p < 0.05$.

in the ocular tissues of a selenite-induced cataract model in the rat. D-pantethine, an antioxidant, partially inhibits these effects of selenite. These results suggest that oxidative stress plays a key role in regulation of CYP gene expression in ocular tissues, particularly in the lens. An earlier study has described the impact of oxidative stress on CYP gene expression, and proposed that such stresses repress CYP1A1 via inactivation of nuclear transcription factor I (NFI).[11] In the present experiments in rats, however, we found that the induction of oxidative stress via an overdose of selenite induces the CYP1A1 gene only in lens tissue. It is thus possible that the induction of this gene is one of the sequential events resulting from an exposure to oxidative stress, as we measured its expression seven days after the injection of selenite. However, our preliminary observations also indicate a slight induction of CYP1A1 in the lens at two days after selenite treatment (data not shown).

The expression of CYP1A1 has been shown to be regulated by the aryl hydrocarbon receptor (AhR), which is activated by xenobiotics such as dioxins or polycyclic aromatic hydrocarbons (PAHs).[11] Orhan *et al*. have also reported increased glutathione *S*-transferase (GST) activity in the lenses of selenite-induced cataract rats.[12] Moreover, the expression of GST isozymes is known to be regulated by AhR.[13] Our unpublished observations further indicate that the AhR gene is expressed in rat ocular tissues, but it is unlikely that selenite directly interacts with this receptor.

Many chemicals including alcohols and other solvents have been shown previously to induce CYP2E1.[14] The regulation of this gene is known to be relatively complex,[15] but it has been found that HNF-1 regulates its transcription in the rat liver.[16] No information is available, however, regarding the regulatory mechanisms underlying CYP2E1 gene expression in ocular tissues, but elevated activity of this gene product might produce increased oxidative stress and deleterious effects at the cellular level as described previously.[14] CYP1A1 and CYP2E1 catalyze the oxidation of many low-molecular weight toxins into more potent electrometabolites.[17] Induction of these CYPs in the lenses would thus probably enhance oxidative stress and potentially damage a range of cellular components. This would in turn be likely to enhance the generation of selenite-induced cataracts.

No obvious induction of CYP genes was evident in the extralenticular tissues (Fig. 2). This suggests that different regulatory mechanisms may exist which are more tolerant of the effects of oxidative stress induced by selenite in these tissues, as suggested in our previous study of region-specific CYP gene expression in rat ocular tissues.[9] Further investigations of the molecular mechanisms underlying CYP induction by selenite will be required to further our understanding of the regulation of the CYP genes in the rat eye.

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