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Structure-toxicity relationship study of *para*-halogenated styrene analogues in CYP2E1 transgenic cells

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ABSTRACT

Styrene is one of the most important industrial intermediates consumed in the world and is mainly used as a monomer for reinforced plastics and rubber. Styrene has been found to be hepatotoxic and pneumotoxic in humans and experimental animals. The toxicity of styrene is suggested to be metabolism-dependent. Styrene-7,8-oxide has been considered as the major metabolite responsible for styrene-induced cytotoxicity. The objective of the study was to investigate the correlation between cytotoxicity of styrene and chemical and biochemical properties of the vinyl group of styrene by development of structure activity relationships (SAR). 4-Fluorostyrene, 4-chlorostyrene and 4-bromostyrene were selected for the SAR study. Cytotoxicity of styrene and the halogenated styrene derivatives with an order of 4-bromostyrene > 4-chlorostyrene > 4-fluorostyrene ≈ styrene was observed in CYP2E1 transgenic cells. Similar orders in the efficiency of the metabolism of styrene and the halogenated styrene analogues to their oxides and in the electrophilicity of the corresponding oxides were observed. Additionally, the order of the potency of cellular glutathione depletion and the degree of protein adduction induced by styrene and the halogenated styrenes were consistent with that of their cytotoxicities. The wild-type cells were less susceptible to the toxicity of the corresponding model compounds than CYP2E1 cells. The present study provided insight into the roles of the biochemical and chemical properties of styrene in its cytotoxicity.

1. Introduction

Styrene is a high production volume chemical with around 20–30 million tons produced each year to make products, such as rubber, plastic, fiberglass, pipes, automobile parts, and food containers. Occupational exposures to styrene occur mainly in the reinforced plastic industry (IARC, 1994, 2002; Leibman, 1975; Scott and Preston, 1994). Human exposure to styrene may also result from cigarette smoke, engine exhausts, heating systems, newly installed carpets, and painting (Hodgson et al., 1993; Teixeira et al., 2010; Huff and Infante, 2011). Styrene has also been detected in the foods and drinking water stored in polystyrene containers (Fleming-Jones and Smith, 2003; Tang et al., 2000). Animal studies showed that intraperitoneal administration of styrene in mice caused elevated activities of γ-glutamyltranspeptidase (GGT) and lactate dehydrogenase (LDH) in bronchoalveolar lavage fluid (BALF) (Carlson, 1997a,b; Gadberry et al., 1996). Multifocal necrosis and cell loss in bronchiolar epithelium were observed in CD-1 mice after exposure to 40 or 160 ppm inhaled styrene (Green et al., 2001). Upon repeated exposures, there was decreased cytoplasmic staining of Clara cells and cell crowding in the terminal bronchioles (Cruzan et al., 1997). Studies in CD-1 and B6C3F1 mice consistently showed cell crowding, decreased staining, and increased cell replication in the Clara cells of the mouse bronchiolar epithelium (Cruzan et al., 1997; Green et al., 2001). Green at el. reported increased proliferation in Clara cells after mice were administrated orally with styrene (Green et al., 2001). Recently, Harvilchuck et al. found elevated reactive oxygen species in Clara cells after exposure to styrene (Harvilchuck et al., 2009).

Styrene is primarily metabolized by P450 isozymes, such as CYP2F2 and CYP2E1 (Guengerich et al., 1991; Nakajima et al., 1994; Carlson, 1997a,b; Green et al., 2001; Cruzan et al., 2012), to form a chemically active metabolite, styrene-7,8-oxide (**5**, Scheme 1),

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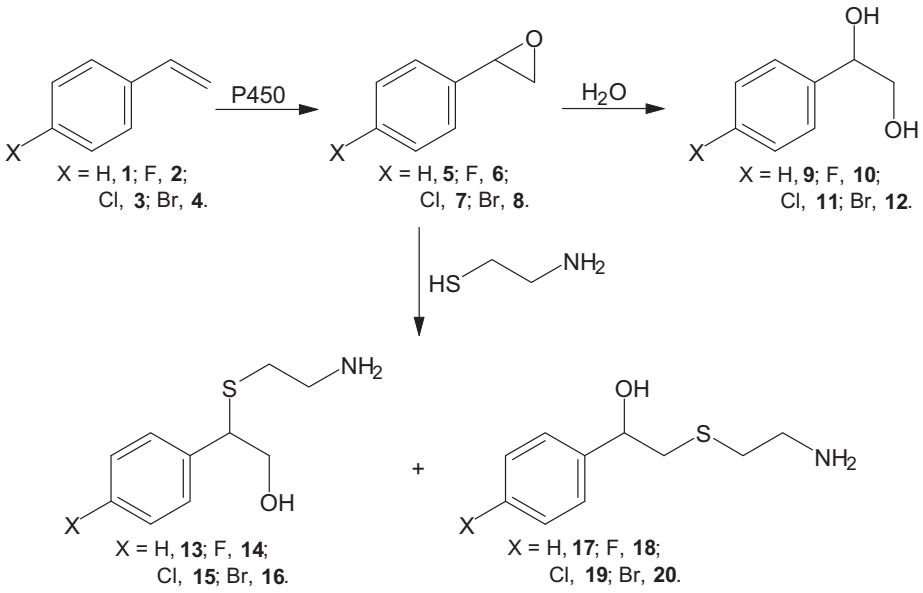

Scheme 1. Metabolism of styrene and halogenated styrenes, and reactions of styrene oxide and halogenated styrene oxides with cysteamine.

which has been suggested to be responsible for styrene toxicity in mice (Bond, 1989). Styrene oxide as an electrophilic species can react with the nucleophilic sites of biomolecules to form adducts. Various DNA adducts have been identified in humans exposed to styrene (Maki-Paakkanen et al., 1991). Styrene was found to induce glutathione depletion in both the liver and lung (Carlson et al., 2006). Styrene oxide derived albumin and hemoglobin modifications were detected in workers exposed to styrene occupationally (Christakopoulos et al., 1993; Fustinoni et al., 1998; Johanson et al., 2000; Osterman-Golkar et al., 1995; Vodicka et al., 1999, 2001, 2003), and the protein modifications correlated with the levels of styrene exposure (Vodicka et al., 1999). Lanosa et al. found a close correlation between metabolic activation of styrene and the sensory irritation response to styrene (Lanosa et al., 2010). Recently, our group reported cellular protein modification by styrene oxide in cultured micro-dissected mouse airways and cells after exposure to styrene (Yuan et al., 2010). Mörbt et al. also identified modified thioredoxin reductase by styrene oxide in cultured A297 cells incubated with styrene (Mörbt et al., 2009).

The objectives of the present study were to develop structure-toxicity relationships of selected halogenated styrenes and to investigate the role of the vinyl group of styrene in cytotoxicity induced by styrene. This allowed us to better understand the biochemical mechanisms of pulmonary toxicity induced by styrene.

2. Materials and methods

2.1. Materials

Styrene (**1**, 99%), 4-fluorostyrene (**2**, 99%), 4-chlorostyrene (**3**, 97%), 4-bromostyrene (**4**, 98%), styrene oxide (**5**, 98%, racemic), 4-fluorostyrene oxide (**6**, 99%), 4-chlorostyrene oxide (**7**, 98%), and 4-bromostyrene oxide (**8**, 98%), trifluoroacetic acid, $CDCl_3$, dimethylformamide (DMF), *N,O*-bis(trimethylsilyl)trifluoroacetamide, cysteamine, β-nicotinamide adenine dinucleotide phosphate (NADPH), and glutathione (GSH) were purchased from Sigma Chemical Co. (St. Louis, MO). Ethyl acetate, anhydrous Na_2SO_4, HPLC-grade acetonitrile and secureseal were obtained from Fisher Scientific Co. (Pittsburgh, PA). DNase kit and RNase I were from Qiagen (Valencia, CA). ^{14}C-Styrene (β-^{14}C), ^{14}C-4-fluorostyrene (β-^{14}C), and ^{14}C-4-bromostyrene (β-^{14}C) were custom synthesized by American Radiolabeled Chemical, Inc. (St. Louis, MO) with chemical purity of 99+% and radioactive purity of 99+%. CellTiter 96 Aqueous One solution kit was from Promega (Sunnyvale, CA). Trypan Blue stain was purchased from Bio Whittaker (Walkersville, MD). Transgenic cell line expressing CYP2E1 (h2E1) and the wild-type cell line (cHo1, human B-lymphoblastoid) were obtained from BD-Gentest (Palo Alto, CA). The cell lines were used in our earlier styrene toxicity studies (Chung et al., 2006; Yuan et al., 2010). Recombinant human CYP2E1 was purchased from BD-Gentest (Palo Alto, CA).

2.2. Synthesis of styrene, 4-fluorostyrene, 4-chlorostyrene, and 4-bromostyrene oxide-derived glycols (**9–12**)

The individual oxides (100 mg each) were mixed with 20 mL of 1.0 N HCl containing 40% acetonitrile, followed by stirring at room temperature overnight. The organic solvent was evaporated by a rotary evaporator, and the remaining aqueous was extracted with acetyl acetate (15 mL × 3). The organic layers were combined, dried over anhydrous Na_2SO_4, and evaporated to dryness in vacuum. The residues were chromatographed in a silica gel column to give the desired compounds. Styrene glycol (**9**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.48–2.82 (br, 2H, OH), 3.65 (dd, *J* = 8.4 and 11.5 Hz, 1H, CH_2), 3.74 (dd, *J* = 3.4 and 11.5 Hz, 1H, CH_2), 4.81 (dd, *J* = 3.4 and 8.4 Hz, 1H, CH), 7.52–7.18 (m, 5H, Ar). 4-Fluorostyrene glycol (**10**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.36–2.68 (br, 2H, OH), 3.65 (dd, *J* = 8.3 and 11.4 Hz, 1H, CH_2), 3.75 (dd, *J* = 3.4 and 11.4 Hz, 1H, CH_2), 4.82 (dd, *J* = 3.4 and 8.3 Hz, 1H, CH), 7.07(dt, *J* = 2 and 8.5 Hz, 2H, Ar), 7.35 (dd, *J* = 5.4 and 8.5 Hz, 2H, Ar). 4-Chlorostyrene glycol (**11**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.22–2.62 (br, 2H, OH), 3.64 (dd, *J* = 8.2 and 11.2 Hz, 1H, CH_2), 3.76 (dd, *J* = 3.2 and 11.2 Hz, 1H, CH_2), 4.82 (dd, *J* = 3.2 and 8.2 Hz, 1H, CH), 7.32(d, *J* = 8.4 Hz, 2H, Ar), 7.36 (d, *J* = 8.4 Hz, 2H, Ar). 4-Bromostyrene glycol (**12**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.32–2.74 (br, 2H, OH), 3.63 (dd, *J* = 8.2 and 11.4 Hz, 1H, CH_2), 3.77 (dd, *J* = 3.4 and 11.4 Hz, 1H, CH_2), 4.81 (dd, *J* = 3.4 and 8.2 Hz, 1H, CH), 7.27(d, *J* = 8.4 Hz, 2H, Ar), 7.51 (d, *J* = 8.4 Hz, 2H, Ar).

2.3. Synthesis of styrene, 4-fluorostyrene, 4-chlorostyrene, and 4-bromostyrene oxide-derived cysteamine adducts (**13–20**)

General procedure: the oxides (5 mmol) were individually mixed with cysteamine (7.5 mmol) in 10 mL of acetonitrile–water solution (6:1) containing triethylamine (10 mmol). The mixtures were stirred at room temperature under an atmosphere of nitrogen for 36 h. The resulting reaction mixtures were diluted with ethyl acetate (30 mL) and then washed with water (10 mL × 3). The organic layers were combined, dried over anhydrous Na_2SO_4, and evaporated under vacuum. The residues were subjected to column chromatography on silica gel to afford the following cysteamine adducts.

The reaction of styrene oxide with cysteamine gave a mixture of 2-(2-aminoethylthio)-2-phenylethanol and 2-(2-aminoethylthio)-1-phenylethanol (**13** and **17**, approximate 1:1) as light yellow oil (197 mg, 20% in yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.48–3.04 (m, 6H+8H), 3.80–3.90 (m, 2H), 3.96 (dd, *J* = 5.7, 7.9 Hz, 1H), 4.78 (dd, *J* = 3.6, 9 Hz, 1H), 5.29 (brs, 2H), 7.20–7.45 (m, 5H × 2); *m/z* = 198.2 $[M+H]^+$.

The reaction of 4-fluorostyrene oxide with cysteamine gave a mixture of 2-(2-aminoethylthio)-2-(4-fluorophenyl)ethanol and 2-(2-aminoethylthio)-1-(4-fluorophenyl)ethanol (**14** and **18**, approximate 1:1) as light yellow oil (194 mg, 18%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.45–3.05 (m, 6H+8H), 3.78–3.90 (m, 2H), 3.95 (dd, *J* = 6, 7.2 Hz, 1H), 4.78 (dd, *J* = 3.48, 8.9 Hz, 1H), 5.30 (brs, 2H), 6.94–7.14 (m, 2H × 2), 7.24–7.42 (m, 2H × 2); *m/z* = 216.3 $[M+H]^+$.

The reaction of 4-chlorostyrene oxide with cysteamine gave a mixture of 2-(2-aminoethylthio)-2-(4-chloroophenyl)ethanol and 2-(2-aminoethylthio)-1-(4-chlorophenyl)ethanol (**15** and **19**, approximate 1:1) as light yellow oil (150 mg, 13%