Modulation of different stress pathways after styrene and styrene-7,8-oxide exposure in HepG2 cell line and normal human hepatocytes

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Received 7 October 2005; Revised 16 January 2006; Accepted 20 January 2006

ABSTRACT: Styrene is one of the most important monomers produced worldwide. IARC classified styrene as a possible carcinogen to humans (group 2B).

Styrene-7,8-oxide (SO) is the main reactive metabolite of styrene, and it is found to be genotoxic in several in vitro test systems.

Styrene and styrene-7,8-oxide (SO) toxicity to HepG2 cells was investigated by evaluating end-points such as heat shock proteins (Hsps), metallothioneins (MT), apoptosis-related proteins, accumulation of styrene within the cells and expression of two isoforms of cytochrome P450. The potential activity of styrene and styrene-7,8-oxide in modulating gene expression was also investigated.

The results showed induction of Hsp70, metallothioneins, BclX S/L and c-myc expression and a decrease in Bax expression in HepG2 after treatments, confirming that these compounds activated protective mechanisms.

Moreover, up-regulation of TGFβ2 and TGFβRIII in HepG2 cells was found after exposure to styrene, while in human primary hepatocytes these genes were down-regulated after both treatments.

Finally, it was found that styrene and SO treatments did not induce CYP1A2 and CYP2E1 protein expression.

In conclusion, both compounds caused toxic stress in HepG2 cells, with SO being more toxic; in the meantime, a different effect of the two compounds in HepG2 cells and primary human hepatocytes was observed regarding their activity in gene modulation. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: styrene, styrene-7,8-oxide, HepG2

Introduction

Styrene is an important organic chemical and is produced mainly to prepare solid polystyrene foam, expanded polystyrene foam and styrene-butadiene rubber. Human exposure to styrene mainly occurs by inhalation of contaminated air during secondary processes, synthesis or degradation of styrene-containing thermoplastics (Goddéris et al., 2004).

Styrene is easily absorbed by the lungs and also through the skin and intestines. It is soluble in blood and accumulates in adipose tissues (Wolff et al., 1977).

In previous studies, it has been demonstrated that styrene plays an important role in promoting cell proliferation and cell-cycle progression (Diodovich et al., 2004); these events are associated with tumour promotion and support the recent classification of styrene as possibly carcinogenic to humans (group 2B) (IARC, 1994).

Styrene-7,8-oxide (SO) is the most important reactive metabolite of styrene, and it is genotoxic in several in vitro test systems (Shield et al., 2004; Laffon et al., 2003a).

Styrene is oxidized by cytochrome P450-dependent monoxygenases to SO that induces DNA adducts, chromosomal aberrations, sister-chromatid exchanges (SCE), micronuclei and DNA damage in several in vitro systems (Laffon et al., 2003b) and moreover it is mutagenic in the Ames test (Wenker et al., 2000). SO is detoxified by hydrolysis catalysed by microsomal epoxide hydrolases (EH), or, to a lesser extent, by conjugation mediated by glutathione S-transferase (GST) (Laffon et al., 2003a, 2003b).

The established human hepatoma cell line (HepG2) is metabolically competent to activate different classes of mutagens and carcinogens into biologically active metabolites (Xin-jiang et al., 2003). Due to their intact and inducible phase I and phase II enzymes, HepG2 cells are able to activate and detoxify xenobiotics. However, there is still a dispute about the extent to which the metabolic profile of HepG2 reflects that of normal human hepatocytes (Alexandre et al., 1999; Mersch-Sundermann...
et al., 2004). Moreover, this cell line is used as a model to study DNA damage (Natarajan and Darroudi, 1991). For these reasons, HepG2 was used as an in vitro system to evaluate end-points of toxicity such as induction of heat shock protein (Hsp), metallothionein (MT) expression and that of two isoforms of cytochrome P450 (CYP450), as well as apoptosis-related proteins, after exposure to styrene and SO.

**Material and Methods**

**Cell Culture**

The human hepatoma cell line HepG2 was grown in a 5% CO₂ in Dulbecco modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U ml⁻¹ penicillin-streptomycin and 5 ml (100×) non-essential amino acids (all from Gibco BRL).

Ten million primary human hepatocytes (from three different donors) were seeded in a flask of 75 cm² using DMEM medium supplemented with 5% FBS, 100 U ml⁻¹ penicillin-streptomycin, 100 nM insulin and 100 nM hydrocortisone. Twenty-four hours after seeding, the medium was changed to DMEM without FBS, but containing the compounds at the chosen concentrations. After 8, 24 and 48 h of treatment, the cells were washed twice with PBS, detached by using a cell scraper, and centrifuged in PBS. The cell pellets were frozen and stored at −80 °C.

**Chemicals**

Styrene (99% of purity) was supplied by the Laboratory of Dr Ehrenstorfer-GmbH (Bgm.-Schlosser, Augsburg, Germany), while styrene-7,8-oxide (>97% of purity) was purchased from Sigma-Aldrich (Italy).

The stock solutions of styrene and styrene-7,8-oxide were diluted in DMSO at a concentration of 1 mM. The final maximum concentration of DMSO, 0.5%, was previously tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to avoid any cytotoxic effect (data not shown).

Dose-range finding was performed using the MTT assay: styrene was tested at concentrations ranging between 0.005 mM and 16 mM (Diodovich et al., 2004), while the styrene-7,8-oxide was tested at concentrations ranging between 0.005 mM and 1 mM (data not shown). Taking into account these results, styrene was used at final concentrations of 0.5 mM and 1 mM while styrene-7,8-oxide was used at final concentrations of 50 µM and 200 µM.

Chloroform (pro analysis 99–99.4% purity) used for gas chromatography-mass spectrometry (GC-MS) analysis was purchased from Merck (VWR International, Italy).

**Total RNA Extraction and Atlas cDNA Expression Array**

Cells were cultured with or without styrene (0.5 mM and 1 mM) and styrene-7,8-oxide (50 µM and 200 µM) for 24 h and RNA was isolated from frozen pellets of 5 × 10⁶ cells. Total cellular RNA was isolated by using TRIzol reagents (Invitrogen, USA). The RNA quality was evaluated by agarose-gel electrophoresis and then DNAase treatment (Ambion, USA) was performed according to the manufacturer’s recommendations. After DNAase treatment, DNA elimination was controlled by PCR, using DNA random primers according to the manufacturer’s protocol, and RNA integrity was confirmed by 2% agarose gel stained with ethidium bromide.

**Atlas Expression Array**

For probe synthesis, 5 µg of total RNA from untreated and styrene treated HepG2 cells was converted into ³²P-labelled first strand cDNA and then purified by column chromatography. Hybridisation with Atlas human oncogene/tumor suppressor array (190 genes, no 7745-1, Clontech UK) was performed following the manufacturer’s recommendations. The results were analysed with AtlasImage™ software, version 2.1. Genes showing an intensity ratio ≥2 between the treated and untreated cells were considered as up-regulated in the analysis, while genes showing a ratio ≤0.5 were considered down-regulated. Moreover, the results were normalised on the basis of the housekeeping genes intensity.

**Quantitative Real-time RT-PCR**

The real-time fluorescence detection method was used to confirm the relative level of genes found overexpressed in the Atlas array. One µg of total RNA was denatured at 65 °C for 10 min and then reverse-transcribed at 42 °C for 40 min in a 50 µl reaction mixture containing 500 µM of each dNTP, 125 units of Superscript II, 40 units of RNase inhibitor, 2.5 µM oligo d(T)₁₆, 5X first strand RT buffer (Invitrogen, USA). Reactions omitting enzyme or cDNA were used as negative controls. Quantitative real-time RT-PCR was performed on an ABI prism 7000 sequence detection system (Applied Biosystem, USA). The primer and probes used (TGFβ2, TGFβRIII, JUN and GAPDH) are commercially available by Applied Biosystem (Assay on Demand). All samples were run in triplicates.

For real-time data analysis, the mean concentration of glyceroldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control for input RNA. GAPDH cDNA levels were determined for each cDNA sample and were
used to normalise all other genes tested from the same cDNA sample. Relative amounts of TGF/β2, TGF/βRIII and JUN cDNAs were calculated by comparison with controls. The relative mRNA expression was also normalised to the correspondent calibrator GADPH. The final results were expressed as the n-fold difference in TGF/β2, TGF/βRIII and JUN expression relative to the GADPH.

Western Blot and ELISA Assay

Parallel flasks of HepG2 cells were cultured with or without styrene (0.5 mM and 1 mM) and styrene-7,8-oxide (50 μM and 200 μM) and collected after 8, 24, 48 and 72 h.

The pellets were washed in cold PBS and the total protein extracts were prepared as described previously (North et al., 2002). Briefly, the cells were lysed in RIPA-like buffer (50 mM Tris, pH 7.4, 0.1% SDS, 250 mM NaCl, 2 mM dithiothreitol, 0.5% Nonidet P-40) in the presence of protease inhibitors. The protein concentrations were determined using Lowry’s method (Lowry et al., 1951).

Forty μg of proteins was separated by 10% NuPAGE Bis-Tris Gel (Invitrogen, USA), and transferred to PVDF membranes (Roche, USA) in a semi-dry system. The first antibodies used were rabbit polyclonal anti-BCL-2 (N-19, Santa Cruz, 1 : 300), rabbit polyclonal anti-BAX (P-19, Santa Cruz, 1 : 300), mouse monoclonal anti-p53 (Ab-2, Oncogene Research, 1 : 300), rabbit polyclonal anti-c-JUN (N, Santa Cruz, 1 : 300), rabbit polyclonal anti-BAX (clone L-19, Santa Cruz, 1 : 300). After washing in rinsing buffer (PBS and 0.1 % Tween 20), the membranes were incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (Pierce, diluted 1 : 3000 for p53) and horseradish peroxidase-conjugated goat anti-rabbit IgG (Oncogene Research, diluted 1 : 3000 for BAX, BCL-2, BCLX S, and e-JUN) for 1 h at room temperature. After further rinsing, the membranes were incubated in ECL detection reagents (Amersham, UK) and exposed to x-ray film (Hyperfilm™ECL, Amersham, UK). Protein loading was detected by Comassie colorimetric assay. The images were captured by using FOTO/analyst™Image Analysis System (FOTO/DYN Inc., USA). For each sample, the amount of protein was determined by densitometry analysis. The NIH Image 1.61 software was then used to estimate the protein signals.

For western blot, two different experiments were carried out for each antibody.

For the ELISA assay, protein extraction from treated (0.5 μM styrene and 200 μM SO) and untreated cells, was made as for the western blots. Samples were analysed with a TGFβ2 kit (Bender Medsystems, USA) according to the manufacturer’s protocol. Two different experiments, each in duplicate, were run.

Microsome Preparation and Immunochemical Analysis

The cells were seeded at a concentration of 5 × 10⁶ cells per 165 cm² flasks in Opti-MEM medium (Invitrogen, USA) containing 10% FBS and 1% 100 U ml⁻¹ streptomycin/penicillin. Three days after seeding, the cells were exposed to styrene or styrene-7,8-oxide for 24 h. HepG2 treated with ethanol (500 mM, 24 h) and phenobarbital (2 mM, 4 days) were used as positive controls for CYP2E1 and CYP1A2 induction, respectively. After treatments, the cells were harvested and resuspended in 20 mM Tris-HCl (pH 7.4) containing protease inhibitors (Sigma, St Louis, MO, USA). Homogenisation was performed by sonication at 4 °C for 30 s at 6 μm amplitude (Soniprep 150, MSE). Microsomes were prepared by differential centrifugation with the first separation at 9000 g for 20 min followed by centrifugation of the supernatant at 105 000 g for 60 min. The resulting pellet (microsomes) was resuspended in 20 mM Tris-HCl (pH 7.4), diluted 1 : 1 in sample buffer (0.25 mM Tris-HCl, pH 6.8, 2% SDS, 30% glycerol, 10% β-mercaptoethanol, 0.01% bromophenol blue) and stored at −20 °C until use. Aliquots of the samples were used for total protein determination by Lowry’s method. Electrophoretic separation of HepG2 microsomes (30 μg) was performed by using 10% NuPAGE gels (Invitrogen, USA). Separated proteins were transferred onto nitrocellulose sheets and processed for antibody staining by using either anti-CYP1A2 (1 : 1000 in PBS + 3% BSA + 0.1% NaN₃) or anti-CYP2E1 (1 : 300 in PBS + 3% BSA + 0.1% NaN₃) (Daichii Pure Chemicals, Tokyo). Anti-rabbit or anti-goat IgG alkaline phosphatase conjugate (1 : 15000) were used as secondary antibody. The binding was localised by addition of the colorimetric substrate BCIP/NBT. Cytochrome P450 expression was quantified by densitometric analysis.

Heat Shock Protein (Hsp-70) and Metallothioneins (MT) Analysis

HepG2 cells (5 × 10⁶ cells per 165 cm² flasks) were exposed to 0.5 and 1 mM styrene or 50 and 200 μM styrene-7,8-oxide. For Hsp-70, the cells were either harvested after 24 h treatment and processed for protein extraction, or left to recover. Recovery was performed by removing the medium, rinsing the cells with PBS, and incubating them in Opti-MEM medium for an additional 24 h at 37 °C. Total protein fractions were obtained by homogenising in sample buffer (0.05 M Tris-HCl, pH 6.8 containing 2% SDS, 10% glycerol, 10% β-mercaptoethanol, 1 mM PMSF). Homogenised proteins (30 μg) were separated on 7% Tris-acetate NuPAGE gels and blotted onto nitrocellulose membrane. The membrane was incubated with mouse anti-Hsp70 monoclonal antibody (1 : 1000, DOI: 10.1002/jat
StressGen Biotechnologies, Canada). After incubation with a goat anti-mouse alkaline phosphatase conjugate antibody (1:15000), the protein expression was visualised by the colorimetric substrate BCIP/NBT.

For MT analysis, the cells were harvested after 24 h treatment and processed for low molecular weight protein separation (Urani et al., 2003). Briefly, the pellets were resuspended in ice-cold 10 mM Tris-HCl (pH 7), 5 mM EDTA, 1 mM PMSF and SDS polyacrylamide gel electrophoresis was performed using 12% NuPAGE gels.

Twenty µg of proteins was processed to enhance membrane transfer and retention of small size proteins (Mizzen et al., 1996). The gels to be transferred were equilibrated with transfer buffer (CAPS: 10 mM 3-cyclohexylamino-1-propanesulfonic acid pH 10.8 in 10% methanol containing 2 mM CaCl₂) for 20 min. After blotting, the nitrocellulose sheet was incubated in 2.5% glutaraldehyde in water for 1 h and washed three times for 5 min in PBS. To remove glutaraldehyde excess, 50 mM monoethanolamine was added to the last washing. The membrane was incubated with 1:1000 mouse anti-metallothionein (Zymed Laboratories Inc., USA) that reacts with both MT-1 and -2 isoforms. The binding was visualised by incubation with a goat anti-mouse alkaline phosphatase conjugate antibody (1:15000) followed by colorimetric reaction (BICP/NBT). Each assay was carried out up to four times.

Gas Chromatographic-Mass Spectrometric (GC-MS) Analysis

The cells were plated onto 100 mm culture dishes (10 000 cells cm⁻²) and treated 24 h after seeding with 1 mM styrene. For accumulation studies, the samples were prepared according to Walter et al. (2002) with some modifications. The cells were harvested after 1, 2, 4, 6 and 24 h of treatment, resuspended in 5 ml ice-cold chloroform and stored at −20 °C until GC-MS was performed. Each sample was injected (2 µl) into a gas chromatography (HP 5890 series II)/electron impact mass spectrometry (HP 5972 series) equipped with a HP-5MS column capillary (30 m length, 0.251 mm inner diameter, 0.25 µm film thickness 95% dimethylpolysiloxan). The injection was in a splitless mode with a temperature of 260 °C. The column was eluted at 60 °C for 5 min, followed by a temperature gradient from 60 °C to 280 °C at 8 °C min⁻¹, and 15 min at 280 °C. The gas carrier was helium (99.9999% purity) at a pressure of 10 psi (1 ml min⁻¹).

The MS fragmentation of standards was: styrene, MS (m/z), data (%) 104 (M, 100), 78 (55), 63 (8), 51 (37); styrene oxide, 119 (M-1, 60), 104 (5), 91 (100), 77 (10), 65 (25), 51(20).

Statistical Analysis

Each assay was carried out 2–4 times and values are expressed as mean ± SD. Data were analysed by using Student’s t-test, and the multiple range test for comparisons. The software package Statgraphics Plus version 5.0 (Statistical Graphics Corp., USA) was used for analysis.

Results

Styrene Accumulation

The GC-MS analyses (Fig. 1) show that the amount of styrene detected in samples obtained after 1, 2, 4 and 6 h of exposure was comparable to the blank value.

![Figure 1. Amount of styrene detected by GC-MS analyses in HepG2 samples after 1, 2, 4, 6 and 24 h of exposure. Initial styrene concentration was always 1 mM. This figure is available in colour online at www.interscience.wiley.com/journal/jat](image-url)
On the contrary, after 24 h of exposure, the area of the peak related to styrene increased about 260-fold (in percentage) as shown by arbitrary units of styrene abundance (Table 1).

### Expression of CYP1A2 and CYP2E1

The expression of CYP1A2 and CYP2E1, the two isoforms involved in vivo in styrene metabolism, was detected in HepG2 microsomes. Basal levels in controls, and in samples exposed to styrene (0.5 and 1 mM) and styrene-7,8-oxide (50 and 200 µM) were visualised by immunochemical reactions (Fig. 2A and B). The content of CYP1A2 and CYP2E1 proteins was comparable between controls, styrene and styrene-7,8-oxide treated samples (Fig. 2A, 2B). Despite styrene not being able to increase cytochrome levels, phenobarbital and ethanol respectively induced CYP1A2 and CYP2E1 in HepG2 cells (Fig. 2A and 2B).

### Hsp70 Expression

As shown in Fig. 3A, the expression of Hsp70, a protein involved in protective mechanisms, was investigated by western blot in controls and cells exposed to styrene and its metabolite styrene-7,8-oxide. Hsp70 levels were comparable to controls after 24 h of 0.5 mM styrene exposure, but significantly \( P < 0.05 \) increased after 24 h of 1 mM styrene treatment. The metabolite, at concentrations of 50 and 200 µM, induced a significant increment \( P < 0.01 \) of the protein level, with 200 µM styrene-7,8-oxide being the higher inducer (Fig. 3A). The recovery of the cells in complete medium for 24 h after treatments restored Hsp70 basal levels in all samples except for 200 µM styrene oxide (Fig. 3B).

### Metallothionein Induction

MT-1 and -2, stress proteins involved in free radical scavenging, were induced at 1 mM styrene and 200 µM styrene-7,8-oxide after 24 h of treatment (Fig. 4). The basal levels of MT were significantly \( P < 0.01 \) increased with 1 mM styrene that produced a mean increment of 69%. Treatment with 200 µM styrene-7,8-oxide determined a higher induction.

### Bax, c-myc and BclXL Expression

The expression of proteins involved in cell cycle regulation and apoptosis were analysed by western blot.
The expression of Bax protein did not change in cells treated with styrene and with styrene-7,8-oxide for 24, 48 and 72 h (data not shown).

Expression of BclX<sub>S/L</sub>, an anti-apoptotic member of the Bcl2 family, changed between controls and cells treated with styrene-7,8-oxide at 50 µM and 200 µM after 8, 24 and 48 h, as shown in Fig. 5A. No differences were seen in the expression of this protein in cells treated with styrene after 72 h of exposure.

The expression of c-myc, a protein involved in cell proliferation, was higher in cells treated 6 and 8 h with styrene-7,8-oxide at 200 µM, decreasing to below the normal levels within 24 h (Fig. 5B).

Gene expression of HepG2 cells exposed to 0.5 mM styrene for 24 h was investigated with an Atlas expression array (oncogene/tumour suppressor genes) and an up-regulation of TGFβ2, TGFβRIII and c-jun proto-oncogene was found (Fig. 7A). These results were verified by real time PCR. Only the TGFβ2 and TGFβRIII up-regulations were confirmed and the m-RNA quantity increased 3.93-fold for TGFβ2 and 14.29-fold for TGFβRIII. C-jun up-regulation was not confirmed by real time PCR, as shown in Fig. 7B.

Primary human hepatocytes were exposed to 0.5 mM styrene and 200 µM styrene-7,8-oxide for 24 h and the c-jun, TGFβ2 and TGFβRIII mRNA levels were analysed by quantitative real time PCR. C-jun was down-regulated 1.8-fold after both exposures; TGFβ2 was significantly down-regulated 3-fold and 5.3-fold after styrene and styrene-7,8-oxide treatment, respectively. TGFβRIII was significantly down-regulated of 50% after styrene-7,8-oxide exposure, while the styrene exerted the 17% of down-regulation (Fig. 8).

Discussion

The HepG2 cell line is a useful in vitro model to study the hepatotoxicity of organic and inorganic compounds.
HepG2 AND HUMAN HEPATOCYTES CHEMICAL EXPOSURE

Figure 7. (A) Atlas human oncogene/tumor suppressor array: comparison of untreated vs 0.5 mM styrene HepG2 cells treated for 24 h (AtlasImage™ software, Clontech, UK). For details see also: http://www.clontech.com/clontech/techinfo/manuals/PDF/PT3381-1.pdf (B) Real-time PCR analysis of TGFβ2, TGFβRIII and c-jun gene expressions from the untreated and 0.5 mM styrene HepG2 cells for 24 h treated. Results are expressed as n-fold difference relative to GADPH and represented as mean ± SD.

It has been shown that these cells express different families of detoxification enzymes (Dehn et al., 2004) as well as drug-metabolising capabilities (Lu et al., 2004).

In our studies, HepG2 cells were exposed to styrene and styrene-7,8-oxide and the induction of Hsp70, MT, CYP1A2 and CYP2E1, apoptosis- and cell cycle-related proteins was evaluated, as well as the accumulation of styrene into the cells.

The GC-MS analysis revealed a very high styrene accumulation in HepG2 after 24 h of exposure. At this time point an increase of Hsp70 and MT proteins was observed, as shown by western blot results.

Hsp70 represents one of the most studied heat shock proteins (Hsps), which plays a key role in either preventing or repairing cellular damage and in protection against cytotoxicity induced by hepato-toxicants (Salminen et al., 1996). Moreover, in addition to their function in restoring protein homeostasis and promoting cell survival, Hsps seem to play a role in the control of apoptosis (Jolly and Morimoto, 2000). The data suggest an induction of protective responses in HepG2 cells, after chemical stress. In addition, the continuous over-expression of Hsp70, after styrene-7,8-oxide removal from culture medium, confirms the higher toxic potential of this metabolite.

Figure 8. Real-time PCR analysis of c-jun, TGFβ2 and TGFβRIII gene expressions from the untreated primary human hepatocytes, 0.5 mM styrene and 200 µM styrene-7,8-oxide (SO) primary human hepatocytes treated cells for 24 h; these data represent the mean of results obtained from three different donors ± SD. Final results were expressed as percentage related to CTRL (100%). * P < 0.05; ** P < 0.01

Metallothionein proteins are rapidly induced in the liver by a range of metals, drugs and inflammatory mediators as well as intervening in the process of toxicity induction or detoxification of certain metals or chemicals (Coyle et al., 2002). Our data showed an induction of MT by styrene-7,8-oxide at 200 µM, that could partially explain the alteration of cellular resistance and proliferation.

The western blot results confirmed that styrene and styrene-7,8-oxide did not induce apoptosis, evidencing an up-regulation of BclXL, an anti-apoptotic member of the Bcl2 family and a decrease in Bax expression, in particular after styrene-7,8-oxide exposure.
Moreover, the expression of c-myc, involved in cell proliferation, was higher in cells treated with styrene-7,8-oxide at 200 µM after 6 and 8 h, while it was decreased after 24 h. The increase in BclX S/L and c-myc protein levels suggested a deregulation of cell growth; in addition, these data could justify a styrene-7,8-oxide role in promoting cell proliferation and cell survival, and in facilitating tumourigenesis in cooperation with other tumour-promoting events.

Human CYP1A2 and CYP2E1 participate in the metabolism of a variety of compounds including the activation of potential carcinogens (Caro and Cederbaum, 2004). The metabolic competence of HepG2 was reported previously (Knasmüller et al., 1998) and the inducibility of CYP1A2 and CYP2E1 was confirmed in this paper. Namely, ethanol and phenobarbital, in vivo specific inducers of the two cytochrome isofoms, highly increased the protein levels in HepG2 as measured by western blot. However, in this study, both styrene and styrene-7,8-oxide were not able to induce CYP1A2 and CYP2E1, even though their importance in the bioactivation of styrene has been demonstrated in the mouse liver (Carlson, 2004).

The effects of styrene and styrene-7,8-oxide on the expression of some cancer related genes in HepG2 and human normal hepatocytes have been investigated by using an Atlas Expression Array. TGFβ2 and TGFβRIII expression was found to be up-regulated.

Transforming growth factor beta (TGFβ) is a prototype of a cytokine family involved in the regulation of various cellular responses including cell growth, differentiation and apoptosis both in vivo and in vitro (Oh et al., 2000). Each member of the TGFβ superfamily binds to a characteristic combination of type I and type II receptors, both of which are needed for signalling.

Moreover, increased expression of TGFβ isoforms is often associated with development and progression of different types of carcinomas including human colon and human breast carcinomas (Reiss, 1999). One mechanism by which TGFβ over-expression may stimulate tumour progression, is apparently its angiogenic activity.

TGFβ type III receptor (RIII) is the major TGFβ binding molecule on the cell surface and binds to all the three mammalian TGFβ isoforms (β1, β2 and β3), with high affinity (Massague, 1990). The TGFβRIII modulates cellular responses to TGFβ but has no signalling sequences.

In this study, an up-regulation of TGFβ2 and TGFβRIII has been found in HepG2 cells after exposure to styrene. In particular the over-expression of TGFβ2 and TGFβRIII could confirm the role exerted by these two genes in the development and progression of carcinomas (Reiss, 1999).

TGFβ2, TGFβRIII and c-jun have been investigated after chemical exposure in human normal hepatocytes. TGFβ2 and c-jun have been found significantly down-regulated after both treatments, while TGFβRIII has been found down-regulated only after styrene-7,8-oxide exposure.

The different gene modulation on hepatocytes and HepG2 exposed to styrene and styrene-7,8-oxide may reflect changes in gene expressions due to the different nature of the cells. Hepatocytes may be more resistant to go in apoptosis and to respond to cytokine induction. Further investigation would clarify the mechanisms of response to these chemicals.

Finally, both compounds caused stress in hepatocytes and the HepG2 cell line, even if to a different extent, confirming the role of the metabolite in carcinogetic and mutagenic activity of styrene.

Acknowledgements—We would like to thank Dr Lysianne Richert, University of Besancon, UFR Medicine — Pharmacie, Besacon, France, for providing us with human hepatocytes and Mr Gerard Bowe, and Dr Marco Mazzara for technical support.

References


