Stereochemical metabolism of styrene in volunteers

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Received: 23 August 2000 / Accepted: 27 January 2001

Abstract Objectives: To study the stereochemistry of styrene metabolism in volunteers, and its interindividual variability. Methods: Twenty healthy male volunteers (aged 18–37 years) were exposed to 360 mg/m³ styrene for 1 h while they performed 50 W physical exercise. Venous blood was drawn during and for up to 2 h after exposure. Urine was collected at time-intervals up to 24 h after exposure. The following parameters were determined: styrene, free and conjugated styrene glycol (SG) in blood, and conjugated SG, mandelic acid (MA) and phenylglyoxylic acid (PGA) in urine. Results: Average pulmonary retention of styrene was 62%. Excretion of the acidic metabolites MA and PGA accounted for 58% of the pulmonary uptake. The average maximum concentration (C_max) and area under the curve (AUC) of free (R)-SG in blood were 1.3 and 1.7 times higher than those of (S)-SG respectively; the half-life of (R)-SG was longer (82 vs 62 min, P < 0.005). C_max and AUC of the conjugated SG enantiomers in blood did not differ, but again half-life for (R)-SG was longer (72 vs 64 min, P < 0.05). Cumulative excretion and renal clearance of conjugated (S)-SG in urine were three and four times higher, respectively, than that of (R)-SG. Cumulative excretion of (S)-MA was 1.6 times higher than (R)-MA. Interindividual differences in the kinetic parameters of the metabolites were two- to threefold. Conclusions: The enantiomeric excess found was different for each metabolite under study, implying different enantioselectivity and/or enantiospecificity of the enzymes and carrier-proteins involved in the biotransformation and excretion. The use of these metabolites as biological indicators for prediction of the enantiomeric excess of the toxic metabolite styrene-7,8-oxide (SO) is therefore not justified. Interindividual differences in the stereochemical metabolism of styrene are moderate.

Key words: Styrene · Stereoisomerism · Styrene glycol · Mandelic acid · metabolism

Introduction

Styrene is a solvent widely used in industry that can cause several adverse effects, including skin and pulmonary irritation at acute exposure, and central nervous system depression after acute as well as chronic exposure. The most extensive exposure to styrene occurs in plants manufacturing glass-reinforced plastics. Entry of styrene into the body occurs mainly via inhalation, and styrene metabolism takes place principally in the liver, although extrahepatic tissues may contribute. The first step in the major metabolic pathway is formation of styrene-7,8-oxide (SO) by cytochrome P450. SO is thereafter hydrolysed to styrene glycol (SG) by microsomal epoxide hydrolase (mEH); this metabolite is subsequently oxidised to mandelic acid (MA) and phenylglyoxylic acid (PGA). These acids are the main urinary metabolites of styrene in humans, and account for 90% of the absorbed dose of styrene [1, 12].

Styrene is not chiral itself, but the first metabolite, SO, possesses an asymmetric centre and thus exists as (R)- and (S)-enantiomers. SG and MA also posses a chiral C-atom, but PGA is optically inactive. The chronic toxicity of styrene is mainly attributable to the reactive metabolite SO [4, 19, 20], and several studies have shown that a difference in toxicity between the enantiomers of SO exists. The (R)-enantiomer showed to be four times more mutagenic to Salmonella typhimurium TA 100 in the Ames test than the (S)-enantiomer [27, 29]. The (R)-enantiomer was also more toxic to lung and liver in mice in vivo [11]. However, another study showed that the (S)-enantiomer caused more sister
chromatid exchanges in vivo in mice than did the (R)-enantiomer [30]. Hence, due to the different toxic potential of the SO enantiomers, determination of the racemic metabolites of styrene, which is the present practice, might not truly reflect the health risk associated with exposure to styrene. A number of studies have addressed the stereometabolism of styrene, but most of them have been performed in vitro [3, 5, 6, 31] or in vivo in laboratory animals, e.g. rats and mice [7, 8, 21, 22, 32]. The few studies performed in humans only measured enantiomers of MA [10, 13, 18]. The question arises, however, whether the enantiomeric composition of MA is representative of that of the ultimate toxic metabolite SO. The metabolism of SO to MA is mediated by a number of enzymes, some of which are known to be stereoselective, i.e. mEH.

Since SO levels in blood are very low in humans, SG in blood has been suggested by Lof et al. [24] as a biomarker to reflect the levels of SO. In analogy, one could speculate whether the R/S ratio of SG in the blood reflects the enantiomeric ratio of SO. Others have suggested the use of R/S ratios of MA or conjugated SG in urine as indicators of the enantiomeric ratio of SO [18]. Direct measurement of SO in blood has been reported, although the enantiomeric composition was not measured [19, 20].

The enantiomeric ratio of styrene metabolites showed considerable interindividual variation [13]. Consequently, persons with a metabolic preference for one of the stereochemical forms could be at higher risk of the toxic effects of SO after styrene exposure. Interindividual variability in the stereochemical metabolism and kinetics of styrene has so far only been quantified in a limited number of styrene-exposed workers, not in volunteers under strictly controlled experimental conditions.

The aim of the present study was, therefore, to gain more insight into the stereochemical metabolism of styrene in humans, and its interindividual variability. For that purpose, we exposed 20 volunteers to styrene by inhalation, and monitored the stereochemy of the blood and urinary metabolites (SG, MA) of styrene.

Methods

Subjects

The volunteers were 20 healthy men, ranging in age from 18–37 years (average 23.3). The body mass index (weight/height²) varied from 18 to 25 kg/m² (average 21.7). The subjects had not been occupationally exposed to solvents, and none had suffered from any respiratory or circulatory disease. Blood was taken for clinical chemistry analyses and haematological screening. All participants were non-smokers, two were alcohol-abstainers and 13 consumed more than 50 g ethanol/week. They were asked to abstain from alcohol during and also 48 h before the study. Each person was informed of the possible effects of styrene, and signed an informed consent prior to the beginning of the study. The study was evaluated and approved by the Medical Ethical Committee of the Amsterdam Medical Center and was performed according to the Declaration of Helsinki 1964.

Exposure to styrene

The volunteers were exposed to 360 ± 20 mg/m³ for 1 h. During exposure, they performed light physical exercise, 50 W, on a bicycle ergometer. The exposures were conducted in an open exposure chamber of 8 m³ volume, with an air-turnover rate of six times/h. Styrene vapour was generated by bubbling a constant flow of medical air through the solvent in two glass bottles connected in series. The solvent vapour was supplied at the fresh-air-side of the ventilation system. The concentration of solvent in the chamber was continuously monitored with a Miran 1B infrared analyser. In order to determine the pulmonary ventilation and concentration of exhaled styrene, we collected exhaled air in a gas-sampling bag for 2 min at four time-periods during exposure. For the calculations the average value was used. Venous blood samples (two 7 ml EDTA containers) were collected at several fixed time-points during exposure and up to 2 h after the end of exposure for determination of styrene, SO and SG. One tube was kept on ice until analysed, to prevent loss of styrene. Blood from the second tube was immediately extracted with hexane (2 x 3 ml), to prevent rapid decomposition of SO in blood [14]. Urine was collected before the exposure and at five time-intervals, 0–2, 2–4, 4–8, 8–12 and 12–24 h, after the end of exposure.

Analytical methods

For the determination of styrene in exhaled air, we withdrew 1 ml of air from the gas-sampling bag with a gas-tight syringe, and injected it into an HP5890 GC with flame-ionisation detection (FID) and a DB1701 (30 m, 0.53 mm, JW Scientific) column. To determine the styrene concentration in blood, we transferred 200 µl of blood into a 4-ml vial and heated it for at least 30 min at 50 °C. Then, 250 µl of headspace air was injected and analysed under the same conditions as styrene in air. m-Xylene was used as internal standard. For the determination of free SG, 1.5 ml of blood was extracted with 5 ml of ethyl acetate. Before extraction, phenylpropylene glycol was added as internal standard. The conjugated SG was determined in the same sample of blood after removal of the ethyl acetate phase and subsequent incubation at 37 °C overnight with β-glucuronidase and sulfatase from Helix pomatia in 0.1 M acetate buffer, pH 4.7. The released SG was extracted with ethyl acetate. Ethyl acetate extracts, from both the free SG and conjugated SG samples, were evaporated to dryness and the residue derivatised with 20 µl of pentfluorpropionic anhydride (PFPA) and 0.5 ml of 1.5% pyridine in dichloromethane. Derivatives were analysed using gas chromatography (GC) with electron capture detection and a chiral column (CP Chirasil-Dex, 25 m, 0.25 mm ID, Chrompack, the Netherlands). Free and conjugated SG in urine were analysed similarly to those in blood. Free SG content in urine was less than 5% of the total SG content. MA and PGA in urine were determined by gas chromatography with FID according to Kečić et al. [15].

Calculation of kinetic parameters

Alveolar ventilation was calculated from the pulmonary ventilation after correction for the anatomical dead-volume of the lungs. We calculated the respiratory intake rate of styrene by multiplying the styrene concentration in air by the alveolar ventilation [26]. We calculated the pulmonary uptake by multiplying the difference between the inhaled and the average exhaled concentration of styrene by the pulmonary ventilation. The kinetic parameters for styrene and SG in blood (t 1/2 and maximum concentration (Cmax)) were obtained from the blood concentration-time course. We calculated the apparent terminal elimination rate constant, k, using the least-squares analysis on the terminal portion of the semi-log blood concentration versus time curves. The t 1/2 was calculated by dividing ln2 by k. The area under the curve (AUC) from time zero to 180 min was calculated by a trapezoidal method (AUC0–180). The extrapolation to time infinity (AUC∞...) was