Environmental and Biological Monitoring of Styrene Exposure: Urinary Excretion of D-Glucaric Acid Compared with Exposure Indices

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Abstract: Environmental and Biological Monitoring of Styrene Exposure: Urinary Excretion of D-Glucaric Acid Compared with Exposure Indices: M.L. SCAPELLA, et al. Istituto di Medicina del Lavoro, Università degli Studi di Padova—Styrene, oxidized by liver microsomal enzymes, can determine a liver enzyme induction. Urinary excretion of D-glucaric acid (DGA), which is believed to estimate this effect, was measured in 27 workers exposed to styrene in a fiberglass plant and in 27 control subjects in order to make a comparison with environmental and biological exposure indices. In exposed workers, airborne concentrations (8-h TWA) of styrene varied between 9 and 415 mg/m³, with styrene metabolites (sum of mandelic acid (MA) + phenylglyoxylic acid (PGA)) ranging from 93 to 2130 mg/g Cr in endshift urine samples collected on a Thursday, and from 45 to 792 mg/g Cr in samples taken before work the following morning. The correlation coefficient (r) between 8-h TWAs and sum of urinary metabolites MA + PGA was 0.92 (y=4.06x−36.05; p<0.001) for Thursday endshift (ES) samples, and 0.84 (y=1.46x + 46.82; p<0.001) for Friday morning samples (beginning of shift: BS). Urinary excretion of MA correlated better with exposure than that of PGA (MA: ES r=0.91; BS r=0.86. PGA: ES r=0.80; BS r=0.76). ES and BS levels of DGA in exposed subjects (equal to 4.41 ± 1.57 and 4.01 ± 1.18 mmol/mol Cr respectively) were both significantly higher than the 2.93 ± 0.88 observed in 27 control subjects (Mann-Whitney test: p<0.001). No significant correlation was found between individual exposure to styrene and urinary excretion of DGA. Furthermore, both ES and BS urinary DGA levels increased across three classes of styrene exposure (<100, 101–200, and >200 mg/m³, respectively, including 10, 7 and 10 workers). The DGA difference between the most exposed and the not exposed subjects was significant (ES-DGA: z=4.03 and p<0.05; BS-DGA: z=3.16 and p<0.05; Kruskall-Wallis test), but individual pairwise comparisons among all other groups were not. In spite of the above results, no significant correlation was found between individual exposure to styrene and urinary excretion of DGA, so that this biomarker cannot be used to monitor the exposure effects on an individual basis.

Key words: D-glucaric acid, Styrene, Enzymatic induction, Biological monitoring, Mandelic acid, Phenylglyoxylic acid, Reinforced plastics industry

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Styrene is an aromatic hydrocarbon widely used in the reinforced plastics industry. It is essentially absorbed by inhalation and metabolized in the liver primarily by the microsomal system of mixed-function oxidases (Cyt-P450) to styrene-7,8-oxide (SO), a relatively reactive intermediate that may cause toxicity by covalent binding to essential cellular macromolecules. Detoxification may occur due to enzymatic hydration to phenylethylene glycol via microsomal epoxide hydrolase, and by glutathione-S-transferase (GST)-catalysed conjugation with cytosolic glutathione (GSH). Mandelic and phenylglyoxylic acids, the main urinary metabolites, are used as dose indicators in biological monitoring of exposed workers. Experimental studies in animals have shown that inhalation exposure to styrene may cause microsomal enzymatic cyt-P450 dependent induction in the liver and glutathione depletion, and epatotoxicity with degenerative or necrotic changes in centrilobular hepatocytes.

Among the several methods which have been proposed to assess enzymatic induction, the mostly used is urinary D-glucaric acid (DGA), which is an end product of carbohydrate metabolism produced via the glucuronic acid pathway. The mechanistic hypothesis linking liver enzyme induction to DGA production could be induction of either UDP-glucose-dehydrogenase with a subsequent increase in UDP-glucuronic acid, or of other enzymes involved in metabolic reactions from UDP-glucuronic acid to D-glucaric acid. Although the glucuronic acid pathway includes enzymes other than those of the microsomal enzyme system, the urinary excretion of D-glucaric acid is increased by many drugs known to stimulate microsomal enzyme activity. In fact urinary excretion of D-glucaric acid is fairly well correlated to the amount of microsomal enzymes in liver specimens during treatment with enzyme-inducing agents.

But the fact that urinary excretion of D-glucaric acid is a non-specific test and influenced by several confounding factors must be considered with particular care when interpreting the results.

Studies concerning the inducing effect of exposure to styrene in man show conflicting results: some authors found a slight increase in the urinary excretion of DGA related to styrene exposure; some others did not confirm this trend, reporting a slight increase in urinary excretion of DGA both in exposed and non-exposed subjects, due to alcohol intake and smoking habit. Other authors observed a statistically significant increase in urinary excretion of DGA in subjects exposed to styrene, even at concentration levels below the TLV.

The aim of the present study was to evaluate the urinary excretion of DGA in workers occupationally exposed to styrene and to verify how some environmental, biological and behavioral variables influence it.

Methods

Subjects

Twenty-seven workers (21 men, 6 women) working in a fiberglass plant were examined. Their average age was 28.5 ± 9.5 years. The mean exposure period of the workers was 5.3 ± 4.4 years.

A control group of 27 subjects (21 men, 6 women), average age 29.3 ± 10.1 years, were also examined. Controls were comparable with workers as regards smoking habit and consumption of alcohol and coffee, and none was exposed to solvents or enzyme-inducing or hepatotoxic substances. This information was obtained by asking all subjects to complete a specially prepared questionnaire.

Environmental investigation

Measurements of time-weighted average (8-h TWA) exposure to styrene were made using Zambelli TK-200 passive personal samplers, each worker being monitored on a Thursday for 4 h in the morning and 4 h in the afternoon. Analysis, after desorption with carbon sulphide, was by gaschromatography according to the NIOSH method.

Biological determinations

In all 27 subjects, the urinary concentrations of mandelic acid (MA) and phenylglyoxylic acid (PGA) were measured in urine samples collected at the end of the workshift on a Thursday and on a Friday morning before the beginning of work. Analyses were carried out by HPLC.

DGA concentrations were determined on the same urine samples. In a smaller number of subjects (15), urine samples were also collected at the beginning of work on two consecutive Mondays, respectively preceding and following the Thursday when environmental and biological investigation was carried out.

DGA urinary concentrations were also determined in all 27 control subjects, by spectrophotometry according to the method of Colombi et al.

For all samples, creatinine (Cr) was assayed by the Jaffè method. MA and PGA values being expressed as mg/g Cr and DGA values as mmol/mol Cr.

Statistical methods

In view of the small number of subjects involved we used non-parametric statistical tests: Mann-Whitney test for two groups or Kruskall-Wallis test for several groups. In the latter test, which corresponds to a one-way analysis of variance, multiple comparisons of all possible pairs of groups were made by computing a statistic “z” according to Hollander and Wolfe. Furthermore, a multiple linear regression equation was fitted to the data with DGA as a dependent variable; and exposure to styrene, sex, age, number of working years, smoking habit, alcohol intake and coffee as predictor variables. All potential predictors were forced into the equation, estimating the regression coefficients, their standard errors, and the F-to-remove tests. If the F-to-remove of a variable is above the acceptable limits, the program computes the proportion of the total variation in DGA accounted for by that variable (R square change) after removing the linear effect of other variables already in the equation. All analyses were performed with the BMDP statistical package.

Results

Individual levels of exposure to styrene, expressed as 8-h TWA, varied from 9 to 415 mg/m³. 18 subjects turned out to be exposed to values exceeding the styrene TLV-TWA adopted by ACGIH for 1997, i.e., 85 mg/m³ or 20 ppm. The sum of urinary metabolites of styrene MA + PGA varied from 93 to 2130 mg/g Cr at the end of the shift (ES) on Thursday and from 45 to 792 mg/g Cr on
Friday at the beginning of the shift (BS), and correlated well with individual styrene exposure: the correlation coefficient \(r\) was 0.92 (\(p<0.001\)) for the Thursday ES samples and 0.84 (\(p<0.001\)) for the Friday BS samples (Fig. 1). The urinary excretion of MA correlated better with exposure than that of PGA (ES \(r=0.91\) for MA and \(r=0.80\) for PGA; BS \(r=0.86\) for MA and \(r=0.76\) for PGA).

Table 1 shows DGA urinary excretion data for both exposed workers and controls. Exposed workers had an average value of 4.41 mmol/mol Cr at Thursday ES and 4.01 mmol/mol Cr at Friday BS. Controls had 2.93 mmol/mol Cr. There was a statistically significant increase in the DGA urinary excretion of exposed workers with respect to controls. Nevertheless, it should be stressed that no significant correlation was found between individual exposure to styrene evaluated by the environmental concentration or by urinary metabolites and urinary excretion of DGA (Fig. 2).

Table 1. Urinary concentrations of D-glucaric acid (DGA) at the end of the shift (ES) and the beginning of the shift (BS) in 27 styrene-exposed subjects and 27 controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (thursday 6:00 p.m.)</th>
<th>Exposed</th>
<th>BS (friday 8:00 a.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>2.93</td>
<td>4.41*</td>
<td>4.01**</td>
</tr>
<tr>
<td>SD</td>
<td>0.88</td>
<td>1.57</td>
<td>1.18</td>
</tr>
</tbody>
</table>

*Exposed ES versus controls (\(p<0.001\); Mann-Whitney test).
**Exposed BS versus controls (\(p<0.001\); Mann-Whitney test).
Table 2. Assessment of variables influencing urinary excretion of DGA by means of the multiple regression analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>R square change</th>
<th>Statistical significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure to styrene</td>
<td>29.8</td>
<td>p&lt;0.005</td>
</tr>
<tr>
<td>Sex</td>
<td>0.1</td>
<td>not significant</td>
</tr>
<tr>
<td>Age</td>
<td>6.8</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>No. working years</td>
<td>1.7</td>
<td>not significant</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>3.6</td>
<td>not significant</td>
</tr>
<tr>
<td>Alcohol intake</td>
<td>2.8</td>
<td>not significant</td>
</tr>
<tr>
<td>Coffee intake</td>
<td>0.2</td>
<td>not significant</td>
</tr>
</tbody>
</table>

*F-to-remove test.

Table 3. Urinary concentrations of DGA at the end of a shift (ES) and before the next shift (BS) in exposed workers, according to level of exposure to styrene

<table>
<thead>
<tr>
<th>No. subjects</th>
<th>DGA mmol/mol Cr mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects non exposed</td>
<td>27</td>
<td>2.93</td>
</tr>
<tr>
<td>Subjects exposed below 100 mg/m³ styrene</td>
<td>3.88 (ES)</td>
<td>1.77</td>
</tr>
<tr>
<td>Subjects exposed to 101–200 mg/m³ styrene</td>
<td>3.72 (ES)</td>
<td>1.58</td>
</tr>
<tr>
<td>Subjects exposed above 200 mg/m³ styrene</td>
<td>5.36 (ES)</td>
<td>1.24*</td>
</tr>
</tbody>
</table>

*Exposed to styrene above 200 mg/m³ versus controls: z=4.03 (ES); z=3.16 (BS); p<0.05 (Kruskall-Wallis test).

Table 4. Urinary concentrations of DGA in styrene-exposed workers during working week

<table>
<thead>
<tr>
<th>Day</th>
<th>No. subjects</th>
<th>DGA mmol/mol Cr mean</th>
<th>SD</th>
<th>Significance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monday BS</td>
<td>15</td>
<td>3.52</td>
<td>0.99</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Thursday ES</td>
<td>15</td>
<td>4.95</td>
<td>1.29</td>
<td>p=0.06</td>
</tr>
<tr>
<td>Following Monday BS</td>
<td>15</td>
<td>4.00</td>
<td>1.24</td>
<td></td>
</tr>
</tbody>
</table>

*Mondays BS versus Thursday ES: p<0.01. Thursday ES versus the successive Monday BS: p=0.06 (Kruskall-Wallis test).

significantly affects DGA excretion.

As exposure is the variable of greatest influence, the data were re-analysed by subdividing subjects into three groups according to the exposure level: under 100 mg/m³, 101–200 mg/m³, and over 200 mg/m³. The corresponding means and standard deviations, as well as those in 27 non exposed subjects are shown in Table 3. As can be seen, DGA values are similar in the first and second exposure groups, and both are near the values obtained in the non exposed. Higher values were observed in subjects working at a styrene concentration above 200 mg/m³. As the Kruskall-Wallis test applied to these data provides a significant result, multiple comparisons of all possible pairs of group were performed. The DGA level found in the non exposed group differs significantly at the 0.05 level from both the ES and BS DGA values observed in workers exposed to above 200 mg/m³ of styrene.

Table 4 reports DGA urinary concentrations in 15 of the 27 workers examined on various days. As can be seen, DGA excretion increases during the week and then falls after the weekend, with statistically significant differences between the Monday BS and Thursday ES (p<0.01), but the difference between the Thursday and the successive Monday BS is of borderline significance (p<0.06).

Discussion

Our group of workers had highly variable exposure to styrene; in two-thirds of them the TLV-TWA recommended by the ACGIH was exceeded. This was also confirmed by the sum of urinary metabolites of styrene (MA + PGA), which in many cases was higher than the ACGIH BEI, i.e., 1040 mg/g Cr ES and 400 mg/g Cr BS. It is well-known that the concentration of styrene in air correlates well with data on urinary metabolites at both ES and BS, so that the latter data are considered to be excellent dose indicators. The correlations we found substantially agree with those in the literature, although it may be stated that the determination of MA alone is a reliable test for the assessment of styrene exposure.

A debate is continuing about the effects of chemical substances usually found in work environments and thus about finding biochemical markers predicting possible liver damage. With specific regard to styrene, experimental studies in animals showed its ability to induce the hepatic mixed oxidase system and to cause, mostly in high
exposure, microscopically degenerative or necrotic changes in centrilobular hepatocytes\(^3\)–\(^10\).

Rare cases of impaired hepatic function and toxic hepatitis have been reported for human styrene exposure\(^1\), but most of the studies are concerned with the inductive effects of styrene in man\(^19\)–\(^22\).

Bergamaschi and Coll.\(^32\) studied a wide group of styrene exposed subjects, aiming at evaluating possible hepatotoxic or inductive effects. When compared to controls, the styrene exposed workers showed statistically significant increases in the activities of alkaline-phosphatase, ornithine-carbamyl-transferase, gamma-glutamyltranspeptidase, urinary D-glucaric acid and 6-beta-OH-cortisol. As they found no significant correlation between the duration or intensity of exposure and biochemical indices of liver damage, they concluded that styrene exposure did not cause hepatotoxic effects.

Induction of microsomial enzyme has several consequences for the organism: proliferation of the smooth endoplasmatic reticulum of cells is the outstanding effect and, since the liver is the principal target of induction, hepatomegaly is a rather common issue in the P-450 type of induction. Moreover, the increase in the activity of microsomal enzymes enhances the rate of biotransformation of the inducer itself and the endogenous and exogenous substances that are metabolized by this system\(^13\),\(^34\). As a consequence, phenomena produced by induction include modification of the biotransformation (inactivation or activation) of other exogenous substances\(^35\).

Our data show that styrene produces a significant increase in DGA concentrations in the urine of styrene-exposed workers in contrast to controls. When exposed workers are subdivided by level of exposure, DGA excretion increases with increasing exposure, reaching statistical significance for exposure exceeding 200 mg/m\(^3\).

Although some authors\(^6\) claim that urinary excretion of DGA is a non-specific test, being affected by biologic and environmental factors as well as by personal habits, the multivariate statistical analysis carried out in our subjects showed that exposure to styrene is the most important predictive factor, followed by age.

We also assessed the number of working years of our exposed subjects, in order to verify whether the role played by age was due to the subjects’ longer exposure or not. Although we found that the number of working years - like all the other variables studied - did not significantly influence DGA excretion, it should be stressed here that most subjects had only been working in the fiberglass sector for a few years.

Our study also showed that the urinary excretion of DGA varies over the working week, being higher at the end of the last shift of the week and then falling after the weekend, this trend being more evident for exposures exceeding 100 mg/m\(^3\).

The above data thus highlight the presence of short-term induction which peaks at the end of the working week and then falls to DGA values which almost completely overlap those of controls - demonstrating a rapid capacity for recovery in the absence of exposure and therefore a subacute, reversible effect. Also for substances other than styrene\(^6\), the literature reports increased urinary excretion of DGA after only 4 h of exposure and then a drop to levels similar to those of controls the following morning. These results also agree with experimental evidence showing proliferation of the smooth endoplasmatic reticulum and therefore a hepatocytic response after 1 h of exposure\(^7\). Instead, other studies on yet other substances show that DGA levels rise during the first two weeks of exposure, reach a plateau, and only after about one month after suspension of exposure fall to levels similar to those of controls\(^8\).

In conclusion, a significant increase in urinary excretion of DGA has been found in workers exposed to styrene above the current TLV\(^28\) when compared to non-exposed subjects; this finding may be regarded as like an effect of the styrene exposure probably arising from an underlining enzyme induction process; nevertheless, new studies in animals are needed to elicite the metabolic pathway connecting enzymatic induction and DGA urinary excretion, particularly to define if there is a direct cause/effect relationship or if this pattern is the result of two distinct mechanisms. Given the fact that no significant correlation was found between individual DGA values and styrene exposure indices, the former may not be used to monitor the exposure effects on an individual basis.

References

1) Leibman KC. Metabolism and toxicity of styrene. Environ Health Persp 1975; 11: 115–119.