

Raised serum bile acid concentrations after occupational exposure to styrene: a possible sign of hepatotoxicity?

C EDLING AND C TAGESSON

From the Departments of Occupational Medicine and Clinical Chemistry, University of Linköping, Linköping, Sweden

ABSTRACT Fasting serum concentrations of conjugated bile acids were investigated in 23 men who had been exposed to styrene and compared with the concentrations in 60 non-exposed individuals. Eleven of the exposed subjects had raised concentrations of either cholic acid or chenodeoxycholic acid or both. There were no indications of alcohol abuse, drug intake, or undiagnosed liver disease. It is possible, therefore, that the raised bile acid concentrations were due to exposure to styrene. This would support the concept that occupational exposure to styrene may affect the liver and point to the possibility that raised serum bile acid concentrations might be a sensitive and early indicator of hepatic injury in individuals exposed to organic solvents.

The liver plays a major part in the biotransformation of environmental agents. Accordingly, the toxic effects of certain chemical agents used in industry—for example carbon tetrachloride¹ and 1, 1, 2, 2-tetrachloroethane²—include serious hepatic injury, and the list of potentially hepatotoxic agents is continually growing. Also, concern among exposed workers has led to a demand for laboratory tests to be used in the early diagnosis and prevention of occupational liver disease. Such tests are, for instance, serum concentrations of “liver enzymes” such as alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and gammaglutamyl transferase (γ -GT). These tests have been used to screen for liver damage in workers exposed to organic solvents.^{3,4} It appears, however, that the frequency of abnormal test results in such screening is low. This could be because the concern for liver injury due to solvent exposure is unwarranted, or because the commonly used laboratory tests are not sensitive enough to detect liver damage. There are reasons, therefore, to investigate the possible use of other, more sensitive and specific laboratory tests. One such test is the measurement of the serum concentrations of bile acids.

With an adequate assay, serum bile acid concentrations appear superior to conventional liver function tests in detecting minor hepatobiliary dysfunction.⁵

Recently, Axelson and Gustavsson proposed that styrene may affect the liver and that this could result in raised serum concentrations of liver enzymes.⁶ We therefore investigated the serum bile acid concentrations in subjects exposed to styrene and compared the levels with those found in healthy, unexposed individuals.

Materials and methods

Subjects—Twenty three men aged 19–58 (mean age 40) processing sewage pipes were investigated. The average duration of their exposure to styrene was 2.5 years. They had no apparent liver disease and took no drugs known to affect the liver. One of the subjects occasionally abused alcohol.

Procedures—On a Friday morning, at the end of a working week, fasting blood samples were taken, centrifuged, and frozen at -20°C until analysed. On the day before environmental sampling of styrene had been performed and urine collected for analysis of mandelic acid. Exposure levels in the breathing zone—measured by personal sampling—ranged from 41 to 202 mg styrene/m³ air.

Analysis—Urinary mandelic acid (MA) was determined by isotachopheresis essentially as

Table 1 Frequency of abnormal liver laboratory test results in 23 men exposed to styrene when processing sewage pipes

Laboratory test	Abnormal test results		
	Definition*	No	%
S-LD	> 7.5 μ kat/l	0	0
S-ALP	> 5.0 μ kat/l	0	0
S- γ -GT	> 0.7 μ kat/l	1	4
S-ASAT	> 0.7 μ kat/l	1	4
S-ALAT	> 0.7 μ kat/l	3	13
S-CA	> 0.8 μ mol/l	9	39
S-CDCA	> 1.1 μ mol/l	9	39

*Upper reference value given by the clinical chemistry laboratory.

described by Sollenberg and Baldesten.⁷ Serum aspartate aminotransferase (S-ASAT), alanine aminotransferase (S-ALAT), gammaglutamyl transferase (S- γ -GT), alkaline phosphatase (S-ALP), and lactate dehydrogenase (S-LD) were determined using standard analytical procedures. Fasting levels of serum cholic acid (S-CA) and chenodeoxycholic acid (S-CDCA) were determined by radioimmunoassays⁸ in which glycine and taurine conjugates were measured with equal sensitivity. Unconjugated CA and CDCA had cross reactivities towards the CA and CDCA antiserum of 20% and 30%, respectively.

Reference values—Fasting reference values for serum bile acids were established using sera from 60 healthy fasting individuals aged 18–61 y (median age 25). As calculated from the mean +2 SD, the upper reference values were 0.8 μ mol/l and 1.1 μ mol/l for S-CA and S-CDCA, respectively.⁸ Table 1 gives the upper reference values for the other liver tests.

Results

Table 1 shows frequency of abnormal results in var-

ious liver laboratory tests. Nine of the 23 individuals had raised fasting levels of S-CA and nine of S-CDCA, and altogether 11 had raised levels of either S-CA or S-CDCA (or both). Three subjects had raised S-ALAT and one raised S-ASAT. Only one subject showed raised S- γ -GT and none increased S-LD or S-ALP.

Altogether, 13 of the 23 subjects investigated had one or more abnormal liver laboratory test results (table 2). Thus it appeared that 10 subjects had increased levels of either S-CA or S-CDCA (or both) but otherwise normal laboratory findings. Two individuals (GH and MN) showed increased liver enzyme activities but normal bile acids while one (KL) had both increased S-ALAT and raised S-CA.

The urinary excretion of mandelic acid (MA) ranged from 0.3 to 17.8 mmol/l (3.44 ± 3.82 , m \pm SD (n = 22)). A correlation ($r = 0.9$) was obtained between urinary MA and styrene exposure; this is in accordance with the concept that urinary MA reflects the degree of styrene exposure.⁹ According to Swedish occupational health practice, urinary MA values of 1.3 mmol/l indicate potentially hazardous styrene exposure. If this MA-value was used to dichotomise the subjects into "high exposed" and "low exposed" (table 3), nine of the ten individuals with raised bile acids were high exposed. Of the five low exposed subjects, one

Table 3 Serum bile acid concentrations in 22 men exposed to styrene when processing sewage pipes

Bile acid* concentration	Group of individuals	
	Low exposed†	High exposed‡
Raised	1	9
Normal	4	8

*CA or CDCA or both.

†< 1.3 mmol mandelic acid/l urine.

‡ \geq 1.3 mmol mandelic acid/l urine.

Table 2 Liver laboratory test results in 13 men exposed to styrene when processing sewage pipes

Subject	Age (y)	S-ASAT (μ kat/l)	S-ALAT (μ kat/l)	S- γ -GT (μ kat/l)	S-CA (μ mol/l)	S-CDCA (μ mol/l)
AB	33	0.22	0.22	0.32	1.4*	2.3*
CD	48	0.37	0.19	0.43	1.3*	1.7*
EF	21	0.38	0.24	0.28	0.9*	1.4*
GH	47	0.84*	0.96*	0.86*	0.7	0.8
IJ	27	0.36	0.43	0.28	0.9*	0.9
KL	48	0.47	0.81*	0.52	1.3*	1.0
MN	34	0.37	0.85*	0.52	0.5	0.6
OP	27	0.28	0.46	0.47	1.8*	2.5*
QR	23	0.42	0.22	0.22	0.6	1.3*
ST	27	0.32	0.24	0.17	1.3*	3.2*
UV	23	0.30	0.25	0.26	0.9*	2.5*
WX	19	0.48	0.38	0.30	0.2	2.5*
YZ	21	0.44	0.14	0.24	2.3*	6.9*

*Abnormal test results.

(20%) had raised serum bile acids. Assuming that the raised bile acids were due to styrene exposure, the bile acid test would have 53% sensitivity and 80% specificity (data from table 3).

Discussion

This study aimed to determine the serum bile acid concentrations after occupational exposure to styrene and to relate these values to those found in healthy non-exposed individuals. In hospital investigations of liver disease as in occupational health screening the results of laboratory tests are related to reference ranges given by the laboratory at hand. The reference values for S-CA and S-CDCA used in this study were based on samples from 60 healthy individuals in the same age span as the exposed subjects. Moreover, there are no indications that fasting serum bile acid concentrations in healthy adults are age- or sex- dependent.¹⁰ With this in mind it seems justified to use the established reference values for comparison with values found in exposed subjects rather than selecting a particular control group.

Direct measurements showed that the investigated subjects were exposed to styrene in the range of 41–202 mg/m³; for comparison, the current Swedish TLV is 170 mg/m³. Also, the urinary excretion of mandelic acid (MA) showed that the subjects were all exposed to styrene and that urinary MA excretion reflected the degree of styrene exposure. As to the general exposure level, previous studies have indicated that 100–300 mg/m³ may have caused raised serum levels of aminotransferases.⁶

The investigation showed that 13 of the 23 subjects (57%) had one or more abnormal liver test results (table 2). Of these, only three had raised liver enzymes whereas 11 had raised bile acids. The reason for this cannot be unequivocally established, but some possibilities may be discussed.

To explain raised aminotransferase levels, alcohol consumption has always to be considered. One of the subjects (GH) was known to abuse alcohol and he also had raised ASAT, ALAT, and γ -GT levels, but normal bile acids. As to the other subjects there were no indications of abnormal alcohol intake and γ -GT values were all normal; (the γ -GT test is claimed to be a sensitive measure of liver damage due to alcohol¹¹). Even normal alcohol consumption together with styrene exposure could have affected the liver, however, since potentiation between alcohol and solvents has been shown in animal experiments^{12,13} and in field studies.¹⁴

Other possibilities might be that the subjects did not fast to obtain proper fasting levels or that they had one or several undiagnosed liver diseases with

concomitant raised bile acid levels. We have no indication, however, to support any of these contentions. None of the subjects took drugs that could have affected the liver on their own or by interacting with solvents.¹⁵ We therefore suggest that in most subjects the raised bile acid concentrations were due to their styrene exposure.

This investigation may lend further support to the concept that occupational styrene exposure adversely affects the liver. It also illustrates that routine monitoring of liver enzymes is a doubtful screening method in occupational health practice, but points to the possibility that raised bile acid concentrations might be a sensitive and early indicator of liver dysfunction.

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