Exposure to toluene increases the urinary excretion of D-glucaric acid*

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Abstract

Workers at a printing plant exposed to low concentrations of toluene (43–401 mg/m³, median 155 mg/m³) had increased urinary D-glucaric acid (3.55–5.12 mmol/mol creatinine) excretion at the end of the shift compared with controls (2.45–3.35 mmol/mol creatinine). No increase was found after the summer holiday (1.92–2.89 mmol/mol creatinine) but excretion had increased two weeks later (4.05–5.55 mmol/mol creatinine). These changes in the excretion of D-glucaric acid were not correlated to levels of exposure, to changes of urinary hippuric acid and o-cresol half lives (three to eight hours), nor to o-cresol/hippuric acid concentration ratios when measured at the end of daily exposure. Since a significant intra and interindividual variability of urinary D-glucaric acid was found in all groups, urinary D-glucaric acid excretion is suitable to monitor group but not individual exposure.

Toluene is used in various industrial processes as a chemical intermediate and solvent.¹ The target organ for toluene toxicity in man and in experimental animals is the central nervous system (extensive review²). In experimental animals, however, exposure to toluene also causes an increase of cytochrome P-450 and of some microsomal enzymes in the liver and other organs.^{3–6} These biochemical effects are associated with an increase in mitochondria and the proliferation of smooth endoplasmic reticulum in hepatocytes suggesting liver microsomal enzyme induction.⁷

Changes in microsomal enzyme activity caused by occupational exposures are usually not accompanied by clinical signs of toxicity but they are regarded as a sensitive index of biological changes. The assessment

of liver microsomal enzyme function by means of non-invasive tests, such as saliva antipyrine half life and D-glucaric acid and 6- β -hydroxycortisol excretion in urine, was therefore proposed for the biological monitoring of occupational exposures to several chemicals.⁸

D-glucaric acid is the end product of the glucuronic acid pathway involving several enzymes present in the cytosol but not in the microsomes. Urinary D-glucaric acid (UDGA) excretion in man was shown to increase after treatment with certain drugs such as phenobarbitone and aminopyrine.⁹ In some species but not in man and guinea pigs D-glucaric acid is converted into ascorbic acid and no UDGA is detectable. In guinea pigs treated with phenobarbitone high correlation was found between liver P-450 content and daily UDGA excretion. When animals were pretreated with an inhibitor of protein synthesis before treatment with phenobarbitone, UDGA excretion decreased with decrease in the level of liver P-450.¹⁰ This observation suggested that measurement of UDGA would provide a quantitative although indirect estimate of hepatic enzyme activity. The rate limiting step of this pathway is unknown even though the enzymes involved in D-glucaric acid formation are increased during enzyme induction.¹¹

We report here on excretions of UDGA and toluene metabolite in workers occupationally exposed to low concentrations of toluene in a rotogravure printing plant.

Materials and methods

SUBJECTS

We studied workers in a printing plant where the ink used in the rotogravure was diluted with pure toluene. Toluene was also used to clean the printing machine. The workers were subdivided as follows.

Group A ($n = 7$) Workers studied for eight days (Monday to Monday) before the summer holidays (four weeks). They were also studied for one day immediately and 50 days after the end of the summer holidays.

Group B ($n = 7$) Workers studied for 15 days immediately after returning from summer holidays and for one day 36 days thereafter.

Group C ($n = 9$) Workers studied for one day,

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one and 28 days after a short holiday (10 days). This group was formed of individuals from the two previous groups.

Control workers ($n = 16$) from the same plant but not exposed to toluene were matched with the exposed workers for age, smoking, and food habits and monitored daily for UDGA and hippuric acid excretion from Monday to Friday for one week only to be compared with group A. In the remaining experiments each group acted as its own control.

ENVIRONMENTAL MONITORING

Environmental exposure to toluene of each worker was assessed as the TWA for the first and second half of the shift on the same days when urine was collected for UDGA, hippuric acid, and o-cresol determinations, except after summer holidays for group A and the 51st day after the summer holiday for group B. Air samples were obtained with activated charcoal personal dosimeters and toluene concentration was measured according to the NIOSH method.¹²

URINE COLLECTION AND ANALYSIS

In groups A and B urine samples were collected before and after the workshift and in group C urine samples were collected for 17 hours after the workshift. Samples were stored at -20°C until analyses were performed. Urinary hippuric acid and o-cresol were measured by HPLC according to De Rosa *et al.*¹³ D-glucaric acid was measured according to Colombi *et al.*¹⁴ All values were then corrected for creatinine content.

Results

Exposure levels have been extensively reported elsewhere.^{15,16} Environmental toluene (range of daily means: $87\text{--}301\text{ mg/m}^3$, median 155 mg/m^3) never exceeded the TLV of 375 mg/m^3 proposed by ACGIH for 1987¹⁷ except for a single day exposure for one worker. Urinary hippuric acid (range of daily means: $0.44\text{--}1.15\text{ mol/mol creatinine}$, median $0.97\text{ mol/mol creatinine}$) and o-cresol (range of daily means: $205\text{--}531\text{ }\mu\text{mol/mol creatinine}$, median $407\text{ }\mu\text{mol/mol creatinine}$) were correlated to the environmental concentration of the solvent.¹⁶ No significant differences were found in exposure between groups A and B.

The table shows the values of postshift UDGA in group A workers measured for a week four weeks before summer holidays and in controls. UDGA in the exposed workers was significantly higher than in the non-exposed workers. Control values were comparable to those already reported.¹⁸ When measured immediately after the holidays, however, UDGA excretion in group A workers did not differ from that of the controls ($2.45 \pm 0.14\text{ mmol/mol creatinine}$, mean $\pm$ SEM, $n = 7$). After 50 days exposure,

UDGA excretion in toluene exposed (group A) and control subjects

Day	UDGA (mmol/mol creatinine)	
	Exposed ($n = 7$)	Controls ($n = 16$)
Monday	3.55 ± 0.40	2.72 ± 0.23
Tuesday	4.44 ± 0.50	2.59 ± 0.21
Wednesday	3.97 ± 0.35	3.35 ± 0.22
Thursday	5.11 ± 0.36	3.05 ± 0.18
Friday	3.98 ± 0.36	2.45 ± 0.16
Saturday	5.12 ± 0.99	—
Monday	5.02 ± 0.33	—

UDGA values of exposed workers are significantly higher than those of control subjects ($p < 0.001$ with analysis of variance for repeated measures). A significant day to day variation (Greenhouse-Geisser probability < 0.002) of UDGA values was found in both groups. (Data are expressed as mean $\pm$ SEM.)

UDGA excretion was again high ($3.79 \pm 0.31\text{ mmol/mol creatinine}$, mean $\pm$ SEM, $n = 7$).

The values of UDGA in group B are reported in the figure. Immediately after the summer holidays, UDGA excretion was comparable to that of non-exposed workers; the values then increased, reaching, in about two weeks, a plateau level comparable with that of group A. No correlation was found in groups A and B between individual postshift UDGA and urinary hippuric acid or o-cresol excretion or environmental exposure as assessed on the same day.

To see whether increased UDGA was associated with a different pattern or time course, or both, of metabolite excretion, urinary hippuric acid and o-cresol were monitored for 17 hours after the end of the workshift in group C workers on two occasions. Immediately after a short holiday UDGA excretion was similar to that of controls ($2.33 \pm 0.12\text{ mmol/mol creatinine}$) and increased by about 25% four weeks later. The time courses of excretion of hippuric acid and o-cresol were the same in each subject.

Urinary D-glucaric acid excretion (mean $\pm$ SEM) in seven workers exposed to toluene (group B) measured after end of summer holidays. Dashed lines represent mean $\pm$ SD of total control values. Data best fitted with a third degree curve ($p < 0.001$, $r = 0.71$).¹⁹

The half lives of excretion for both metabolites and postshift o-cresol/hippuric acid ratios differed among individuals (ranging from three to eight hours and from 12×10^{-5} and 84×10^{-5} , respectively). None of these parameters, however, changed significantly after four weeks of exposure to toluene.

Discussion

High doses of toluene have been shown to increase phase I and phase II microsomal enzymes.³⁻⁶ The effect was measurable as soon as one to three days after exposure,^{4,5} was reversible within one to four days,⁴ and was associated with an induction of the metabolism of toluene itself.⁶

UDGA excretion may correlate with liver cytochrome P-450 levels, but it is possible that it represents an index of phase II drug metabolism rather than being determined by the activity of phase I enzymes.²⁰ Furthermore, several of the enzymes in the glucuronic pathway are not localised in microsomes. It is therefore clear that an increased UDGA excretion and the induction of liver microsomal enzymes might be associated but not necessarily consequential.²¹

UDGA excretion, for instance, increases dramatically (up to 20-fold) in patients chronically administered various anticonvulsant drugs and this increase was found to be related to induction of liver enzymes.²²

Workers exposed to a mixture of toluene, xylene, and various pigments, but not those exposed to hexane, octane, ethyl, benzyl, and butyl alcohols, and pigments, had a twofold increase of UDGA above control levels. They also had an increased blood antipyrine half life. The authors suggested the causative role of toluene and pigments.²³ Pigments were in fact able to inhibit hepatic N-demethylase activity when administered to rats. UDGA was also studied in workers exposed to low concentrations of toluene only²⁴ but, unlike in our study, no difference was found between exposed and control workers. It may be that the exposure of their workers was more variable than ours, and their controls included more smokers and drinkers.

It is not clear whether the observed increase of UDGA is a sign of liver enzyme induction. Certainly, UDGA increased excretion is not associated with an accelerated metabolism of toluene itself as shown by the pattern of hippuric acid and o-cresol excretions. In rats only excessively high doses of toluene induced its own metabolism²⁵ and after experimental and occupational exposure to toluene (about 375 mg/m³) no changes in saliva antipyrine clearance were detected.²⁶ Taken together these data suggest that the increased UDGA excretion caused by low concentrations of toluene does not result from liver enzyme induction.

In conclusion, occupational exposure to low

concentrations of toluene (below ACGIH limits) increases UDGA excretion. The increase is moderate, reversible, and not associated with induction of toluene metabolism. Owing to the high interindividual variation of UDGA excretion both in control and exposed subjects, this test is not suitable for monitoring the occupational exposures to toluene of individuals considering also that the significance of such an increase is unknown. Nevertheless, the measurement of UDGA excretion may be an index of exposure on a group basis.

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