Interactions between drugs and chemicals in industrial societies.
G.L. Plaa, P. du Souich, S. Erill, editors.

URINARY THIOETHERS AS BIOLOGICAL MONITORS OF ENVIRONMENTAL EXPOSURE TO CHEMICALS

JULES BRODEUR

Département de médecine du travail et d'hygiène du milieu, Faculté de médecine, Université de Montréal, C.P. 6128, Succ. "A", Montréal, Québec, Canada H3C 3J7.

INTRODUCTION

Glutathione conjugation of reactive electrophiles originating from xenobiotics results in the formation of thioethers which are ultimately excreted mainly as mercapturic acids in the urine, but also as cysteine conjugates and other thioethers (e.g. thiodiglycolic acid with vinylidene chloride and vinyl chloride).

Many, but not all chemicals that undergo this metabolic transformation are potential alkylating or arylating substances possessing mutagenic, carcinogenic, necrogenic or immunotoxic properties. Conjugation of reactive electrophiles is therefore generally considered to be a detoxification mechanism, as it can prevent covalent binding of these substances to cellular macromolecules.

NON-SPECIFIC DETERMINATION OF THIOETHERS.

Non-specific determination of thioethers can be perfomed after alkaline hydrolysis and measurement of the liberated thiols (1) according to the colorimetric method of Ellman (2). Improvements to this general procedure have been proposed. Thus, ethyl acetate extraction of acidified urine samples before alkaline hydrolysis by removing the disulfide cystine, partly reduces the high background values and large interindividual variations (3). This is the most often used method and one found to work quite satisfactorily in our laboratories. Other modifications have been proposed to remove yellow urinary pigments and prevent oxidation of liberated thiol groups (4,5).

Using such non-specific procedures, exposure to complex mixtures of chemicals in chemical (1) and rubber (6-8) industry, near chemical waste incinerators (9), and during cigarette smoking (3-5) has been found to result in higher excretion of urinary thioethers. Similarly, exposure to known industrial chemicals (styrene, vinyl chloride and dimethylformamide has also resulted in higher excretion of thioethers (10).

It has been therefore suggested that the determination of urinary thioethers can be used to evaluate absorption of electrophilic chemicals (10-13). However, the application of such procedures to the biological monitoring of internal exposure to reactive intermediates on an individual basis has been limited by the fact that they lack chemical specificity and suffer from relatively high

background values. Contribution to the background is provided by urinary excretion of other thioethers resulting from the conjugation of glutathione with endogenous steroids and unsaturated acyl-CoA thioesters, and with food additives, tobacco smoke components and various xenobiotics (14). In addition, background values undergo considerable intra- and interindividual variations (15). In spite of this, non-specific determination of thioethers represents a useful means to assess group exposure to mixtures of chemicals.

SPECIFIC DETERMINATION OF THIOETHERS

When the nature of exposure is known and appropriate standards of specific thioethers are available, specific determination of individual mercapturic acids and/or respective cysteine conjugates becomes feasible. Over the years, separation and determination of specific thioethers has been performed using paper chromatography (16,17), gas chromatography (18), gas chromatography coupled to mass spectrometry (19), and high pressure liquid chromatography (20).

In our laboratories, a gas chromatography procedure has been proposed for the determination of the ortho, meta and para isomers of bromophenylmercapturic acid derived from bromobenzene (21). More recently, high pressure liquid chromatography procedures have been developped for the specific determination of thioethers derived from a series of industrial solvents, using ultra-violet detection (bromobenzene, styrene and benzene) or fluorescence detection (ethylene oxide (22), bromoethanol, acrylonitrile, vinylidene chloride and 1,2-dichloroethane). Using such specific analytical procedures, we have undertaken to study the toxicological significance of urinary thioethers, such as the relevant question of the relationship existing between exposure to chemicals and amounts of urinary thioethers excreted.

Previous studies conducted in our laboratories, using a non-specific colorimetric method for analysis of thioethers (23), have shown that determination of urinary thioethers does not always reflect exposure to bromobenzene (Figure 1, ref. 23). This phenomenon was evident when the animals were exposed acutely to hepatotoxic doses of bromobenzene and it reflected dose-dependent depletion of liver glutathione. In other words, administration of progressively increasing doses of bromobenzene resulted in a lower percentage of the administered dose being excreted as thioethers.

This was recently confirmed with similarly treated rats given acute doses of bromobenzene; this time, urine was analysed specifically for bromophenylmercapturic acids. Results are presented on Table I.

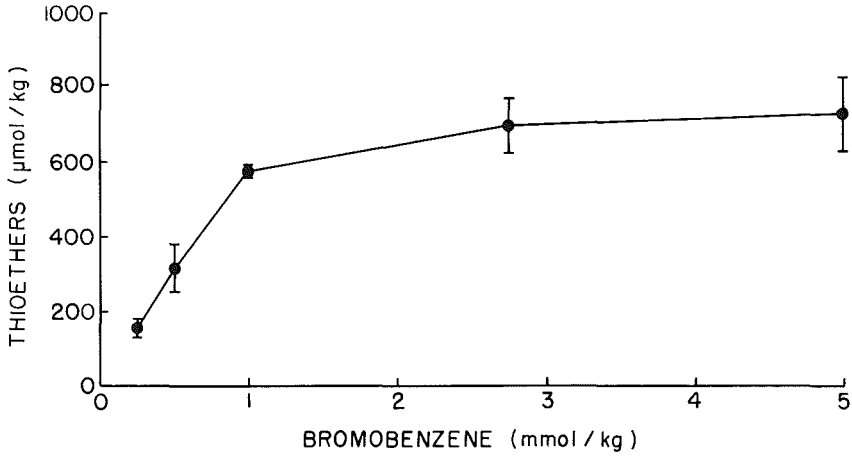

Fig. 1. Urinary excretion of thioethers in rat during a 24-hour period after intraperitoneal administration of increasing doses of bromobenzene. Results are means ± S.E.M. for 6 animals per dose. Control value for thioethers is 16.1 ± 1.8 µmol/kg. Data taken from ref. 23.

TABLE I

EXCRETION OF BROMOPHENYLMERCAPTURIC ACIDS DURING A 24-HOUR PERIOD AFTER ACUTE INTRAPERITONEAL ADMINISTRATION OF INCREASING DOSES OF BROMOBENZENE

Dose of bromobenzene (mmol/kg)	Percent of dose excreted as bromophenylmercapturic acids
0.5	26
1.0	23
2.5	19
5.0	11

The observation that urinary thioether excretion does not correlate with exposure after acute administration of high doses of bromobenzene was extended to the situation of repeated administration of smaller doses of the toxicant. Thus repeated intraperitoneal injection of minimally toxic or sub-toxic acute doses of bromobenzene to mice resulted, after 18 days of treatment, in a dose-dependent decrease of the percentage of the daily administered dose excreted as mercapturic acids (Table II, ref. 21); this was accompanied by similarly dose-dependent important increases of enzymatic parameters of hepatotoxicity.

TABLE II
EXCRETION OF BROMOPHENYLMERCAPTURIC ACIDS DURING A 24-HOUR PERIOD AFTER DAILY REPEATED INTRAPERITONEAL ADMINISTRATION OF INCREASING DOSES OF BROMOBENZENE

Dose of bromobenzene (mmol/kg/day)	Percent of dose excreted as bromophenylmercapturic acids on day of treatment	
	Day 1	Day 18
0.5	26	13
0.75	22	11
1.0	21	6

Ethylene oxide is a chemical of considerable importance: it is widely used as a sterilant in hospitals and the pharmaceutical industry and as a synthesis intermediate in the chemical industry. 2-Hydroxyethylmercapturic acid represents an important urinary metabolite of ethylene oxide in rats (24). Studies conducted by our colleagues have shown that the urinary excretion of 2-hydroxyethylmercapturic acid in rats also becomes relatively less important as the administered intravenous dose of ethylene oxide increases from 10 to 100 mg/kg (Table III, ref. 22).

TABLE III
EXCRETION OF 2-HYDROXYETHYLMERCAPTURIC ACID DURING A 12-HOUR PERIOD AFTER ACUTE INTRAVENOUS ADMINISTRATION OF INCREASING DOSES OF ETHYLENE OXIDE*

Dose of ethylene oxide (mg/kg)	Percent of dose excreted as 2-hydroxyethylmercapturic acid
1	30
10	29
100	16

*Data taken from ref. 22

However, when exposure to ethylene oxide was carried out by inhalation over a period of 6 hours, the excretion of 2-hydroxyethylmercapturic acid varied linearly with the concentration of the chemical over the entire range of concentrations. Thus, although exposure to the highest concentration (200 ppm) of

ethylene oxide during a period of 6 hours theoretically allowed for the absorption of 76 mg/kg of the chemical (25), the excretion of mercapturic acid averaged 0.27 mmol/ppm for all 6 concentrations studied: 1, 5, 10, 25, 50 and 200 ppm (22). Thus temporal extension of the absorption of a given dose of ethylene oxide imposes a smaller stress on the glutathione detoxification system and allows for a more complete conjugation of the reactive electrophile.

Since the metabolic formation of mercapturic acid, under certain conditions of exposure, is intimately dependent upon the availability of glutathione in tissues, it was hypothesized that repletion of glutathione content would facilitate conjugation and favor more complete excretion of an electrophile as a mercapturic acid. L-2-Oxothiazolidine-4-carboxylic acid (OTCA) is an example of a chemical that can promote such repletion. OTCA is a substrate for 5-oxoprolinase in vivo and thus leads to the formation of cysteine which is then used for the intracellular synthesis of glutathione (26). N-acetylcysteine (NAC) is another cysteine precursor and as such is also able to promote the biosynthesis of glutathione (27).

We have conducted a series of experiments with OTCA and NAC in animals given bromobenzene. In mice, the acute administration of bromobenzene at doses ranging from 1 to 5 mmol/kg did not result in a dose-dependent relative decrease of the urinary excretion of bromophenylmercapturic acids, as seen previously with the rat (Figure 1, Table I). The intraperitoneal injection of OTCA (2-16 mmol/kg) or NAC (5 mmol/kg) resulted in a moderate but significant increase in mercapturic acid excretion for all doses tested (Table IV, ref. 28).

TABLE IV

INFLUENCE OF OTCA AND NAC ON THE EXCRETION OF BROMOPHENYLMERCAPTURIC ACIDS DURING A 24-HOUR PERIOD AFTER ACUTE INTRAPERITONEAL ADMINISTRATION OF INCREASING DOSES OF BROMOBENZENE

Dose of bromobenzene (mmol/kg)	Percent of dose excreted as bromophenylmercapturic acids		
	without OTCA/NAC	with OTCA	with NAC
1	18	26	--
4	16	23	--
5	18	--	24

OTCA was also effective in increasing the percentage of the daily administered dose of bromobenzene excreted as mercapturic acids, under conditions of repeated exposure. Thus, in mice given bromobenzene at daily doses of 0.5, 0.75 and 1.0 mmol/kg for 18 days, the relative excretion of mercapturic acids decreased in a dose-dependent manner as already shown on Table II. Administration of OTCA (1 or 2 mmol/kg) prevented such reduction in metabolite excretion, allowing for 17 to 22% of the administered dose to be excreted as mercapturic acids (21). This was accompanied by a protection against the hepatotoxicity of bromobenzene.

Although optimal conditions for treatment with OTCA and NAC were not explored in these studies, administration of glutathione precursors tended to restore the urinary excretion of bromophenylmercapturic acids to higher levels as expected from the degree of exposure. Dosimetry of internal exposure to electrophilic metabolites of bromobenzene is therefore apparently improved as a result of OTCA and NAC pretreatment.

CONCLUSION

Determination of urinary thioethers represents a means by which the absorption of electrophilic alkylating or arylating chemicals can be evaluated; it therefore may exert an important warning function. However, there are some limitations to the widespread use of the existing analytical procedures for the biological surveillance of persons exposed to chemicals: they lack specificity and cannot be used to assess individual exposure to chemicals. In addition, the relationship between exposure and thioether urinary excretion is not always linear, as has been shown with bromobenzene and ethylene oxide. Administration of OTCA and NAC, two cysteine prodrugs and stimulators of glutathione biosynthesis in the liver, improves the relationship.

Studies conducted in our laboratories point to the usefulness of specific thioether determination for a better understanding of the toxicological significance of urinary thioethers. They provide some indications that under proper circumstances exposure to and absorption of potential electrophilic reactants can be more correctly assessed. Biological monitoring of environmental exposure to chemicals by the determination of urinary thioethers may therefore become an important tool to evaluate the influence of environment on various bodily functions, including drug absorption, distribution and elimination.

ACKNOWLEDGEMENTS

The author acknowledges the financial support of Institut de recherche en santé et en sécurité du travail du Québec (Group grant and operating grant RS-83-27) and Medical Research Council of Canada (operating grant MA-8943).

REFERENCES

1. Seutter-Berlage F, Van Dorp HL, Kosse HGJ, Henderson PT (1977) Int Arch Occup Environ Health 39:45-51
2. Ellman GL (1959) Arch Biochem Biophys 82:70-77
3. Van Dorn R, Bos RP, Leijdekkers CM, Wagenaas-Zegers MAP, Theuws JLG, Henderson PT (1979) Int Arch Occup Environ Health 43:159-166
4. Buffoni F, Coppi C, Santoni G (1982) 28:248-250
5. Heinonen T, Kytöniemi V, Sorsa M, Vainio H (1983) Int Arch Occup Environ Health 52:11-16
6. Savolainen H, Vainio H, Kilpikari I (1977) Acta Pharmacol Toxicol 41(Suppl):33
7. Vainio H, Savolainen H, Kilpikari I (1974) Brit J Ind Med 35:232-234
8. Kilpikari I, Savolainen H (1982) Brit J Ind Med 39:401-403
9. Van Doorn R, Leijdekkers CM, Bos RP, Brouns RME, Henderson PT (1981) Brit J Ind Med 38:187-190
10. Malonova H, Bardodej Z (1983) J Hyg Epidemiol Microbiol Immunol 27:319-328
11. Van Doorn R, Leijdekkers CM, Bos RP, Brouns RME, Henderson PT (1981) Ann Occup Hyg 24:77-92
12. Sorsa M, Hemminki K, Vainio H (1982) Teratogen Carcinogen Mutagen 2:137-200
13. Vainio H, Kilpikari I (1984) In: Aitio A, Riihimaki V, Vainio H (eds) Biological monitoring and surveillance of workers exposed to chemicals. Hemisphere Publishing Corporation, Washington, pp 247-252
14. Summer KH, Rozman K, Coulston F, Greim H (1979) Toxicol Appl Pharmacol 50:207-212
15. Pentz R (1978) Naunyn-Schmiedebergs Arch Pharmacol 302(Suppl): R20
16. Grenby TH, Young L (1960) Biochem J 75:28-33
17. Bray HG, Caygill JC, James SP, Wood PB (1964) Biochem J 90:127-132
18. James SP, Waring RH, White DA (1967) J Chromat 26:255-258
19. Niederwieser A, Steinman B, Matasovic A (1978) J Chromat 147:163-176
20. Hernandez O, Yagen B, Cox RH, Bend JR, McKinney JD (1982) J Liq Chromat 5:345-365
21. Goyal R, Brodeur J (1986) J Toxicol Environ Health (in press)
22. Gêrin M, Tardif R (1986) Fund Appl Toxicol 7:419-423
23. Chakrabarti S, Brodeur J (1984) J Toxicol Environ Health 14:379-391
24. Jones AR, Wells G (1981) Xenobiotica 11:763-770
25. Ehrenberg L, Hussain S (1981) Mutat Res 86:1-113
26. Williamson JM, Meister A (1981) Proc Natl Acad Sci USA 78:936-939
27. Lauterburg BH, Corcoran GB, Mitchell JR (1983) J Clin Invest 71:980-991
28. Brodeur J, Goyal R (1986) Proc Can Fed Biol Sci 29:137

Discussion - Urinary thioethers as biological monitors of environmental exposure to chemicals.

G. Zbinden

I believe that there is some circadian rythm in the production of glutathione. Does this reflect on changes in toxicity of compounds that are conjugated with glutathione, according to the time of administration?

J. Brodeur

There is variation, up to 30%, in the synthesis of glutathione both in laboratory animals and in man, and this has something to do with the feeding habits, but I am not aware of any study in which the toxicological importance of these changes in glutathione concentration has been specifically investigated.

L.F. Prescott

You have shown that repeated administration of increasing doses of bromobenzene leads to decreased elimination of its mercapturic acid conjugates. In experimental models, acute glutathione depletion is followed by an increased synthesis and an overshoot phenomenon is observed. I wonder why this is not evident in the case of bromobenzene. Could it be due to the magnitude of the doses which are close to those producing toxicity?

J. Brodeur

Yes, this is probably the reason, in addition renal toxicity may contribute to the decreased excretion of the conjugates.

P. Moldeus

I presume that one of the limitations of the measurement of thioethers as biological monitors of environmental exposure to dangerous chemicals is that many carcinogens do not form glutathione conjugates to any great extent.

J. Brodeur

Yes, of course. The sensitivity of a HPLC method with fluorescence detection is high but not all reactive chemicals do produce thioethers.

P.G. Watanabe

You mentioned that increased excretion of thioethers has been reported in individuals exposed to 1,1,1-trichloroethane, but I am not aware of thioethers being produced from 1,1,1-trichloroethane.

J. Brodeur

The investigators reporting this increase used a non-specific method and it is not clear that the subjects studied were exposed to single agents. This weakens their conclusions but, on the other hand, the formation of glutathione conjugates from some haloalkanes other than 1,1,1-trichloroethane has been recently reported.

P.G. Watanabe

I think that it should be stressed that the use of non-specific methods for the determination of thioethers, as in the study just mentioned, can lead to false conclusions due to many potential interferences.