Interactions of m-xylene and aspirin metabolism in man

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ABSTRACT In a series of experiments to investigate interactions between industrial solvents and common medications the interaction between m-xylene and aspirin was studied. As both these substances are metabolised and excreted as glycine conjugates there would possibly be competition for this conjugation pathway. Five male volunteers were exposed on separate occasions to m-xylene by inhalation (100 ppm), aspirin (1500 mg) by mouth, and m-xylene and aspirin together under controlled conditions in an exposure chamber. Urine and blood samples were collected and analysed for m-xylene, aspirin, and their metabolites. The amounts of the major glycine conjugates produced from m-xylene (m-methylhippuric acid) and aspirin (salicyluric acid) were significantly reduced by about 50% when m-xylene and aspirin were coadministered. There appears to be a mutual inhibition on the formation of the respective glycine conjugates. It is suggested that the inhibition is due to competition for either the enzymes, acyl-CoA synthetase, or glycine N-acylase. These findings have implications in the biological monitoring of workers exposed to m-xylene.

The interaction between solvents and ethanol is well known¹⁻³ but there have been few reports on the interactions between solvents and drugs.⁴ The use of non-prescription medication may influence the disposition of solvents in occupationally exposed workers.

Drug interactions are well established but are not necessarily predictable and may have various consequences.⁵ These include (1) the enhancement of the effect of one drug by another, (2) a new effect that is not seen when either drug is used alone, (3) inhibition or antagonism of one drug by another, (4) there may be no change in the net effect but the kinetics and metabolism may be altered substantially. Clearly these effects are not limited to drug-drug interactions but may extend to drug-solvent interactions since the same systems are concerned in the biotransformation and excretion.

Xylene is an organic solvent widely used in the paint industry (United Kingdom exposure limit 100 ppm (435 mgm⁻³)).⁶ The major metabolic pathway in man involves microsomal oxidation followed by glycine conjugation to produce methylhippuric acid that is excreted in the urine.⁷

Occupational exposure to high atmospheric concentrations of m-xylene can cause headaches and nausea

that may lead to taking analgesics containing aspirin (acetylsalicylic acid). Aspirin is rapidly hydrolysed to salicylic acid and after conjugation with glycine is eliminated as salicyluric acid in the urine.⁸ Glycine conjugation is thus common to both m-xylene and aspirin metabolism (fig 1). As there is competition for glycine from salicylic acid and methylbenzoic acid, saturation might occur that would lead to changes in the metabolism of xylene or aspirin or both.

This volunteer study was designed to investigate the possibility that the use of aspirin during exposure to xylene may alter the kinetics and metabolism of this solvent. Metabolic interactions may have implications in biological monitoring when m-methylhippuric acid is used to assess the uptake of xylene in exposed workers.

Materials and methods

STUDY DESIGN

This study was part of a series of experiments approved by the Northwick Park Hospital Ethical Committee and all the volunteers gave their informed consent.⁹

Five men, aged between 25 and 50, who were not occupationally exposed to solvents volunteered for the study. Four were non-smokers and the consumption of alcohol in all five was moderate. They were

Fig 1 Major metabolic pathways for xylene and aspirin.

informed of the details of the experimental programme and were given a full clinical examination that included biochemical and haematological tests before the study started.

Each volunteer was studied on three occasions according to the experimental design summarised in table 1.

All experiments, including those in which there was no exposure to solvent—that is, “aspirin only” started at 0930 in an exposure chamber that was maintained at 50% humidity and 22°C. The construction and operation of the chamber has been described previously.³ The volunteers were offered non-alcoholic beverages during the experiment and given a light sandwich

lunch about three hours after entering the chamber. They were asked to refrain from taking any medication or alcohol for 24 hours before the experiments.

M-XYLENE EXPOSURE

Each volunteer was exposed to m-xylene (100 ± 5 ppm) in the exposure chamber for four hours.

ASPIRIN STUDY

Each volunteer was given the normal therapeutic dose of aspirin (600 mg) 30 minutes before entering the chamber and a further 300 mg at one, two and a half, and four hours after entering the chamber. This dose regimen was calculated to give a steady state plasma salicylate concentration of about 60 mg/l¹⁰ for a period of six to seven hours.

M-XYLENE + ASPIRIN STUDY

Each volunteer was given the normal therapeutic dose of aspirin (600 mg) 30 minutes before entering the chamber. He then entered the chamber where the concentration of m-xylene was stabilised at 100 ± 5 ppm. A further 300 mg of aspirin was given at one, two and a half, and four hours after entering the chamber. The volunteers left the exposure chamber after four hours.

SAMPLING PROCEDURE

Volunteers were asked to provide a urine sample before the experiment began and then samples were collected at hourly intervals during and after the study for a total of seven hours. The urine volumes were measured and the samples stored (–20°C) in polycarbonate bottles.

A cannula was inserted into a forearm vein 15 minutes before the subjects left the exposure chamber. Blood samples (10 ml) were taken and transferred into EDTA bottles at 0, 0.5, two, and three hours after leaving the chamber. Duplicate 1 ml samples were dispensed into headspace vials, sealed, and analysed immediately. The remaining blood was centrifuged at 2500 rpm for 20 minutes and the plasma collected and stored at –20°C.

Breath samples were taken at roughly 15 minute intervals throughout the postexposure period. Xylene in breath (alveolar air) was measured directly by respiratory mass spectrometry.¹¹

SYNTHESIS OF M-METHYLHIPPURIC ACID

m-Methylhippuric acid was synthesised by the general method of Vogel¹² for hippuric acid. White crystals were obtained, mpt 138–139°C, mass spectral and infrared data were consistent with m-methylhippuric acid: m/z = 175 (M⁺ – H₂O), 119 (C₆H₅CON). $\nu_{\max}$ 3400 (amide N-H), 3000 (broad OH), 1650, 1700 (C=O).

Table 1 Experimental design

Volunteers	Experimental sessions		
	1	2	3
1	Xylene only	Xylene + aspirin	Aspirin only
2, 3	Aspirin only	Xylene only	Xylene only
4, 5	Xylene + aspirin	Aspirin only	Xylene + aspirin

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ANALYTICAL METHODS

Analysis of methylbenzoic acid, m-methylhippuric acid, salicylic acid, and salicyluric acid as free acids

One millilitre samples of urine or plasma were acidified with 1.5 M HCl (1 ml) and extracted into diethyl ether (8 ml). The ether extracts were evaporated to dryness and silylated using bis-trimethylsilyl-trifluoroacetamide at 60°C for 30 minutes.

The silylated metabolites of both xylene and aspirin were detected and quantified using capillary gas chromatography with flame ionisation detection (25 m BP1 column, 0.25 µm). The compounds were injected at an oven temperature of 130°C. After an initial hold of 30 seconds, the oven temperature was programmed at 30°C/min until 180°C and held at this temperature for three minutes. It was then programmed at 20°C/min until 300°C. The final oven temperature was held for one minute.

o-Methylhippuric acid (purchased from Koch Light Laboratories) was used as an internal standard.

Within day and day to day coefficient of variation for these four metabolites was below 10%.

Assay of methylbenzoic acid glucuronide and salicyluric acid glucuronide

Urine (1 ml) was adjusted to pH 1 with 1.5 M HCl (1 ml) and extracted with diethyl ether (8 ml) to remove free acids and glycine conjugates. The pH of the aqueous phase was then adjusted to 5 with 0.2 M acetate buffer (1 ml) and treated with β-glucuronidase (Glucurase, from bovine liver, 5000 units/ml Aldrich Chemical Co). This solution was incubated at 37°C for 16 hours and then re-extracted into diethyl ether. The analysis for methylbenzoic and salicylic acid in this extract was performed as described above.

As a positive control, a tube was incubated containing 1 mg phenolphthalein diglucuronide dissolved in 1 ml of urine. After incubation, the appearance of a purple colour on addition of alkali confirmed enzyme activity.

Xylene in blood

Headspace gas chromatography was used to measure the concentration of xylene in blood. The blood samples (1 ml) were incubated at 60°C in a Perkin Elmer HS6 unit for 15 minutes. The headspace (0.5 ml) was injected on to an SP1000 column and detected using a flame ionisation detector. The blood was spiked with *n*-propyl benzene (Aldrich) which was used as an internal standard. The detection limit for xylene was 0.5 µmol/l with a coefficient of variation of less than 10%.

Xylene in breath

Expired xylene was measured directly using a respiratory mass spectrometer. As the subject exhales

Fig 2 Mean excretion of *m*-methylhippuric acid in urine. Results are expressed as means and SDs of five volunteers.

through a Haldane Priestly tube, a sample of breath is continuously drawn through the inlet tube to the mass spectrometer.¹¹ The most abundant ion *m/z* 91 was used for monitoring.

Pharmacokinetic calculations

The blood and breath pharmacokinetic parameters were estimated by linear regression of the log concentrations against time and the exponentials were separated by curve stripping.¹³

Results

GLYCINE CONJUGATES OF XYLENE AND ASPIRIN

In the xylene only and aspirin only exposures there was an increasing excretion of the glycine conjugate of the parent compound, with maximum excretion occurring three to four hours after the volunteer entered the chamber. The excretion of *m*-methylhippuric acid and salicyluric acid was similar (about 1 mmol/h) during the second half of the exposure period. When xylene and aspirin were administered separately the mean total excretion during the course of the experiment was 4.8 mmol of *m*-methylhippuric acid and 4.2 mmol of salicyluric acid respectively. This accounts for 90% of the dose of xylene (assuming 60% uptake and a respiration rate of 10 l/min)⁷ and 60% of the dose of aspirin. After co-administration of xylene and aspirin a pronounced decrease was observed in the hourly excretion of both *m*-methylhippuric and salicyluric acid (figs 2 and 3). The excretion of both metabolites (salicyluric acid and *m*-methylhippuric acid) was reduced to 1.8 mmol of each, which accounts for only

Fig 3 Mean excretion of salicylic acid in urine. Results are expressed as means and SDs of five volunteers.

35% and 25% of the respective doses. No m-methylhippuric acid or salicylic acid was detected in the volunteers pre-exposure urine.

The sum of the two glycine conjugates excreted was about the same in each of the three exposures over the seven hour period—that is, the same total amount of glycine conjugates is excreted in single or co-administered exposures.

FREE ACIDS AND GLUCURONIDE CONJUGATES IN URINE

The proportion of salicylic acid excreted as the free acid varied greatly between the five volunteers, accounting for between 1.6% and 23% of the dose. Excretion of salicylglucuronides was also highly variable, between 1.3% and 14% of the dose. No differences in either free acids or glucuronides were noted whether the xylene and aspirin were administered separately or together.

Methylbenzoic acid was only detected in urine as its ester glucuronide and not as the free acid. The amount of this conjugate was increased when xylene and aspirin were co-administered but it did not account for a significant part of the dose (less than 5%).

BLOOD AND BREATH CONCENTRATIONS OF XYLENE

Figure 4 shows the mean concentrations of blood and breath xylene against time during the postexposure period. There were no significant changes in the half life of blood or breath xylene when xylene or xylene and aspirin is administered. Table 2 shows the half life for blood and breath concentrations.

Fig 4 Mean concentration from five volunteers of m-xylene in (a) blood and (b) expired air after exposure.

Table 2 Half life (hours) for blood and breath xylene

	Xylene only	Xylene + aspirin
Blood (n = 5)	1.89	2.08
Breath (n = 5)	1.90	2.03

PLASMA CONCENTRATIONS OF METABOLITES

No m-methylhippuric acid, salicylic acid, or methylbenzoic acid was detected in the plasma. The concentrations of salicylic acid did not change significantly when aspirin was given alone or with xylene. There was large interindividual variability in plasma salicylate concentrations (40–100 mg/l) although each individual had a relatively consistent plasma salicylate concentration over the postexposure period.

Discussion

The ingestion of aspirin (1500 mg over five hours) by volunteers during a four hour inhalation of m-xylene (100 ppm) produced pronounced alterations in m-xylene metabolism. The inhalation of m-xylene had similar effects on aspirin metabolism. The excretion of the major metabolites of both m-xylene (m-methylhippuric acid) and aspirin (salicylic acid) were reduced

by about 50% over the seven hour experimental period. The greatest reduction in excretion appeared to be during the initial four hour exposure period, with excretion being reduced to 25–50%. Although we have observed considerable interindividual variation in aspirin metabolism that is in agreement with earlier work,¹⁴ a consistent proportional decrease in the excretion of salicyluric acid was found in each volunteer.

Aspirin could act at any one of four stages in the uptake, metabolism, and excretion of xylene—that is, on the respiratory uptake, the oxidation to methylbenzoic acid, conjugation with glycine, or the excretion of the conjugates. From the results presented here aspirin is unlikely to affect the uptake of the solvent; neither blood or breath xylene concentrations differed significantly when the volunteers were exposed to xylene only and when xylene and aspirin were co-administered. Similarly, it seems unlikely that the inhibition of xylene metabolism is at the oxidative step. Riihimäki *et al* have shown that a moderate dose of ethanol can affect xylene metabolism²: the blood concentration of xylene is raised and the urinary excretion of m-methylhippuric acid is reduced. This inhibition is probably at the oxidative step due to a changed hepatic NADH/NAD⁺ ratio produced by the oxidation of ethanol by alcohol dehydrogenase. This is an unlikely mechanism for the inhibition of xylene metabolism by aspirin since in our study we did not observe an increase in blood xylene concentrations when xylene and aspirin were co-administered. As only a small proportion (about 1%) of a dose of aspirin is oxidised, the inhibition of aspirin metabolism by xylene is unlikely to be at an oxidative step.

The inhibition probably occurs at some stage associated with glycine conjugation—either due to a limited supply of glycine or competition by the two organic acids for the conjugating mechanism. The rate of conjugation of benzoic acid with glycine is rapid and is limited by the mobilisation of endogenous glycine¹⁵ but only at high concentrations of benzoic acid.¹⁶ If it is assumed that the maximum rate of glycine mobilisation is about 11 mmol/hour⁸ then under normal industrial exposure conditions the capacity for the formation of m-methylhippuric acid should not be limited by the availability of glycine. In these experiments the maximum separate or combined excretion of hippuric acid and salicylic acid was 2.3 mmol/h, indicating that even with co-administration the glycine capacity should not have been exceeded.

Man has a limited capacity for the synthesis of salicylurate from salicylate and glycine¹⁵. Nelson *et al* have shown that the administration of glycine has no effect on the metabolism of salicylate.¹⁷ It appears therefore that in the case of salicylurate formation the mobilisation of glycine is not the rate limiting process.

Evidence that the mutual inhibition of the conjugate formation is not due to glycine limitation comes from a further experiment (unpublished data) in which one of the volunteers was given a dose (3×2 g) of glycine before and during a repeat combined exposure. There was no apparent effect on the excretion of m-methylhippuric acid or salicyluric acid even though the plasma glycine concentration was increased by about 50%.

The conjugation of organic acids with glycine is located in the mitochondria.¹⁸ Competition between methylbenzoic and salicylic acids might occur during transport into the mitochondria or at an enzymic level. Most organic acids cross the mitochondrial membrane freely and competition probably involves one of the two enzymic steps necessary in glycine conjugation—that is, acyl CoA synthetase or acyl CoA-glycine N-acyl transferase (glycine N-acylase). There is no basis for distinguishing where inhibition takes place from the data obtained in this study.

It is unclear at the moment as to the fate of that fraction of the xylene and aspirin doses that is unaccounted for when they are co-administered. Excretion may be delayed, although this seems unlikely since there is no considerable build up of the free acids—that is, methylbenzoic or salicylic acid. Some metabolites may have been excreted in the bile or a different pathway used.

Attention should be drawn to the practical implications of this study. The measurement of m-methylhippuric acid in urine is useful for the biological monitoring of workers exposed to m-xylene. If a worker is exposed to xylene and has taken aspirin then the m-methylhippuric acid concentration will be artefactually reduced. The occupational physician will need to know if the workers have taken any analgesic, in particular, aspirin. Ideally, the analytical method used for m-methylhippuric acid will show if there has been appreciable ingestion of aspirin and this information can be reported with the analytical result.

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References

- 1 Muller G, Spassowski M, Henschler D. Interaction of trichloroethylene and ethanol. *Arch Toxicol* 1975;33:173–89.
- 2 Riihimäki V, Savolainen K, Pfaffli P, Pekari K, Sipil HW, Laine A. Metabolic interactions between m-xylene and ethanol. *Arch Toxicol* 1982;49:253–63.
- 3 Wilson HK, Robertson SM, Waldron HA, Gompertz D. Effect of alcohol on the kinetics of mandelic acid excretion in volunteers exposed to styrene vapour. *Br J Ind Med* 1983;40:75–80.
- 4 Dossing M, Baelum J, Hansen SH, Lundqvist GR. Effect of

- ethanol, cimetidine and propranolol on toluene metabolism in man. *Int Arch Occup Environ Health* 1984;4:309-15.
- 5 Murad F, Gilman AG. Drug interactions. In: Gilman AG, Goodman LS, Rall TW, Murad F, eds. *The pharmacological basis of therapeutics*. 7th ed. London: Macmillan, 1985:1734-6.
 - 6 Health and Safety Executive. *Occupational exposure limits*. London: HMSO, 1987. (Guidance note EH40.)
 - 7 Riihimäki V, Pfaffli P, Savolainen K, Pekari K. Kinetics of m-xylene in man. *Scand J Work Environ Health* 1979;5:217-31.
 - 8 Amsel LP, Levy G. Drug biotransformation interactions in man: II. A pharmacokinetic study of the simultaneous conjugation of benzoic and salicylic acids with glycine. *J Pharm Sci* 1969;58:321-6.
 - 9 Denham MJ, Forster A, Tyrrell DAJ. Work of a district ethical committee. *Br Med J* 1979;iii:1042-5.
 - 10 Saunders L, Ingram D, Dickinson CJ, Sheffiff M. A comprehensive computer simulation of drug metabolism and pharmacokinetics. *Comput Educ* 1982;6:243-52.
 - 11 Wilson HK, Ottley TW. The use of a transportable mass spectrometer for the direct measurement of industrial solvents in breath. *Biomed Mass Spectrom* 1981;8:606-10.
 - 12 Vogel AI. *A textbook of practical organic chemistry*. 4th ed. London: Longmans, 1967:885.
 - 13 Johnston A, Woollard RC. STRIPE. An interactive computer program for the analysis of drug pharmacokinetics. *J Pharmacol Methods* 1983;9:193-9.
 - 14 Hutt AJ, Caldwell J, Smith RL. The metabolism of aspirin in man: a population study. *Xenobiotica* 1986;16:239-49.
 - 15 Levy G, Amsel LP. Kinetics of competitive inhibition of salicylic acid conjugation with glycine in man. *Biochem Pharmacol* 1966;15:1033-8.
 - 16 Wan SH, Riegelman S. Renal contribution to overall metabolism of drugs I: conversion of benzoic acid to hippuric acid. *J Pharm Sci* 1972;61:1278-84.
 - 17 Nelson E, Hanano M, Levy G. Comparative pharmacokinetics of salicylate elimination in man and rats. *J Pharmacol Exp Ther* 1966;153:159-66.
 - 18 Killenberg JB, Webster LT. Conjugation by peptide bond formation. In: Jakoby WB, ed. *Enzymatic basis of detoxication*. Vol II. London: Academic Press, 1980:141-67.