Short Communication

Determination of Urinary Phenolic Metabolites from Rats Treated with 1,2,3- and 1,3,5- Trimethylbenzenes

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Trimethylbenzene (TMB) is widely used as a solvent in the print and paint industries¹, and for products such as paint and varnish². Hence it causes environmental contamination not only in work places¹ but also inside houses^{3, 4}. Toluene and xylene are regarded as 'safe replacements' for benzene⁵, due to the well-known finding⁶ that these less methyl-substituted benzenes are oxidized principally at a methyl substituent without substantial aromatic ring oxidation during *in vivo* metabolism. Although toxicological data on TMB are not abundant, some critical toxicities have been reported such as genotoxicity of TMB^{7, 8} and haematotoxicity of 1,2,3-TMB⁹. These data strongly suggest that TMB has 'benzene-like' toxicities. It is therefore necessary to obtain information¹⁰ on ring oxidation during metabolism of TMB. We describe here urinary excretion of phenolic metabolites in rats administered 1,2,3- and 1,3,5-TMBs, as a first investigation on ring oxidative metabolism of TMB.

Materials and Methods

3,4,5- and 2,4,6-trimethylphenols (TMPs) were purchased from Wako Chemicals Inc. (Kyoto, Japan). 2,3,4-TMP was synthesized according to the previously reported method¹¹. Naphthalene-d₈ and acenaphthene-d₁₀ for GC/MS were obtained from CIL (Cambridge

Isotope Laboratory, England) and β -glucuronidase/aryl sulphatase (EC 3.2.1.31, H-2, extract of *Helix pomatia*) was obtained from Sigma Co (St.Louis, MO, USA). All other chemicals were of special or analytical reagent grade.

Male Wistar rats (7 weeks old; SLC, Hamamatsu, Japan) were housed in individual stainless steel metabolism cages with free access to water and food. 1,2,3- or 1,3,5-TMB was given intraperitoneally in doses of 0.3, 1 and 3 mmol per kg of weight (administered in 2.5 ml of olive oil per kg of body weight). Rats receiving an olive oil only served as a control group. Four rats were used in each TMB treatment group as well as the control group. Urine samples were collected daily for 2 days and stored at -20°C until the analysis.

Urine samples were hydrolyzed enzymatically using an extract of *Helix pomatia* (β -glucuronidase/aryl sulphatase mixture) according to the previously reported method¹⁰. Extraction and GC-MS determination of the TMPs were carried out from the hydrolyzates similarly as previously described¹² for the analysis of urinary 3,4-dimethylphenol. The recovery of each TMP was satisfactory when 50 μ g of it was added to 0.5 ml of a hydrolyzed control urine sample ($109.8 \pm 11.3\%$ for 2,3,4-TMP, $103.7 \pm 10.6\%$ for 3,4,5-TMP and $118.8 \pm 9.1\%$ for 2,4,6-TMP; n=5).

Results and Discussion

The target phenolic metabolites were 2,3,4- and 3,4,5-TMPs from 1,2,3-TMB, and 2,4,6-TMP from 1,3,5-TMB (Fig 1). As the objective of this preliminary study is to show the extent of urinary phenolic metabolites excretion from the TMBs, the determination of urinary TMPs were undertaken using hydrolyzed urine samples. Urinary excretion of these TMPs in the 48 h after dosage of the TMBs is summarized in Table 1. The cumulative excretion was approximately 5–10% of the administered dose, with the excretion occurring mostly in the first 24 h after the treatment. 2,3,4-TMP was found to be the main phenolic metabolite in 1,2,3-TMB and the excretion of another isomer 3,4,5-TMP was very minor (<0.3% of dose). The excretion (% of dose) of phenolic metabolites decreased with increasing dose of 1,3,5-TMB, while this trend was not observed in 1,2,3-TMB treated rats. The degree of urinary excretion of the TMPs in 1,2,3- and 1,3,5-TMBs was close to that reported for 1,2,4-TMB¹⁰.

Toluene and xylene are metabolized almost exclusively via the hippuric acid pathway⁶, although mercapturic acid metabolism is also involved to a considerable degree following an initial side chain oxidation in o-xylene¹³. It was therefore assumed that toluene and xylene are 'safe replacements' for benzene⁵. The urinary excretion of phenolic metabolites is very limited in the metabolism of these less methyl-substituted benzenes *in vivo*. Bakke and Scheline¹⁴ reported 0.1–1.1% of dose for excretion

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Fig. 1. Phenolic metabolites from 1,2,3- and 1,3,5-TMBs.

Table 1. Urinary excretion (% of dose)^{a)} of the TMPs in 1,2,3- and 1,3,5-TMB treated rats
<1,2,3-TMB>

dose mmol/kg	2,3,4-TMP			3,4,5-TMP		
	0–24 h	24–48 h	total	0–24 h	24–48 h	total
0.3	5.90 ± 2.62	0.46 ± 0.34	6.36 ± 2.92	ND	ND	ND
1.0	7.93 ± 5.00	0.35 ± 0.16	8.28 ± 4.85	≤0.24	ND	≤0.24
3.0	6.20 ± 3.45	0.57 ± 0.34	6.77 ± 3.60	≤0.19	≤0.04	≤0.19

<1,3,5-TMB>

dose mmol/kg	2,4,6-TMP		
	0–24 h	24–48 h	total
0.3	7.04 ± 1.24	0.53 ± 0.29	7.57 ± 0.99
1.0	4.39 ± 0.61	0.51 ± 0.12	4.90 ± 0.64
3.0	3.32 ± 0.58	0.82 ± 0.34	4.14 ± 0.67

a) Each figure represents Mean ± SD for four rats or highest value in four rats (3,4,5-TMP).

of urinary methyl or dimethylphenols from rats administered toluene or xylene. Šedivec and Flek¹⁵⁾ found that ring hydroxylated metabolites excreted in urine corresponded to 0.05–1.98% of a dose administered to volunteers exposed to isomeric xylenes. Contrary to this, the present study as well as another investigation¹⁰⁾ on 1,2,4-TMB indicate that the degree of phenolic metabolite excretion from TMB is at least one order of magnitude higher than those reported for toluene and xylene.

The higher excretion of phenolic metabolites from *in vivo* metabolism of TMB is to be noted, especially in relation to the genotoxicity of TMB^{7, 8)} and the

haematotoxicity of 1,2,3-TMB⁹⁾ previously reported. Toluene and xylene have been shown¹⁶⁾ not to elicit genotoxic action. There is no report on the haematotoxicity of toluene and xylene, to our knowledge. TMB may be regarded as a ‘benzene-like’ compound because its reported toxicities are known to be characteristic¹⁷⁾ of benzene. Although the higher excretion of phenolic metabolites may suggest the easier oxidation of the aromatic ring, these toxicities cannot be directly explained in terms of the quantity of phenolic metabolites. Further research is needed to elucidate the ring oxidative metabolism of TMB and to clarify its

relation to such toxicities.

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