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PRELIMINARY COMMUNICATIONS

OXIDATIVE METABOLISM OF SOME N-METHYL CONTAINING XENOBIOTICS CAN LEAD TO STABLE PROGENITORS OF FORMALDEHYDE

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N-Methyl groups occur in many xenobiotics and for most of these the major metabolic biotransformation involves N-demethylation to the desmethylamine and formaldehyde, a reaction catalysed by mixed functional oxidases. This oxidation is considered to occur either *via* direct oxidation of the methyl group carbon to generate a carbinolamine (Figure 1,B) or *via* N-oxide formation and re-arrangement to the carbinolamine. The carbinolamines are generally considered to be transient species which rearrange to eliminate formaldehyde[1]. The formaldehyde is rapidly detoxified by oxidation to formate at a rate which exceeds the rate of its production by N-demethylation reactions[2]. This oxidation is performed by aldehyde oxidases (EC 1.2.1.3) which have been found in the cytosol, mitochondria and microsomes of hepatocytes[3]

We have recently reported the synthesis of triazene carbinolamines (Figure 1,H) and found them to be surprisingly stable[4]. A synthesis of the carbinolamine of pentamethylmelamine (Figure 1,E) also yields a stable compound[5]. The stability of these carbinolamines raised the possibility that during the N-demethylation *in vivo* of the antineoplastic N-methyltriazenes and hexamethylmelamine (Figure 1,G and D) stable carbinolamines may be produced which might liberate formaldehyde in extra hepatic tissues if they leave the liver, or may be metabolised further within the liver to other species. Previous investigations on the metabolism of the triazenes and hexamethylmelamine have reported their N-demethylation by liver fractions *in vitro* [6,7] as judged by the colorimetric estimation of formaldehyde by the Nash reagent[8]. However, we have found that the synthetic carbinolamines react both qualitatively and quantitatively like formaldehyde with Nash reagent and so this method is unable to distinguish between free formaldehyde and the carbinolamines formed by N-demethylation reactions.

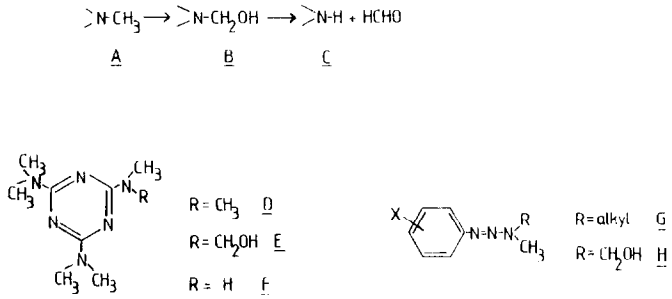

Figure 1: Metabolic pathway for N-methyl compounds and structures of antineoplastic agents with N-methyl moieties.

If, under the conditions of an N-demethylation by a liver homogenate in vitro, the carbinolamines are only transient species it would be expected that the formaldehyde they release would be oxidised to formate, which is not Nash positive, at a rate comparable to that for formaldehyde itself. The disappearance of the Nash positive species of formaldehyde and the carbinolamine of pentamethylmelamine (Figure 1,E) with time on incubation with a whole mouse liver homogenate was determined (Figure 2). After 16 minutes 50% of the initial amount of formaldehyde had been removed, presumably by oxidation to formate, whereas only 2% of the

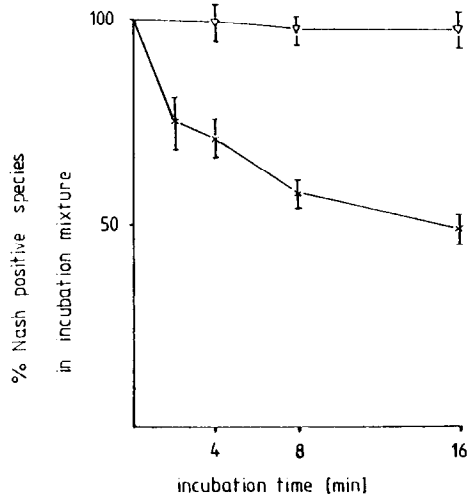

Fig.2. Metabolism of N-hydroxymethylpentamethylmelamine (▽) and formaldehyde (×) by mouse liver homogenates. Formaldehyde and N-hydroxymethylpentamethylmelamine were incubated at a concentration of 0.1 mM with 0.5 ml of a 10% mouse (male CBA Lac) liver homogenate in 0.25 M sucrose solution. 1 mM NAD was added and the final incubation volume was made up to 2.5 ml with Earl's buffer. At the end of the incubation the samples were deproteinized with 0.6 ml 20% trichloroacetic acid solution. 2 ml of the supernatant were mixed with 1 ml Nash reagent for colour development. Values are given as means ± s.e.m. of at least four experiments.

carbinolamine had been removed, as judged by the Nash positive component. This shows that this carbinolamine is stable and that this method is able to distinguish between free formaldehyde and the carbinolamine.

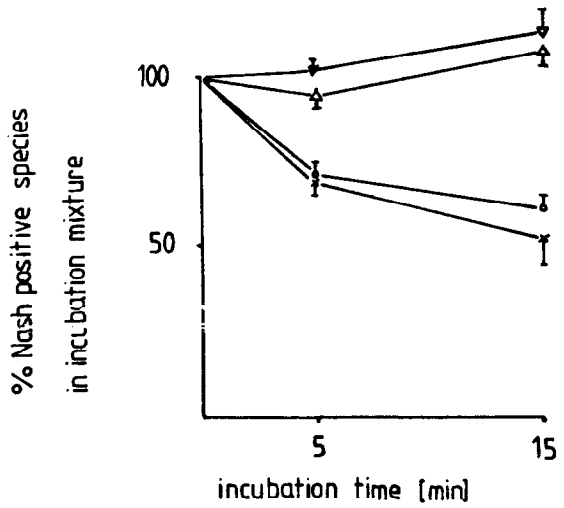

Fig.3. Metabolism Of Nash positive microsomal metabolites of hexamethylmelamine (▽), pentamethylmelamine (△) and aminopyrine (○) by mouse liver homogenates freed from microsomes; (×) indicates disappearance of formaldehyde. For the generation of Nash positive metabolites drugs were incubated with microsomes equivalent to 100 mg of liver/ml of incubation medium (Earl's buffer). Microsomes were prepared from livers of 3-week old male CBA Lac mice by centrifugation of the 9000g fraction of a 10% homogenate in 0.25 M sucrose after addition of $CaCl_2$(10). Drug concentrations were 5 mM for aminopyrine and 0.5 mM for hexamethyl melamine and pentamethylmelamine. The incubation mixtures were fortified with cofactors which generated 1 mM NADPH. After 30 min incubation microsomes were removed by centrifugation. 1.9 ml aliquots of the supernatant containing metabolites and parent drugs were then incubated with microsome free liver homogenate equivalent to 50 mg of mouse liver and with 1 mM NAD in a final volume of 2.5 ml. After 5 and 15 min the mixtures were deproteinized and assayed as described in the legend to Fig.2. 100% refers to the amount of Nash positive species generated during the microsomal incubation. The concentration of formaldehyde added to the microsome free homogenate was 0.1 mM. Values are expressed as means ± s.e.m. of at least 5 groups of animals.

The stability of the carbinolamine which may be formed during the N-demethylation of hexamethylmelamine (Figure 1,D) was then confirmed further by first incubating hexamethylmelamine with mouse liver microsomes and an NADPH generating system, conditions which N-demethylate the drug as judged by the production of a Nash positive species. The microsomes were then precipitated by centrifugation and the supernatant, which contains the unchanged drug and metabolites, was then incubated with microsome-free mouse liver homogenate. The microsome-free homogenate was found to be unable to N-demethylate hexamethylmelamine or other N-methyl containing drugs but was able to metabolise formaldehyde at the same rate as a whole liver homogenate, as described above. The disappearance of Nash positive species was monitored after 5 and 15 minutes incubation, with the sum of the metabolites which gave a Nash positive reaction at the end of the microsomal incubation taken to be 100% (Figure 3).

Exogenous formaldehyde disappeared rapidly when incubated with the microsome free homogenate whereas the Nash positive metabolite(s) of hexamethylmelamine did not disappear. In a separate experiment pentamethylmelamine (Figure 1,F), a major metabolite of hexamethylmelamine, did also yield Nash positive microsomal metabolites which were not metabolised by the microsome-

free homogenate. Both hexamethylmelamine and pentamethylmelamine did not themselves alter the rate of formaldehyde disappearance and so do not inhibit aldehyde oxidases. The N-methyl group containing compounds aminopyrine, doxepine and N,N-dimethylaniline were investigated under identical conditions and the Nash positive component(s) from their microsomal metabolism disappeared at a rate comparable to formaldehyde on addition of the microsome free homogenate (Figure 3).

The stability of the carbinolamines described suggests that certain N-methyl group containing xenobiotics may form nascent forms of formaldehyde which may escape the detoxification mechanisms of the liver and perhaps have serious toxicological consequences for the host. It is of interest that stable carbinolamines, or derivatives, from anti-neoplastic triazenes, hexamethylmelamine and procarbazine have all recently been described and may be important to their mechanisms of cytotoxicity[9]

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