Pharmacokinetics of biliary excretion of N-nitrosodimethylamine in rats fed diets containing levels of protein

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ABSTRACT

The excretion of N-nitrosamines (including NDMA) in the bile of humans and of animals has been reported, but the influence of the nutritional status, which is an important denominator in the metabolism and disposition of foreign compounds (xenobiotics) in this regard, especially with respect to dietary protein energy malnutrition (PEM) and over nutrition, has not been previously investigated.

Volatile and non-volatile N-nitrosamines have been found to be toxic, and versatile carcinogens in animals, and they contaminate the human environment. Food stuff, urban air, water and soil. In addition, they can be mutagenic, and a small dose of a nitrosamine, known to be capable of producing a malignant tumour in rats, can be formed from simple substances of plant and animal origin, such as nitrates, nitrites and secondary amines. The elaboration of N-nitrosamines, in vitro and in vivo, occurs either spontaneously in acid medium, or are mediated by appropriate enzymes. These compounds require prior metabolic transformation for their activity, and they can have cumulative effects which are attributable to alkylating entities.

NDMA is the only N-nitrosamine that has been constantly detected in the human environment. Like other nitrosamines species, it is organ specific in its toxicity and carcinogenicity, causing haemorrhagic centrilobular cell necrosis of the liver. The latter lesion is specific and a characteristic property which distinguishes the action of N-nitrosamines from other hepatotoxic chemicals, such as carbon tetrachloride and the aflatoxins.

The elimination of unchanged NDMA through the expired air, faeces and body fluids including urine and bile, is of immense pharmacological and toxicological importance, since certain drugs in clinical use are secondary amines and are known to form nitrosamines, in vivo, and the excretion of pure NDM is a detoxication mechanism. In particular, the toxicity and carcinogenicity of a given nitrosamine is bound to be enhanced by its prolonged presence in the blood circulatory system through a recycling process, in the event of enterohepatic circulation of the compound.

In the tropics of Africa, Asia and Latin America, well known for their high incidences of PEM, the population that would be exposed to nitrosamines through frequent ingestion of contaminated food, beverages, water and drugs can be divided into two nutritional groups, namely; the peasantry, who subsists or marginal protein diets, and the well-to-do, who have affordable high protein meals. In view of this wide difference in dietary habits, and the dependence of drug metabolizing enzymes of liver and other tissues on the quality and quantity of protein, it would seem desirable to examine the biliary excretion of N-nitrosamines in relation to dietary protein availability.

The aim of this study was to establish, comparatively whether or not there are differences in the kinetics and mode of biliary elimination of NDMA (a representative N-nitrosamine and a prime suspect in the causation of urban cancers) as between the nutritional states of chronic protein malnourishment, a cute protein deprivation, protein sufficiency and protein over nourishment using model experimental rats.

Material and Methods

Pure N-Nitrosodimethylamine (NDMA) >99%, mol. wt. 74.08, and boiling point (b.p.) 153° was obtained from our stock in the Biochemical Toxicology laboratory of the Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan (Nigeria). Urethane (ethylcarbamate) and other chemicals reagents and solvents were of analytical grade and purchased from either May & Baker Ltd, or BDH Chemical Ltd, London.

Animals: Albino Wistar rats (littermates) weaned at 22-24 days of age were obtained from pre-clinical animal house of Ibadan College of Medicine, and they weighed between 30g and 40g. The rats were allocated on the basis of weight and litter origin to groups of 8 rats each. They were housed in warm cages (25°C) and fed on semi-purified test diets containing different levels of casein as protein source (Table 1), and drinking water, for a period of 30 days, ad libitum. This dietary regimen established the model test animals.

Induction of kwashiorkor in rats: Weanling rats given the kwashiorkorogenic diet for 30 days were established using anatomical prognostic indicators and the biological statuses of kwashiorkor rats. These parameters were monitored in the model animals against control animals on the normal protein diet.
The dietary animal models established were as follows: High protein (HPD), Normal protein (NPD), low protein (LPD) and kwashiorkor (KWD) rats.

Cannulation of bile duct: The experimental animals were anaesthetised with a 25% urethane solution given intraperitoneally (i.p.) at a rate of 1.5g/kg. A small midline abdominal incision was created, and the bile duct was exposed at its junction with the duodenum. Bile was collected in clean glass tube through a suitable polythene cannula surgically inserted into the bile duct about 1.5cm from the duodenum junction. The cannula was held in position with a tied thread to avoid its dislodgement.

Administration of DNMS

Four test rats from each dietary were used. NMDA was injected i.p. at the rate of 20mg/kg following a 10-minute collection of pure (control) bile for baseline evaluation. Subsequently, bile was collected at 10, 20, and finally in 30 min. intervals until the animal became moribund. The bile samples were immediately stored in a refrigerator at 4°C, until analysed. As much as possible, analysis of bile was performed within 24hrs of collection of the fluid.

Analysis of the bile

The bile samples were extracted of NDMA, using dichloromethane as solvent, and then concentrated to 1ml on an Extrelut column.

Qualitative analysis of NDMA in the dichloromethane extract concentrate of bile was by thin layer chromatography (t.l.c.) with positive response (purple spot) to two recommended spray reagents as an identification test for the presence of nitrosamines. The solvent system was n-hexane: diethyl ether: dichloromethane (4:3:2). The treated plates were first exposed to shortwave UV light for 3 minutes, to undergo photochemical denitrosation before their development.

Quantitative estimation for unchanged NDMA content was by gas liquid chromatography (g.l.c.) using flame ionisation detection (F.I.D) and the appropriate standard reference compounds.

Computation of kinetic parameters

- Elimination rate constant (K) was calculated from the semi-log plot of bile excreted NDMA: time relationship using the equation: Slope = -K/2.303
- Biological half-life (t1/2) of NDMA was derived using the equation t1/2 = 0.693/K where K = rate constant

Results

The data in Table 2 suggest that rats on high protein diet (HPD) and normal (NPD) were the highest excretors of unchanged NDMA in bile, while the kwashiorkor rats (KWD) and the low protein rats (LPD) were the least excretors of the compound, in that order. The corresponding values for biological half-life (t1/2) were 0.013 hr, 0.014hr, 0.031hr, and 0.029hr and for eliminating rate constant, 54.05Kh', 48.8Kh', 23.01Kh', and 23.76Kh'. This set of data shows that the rate at which ndma is eliminated in rat bile is dependent on the protein status, but does not appear to discriminate between the varying degrees of dietary protein derivation or protein sufficiency.

Figure 1 and Figure 2 show, respectively, the time courses of biliary excretion of NDMA and the semi-log plot of the data for the different dietary protein model rats used in this study.

Discussion

The time courses of the biliary excretion of NDMA by the various dietary protein rat models investigated (Figure 1) show that biphasic kinetics is operational in each case. A semi-log plot of this kinetics (Figure 2) supports a possible pharmacokinetic classification of effect of dietary protein on biliary excretion of nitrosamines into two significantly distinct groups, namely; fast and slow excretors as exhibited by the well-nourished rats (HPD & NPD) and the malnourished animals (KWD & LPD) respectively.

Biological half-life (t1/2) values decreased with protein nourishment, while the converse was the case for rate constant (K). The malnourished rats exhibited the slowest clearance rate of NDMA. It is known that a low protein diet ameliorates the toxic
effects of NDMA by slowing down hepatic metabolism of compound into harmful products, and invariably enhancing its elimination from the body. Therefore, the results of this study which show that a protein depleted diet diminished the rate of elimination of NDMA via bile, and increases half-life of the compound, would suggest that the biliary route of excretion of nitrosamines, on the one hand, and the urinary route, on the other hand, could be mutually exclusive. There is also the possibility that excretion of such a small but highly polar molecule as NDMA in bile, in apparent violation of the rule that only large molecular weight compounds (molecular weigh >250-300) are excretable to any large extent, could entail a supportive role of high protein diet in the biliary elimination of nitrosamines.

The biphasic kinetics (Figure 1) in the elimination of NDMA in bile in protein malnourished rats is in line with the pharmacokinetics of NDMA peak (ug) half-life of the compound (hr) in rats.

### Table 2: Composition of experimental diets

<table>
<thead>
<tr>
<th>Components</th>
<th>Amounts of component in experimental diet %</th>
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<tbody>
<tr>
<td></td>
<td>(KWD)</td>
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<tr>
<td>Cassia</td>
<td>3.5</td>
</tr>
<tr>
<td>Corn starch</td>
<td>81.5</td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td>8.0</td>
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<tr>
<td>Salt mixture</td>
<td>4.0</td>
</tr>
<tr>
<td>All vitamin</td>
<td>3.0</td>
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<tr>
<td>Supplement</td>
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</table>

### Table 2: Effects of dietary protein insufficiency and oversufficiency on the pharmacokinetics of excretion of N-nitrosodimethylamine in rat bile.

<table>
<thead>
<tr>
<th>Dietary Regimen</th>
<th>Protein Metabolism</th>
<th>Biological Half Life (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDMA Excretion (ug)</td>
<td>NDMA peak Excretion (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peak Excretion time (min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Constant (Kb-1)</td>
</tr>
<tr>
<td>3.4% (KWD)</td>
<td>82.33</td>
<td>2.74</td>
</tr>
<tr>
<td>8% (LPD)</td>
<td>118.28</td>
<td>2.96</td>
</tr>
<tr>
<td>27% (NPD)</td>
<td>164.19</td>
<td>4.11</td>
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<tr>
<td>64% (HPD)</td>
<td>175.20</td>
<td>4.38</td>
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</table>

*values are arithmetic mean of 3 successful measurements.

References

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