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

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ABSTRACT

Albino Wistar rats (*Rattus norvegius*) fed semi-purified diets containing 3.5%, 8%, 27%, and 64% casein, respectively, as the protein source, were poisoned with an intraperitoneal dose of 20mg N-nitrosodimethylamine (NDMA)/kg, following cannulation of the bile duct, *in vitro*, under urethane anaesthesia. Bile exudates was collected at designated time intervals and analysed for unchanged NDMA using thin layer chromatography and gas liquid chromatography methods. Rats on 64% high protein diet (HPD) were the highest excretors of NDMA, followed by rats on the 3.5% kwashiorkorigenic diet (KWD), 8% low protein diet (LPD) and 27% normal protein diet (NDP) as the least excretors, in that order. The corresponding values for culmulative excretions of NDMA were 4.38%, 2.74%, 2.96% and 4.11%, and for elimination rate contents they were 54.05Kh^{-1}, 23.01Kh^{-1}, 23.76Kh^{-1} and 48.88Kh^{-1}, while the respective elimination half-life values were 0.013h, 0.031h, 0.029h and 0.014h. The toxicological and pharmacological implication of the pharmacokinetic findings are discussed.

Introduction

The excretion of N-nitrosamines (including NDMA) in the bile of humans[1] and of animals[2,3] 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 malnourishment (PEM) and over nourishment, has not been previous investigated.

Volatile and non-volitile N-nitrosamines have been found to be toxic[4], and versatile carcinogens[5] in animals, and they contaminate the human environment,[6-12] food stuff, urban air, water and soil. In addition, they can be mutagenic[13], and a small dose of a nitrosamine, known to be capable of producing a malignant tumour in rats[5], 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[14] for their activity, and they can have cumulative effects which are attributable to alkylating entities.[15-17]

NDMA is the only N-nitrosamine that has been constantly detected in the human environment. Like other nitrosamine 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 the aflatoxins.

The elemination 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*,[18] 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 presencein 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 their high incidences of PEM, the population that would be exposed to nitrosamines through frequent ingestion of contaminated food, bverages, water and drugs can be divided into two nutritional groups, namely; the peasantry, who susbsist 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.) 153^0 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 purchases 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^{0}C) and fed on semi-purified test diets containing different levels of casein as protein source (Table1), 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 kwashiorkorigenic diet[19] for 30 days were established using anatomical prognostic indicators and the biological statuses of kwashiorkor rats.[20] 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 anaesthesized with a 25% urethane solution given intraperitonealy (i.p.) at a rate of 1.5g/kg[21]. A small midline abdominal incision was created, and the bile duct was exposed at its junction with the duodeneum. 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.[22] 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. Subsquently, 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.[23]

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[24] spray reagents as an identification test for the presence of nitrosamines. The solvent system was n-hexane: diethylether: 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.)25 using flame ionisation detection (F.I.D) and the appropriate standard reference compounds.

Computation of kenetic 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) pf 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 ($t^1/_2$) were 0.013 hr, 0.014hr, 0.031hr, and 0.029hr and for eliminating rate constant, 54.05Kh^{-1}, 48.8Kh^{-1}, 23.01Kh^{-1} and 23.76Kh^{-1}. 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 deprivation 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.

Figure 1

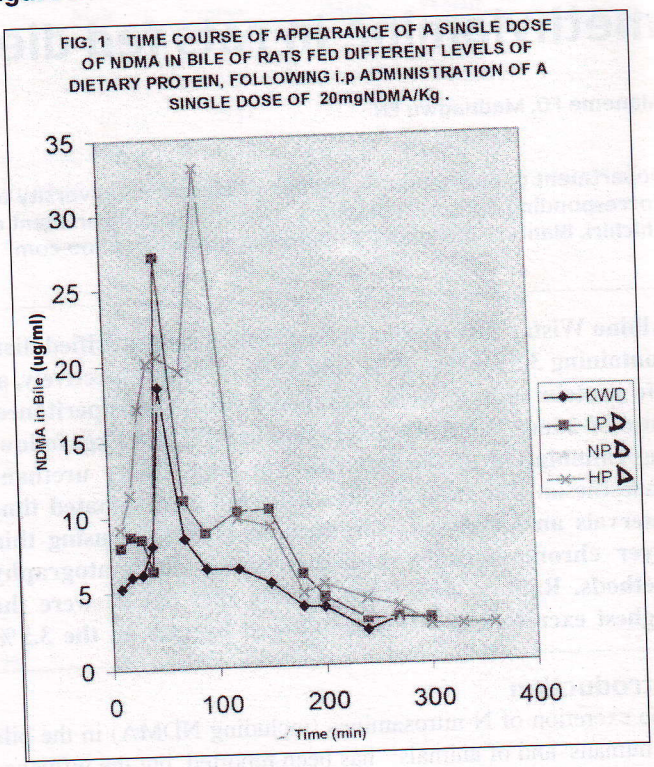
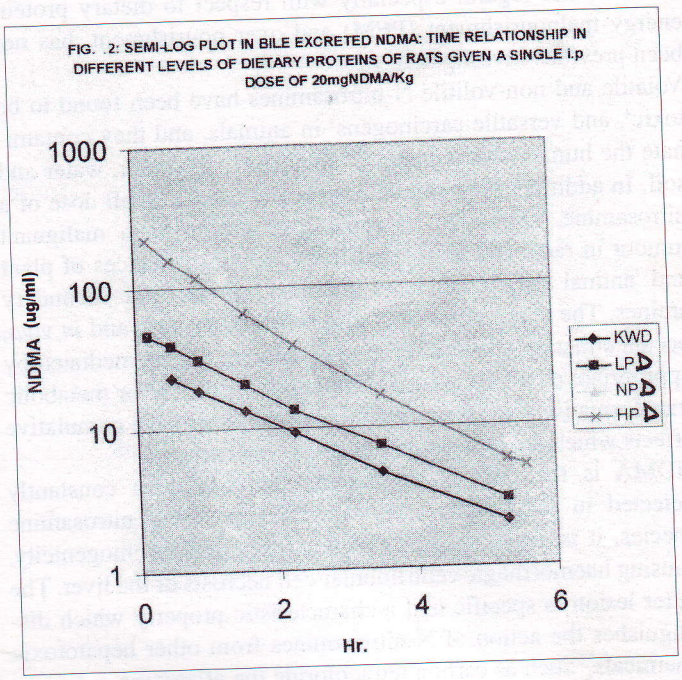


Figure 2



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 exihibited by the well-nourished rats (HPD & NPD) and the malnourished animals (KWD & LPD) respectively.

Biological half-life ($t^1/_2$) 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 toxi

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[26] that only large molecular weight compounds (molecular weigh >250-300) areexcretable 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[27,28] of many chemical compounds in biological fluids.

The study emphasizes the need for the dtermination of the nutritional status of humans in the pharmacokinetic evaluation of drugs and other environmental chemicals, before a correct toxicological and pharmacological assessment of a xenobiotic can be obtained.

Table 1: Composition of experimental diets

Diet	Amounts of component in experimental diet%			
Components	Kwashiorkorigenic (KWD)	Low protein (LPD)	Normal protein (NPD)	Highprotein (HPD)
Casein	3.5	8.0	27.0	64.0
Corn starch	81.5	77.0	58.0	21.0
Vegetable Oil	8.0	8.0	8.0	8.0
Salt mixture	4.0	4.0	4.0	4.0
All vitamin Supplement	3.0	3.0	3.0	3.0

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

Dietry Protein Regimen	Culmulative NDMA excretion(ug)	Culmulative NDMA Excretion(%)	Excretion peak (ug)	Peak time (min)	Elimination rate Constant (Kh-1)	Biological half-life($t^1/_2$) (hr)
3.4%(KWD)	82.33	2.74	18.49	50	23.01	0.03
8%(LPD)	118.28	2.96	27.14	50	23.76	0.029
27%(NPD)	164.19	4.11	31.19	90	48.8	0.014
64%(HPD)	175.20	4.38	33.09	90	54.05	0.013

*values are arithmetic means of 3 successful cannulations.

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