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EFFECTS OF THIAMINE DEFICIENCY ON THE METABOLISM AND ACUTE TOXICITY OF DIMETHYLNITROSAMINE IN THE RAT

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Dimethylnitrosamine (DMN) is a potent hepatotoxin and carcinogen which requires metabolic activation for its toxic and carcinogenic actions [1]. DMN is oxidatively demethylated by the enzyme system located mainly in the hepatic microsome to give as one product, formaldehyde, and as the second product, monomethylnitrosamine which is presumed to be unstable and decomposes to methonium ion and nitrogen gas; the formaldehyde is oxidized to CO_2. The conversion of DMN to CO_2 has been considered an indirect measure of the activation of DMN to its toxic and carcinogenic derivative methonium ion [2, 3]. Recently, evidence has been reported to suggest that metabolism of DMN measured by conversion of the substrate to CO_2, both in vitro and in vivo, does not correlate well with acute toxicity [4]. Methonium ion, CH_3^+, alkylates proteins and nucleic acids and conversion of DMN to this strong electrophile is thought to be responsible for the toxic properties of this compound [1] although there is no direct evidence to support this. It has been shown that nutritional status of the animals, e.g. protein deficiency, at the time of administration of DMN plays an important role in its metabolism and in determining the site of acute and chronic injury [5, 6, 7, 8]. An objective of this investigation is to study the alteration in DMN metabolism under various nutritional states and to examine the relationship between metabolism and toxicity of this compound. Thiamine deficiency or excess has been shown to alter the metabolism of many drugs including aniline, aminopyrine, ethylmorphine and zoxazolamine [9, 10, 11]. We report here the effect of thiamine deficiency on 1) DMN metabolism measured as rate of disappearance of DMN from serum and in vitro conversion of DMN to formaldehyde and CO_2 and 2) acute toxicity of DMN estimated by the LD_{50} doses.

Materials and Methods

Chemicals: DMN was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wisconsin. Radioactive ^{14}C-DMN was obtained from Amersham/Searle Corporation, Ill. All dietary ingredients, vitamins and Rogersand Harper salt mixture were obtained from NBCo. Biochemicals Cleveland, Ohio.

Animals and Diets: Weanling, male Fischer rats derived from the Fischer 344 strain (Faculty of Science, Mahidol University, Thailand) aged 21 days were housed individually and food and water were provided ad libitum. Semipurified agar-gel diet [12] was used throughout the study. The control diet contained all vitamins and minerals required by rats. In the thiamine deficient diet, thiamine hydrochloride was omitted from the vitamin mix. All animals were fed control diet until 30 days old; they were then divided into two groups and fed either control or thiamine deficient diet for another 21 days.

Methods: The level of unchanged DMN in the serum was estimated as nitrite by the method of Daiber and Preussmann [13] as modified by McLean and Day [14]. The conversion of DMN to formaldehyde was assayed in a standard incubation system containing 2 ml of 9000 g supernatant fraction of rat liver equivalent to 40 mg of protein, NADP (2.5 μmoles), G-6-P (25 μmoles), G-6-P-D (3 I.U.), semicarbazide hydrochloride (45 μmoles) nicotinamide (72 μmoles), $MgCl_2$ (25 μmoles), DMN (90 μmole, 15 mM) and potassium phosphate buffer (0.5 M, pH 7.4) to make the final volume of 6 ml. The reaction mixture was

incubated for 30 min at 37° under oxygen. The reaction was stopped by the addition of $ZnSO_4$ (15% W/V, 2 ml). The formaldehyde formed was determined according to Nash [15]. The in vitro production of CO_2 from ^{14}C-DMN by 200 mg liver slices was measured by a modification of the method described by Shank [16]. Determination of the LD_{50} doses was done by administration of single doses of DMN to groups of 10 animals per dose level. The 7-day-LD_{50} values with 95% confidence limits and slope function were calculated according to Litchfield and Wilcoxon [17]. The erythrocyte transketolase activity was measured by the method of Brin et al [18].

Results and Discussion:

The erythrocyte transketolase activity was used as a criteria for detecting marginal thiamine deficiency before clinical signs and symptoms appeared. A TPP stimulation of 20% indicates a borderline deficiency [19]. In rats fed the deficient diet for 21 days, the percent TPP effect was 54.80 ± 24.58 in comparison with 7.4 ± 1.50 in the control animals (n = 6) which indicated thiamine deprivation.

Dimethylnitrosamine metabolism was first measured as the rate of DMN disappearance from serum. The in vivo disappearance rate was markedly increased in thiamine deficient rats (Table I) suggesting that these animals removed DMN from the circulation at a rate much faster than did the control. This may reflect a greater rate of DMN metabolism and/or excretion in the thiamine deficient rat.

TABLE I

Effect of thiamine deficiency on the rate of disappearance of DMN from serum.

Group	No. of Rats	Serum DMN Level (μg/ml serum) mean ± S.D.
Control	4	14.84 ± 2.96
Thiamine Deficient	4	3.07 ± 2.02*

At 5 hours after a single dose of DMN (32 mg/kg body weight) was injected to each rat, blood samples were collected by cardiac puncture technique. Serum DMN was estimated as nitrite by the method of Daiber and Preussman [13], as modified by McLean and Day [14].

*Significantly different $p < 0.005$ from the control value.

The optimum substrate concentrations for the in vitro oxidation of DMN were determined for control and thiamine deficient rats, in both cases these concentrations were 15 mM and 0.4 mM when measuring formaldehyde formation and conversion of DMN to CO_2, respectively. The level of formaldehyde formed from DMN in thiamine deficient rats was slightly higher than that in the control animals but not statistically significant (Table 2). However, the rate of conversion of ^{14}C-DMN to CO_2 was not altered in thiamine deficient rats in comparison with the control values (Table 3). These results suggest that thiamine deficiency may not have an effect on the enzymes responsible for oxidative demethylation of DMN to formaldehyde with the subsequent oxidation to form CO_2. Table 4 illustrates that the acute toxicity of DMN estimated by the LD_{50} doses was significantly enhanced in thiamine deficient animals.

TABLE 2

Effect of thiamine deficiency on the in vitro conversion of DMN to formaldehyde

Group	Number of animals	HCHO (nmoles/mg protein/hr) mean ± S.D.	Liver protein (mg/g liver) mean ± S.D.
Control	12	17.50 ± 5.97	227.11 ± 21.78
Thiamine Deficient	12	20.19 ± 6.81*	233.66 ± 21.46*

Duplicate samples of 9000 g supernatant from thiamine deficient and control animals were incubated for 30 min at 37°C in media as described in the method section. Formaldehyde formed was estimated by the method of Nash [15].

*Not significantly different from the control value.

TABLE 3

Effect of thiamine deficiency on the in vitro conversion of DMN to CO_2

Group	Number of animals	%Conversion/200 mg liver/hr
Control	4	4.79 ± 3.15
Thiamine Deficient	5	4.31 ± 1.25

Duplicate samples of liver slices (200 mg) from thiamine deificient and control animals were incubated at 37°C for 1 hour in Krebs-Ringer Phosphate buffer (pH 7.4) containing 0.4 mM (0.1 μCi/0.25 μmole) DMN and fortified with 0.01 M glucose.

TABLE 4

Effect of thiamine deficiency on the LD_{50} levels of DMN in the rat

Group	LD_{50} (mg/kg bw)	95% confidence limit	slope function
Control	50.0	43.5 - 57.5	1.17
Thiamine Deficient	41.0	37.8 - 46.6	1.11

Groups of 10 rats fed on either control or thiamine deficient diet for 21 days were injected with DMN (i.p.) at various dose levels. The 7-d-LD_{50} values were determined according to Litchfield and Wilcoxon [17].

The present results suggest that thiamine deficiency markedly stimulates the metabolism and/or excretion of DMN; and increases the sensitivity of the rat to an acutely toxic dose of this compound. Although the rate of disappearance of DMN from serum was greatly enhanced in thiamine deficient rats,

the oxidative N-demethylation pathway responsible for the conversion of DMN to formaldehyde and to CO_2 was not equally affected. These suggest that another pathway of DMN metabolism, possibly the conversion of DMN to the methonium ion (CH_3^+) which alkylates the nucleic acids and proteins, may be stimulated under such circumstance. These present observations also confirm the previous finding of Ruchirawat and Shank [4] that the conversion of DMN to CO_2 was not well correlated with acute toxicity.

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