TOXICITY OF 1, 2-DIMETHYLHYDRAZINE IN MICE: EFFECT OF DIET AND DEVELOPMENT OF TOLERANCE

N. J. TEMPLE[1] and S. M. EL-KHATIB

Department of Biochemistry, Universidad Central del Caribe
School of Medicine, Box 935, Cayey, Puerto Rico 00634

Received for Review November 22, 1984
Accepted for Publication February 15, 1985

ABSTRACT

The effect of diet on the lethality of 1, 2-dimethylhydrazine (DMH) injections was investigated using Swiss mice. Mortality was highest when mice were fed a nutrient-diluted/high-fat diet, was intermediate with a nutrient-diluted/low-fat diet and was lowest with laboratory chow. Wheat bran was not protective. Mice developed tolerance to DMH after receiving several injections of progressively increasing dose.

INTRODUCTION

During recent years, considerable attention has been focused on interactions between diet and xenobiotics. Thus, for example, diet greatly affects the body's reaction to drugs (Theur and Vitale 1977) and toxic heavy metals (Levander *et al.* 1975; Muto and Omori 1977; Omori and Muto 1977; Stoewsand *et al.* 1974; Suzuki and Yoshida 1978).

In this report, we describe some dietary effects on the toxicity of 1, 2-dimethylhydrazine (DMH), a colon carcinogen, in mice. In addition, we report on the development of tolerance to the chemical.

MATERIALS AND METHODS

The experimental diets (Table 1) were a control diet (chow/5.5% fat), diet F6 (nutrient-diluted/5.5% fat), diet F23 (nutrient-diluted/23% fat) and diet B (diet F23 with bran added to restore the dietary fiber content to the level of the control diet).

Swiss mice were used from a colony maintained in our animal house. They were randomized into diet groups at age 4-7 weeks and were fed the

[1]Current address: Department of Foods and Nutrition, The University of Alberta, Edmonton, Alberta, T6G 2M8, Canada.

Table 1. Composition of diets (g/100 g)

Diet Component[1]	Control	F6	F23	B
Chow[2]	100	67	81	69
Corn oil[3]	-	1.9	18.5	15.8
Oleic acid[4]	-	-	0.28	0.24
Wheat starch[5]	-	31	-	-
Wheat bran	-	-	-	14.4
Total fat[6]	5.5	5.5	23	21
Total fiber[6]	16	9.8	9.8	17.4
Nutrients[7]	100	61	61	57-92

[1] The diet composition is on a dry weight basis. The diets (except the control diet) were prepared approximately once per week by mixing the ingredients with water until a soft consistency was obtained. They were stored at 4°C.
[2] Rodent Laboratory Chow from Ralston Purina Co., St. Louis, Missouri.
[3] "Mazola" from Best Foods, San Juan, Puerto Rico.
[4] From Fisher Scientific Co., Fair Lawn, New Jersey.
[5] From United States Biochemical Corp., Cleveland, Ohio.
[6] Final content of fat and dietary fiber in diet.
[7] Relative content of vitamins, minerals and protein per 100 cal.

specified diet for 7 weeks before being used. Food and water were provided *ad libitum*. The animal room was temperature-controlled and a 12-h light/dark cycle was used.

Just before use, DMH dihydrochloride (Sigma, St. Louis, MO) was dissolved in 1mM EDTA and neutralized with a saturated solution of sodium bicarbonate. Mice were injected sc with the indicated dose. Statistical analyses were made using a 2x2 contingency table (Zar 1974).

RESULTS

At the time of DMH treatment, mice fed diets F6, F23 and B weighed slightly (about 7%) less than those fed the control diet.

Preliminary experiments indicated that nutrient-diluted diets (F6 and F23) increased the lethality of DMH in mice. To further clarify this, the experiment reported in Table 2 was carried out. A greater mortality occurred in mice fed diet F23 than in those fed the control diet. This was highly significant in both sexes. Diet F6 apparently produces an intermediate mortality rate.

To determine whether lack of dietary fiber is pertinent to the above results diet B was also used. This did not reduce the mortality rate and might even increase it (compared to diet F23).

Mice on the control diet were given weekly injections of DMH. The first injection was at a dose roughly 50% of the lethal dose with successive doses being increased by a factor of 1.25-1.80. In both sexes it was observed that this induced tolerance to DMH. Consequently, the dose required to induce a particular percent mortality rose approximately 7- to 12-fold in comparison to similar mice previously unexposed to DMH (data not shown).

Table 2. Mortality in mice given various diets and injected with DMH

Diet	Sex	Dose[1]	Mortality[2]	Number of Mice
Control	M	28	8^{bfg}	53
F6	M	28	26^{bc}	50
F23	M	28	41^{g}	41
B	M	28	52^{cf}	44
Control	F	34	21^{ef}	53
F6	F	34	30^{ad}	64
F23	F	34	49^{ae}	65
B	F	34	57^{df}	72

[1] The dose of DMH is mg DMH dihydrochloride/kg b. wt.

[2] The mortality is expressed as a %. Statistical comparisons were made only between groups of the same sex. Groups sharing the same suffix are significantly different. a: $p < 0.05$; b, c: $p < 0.025$; d, e: $p < 0.005$; f, g: $p < 0.001$.

DISCUSSION

The results indicate that diet can alter the toxicity of a xenobiotic. When the same sc dose of DMH was administered to Swiss mice, a lower mortality rate was observed when feeding a control diet (laboratory chow; 5.5% fat) than with a nutrient-diluted/high-fat diet (F23; 23% fat). An intermediate mortality rate was generally seen when using a nutrient-diluted/low-fat diet (F6; 5.5% fat). This was seen in both sexes. Thus there appear to be two major factors which increase the mortality rate: (1) a high-fat intake and (2) nutrient dilution (i.e., the 39% drop in content of vitamins, minerals, protein, fiber and also nonnutrients per 100 cal. in diets F6 and F23 compared to the control diet).

A modifying effect of dietary fat on DMH metabolism has been previously reported (Glauert and Bennink 1982; Wargovich and Felkner 1982). Raising the level of dietary fat appears to reduce toxicity caused by aflatoxin in chicks and by dinitrotoluene in mice but, on the other hand, increases toxicity (leukopenia) in rats induced by benzene (Calabrese 1981).

Lack of dietary fiber was suspected of being the factor responsible for the nutrient dilution effect. Ershoff and others (Calabrese 1981) have reported that fiber reduces the toxicity caused by various chemicals, particularly food additives. Our data show that addition of wheat bran to diet F23 did not provide any protective effect on DMH toxicity.

After several weekly injections, using a progressively greater dosage, the dose needed to kill a given proportion of the mice increased about 7- to 12-fold in each sex. Thus Swiss mice developed tolerance to DMH. We considered this a most fortuitous finding. DMH-treated mice are a valuable model for colon cancer. The standard procedure is to avoid severe toxic effects by dividing the total dose into 10-20 weekly injections. Our findings indicated that if the DMH is given not as a series of identical injections (as appears to be the universal practice) but with the weekly dose steadily rising, the total number of injections can be cut by about half. Preliminary studies in our laboratory indicated that such a regimen is distinctly advantageous.

ACKNOWLEDGEMENTS

We wish to thank Pablo D. Burgos, Fernando Ortiz and Arnaldo Martinez for skilled technical assistance.

REFERENCES

CALABRESE, E. J. 1981. *Nutrition and Environmental Health, Vol. 2, Minerals and Macronutrients,* pp. 386—389, 436—437, J. Wiley & Sons, New York.

GLAUERT, H. P. and BENNINK, M. R. 1982. Effect of diet on the metabolism of 1, 2-dimethylhydrazine (DMH) in the rat. Fed. Proc. *41,* 356.

LEVANDER, O. A., MORRIS, V. C., HIGGS, D. J. and FERRETTI, R. J. 1975. Lead poisoning in vitamin E deficient rats. J. Nutr. *105,* 1481—1485.

MUTO, Y. and OMORI, M. 1977. Nutritional influence of the onset of renal damage due to long-term administration of cadmium in young adult rats. J. Nutr. Sci. Vitaminol. *23,* 349—360.

OMORI, M. and MUTO, Y. 1977. Effects of dietary protein, calcium, phosphorus and fiber on renal accumulation of exogenous cadmium in young rats. J. Nutr. Sci. Vitaminol. *23,* 361—373.

STOEWSAND, G. S., BACHE, C. A. and LISK, D. J. 1974. Dietary selenium protection of methylmercury intoxication of Japanese quail. Bull. Environ. Contam. Toxicol. *11,* 152—156.

SUZUKI, T. and YOSHIDA, A. 1978. Long-term effectiveness of dietary iron and ascorbic acid in the prevention and cure of cadmium toxicity in rats. Am. J. Clin. Nutr. *31,* 1491—1498.

THEUR, R. C. and VITALE, J. J. 1977. Drug and nutrient interactions. In *Nutritional Support of Medical Practice,* (H. Schneider, C. Anderson and D. Coursin, eds.), pp. 297—317, Harper and Row, New York.

WARGOVICH, M. J. and FELKNER, I. C. 1982. Metabolic activation of DMH by colonic microsomes: A process influenced by type of dietary fat. Nutr. Cancer *4,* 146—153.

ZAR, J. H. 1974. *Biostatistical Analysis,* pp. 59—69, Prentice-Hall, Englewood Cliffs, NJ