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INHIBITORY EFFECTS ON SOMATIC AND GERM CELL GENOTOXICITY OF NITROSAMINES

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ABSTRACT

Dimethylnitrosamine, diethylnitrosamine, dipropylnitrosamine, dibutylnitrosamine and N-nitrosopyrrolidine were shown to be genotoxic not only to somatic cells but also to germ cells. Genotoxicity to somatic cells was revealed by the micronucleus test. Genotoxicity to germ cells was indicated by the results of the dominant lethal test.

These nitrosamines induced the formation of micronucleated polychromatic erythrocytes in bone marrow cells. They also induced a reduction in fertility index, gestation index and implantation index. The % dead implants and females with resorptions were increased.

Vitamin A, vitamin C, vitamin E, niacin, riboflavin, thiamine, pyridoxine, biotin, folic acid and vitamin B_{12} reduced the genotoxicity to somatic and germ cells. The same observation was recorded of calcium, magnesium, manganese, copper, iron and zinc.

The genotoxicity of dimethylnitrosamine was reduced by expressions of fruits and vegetables.

Introduction

Nitrosamines have been found in tobacco smoke (1) alcoholic beverages (1), mushrooms (2). and foods such as grains, pasteurized milk and cheese (3) (4) (5) and nitrite treated cheese, fish, smoked fish and meat (2) (6). The occurrence of fairly high levels of nitrosamines in certain types of meat curing mixtures containing spices and nitrite indicates that some of the nitrosamines in cured meat products may originate from these curing mixtures (7). Nitrosamines in these premixes are apparently formed under dry conditions because of the interaction of amines in spices and nitrites both of which are major components of these formulations (8). There is evidence that man is exposed to nitrosamines either preformed in foods prepared with nitrite such as bacon and sausages or formed in the gastrointestinal tract (9). It has also been reported that nitrosamines may be formed from nitrite and secondary amines under the acidic conditions of the stomach.

Nitrosamines are highly carcinogenic in experimental exposures to animals and may represent an important cancer hazard to man (10). These are metabolized mainly by microsomal mixed function oxidases into reactive intermediates which react with DNA. Nitrosamines have to be metabolized to a reactive intermediate by microsomal mixed function oxidases in order to have mutagenic and carcinogenic effect (11). There is a high specificity among various agents, tissues and cells in their capacity to metabolize nitrosamines.

Dimethylnitrosamine labelled with ^{14}C interacted with rat liver nucleic acids especially DNA in vivo to form 7-methylguanylic acid residues that could be released as 7-methyl guanine by hydrolysis (12).

Diethylnitrosamine has induced tumors of the respiratory tract, upper alimentary tract, and liver in mice, rats, hamsters, fish, birds, rabbits, dogs, pigs, guinea pigs and monkeys (13).

N-nitrosopyrrolidine, a carcinogen, is formed by cooking foods containing non-carcinogenic nitrosoproline (14). N-nitrosopyrrolidine occurs at levels of 1-80 ppm in fried but not uncooked bacon (6).

Experimental Methods

Somatic cell genotoxicity was studied using the micronucleus test (15). Germ cell genotoxicity was investigated using the dominant lethal test (16).

The experimental mice used were of Swiss Webster strain.

The vitamins were obtained from Sigma Chemical Co. Copper chloride, zinc chloride, manganese chloride, magnesium chloride, calcium chloride and ferrous sulfate, analytical grade, were obtained from Mallincdrott.

The nitrosamines and the vitamins were introduced simultaneously by oral gavage, in the micronucleus test. Simultaneous administration was also used for the mineral ions.

In the dominant lethal test simultaneous administration was done with nitrosamines, vitamins and mineral ions.

Assessment of somatic cell genotoxicity was based on the formation of micronucleated polychromatic erythrocytes in bone marrow cells. Inhibitory effects were based on the reduction of the formation of micronucleated polychromatic erythrocytes. Assessment of germ cell genotoxicity was based on the reduction of fertility index, gestation index, implantation index and increase in percentage dead implants and females with resorptions.

Results and Discussion

Table 1 shows that dimethylnitrosamine, diethylnitrosamine, dipropylnitrosamine, dibutylnitrosamine and N-nitrosopyrrolidinc induced the formation of micronucleated polychromatic erythrocytes in bone marrow cells of mice. Diethylnitrosamine induced the formation of more micronucleated polychromatic erythrocytes than dimethylnitrosamine because the carbocation released from diethylnitrosamine is more stabilized than the released from dimethylnitrosamine. This enhances the alkylating ability of diethylnitrosamine for DNA.

Table 1. Somatic cell genotoxicity of nitrosamines

	No. of micronucleated polychromatic erythrocytes
Negative control (distilled water)	1.22 ± 0.05
Dimethylnitrosamine, 10 mg/kg	18.75 ± 1.21
Diethylnitrosamine, 10 mg/kg	25.22 ± 1.37
Dipropylnitrosamine, 10 mg/kg	9.64 ± 1 06
Dibutylnitrosamine, 10 mg/kg	9.72 ± 0.91
Nitrosopyrrolidine, 10 mg/kg	9.67 ± 0.89

Table 2. Dose of vitamins and mineral ions administered to experimental mice

	Dose per kilogram weight
Vitamin A	150 mg
Vitamin C	150 mg
Vitamin E	150 mg
Niacin	150 mg
Riboflavin	150 mg
Thiamine	150 mg
Pyridoxine	150 mg
Biotin	150 ug
Folic Acid	150 ug
Vitamin B 12	150 ug
Calcium	150 mg
Magnesium	150 mg
Copper	15 mg
Iron	15 mg
Manganese	15 mg
Zinc	15 mg

Table 3 depicts data on germ cell toxicity of the five nitrosamines. All of them reduced the fertility index, gestation index, implantation index and increased percentage of dead implants and females with resorptions. They also induced the reduction in fetal weight. These effects can be a consequence of the metabolism of the nitrosamines to agents that alkylate DNA of the germ cells.

From the data in Table 4, it can be seen that the vitamins and mineral ions reduced the formation of micronucleated polychromatic erythrocytes induced by dimethylnitrosamine. This means that the fragmentation of the chromatin

Table 3. Germ cell genotoxicity of nitrosamines

	FI	*GI*	*II*	*DI*	*FR*	*FW*
Control	94.2	96.1	10.2	1.4	1.6	1.3 gm
Dimethylnitrosamine	22.1	58.3	7.3	27.4	100	0.8
Diethylnitrosamine	17.6	44.5	7.1	38.6	100	0.8
Dipropylnitrosamine	33.7	65.2	7.3	21.8	100	0.8
Dibutylnitrosamine	32.9	63.4	7.4	22.8	100	0.8
N-nitrosopyrrolidine	33.9	68.2	7.0	19.6	100	0.8

FI = fertility index = No. of females pregnant/No. of females mated X 100
GI = gestation index = No. of live implants/Total no. of implantations X100
II = implantation index = Total implantations/no. of females pregnant
DI = percentage dead implants
FR = percentage females with resorptions

Table 4. Effect of vitamins and mineral ions on somatic cell genotoxicity of nitrosamines

	Number of micronucleated polychromatic erythrocytes per thousand
Negative Control, distilled water	1.31 ± 0.12
Dimethylnitrosamine alone	18.75 ± 1.22
plus vitamin A	0.89 ± 0.08
plus vitamin C	0.32 ± 0.04
plus vitamin E	0.12 ± 0.05
plus niacin	1.12 ± 0.78
plus riboflavin	0.00
plus thiamine	1.22 ± 0.08
plus biotin	1.12 ± 0.03
plus pyridoxine	1.18 ± 0.09
plus folic acid	0.98 ± 0.04
plus vitamin B 12	0.98 ± 0.07
plus calcium	1.33 ± 0.08
plus magnesium	1.78 ± 0.06
plus copper	1.56 ± 0.13
plus iron	1.44 ± 0.07
plus manganese	2.22 ± 0.67
plus zinc	1.78 ± 0.06

material was reduced in the presence of vitamins and mineral ions. The alkylating tendency of dimethylnitrosamine for DNA was inhibited by vitamins and mineral ions. The same observation is recorded for diethylnitrosamine (Table 5) dipropylnitrosamine (Table 6), dibutylnitrosamine (Table 7), and N-nitrosopyrrolidine (Table 8).

Table 5. Effect of vitamins and mineral ions on somatic cell genotoxicity of diethylnitrosamine

	No. of micronucleated polychromatic erythrocytes per thousand
Negative Control, Distilled water	1.12 ± 0.08
Diethylnitrosamine alone	25.22 ± 1.37
plus vitamin A	1.53 ± 0.09
plus vitamin C	1.06 ± 0.11
plus vitamin E	1.40 ± 0.23
plus niacin	1.43 ± 0.05
plus riboflavin	1.22 ± 0.04
plus thiamine	1.60 ± 0.06
plus biotin	1.17 ± 0.09
plus folic acid	1.52 ± 0.07
plus vitamin B 12	1.33 ± 0.08
plus calcium	2.47 ± 0.30
plus magnesium	2.28 ± 0.46
plus copper	1.82 ± 0.09
plus iron	1.27 ± 0.04
plus manganese	1.40 ± 0.04
plus zinc	1.40 ± 0.23

Tabie 6. Effect of vitaminsand mineral ions on somatic cell genotoxicity of dipropylnitrosamine

	No. of micronucleated polychromatic erythrocytes per thousand
Negative control, distilled water	1.00 ± 0.12
Dipropylnitrosamine alone	9.64 ± 0.98
plus vitamin A	0.87 ± 0.03
plus vitamin C	0.98 ± 0.08
plus vitamin E	0.78 ± 0.11
plus niacin	1.22 ± 0.09
plus riboflavin	0.52 ± 0.05
plus thiamine	1.11 ± 0.07
plus biotin	1.22 ± 0.09
plus folic acid	1.32 ± 0.08
plus vitamin B 12	1.28 ± 0.06
plus pyridoxine	1.22 ± 0.07
plus calcium	0.86 ± 0.04
plus magnesium	0.79 ± 0.12
plus copper	1.11 ± 0.08
plus iron	1.02 ± 0.07
plus manganese	1.21 ± 0.09
plus zinc	0.82 ± 0.05

Table 7. Effect of vitamins and mineral ions on somatic cell genotoxicity of dibutylnitrosamine

	No. of micronucleated polychromatic erythrocytes per thousand
Negative control, distilled water	1.18 ± 0.05
Dibutylnitrosamine alone	9.72 ± 0.91
plus vitamin A	0.97 ± 0.03
plus vitamin C	1.21 ± 0.09
plus vitamin E	0.87 ± 0.08
plus niacin	1.11 ± 0.13
plus riboflavin	1.35 ± 0.08
plus thiamine	1.27 ± 0.06
plus biotin	1.09 ± 0.06
plus folic acid	1.42 ± 0.09
plus vitamin B 12	1.47 ± 0.23
plus pantothenic acid	1.22 ± 0.12
plus calcium	1.02 ± 0.04
plus magnesium	1.02 ± 0.12
plus copper	1.21 ± 0.09
plus iron	1.26 ± 0.07
plus manganese	2.11 ± 0.09
plus zinc	0.98 ± 0.08

Table 8. Effect of vitamins and mineral ions on the somatic cell genotoxicity of N-nitrosopyrrolidine

	No. of micronucleated polychromatic erythrocytes per thousand
Negative control, distilled water	1.22 ± 0.06
N-nitrosopyrrolidine alone	9.67 ± 0.78
plus vitamin A	1.23 ± 0.06
plus vitamin C	1.73 ± 0.05
plus vitamin E	1.87 ± 0.06
plus niacin	0.93 ± 0.05
plus riboflavin	0.89 ± 0.05
plus thiamine	1.33 ± 0.24
plus pyridoxine	1.01 ± 0.06
plus biotin	1.82 ± 0.09
plus folic acid	1.12 ± 0.08
plus vitamin B 12	1.11 ± 0.08
plus calcium	1.67 ± 0.07
plus magnesium	1.55 ± 0.33
plus copper	1.22 ± 0.07
plus iron	0.93 ± 0.09
plus manganese	1.10 ± 0.05
plus zinc	1.08 ± 0.08

Germ cell genotoxicity of dimethylnitrosamine was reduced by vitamins and mineral ions. This was shown in the increase of fertility index and gestation index and in the reduction of % dead implants and females with resorption (Table 9). The same observation is recorded of diethylnitrosamine (Table 10), dipropylnitrosamine (Table 11), dibutylnitrosamine (Table 12) and N-nitrosopyrrolidine (Table 13).

Expressions from fruits and vegetables reduced the somatic cell genotoxicity of dimethylnitrosamine (Table 14 and Table 15). Fruits and vegetables are natural sources of vitamins and mineral ions.

Summary

Dimethylnitrosamine, diethylnitrosamine, dipropylnitrosamine, dibutylnitrosamine and N-nitrosopyrrolidine are genotoxic to both somatic and germ cells. This genotoxicity can be reduced by vitamin A, vitamin C, vitamin E, niacin, riboflavin, thiamine, folic acid, biotin, vitamin B 12, pyridoxine, and pantothenic acid. Genotoxicity can also be reduced by calcium, magnesium, copper, iron and manganese. Expressions from fruits and vegetables can also reduce the genotoxicity of nitrosamines.

Table 9. Effect of vitamins and mineral ions on germ cell genotoxicity of dimethylnitrosamine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
Dimethylnitrosamine alone	22.1	58.3	27.4	100
plus vitamin A	88.2	90.6	2.0	2.1
plus vitamin C	91.4	88.4	1.6	2.0
plus vitamin E	87.6	87.9	1.4	1.6
plus niacin	93.1	92.3	1.8	2.0
plus riboflavin	92.6	93.4	1.3	1.6
plus thiamine	89.6	91.5	1.8	1.8
plus pantothenic acid	87.9	88.7	1.8	1.8
plus calcium	88.8	89.3	1.5	1.6
plus magnesium	91.2	88.4	2.0	2.0
plus iron	86.5	91.1	2.0	1.9
plus zinc	87.9	92.0	1.4	1.3

Table 10. Effect of vitamins and mineral ions on germ cell genotoxicity of diethylnitrosamine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
Diethylnitrosamine alone	17.6	44.5	38.6	100
plus vitamin A	92.2	89.9	2.1	2.1
plus vitamin C	88.9	92.3	1.6	1.4
plus vitamin E	87.5	88.6	2.1	1 9
plus biotin	91.6	87.6	1.7	1.8
plus thiamine	93.2	90.8	1.5	1.4
plus pyridoxine	94.2	91.8	1.7	1.5
plus calcium	96.2	93.1	1.2	1.5
plus magnesium	92.3	94.2	1.5	1.6
plus manganese	85.7	88.5	1.9	1.8
plus iron	83.9	98.1	1.4	1.3
plus zinc	89.7	91.8	1.5	1.6

Table 11. Effect of vitamins and mineral ions on the germ cell genotoxicity of dipropylnitrosamine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
Dipropylnitrosamine alone	33.7	65.2	21.8	100
plus vitamin A	86.7	88.6	1.4	2.1
plus vitamin C	88.5	89.7	2.1	2.2
plus vitamin E	91.1	91.5	1.2	1.5
plus niacin	92.3	88.9	1.9	1.6
plus riboflavin	90.1	89.7	1.9	1.6
plus folic acid	86.4	92.0	1.8	1.9
plus vitamin B 12	85.9	88.5	1.4	1.3
plus calcium	83.9	88.1	2.1	2.1
plus magnesium	86.7	91.2	1.9	2.1
plus copper	91.1	87.2	1.6	1.9
plus iron	88.4	85.8	1.9	2.1

Table 12. Effect of vitamins and mineral ions on germ cell genotoxicity of dibutylnitrosamine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
Dibutylnitrosamine	32.9	63.4	22.8	100
plus vitamin A	88.6	86.7	1.2	2.2
plus vitamin C	89.3	88.6	2.3	2.4
plus vitamin E	87.6	89.6	2.1	2.1
plus riboflavin	90.1	86.8	2.1	2.2
plus pyridoxine	91.8	90.3	1.8	1.8
plus pantothenic acid	87.6	88.9	1.9	2.1
plus biotin	86.7	86.9	2.1	2.2
plus calcium	89.6	94.1	1.1	1.8
plus copper	89.7	94.3	2.1	2.1
plus manganese	87.6	89.7	1.9	2.1
plus zinc	91.1	95.6	1.3	1.4

Table 13. Effect of vitamins and mineral ions on germ cell genotoxicity of N-nitrosopyrrolidine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
N-nitrosopyrrolidine	33.4	68.1	19.3	100
plus vitamin A	93.1	92.5	2.1	2.1
plus vitamin C	92.3	91.1	2.3	2.2
plus vitamin E	93.2	90.4	1.8	2.0
plus niacin	91.1	89.7	2.3	2.1
plus pantothenic acid	86.7	87.8	2.3	2.1
plus biotin	89.4	88.6	2.2	2.2
plus folic acid	88.7	90.7	1.9	1.8
plus calcium	90.1	89.8	2.1	2.1
plus magnesium	92.3	91.1	1.5	1.6
plus copper	93.1	89.7	2.1	2.2
plus iron	91.1	88.3	2.2	2.1
plus zinc	90.4	90.1	1.7	1.9

Table 12. Effect of vitamins and mineral ions on germ cell genotoxicity of dibutylnitrosamine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
Dibutylnitrosamine	32.9	63.4	22.8	100
plus vitamin A	88.6	86.7	1.2	2.2
plus vitamin C	89.3	88.6	2.3	2.4
plus vitamin E	87.6	89.6	2.1	2.1
plus riboflavin	90.1	86.8	2.1	2.2
plus pyridoxine	91.8	90.3	1.8	1.8
plus pantothenic acid	87.6	88.9	1.9	2.1
plus biotin	86.7	86.9	2.1	2.2
plus calcium	89.6	94.1	1.1	1.8
plus copper	89.7	94.3	2.1	2.1
plus manganese	87.6	89.7	1.9	2.1
plus zinc	91.1	95.6	1.3	1.4

Table 13. Effect of vitamins and mineral ions on germ cell genotoxicity of N-nitrosopyrrolidine

	FI	*GI*	*DI*	*FR*
Control	94.2	96.1	1.4	1.6
N-nitrosopyrrolidine	33.4	68.1	19.3	100
plus vitamin A	93.1	92.5	2.1	2.1
plus vitamin C	92.3	91.1	2.3	2.2
plus vitamin E	93.2	90.4	1.8	2.0
plus niacin	91.1	89.7	2.3	2.1
plus pantothenic acid	86.7	87.8	2.3	2.1
plus biotin	89.4	88.6	2.2	2.2
plus folic acid	88.7	90.7	1.9	1.8
plus calcium	90.1	89.8	2.1	2.1
plus magnesium	92.3	91.1	1.5	1.6
plus copper	93.1	89.7	2.1	2.2
plus iron	91.1	88.3	2.2	2.1
plus zinc	90.4	90.1	1.7	1.9

Table 14. Effects of expressions from fruits on the somatic cell genotoxicity of dimethylnitrosamine

	No. of micronucleated polychromatic erythrocytes per thousand
Dimethylnitrosamine, 10 mg/kg	15.67 ± 1.56
plus Atis	1.66 ± 0.66
plus Avocado	2.10 ± 0.69
plus Duhat	1.86 ± 0.38
plus Kamatsile	2.49 ± 0.77
plus Lansones	3.08 ± 0.98
plus Melon	3.22 ± 0.18
plus Papaya	2.11 ± 0.21
plus Sineguelas	3.55 ± 1.01
plus Suha	2.33 ± 0.33
Control	1.76 ± 0.07

Table 15. Effects of expressions from vegetables on the somatic cell genotoxicity of dimethylnitrosamine

	No. of micronucleated polychromatic erythrocytes per thousand
Dimethylnitrosamine, 10 mg/kg	15.67 ± 1.56
plus Ampalaya fruit	3.58 ± 0.31
plus Ampalaya leaves	3.66 ± 0.33
plus Bell pepper, green	3.08 ± 0.16
plus Bell pepper, red	2.77 ± 0.38
plus Garlic	2.01 ± 0.09
plus Kamias	3.77 ± 0.68
plus Green mongo	2.57 ± 0.84
plus White onions	2.44 ± 0.55
plus Raddish	3.22 ± 0.18
plus Squash fruit	3.21 ± 0.76
plus Squash leaves	3.55 ± 0.69
plus Tomatoes	2.88 ± 0.76
Control	1.76 ± 0.07

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