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METHIONINE-CYSTEINE DEFICIENCY AND ALKYLATION OF DNA IN LIVER, KIDNEY AND LUNG OF MICE ADMINISTERED DIMETHYLNITROSAMINE

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Abstract—The effect of methionine-cysteine deficiency on the methylation of DNA purines by dimethylnitrosamine metabolites was studied in subadult and adult mice. In liver, no dietary effect on the specific methylation of 7-methylguanine was observed, while that of 3-methyladenine decreased in the adult animals. The specific methylation of guanine in the O^6-position and the ratio of O^6-methylguanine to 7-methylguanine increased significantly after methionine-cysteine deficiency. Methylation in kidney decreased in subadult but increased in adult mice. In lung, the amount of 7-methylguanine was significantly elevated after methionine-cysteine deficiency in both the subadult and adult mice. The results demonstrate an increase in the specific methylation in liver of guanine in the O^6-position by the methionine-cysteine deficient diet, together with differences in the methylation pattern between organs of the two age groups.

N-nitroso compounds act in the body via their conversion in the cells to alkylating agents reacting with DNA, RNA and protein. The DNA adducts have been most extensively studied because alkylation in one or several positions will weaken the base pairing properties and give rise to an incorrect nucleotide insertion during replication. Misreadings of the DNA template are thought to be the basis of mutagenic and carcinogenic effects by the alkylating agents [1–4].

Certain differences in the age dependent sensitivity to carcinogenic stimuli have been observed [5]. The results suggest that in addition to the accumulating effects of exposure to carcinogens over a period of years, changes occur in the cells of the senescent tissues.

Enzyme systems participating in the metabolism of toxic components as well as in the repair of damaged DNA may be affected by protein deficiency. Deficiency of methionine reduces the capacity for protein synthesis [6], diminishes its availability as a substrate in *S*-adenosylmethionine reactions [7], and limits its use as a precursor for cysteine. Cysteine is an acceptor of reactive methylgroups obtained from the procarcinogen dimethylnitrosamine (DMN). It has been reported that high dosages have a protective effect against DMN [8].

Renal tumour formation increases in rats fed on a protein deficient diet prior to administration of DMN [9]. The induction of hepatic tumours is depressed, and the ability of the liver to metabolize DMN is decreased [10]. This raises the question of whether or not, at the initial stage of carcinogenesis, there are differences in the level of alkylation in organs other than liver during amino acid deficiency, and especially during deficiency of methionine and cysteine.

In this paper we describe the effect of methionine-cysteine deficiency on the methylation of DNA purines by DMN metabolites in subadult and adult mice. The response in liver of the specific methylation of guanine in the O^6-position was diet dependent. Methylation distribution between liver, kidney and lung showed differences between the age groups that were fed on the deficient diet.

MATERIALS AND METHODS

Materials. The sources of materials have been listed previously [11]. L-Amino acids obtained from Tanabe Seiyaka Co. Ltd. (Osaka, Japan), were a gift from KabiVitrum (Stockholm, Sweden).

Animals and diets. White male outbred mice of the Naval Medical Institute strain were used. Subadult mice of 20 g body weight were 30 days old and adult mice of 35 g body weight were 60 days old. The

Table 1. Dietary composition

Ingredients	Control diet (g/kg)
Amino acid mixture*	120
Glucose	200
Sucrose	100
Potato starch	380
Corn oil	100
Salt mixture	50
Vitamin mixture	5
Cellulose	45

* *Control diet.* Composition of the complete amino acid mixture in mg/g N was that of whole egg [20] and as follows: alanine, 370; arginine, 381; aspartic acid, 601; asparagine, 381; cysteine 152; glutamic acid 796; glutamine, 600; glycine 207; histidine, 152; isoleucine, 393; leucine 551; lysine, 436; methionine, 210; phenylalanine, 358; proline, 260; serine, 478; threonine, 320; tryptophan, 93; tyrosine, 260; valine 428.

Experimental diet. In the cysteine–methionine deficient diet cysteine was reduced to 15 mg and methionine to 21 mg and the corresponding nitrogen was added as glycine.

animals were kept in pairs in each cage. Food and water was given *ad libitum*. The composition of the control and experimental diet is given in Table 1. The dietary mixtures were supplemented with water and then dried into pellets at 40°. The two age groups of mice were fed either the diet containing the control amino acid mixture or the mixture deficient in methionine and cysteine. After 6 days the mice received a single intraperitoneal injection of 1.5 μCi (^{14}C)DMN (0.5 mg/kg body weight) and were killed 45 min later.

Isolation of DNA. The liver, kidneys and lungs were removed from the animals and tissues from 4 subadult or 3 adult mice were pooled. Nuclei of the fresh liver were isolated with the use of 2.3 M sucrose – 1 mM $CaCl_2$ [12]. DNA was precipitated in ethanol after incubation with RNase A (EC 3.1.4.22) and pronase K (EC 3.4.24.4) [11]. Kidneys and lungs were frozen in liquid nitrogen and stored at −80°. DNA of these organs was isolated by the phenol method [13].

Analysis of DNA. DNA was hydrolysed in 0.1 M HCl at 70° for 30 min. The hydrolysates (3 mg of liver DNA, 1 mg of kidney or lung DNA) were analysed by column chromatography with the use of Sephadex G 10 (1.5 or 1.0 × 90 cm), eluting with 0.05 M ammonium formate (pH 6.8) containing 0.02% Na azide [14]. Fractions of 4.5 ml were collected in scintillation vials, the absorbance at 260 nm was measured, and the fractions were then evaporated to dryness. Distilled water and a scintillation solution were added, and then the level of radioactivity was measured [11].

Statistical evaluation The data of Table 2 were computed by means of one way analysis of variance. The data of Table 3 were analysed by using two ways analysis of variance.

RESULTS

Dietary effect on growth of subadult and adult mice

Male mice were fed a diet containing a complete mixture of amino acids or a mixture low in methionine and cysteine (10% that of the control level, Table 1). The nitrogen content present in the diet corresponded to 12% protein. In both age groups the change in body weight was diet-dependent, showing a loss in weight associated with the methionine–cysteine deficient diet (Table 2). The wet weights of liver and kidney, but not lung, were significantly diminished in both age groups fed the deficient diet.

Methylation of DNA purines in liver, kidney and lung

Methylation was determined in DNA of liver, kidney and lung 45 min after injection of (^{14}C)DMN (Table 3). In the subadult animals the methionine–cysteine deficient diet had no effect in liver on the specific methylation of adenine in the N-3 and guanine in the N-7 position. O^6-methylguanine increased in two out of three experiments and the ratio of O^6-methylguanine to 7-methylguanine increased following methionine–cysteine deficiency. In kidney, the relative decrease in 3-methyladenine was more pronounced than that of 7-methylguanine. This was also seen in the ratio of 3-methyladenine to 7-methylguanine. O^6-methylguanine was low and not used for further calculations. In lung, 7-methylguanine increased twofold after methionine–cysteine deficiency. The values for 3-methyladenine and O^6-methylguanine were too low to be interpreted.

In adult mice, the methionine-cysteine deficient diet had no effect in liver on the level of 7-methylguanine, while 3-methyladenine decreased slightly and O^6-methylguanine increased markedly. The ratio of 3-methyladenine to 7-methylguanine confirmed the decrease in 3-methyladenine, and that of O^6-methylguanine to 7-methylguanine confirmed the increase in O^6-methylguanine. In both kidney and lung, 7-methylguanine increased in the deficient group and in kidney 3-methyladenine also increased. The ratio of 3-methyladenine to 7-methylguanine in experiment 5 was lower in the control diet; this was due to the marked increase in 7-methylguanine after methionine–cysteine deficiency.

Comparison of the specific methylations in liver between subadult and adult mice showed similar levels except for 3-methyladenine which was lowest in the adult liver after methionine–cysteine deficiency. The increase of O^6-methylguanine in the liver of subadult and adult animals considered together, following methionine–cysteine deficiency, was statistically significant ($P < 0.05$). Likewise, the increased ratio of O^6-methylguanine to 7-methylguanine was significant ($P < 0.025$). In lung, the increase in 7-methylguanine after methionine–cysteine deficiency was significant ($P < 0.01$) in the subadult and adult animals considered together. Differences in methylation between the two age groups were seen in kidney, with a decrease in the subadult

Table 2. Change in body weight and tissue wet weights per animal after 6 days on the control or experimental diet*

Mice	Diet	Change in body weight (g)	Wet weight of tissue (g) Liver	Kidney	Lung
Subadult	Control	4.66 ± 0.81	1.45 ± 0.06	0.375 ± 0.03	0.224 ± 0.01
	Met and cys deficient	−3.72 ± 0.80†	0.80 ± 0.05†	0.242 ± 0.03†	0.165 ± 0.02
Adult	Control	1.18 ± 0.82	1.84 ± 0.01	0.484 ± 0.02	0.193 ± 0
	Met and cys deficient	−6.68 ± 0.14†	1.13 ± 0.02†	0.343 ± 0.02†	0.272 ± 0.02

* The results are mean values ± S.E.M. of at least 16 animals per dietary group.
Data were computed with the use of one way analysis of variance.
† Significant difference ($P < 0.05$) from control diet within one age group.

Table 3. Diet and age dependent methylation of DNA-purines from (^{14}C)DMN*

Mice	Tissue	Expt	Methylation (μmole/mole of parent base)						Ratios			
			3-methyladenine		7-methylguanine		0^6 methylguanine		3-methyladenine/ 7-methylguanine		0^6-methylguanine/ 7-methylguanine	
			Contr	Def	Contr	Def	Contr	Def	Contr	Def	Contr	Def
Subadult	Liver	1	7.8	6.3	116.1	93.8	5.6	5.0†	0.067	0.068	0.048	0.053‡
		2	6.5	8.0	96.2	109.6	1.7	4.0†	0.067	0.073	0.018	0.037‡
		3	9.6	10.0	123.0	129.8	5.5	8.8†	0.078	0.077	0.045	0.067‡
	Kidney	1	6.0	3.0	23.6	17.4	1.3	2.1	0.252	0.170		
		2	3.6	1.7	27.2	30.3	UD	4.2	0.134	0.055		
		3	3.5	2.2	30.1	17.6	1.7	1.3	0.115	0.125		
	Lung	2	1.4	6.7	8.2	20.6§	5.1	UD	0.168	0.323		
		3	1.1	1.4	5.2	14.0§	2.0	1.9	0.204	0.097		
Adult	Liver	4	8.7	5.0	106.0	91.9	2.7	5.5†	0.082	0.055	0.025	0.060‡
		5	5.7	5.6	121.4	133.8	1.7	5.4†	0.047	0.042	0.014	0.040‡
	Kidney	4	3.1	5.4	15.6	33.4	0.9	2.2	0.201	0.160		
		5	0.6	1.4	18.0	25.7	1.5	0.7	0.033	0.055		
	Lung	4	1.2	1.0	1.7	15.0§	1.2	UD				
		5	UD	3.9	4.8	15.9§	UD	UD				

* Mice were injected with (^{14}C)DMN, 0.5 mg/kg body weight and killed 45 min later. For isolation of DNA-purines see Materials and Methods. UD, under detection limit. Data were computed with the use of two ways analysis of variance.

† Significantly different from controls, subadult and adult mice considered together, $P < 0.05$.

‡ Significantly different from controls, subadult and adult mice considered together, $P < 0.025$.

§ Significantly different from controls, subadult and adult mice considered together, $P < 0.01$.

and an increase in the adult mice fed on the methionine–cysteine deficient diet. In addition, in kidney the control subadults showed higher values of 7-methylguanine than the control adult animals.

DISCUSSION

The present study demonstrates that a pronounced decrease in dietary methionine and cysteine to a 10% level of the control diet changes the metabolic effects of DMN on DNA. The increase in the level of O^6-methylguanine in liver, and the elevated level of the ratio O^6-methylguanine to 7-methylguanine, suggest differences in the methylation pattern of guanine.

The enzymes repairing methylation in the O^6-position of guanine may be quantitatively decreased by the amino acid deficiency, because overall protein synthesis in liver is diminished by such a diet [6]. The repairing enzyme is irreversibly inactivated after acceptance of a single methylgroup to its cysteine residue [15]. The increase in O^6-methylguanine may also be explained by changes in the protein concentration of chromatin [11]. Proteins that regulate gene activity are bound to the major grove of the DNA helix, specifically to O^6-guanine and 7-*N*-guanine [16]. A physiological alteration in the binding capacity of the proteins, induced by methionine–cysteine deficiency, may expose one position of guanine more than the other [16] and favour the methylation in the O^6-position of guanine.

In a previous study, the methionine–cysteine content of the diet was lowered to 40% of the control food [11]. This moderate reduction had no effect on the concentration of O^6-methylguanine in the liver; while the incorporation of (^{14}C) from (^{14}C)DMN into total nuclear protein was decreased. In the previous study [11] a dosage of 5 mg DMN/kg body weight was injected into mice instead of the 0.5 mg used here. In rats, the dose response of the O^6-methylguanine repair is not strictly linear between 0.5 and 5 mg of DMN indicating a diminished activity at higher dosages [17]. The moderate reduction in dietary methionine–cysteine used previously at the 5 mg DMN-dosage was not sufficient to provoke a change in the level of O^6-methylguanine in the mice [11]. The lower dosage of DMN used here will ensure an optimal repair activity of O^6-methylguanine in the controls [17]. Comparison was made with the deficient mice under the more favourable repair conditions of the controls, and measurable differences in the methylation of liver guanine in the O^6-position were obtained.

By diminishing the dosage from 5 mg to 0.5 mg of DMN isotope dilution became less and radioactivity values increased. This made possible a significant detection of 7-methylguanine in kidney and lung. In these tissues, no diet dependent changes in O^6-methylguanine were noticeable. This may to some extent result from the technical problem that after column chromatography O^6-methylguanine is distributed over a number of fractions, thus making evaluation of low radioactivity levels uncertain. However, 3-methyladenine elutes ahead of the other methylated purines [14] and is collected in a few fractions; this makes reliable radioactivity measurements possible. In liver, no such problems arise because there is a higher level of radioactivity than in kidney and lung.

The high level of 7-methylguanine in kidneys of the control subadult mice was decreased by the methionine–cysteine deficiency. In contrast, the low level in the control adults seemed to allow for a further increase after methionine–cysteine deficiency.

The age dependent differences in the methylation of kidney DNA indicate an effect of methionine–cysteine deficiency on the metabolic capacity of the tissue. Preliminary experiments show an increase in the level of protein synthesis in kidney following methionine–cysteine deficiency. The increase was more pronounced in adult than subadult mice. This suggests differences in enzyme activities, between the age groups, which might be interpreted as a diet-dependent reduced DMN metabolizing activity in the subadult mice.

Methylation of DNA in lung by *N*-methyl-*N*-nitrosurea has been reported [18]. The present results confirm the methylation using DMN as a precursor. Lung was more responsive than liver to dietary changes in the specific methylation of guanine in the N-7 position. In lung, an enhanced level was observed after methionine–cysteine deficiency.

The major metabolic functions of methionine are utilization for protein synthesis, and conversion to *S*-adenosylmethionine which serves as a biological methylgroup donor [7]. Methionine may also be converted to cysteine, which as a free amino acid acts as a trap for activated methylgroups [8]. Alternatively, the amino acid cysteine is part of the repairing enzyme O^6-methylguanine transferase, and will accept the guanine-bound methylgroup [15].

Methionine is thought to have a protective effect by preventing carcinogen induced methylation changes in the genome [19]. A methionine-induced reversal of methylation by carcinogens has been suggested. The amino acid is the substrate for the *S*-adenosyl-methionine mediated normally occuring methylation of DNA [7, 19].

The present results demonstrate the requirement for methionine and cysteine in the diet. Deficiency of these amino acids changed the methylation pattern of DNA purines, leading to an increased specific methylation of liver guanine in the O^6-position, an elevated O^6-methylguanine to 7-methylguanine ratio, and an increase in 7-methylguanine in the lung. Age-related differences in liver showed the lowest content of 3-methyladenine in adult mice after methionine-cysteine deficiency. The difference between subadult and adult animals of the 7-methylguanine content in kidney suggests a change with age in the capacity for DMN metabolism which becomes strengthened by enforcing a methionine-cysteine dietary regimen.

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