Status of Drug-Metabolizing Enzymes after Treatment of Fat-Deficient Mice with N-Nitrosodiethylamine

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Summary Dietary deprivation of fat in male Swiss mice for 6 weeks resulted in a decrease in cytochrome *c*-reductase (Cyt.*c*-red.) activity without alteration of the activities of arylhydrocarbon hydroxylase (AHH), aminopyrene demethylase (APD), and cytochrome P-450 (Cyt. P-450) in the liver, whereas levels of glutathione-S-transferase (GST) and reduced glutathione (GSH) were significantly increased by fat-free diet feeding. Following N-nitrosodiethylamine (NDEA) administration, AHH, APD, and Cyt.P-450 significantly declined, whereas GST and GSH were stimulated in the liver of control as well as fat-deficient mice. NDEA treatment decreased the activities of AHH and APD, and increased the GST activity in the lungs of fat-deficient mice only. The higher levels of GST and/or GSH in fat deficiency may have a protective role in chemical carcinogenesis.

Key Words: arylhydrocarbon hydroxylase, aminopyrene demethylase, cytochrome P-450, glutathione-S-transferase, reduced glutathione

Several epidemiological and laboratory studies indicate that diet is an important factor in controlling the development and progression of many types of cancers. Increased intake of saturated fatty acids has been shown to be associated with the increased risk of cancer of colon and rectum, and possibly of the prostate, bladder, and lung as well [1]. Studies on experimental animals have also revealed that ingestion of high amounts of fat can stimulate spontaneous [2], transplanted [3] and chemically induced [4] tumors. Conversely, low dietary intake of fat may provide protection against tumorigenesis induced by various means, including chemical ones. In this regard we have found that dietary deprivation of fat was able to inhibit lung tumors induced in mice by N-nitrosodiethylamine (unpublished work).

NDEA is one of the powerful carcinogenic nitrosoamines found in tobacco, the environment, smoke, and also in food products [5]. Administration of this carcinogenic compound to animals altered the status of Cyt.P-450-dependent drug-metabolizing enzymes that are responsible for the metabolic activation of NDEA. The metabolic activation of NDEA might be achieved by Cyt.P-450, catalyzing hydroxylation (AHH), demethylation (APD), etc. of various substrates. Earlier, we found that the alterations in AHH activity induced by NDEA were modulated by the diet [6]. Since dietary deprivation of fat has a protective role in carcinogenesis, it is likely that changes in drug-metabolizing enzymes after the carcinogen administration have some relevance to the tumor induction process. This paper reports experiments aimed at finding such relevance.

MATERIALS AND METHODS

NADPH, benzo(*a*)pyrene, cytochrome *c*, 5,5'-dithiobisnitrobenzene, 1-chloro 2,4-dinitrobenzene, and *p*-nitrophenol were purchased from Sigma Chemical Co., St. Louis, MO. 3-Hydroxybenzo(*a*)pyrene was a gift from Chemical Carcinogen Repository, NCI, Bethesda, MD. All other chemicals, of analytical grade, were purchased locally.

Male Swiss NMRI mice (25–30 g) from the Institute's colony were classified into two groups, control and fat-deficient. Each group consisted of 10 to 12 animals that were fed *ad libitum* either a fat-deficient or control diet for 6 weeks. The experimental diets were prepared in the laboratory. The composition of the diets is given in Table 1. Weight gain and diet consumption were recorded at weekly intervals.

After 6 weeks of diet feeding, 5 to 6 animals from each group were administered NDEA intraperitoneally at a dose of 40 mg/kg body weight in 0.1 ml of sterilized saline for three consecutive days. After carcinogen administration, the animals were fasted overnight, and then killed under ether anesthesia. Liver and lungs were removed and perfused immediately with cold 0.15 M KCl containing 2 mM EDTA (pH 7.4), minced with scissors, and homogenized in a glass-Teflon

Table 1. Composition of diet (%).

Ingredients	Control	Fat-deficient
Casein	18.0	18.0
Starch	59.8	72.8
Sucrose	4.0	4.0
Fat (peanut oil)	6.0	—
Salt mixture	4.0	4.0
Choline chloride	0.2	0.2
Fiber	7.0	—
Vitamin mixture	1.0	1.0
kcal/100 g	398	394

homogenizer to a concentration of 1 g wet wt/4 ml of the buffer solution. Homogenates were centrifuged at $12,000 \times g$ for 30 min, and the post-mitochondrial supernatant (PMS) was used for the estimation of drug-metabolizing enzymes.

Cyt.P-450 was estimated as describe previously [6], and AHH activity was measured by the method of Yang *et al.* [7]. Determination of APD was done according to Cochin and Axelrod [8]. The method of Mazel [9] was used for the estimation of NADPH-Cyt.*c*-red. with cytochrome *c* as a substrate. GST was estimated as described by Habig *et al.* [10]. The method of Moron *et al.* [11] was used for the estimation of GSH. Protein was determined according to Lowry *et al.* [12] using bovine serum albumin as a standard.

Determination of the statistical significance of the difference between groups was done by Student's *t*-test.

RESULTS

Fat deficiency for 6 weeks and/or NDEA treatment did not lead to any change in body weight, food consumption, organ weights (liver and lungs), or soluble protein contents in liver and lung of mice (data not shown).

Table 2 shows that dietary deprivation of fat did not have any significant effect on the activities of AHH, APD, and Cyt.P-450 content in the liver. The activities of AHH, APD, and Cyt.P-450 were lowered in control and fat-deficient groups after NDEA administration. The basal level of Cyt.*c*-red. (36.2 ± 1.1 nmol/mg protein) was significantly decreased in the control NDEA-treated group (28.1 ± 1.8 nmol/mg protein) but remained unchanged in the fat-deficient NDEA-treated group. Feeding of the fat-free diet to the mice enhanced hepatic GST activity from $1,292 \pm 118$ to $2,958 \pm 188$ nmol/mg protein. NDEA treatment also led to 2.2- and 1.25-fold increases in GST activity of control and fat-deficient

Table 2. Effect of N-nitrosodiethylamine treatment on hepatic drug-metabolizing enzymes in control and fat-deficient mice.

Parameter*	Control	Control NDEA-treated	Fat-deficient	Fat-deficient NDEA-treated
AHH	10,505 $\pm$ 961	8,150 $\pm$ 889 ^a	11,498 $\pm$ 1,108	7,784 $\pm$ 227 ^b
APD	271 $\pm$ 30	234 $\pm$ 9.2 ^a	260 $\pm$ 5.3	215 $\pm$ 21 ^b
Cyt.P-450	15.5 $\pm$ 1.3	10.2 $\pm$ 1.2 ^a	16.0 $\pm$ 1.2	7.4 $\pm$ 0.7 ^b
Cyt. <i>c</i> -red.	36.2 $\pm$ 1.1	28.1 $\pm$ 1.8 ^a	29.8 $\pm$ 1.3 ^a	29.6 $\pm$ 1.7
GST	1,292 $\pm$ 118	2,930 $\pm$ 198 ^a	2,958 $\pm$ 188 ^a	3,714 $\pm$ 213 ^b
GSH	6.4 $\pm$ 0.5	9.4 $\pm$ 1.0 ^a	7.8 $\pm$ 0.5 ^a	9.8 $\pm$ 0.7 ^b

Values are mean $\pm$ SD, $n = 5$. *Units: AHH, pmol/g tissue/min; APD, nmol of formaldehyde formed/g tissue/30 min; Cyt. P-450, nmol/g tissue; Cyt. *c*-red., nmol of cytochrome *c*-reduced/min/mg microsomal protein; GST, nmol of 1-chloro 2,4-dinitrobenzene conjugated/min/mg protein; GSH, μ mol/g tissue. ^aCompared with corresponding control group value, $p < 0.005$. Fat-deficient group compared with control group. ^bCompared with corresponding fat-deficient group value, $p < 0.005$.

Table 3. Effect of N-nitrosodiethylamine treatment on pulmonary drug-metabolizing enzymes in control and fat-deficient mice.

Parameter*	Control	Control NDEA-treated	Fat-deficient	Fat-deficient NDEA-treated
AHH	197 ± 17	145 ± 11 ^a	186 ± 24	125 ± 14 ^c
APD	166 ± 25.5	132 ± 13 ^b	148 ± 22.3	110 ± 19.2 ^c
Cyt. P-450	6.1 ± 0.55	8.1 ± 0.67 ^a	6.9 ± 0.7	6.4 ± 0.4
Cyt. c-red.	12.2 ± 1.3	12.2 ± 1.6	11.0 ± 1.2	11.6 ± 1.6
GST	345 ± 43	303 ± 31	334 ± 34	385 ± 34 ^d
GSH	1.49 ± 0.66	1.0 ± 0.06 ^a	0.99 ± 0.05 ^a	0.93 ± 0.08

Values are mean ± SD, *n* = 5. *Units: Same as in Table 2. ^aCompared with corresponding control group value, *p* < 0.005. ^bCompared with corresponding control group value, *p* < 0.05. ^cCompared with corresponding fat-deficient group value, *p* < 0.005. ^dCompared with corresponding fat-deficient group value, *p* < 0.05.

groups, respectively. The hepatic GSH level was increased by 21.8% in fat-deficient, 46.8% in control-NDEA-treated, and 53.1% in fat-deficient NDEA-treated groups in comparison with the level in the control group.

Feeding of fat-depleted diet to animals did not significantly change the activities of AHH and APD, but decreased the Cyt.c-red. activity in the lung (Table 3). On the other hand, Cyt.P-450 contents were enhanced from 6.1 ± 0.55 to 6.9 ± 0.7 nmol/g tissue. GST activity remained unaltered in fat deficiency. The GSH level was decreased by 33.6% in the lungs of fat-deficient mice. NDEA treatment lowered the AHH activity in both control and fat-deficient groups, but induced Cyt.P-450 in the control group only. Control NDEA-treated animals showed a decline in GSH level, whereas treatment of fat-deficient animals with NDEA did not alter the GSH level. However, GST activity was increased from 334 ± 34 to 385 ± 34 nmol/mg protein (*p* < 0.05) in the lung.

DISCUSSION

Our observation of decreased hepatic activities of AHH, APD, and Cyt.P-450 in control and fat-deficient groups after NDEA treatment may be suggestive of some inhibitory effect of NDEA administration on these enzymes. The inhibitory effect of NDEA was more pronounced in the fat-deficient group than in the control group. The reason for this observation is not simple to explain. However, one possibility could be the labilization or disorganization of the microsomal membranes by nitrosoamine administration leading to the inhibition or inactivation of some components of the mixed function oxidase system [13].

The tripeptide glutathione accounts for over 90% of the nonprotein thiol of the liver. The ubiquitous distribution and high concentration of GSH in tissues have given rise to considerable speculation as to its physiological role. Through the action of GST, and possibly nonenzymatically, GSH can be conjugated with numerous electrophilic xenobiotics or their metabolites [14]. This function is

thought to be a major protective mechanism for the liver and other organs against the toxic and carcinogenic [15] activity of many metabolically activated xenobiotics. Thus, any impairment in the levels of GST and/or GSH may lead to increased chances for the binding of reactive metabolites with DNA [16]. Elevated GSH levels in fat-deficient animals after NDEA treatment might be a favorable condition for protection of liver against the adverse effects of active alkylating metabolites, and might also protect other organs from these reactive species transported to them through the systemic circulation.

The biological roles that GST-mediated conjugation of reactive metabolites of certain chemical carcinogens and detoxification of free radical-mediated lipid peroxides play in the process of chemical carcinogenesis are not yet clearly understood, but an inverse correlation between GST activity and incidence of chemically induced tumors has been found [17]. In the present study, we have found an increase in hepatic GST activity due to fat deficiency. This could be one of the important factors responsible for the tumorigenesis inhibition due to fat deficiency recorded by various workers [18, 19]. The stimulation in GST activity by NDEA administration may represent a defense response of the organism. Therefore, the overall higher inducibility of GST in NDEA-treated, fat-deficient animals appears to be of relevance from carcinogenesis point of view.

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