γ-GLUTAMYLTRANSPEPTIDASE IN CHEMICALLY INDUCED RAT MAMMARY GLAND CARCINOGENESIS IN SPECIFIC DIETARY STATES

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The levels of γ-glutamyltranspeptidase (GGTP) (EC 2.3.2.2) were measured in 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced mammary adenocarcinomas, in control mammary gland tissue, and in sera from tumor-bearing and control rats. The carcinogenic process was modulated by diets differing in the content and degree of unsaturation of their lipid component. GGTP activity levels were elevated up to 18-fold in mammary tumors as compared to corresponding control mammary gland tissue. Also, GGTP activity in sera from tumor-bearing rats was up to 4-fold higher than in the sera of the corresponding controls. GGTP activity levels in the mammary gland and in the sera of control rats from low-fat dietary group were up to 6-fold higher than the control values in either of two high-fat dietary groups.

INTRODUCTION

It is now generally accepted that there is a positive correlation between the amount of fat in the diet and the incidence of human cancer in various organs, especially carcinomas of the breast (1). A similar correlation has been demonstrated in studies using the 7,12-dimethylbenz(*α*)anthracene (DMBA)-induced rat mammary tumor model (2). Recent investigations by Carroll and associates (3,4) and by others (5,6) have also shown that rats fed high-fat diets, especially high polyunsaturated fat, gave a higher tumor incidence and developed a greater number of tumors than animals on a low-fat diet. The mechanism through which a low-fat diet provides protection against tumorigenesis is not known. The protective effects of the low-fat dietary regime against chemically induced carcinogenesis may well be due to altered metabolism of the carcinogen resulting in accelerated excretion of reactive intermediates of the carcinogen, to an inhibition of its activation, or both. The increased excretion of carcinogen possibly occurs via a carcinogen-glucuronic acid conjugate and/or as mercapturic acid derivatives of the carcinogen. γ-Glutamyltranspeptidase (GGTP), a glycoprotein which catalyzes the transfer of the γ-glutamyl moiety from glutathione and several other γ-glutamyl derivates to various amino acid and peptide acceptors, or to water, is known to play an important role in the detoxification of compounds such as carcinogens by the formation of mercapturic acid derivatives (7). Recently, GGTP has been suggested as a putative marker for hepatic neoplasia (8); whether GGTP is also a marker for other neoplastic systems remains to be established.

In the present study we investigated whether dietary fats, which affect chemical carcinogenesis (3-6), influence levels of GGTP activity in normal mammary gland and whether enhanced levels of GGTP activity, as reported earlier for chemically induced hepatomas (9), are present in DMBA-induced mammary adenocarcinomas and in the sera of tumor-bearing rats.

MATERIALS AND METHODS

L-γ-Glutamyl-*p*-nitroaniline and glycylglycine were purchased from Calbiochem-Behring Corporation, La Jolla, CA.; DMBA was obtained from Sigma Chemical Company, St. Louis, MO. All other chemicals were of reagent grade quality.

Diets: The compostion of the high polyunsaturated fat diet was as follows: casein 23%, corn oil 20%, sucrose 46%, Alphacel (non-nutrient bulk) 6%, salt mixture (10) 4%, and ICN vitamin fortification mixture (without *α*-tocopherol) 1%. This provided a *α*-tocopherol-unsupplemented basal diet. The high saturated-fat diet had identical composition except that a mixture of 18% coconut oil plus 2% linoleic acid was substituted for the corn oil. The low-fat diet, on the other hand, contained only 2% linoleic acid (to prevent essential fatty acid deficiency) and the sucrose content was increased to 64%.

Comparison of the growth rate curves indicated no significant differences between the dietary groups (5,6). All diets were supplied by ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, OH.

Mammary Tumor Induction

Weanling (21 days old) female Sprague-Dawley rats were placed on the synthetic diets described above. At 50 days of age, the experimental animals were given a single 10-mg dose of DMBA in 1 m*l* of corn oil by stomach intubation; control animals received 1 m*l* of the oil. All animals were kept on their respective diets and palpated for mammary tumors once a week. Both control and tumor-bearing rats were euthanized in a CO_2 chamber, and blood was obtained by cardiac puncture from both control and mammary tumor-bearing animals. Mammary tumors were identified histologically as adenocarcinomas (89%) and fibroadenomas (11%) (5). Only adenocarcinomas that were 0.5 to 1.5 cm in diameter and with no visible signs of necrosis were used for these studies. Control mammary gland tissue was obtained from rats of the same age and fed the same diet as the tumor-bearing rats. Connective tissue and other extraneous tissue was carefully removed before homogenization.

Enzyme Assay

GGTP activity was assayed on freshly obtained tissue and serum; homogenization and isolation of tissue fractions were carried out at 0-4 C. The homogenates, prepared in 0.25 *M* sucrose (1:4 w/v) using a Waring Blendor, full speed, 1 min were centrifuged for 15 min at 800 *g* to remove the nuclear fraction and the postnuclear supernate was then centrifuged for 1 hr at 105,000 *g* in order to separate the soluble fraction from the particulate fraction. The particulate fraction was suspended in water before measuring GGTP activity. Both membrane-bound (particulate) and soluble (cytosolic) GGTP activities were assayed using γ-glutamyl-*p*-nitroanilide (4 mM) and glycylglycine (30 mM) in 0.1 *M* Tris-HCl buffer, pH 8.2, at 37°. No measurable GGTP activity was detected in the nuclear fraction. Serum GGTP was assayed under similar experimental conditions but at pH 9.0. The *p*-nitroaniline released by enzyme action was measured at 405 nm using a Gilford 2400 recording spectrophotometer. The enzyme activity (expressed as μmoles/hr/mg protein for the tissular enzyme and μmoles/min/*l* of rat serum for the circulating enzyme) was calculated using a *p*-nitroaniline molar extinction coefficient of 10,200 $M^{-1}cm^{-1}$ at 405 nm (11). The degrees of significance of the differences between control and tumor values were determined by the Student's t-test.

RESULTS AND DISCUSSION

γ-Glutamyltranspeptidase activity in the normal mammary gland tissue was primarily localized in the particulate fraction, indicating the membrane-bound nature of the enzyme. The membrane-bound activity, however, depended on the amount and type of dietary fats. Thus the GGTP activity in normal mammary tissue of low-fat-fed rats was considerably higher that that observed for normal mammary tissues of rats fed either of the two high-fat diets (Table 1). As in the case of mammary tissue, the sera of control rats fed a low-fat diet had higher GGTP activity than that observed in the sera of control rats receiving the high-fat diets (Table 2). The differences between the control enzymatic levels in the low-fat group and those of the corresponding controls from the two high-fat dietary groups were found to be statistically significant ($p < 0.025$). This suggested a reverse correlation between GGTP activity levels and tumor incidence, which has been shown to be highest in rats fed a high polyunsaturated fat diet, intermediate with high saturated fat diet, and lowest in the low-fat group (3-6). Since GGTP is involved in the detoxification of carcinogens through mercapturic acid formation (7), the elevated levels of GGTP activity observed in the low-fat dietary group may lower tumor incidence through accelerated carcinogen detoxification.

The levels of particulate GGTP activity in mammary adenocarcinomas were found to be elevated (e.g., up to 18-fold in high polyunsaturated fat group, Table 1), over control mammary gland tissue of animals in the same dietary group. Also levels of GGTP activity in the noncancerous mammary tissue of the tumor-bearing rats were identical to that observed for the control mammary tissue. Unlike the normal mammary tissue where

TABLE 1. *γ-Glutamyltranspeptidase activity levels in control mammary tissue and in adenocarcimas of rats fed α-tocopherol-unsupplemented basal diets*

Diet	GGTP activity[a] Control	GGTP activity[a] Tumor
High-polyunsaturated-fat	0.12 ± 0.02 (8)	2.19 ± 0.62 (7)[b]
High-saturated-fat	0.22 ± 0.03 (4)	1.11 ± 0.14 (4)[b]
Low-fat	0.75 ± 0.15 (4)	1.20 ± 0.23 (4)[c]

[a] Assayed as described under Materials and Methods. The results are expressed as mean ± S.E.M. Numbers in parenthesis indicate the number of determinations. The differences between the tumor and corresponding control values were statistically significant
[b] $p < 0.005$
[c] $p < 0.05$

TABLE 2. *Levels of γ-glutamyltranspeptidase in sera from DMBA-induced mammary tumor-bearing rats fed α-tocopherol-unsupplemented basal diets.*

Diet	GGTP activity[a] Control rats	GGTP activity[a] Mammary gland tumor-bearing rats
High-polyunsaturated-fat	0.55 ± 0.06 (7)	2.34 ± 0.65 (5)[b]
High-saturated-fat	0.68 ± 0.08 (6)	1.48 ± 0.31 (5)[b]
Low-fat	2.70 ± 0.35 (3)	5.05 ± 0.74 (8)[c]

[a] Assayed as described under Materials and Methods. The results are expressed as mean ± S.E.M. Numbers in parenthesis indicate the number of determinations. The differences between the tumor-bearing and corresponding control values were statistically significant
[b] $p < 0.005$
[c] $p < 0.05$

GGTP activity was primarily located in the particulate (membrane-bound) fractions, total activity in the homogenates of neoplastic mammary tissue was distributed (about 50%) in each of the particulate and cytosolic fractions (data not shown). It is possible that GGTP in the neoplastic mammary tissue has a loose association with membrane matrix and that a portion of the total GGTP activity gets detached from the membranes during homogenization of the tissue. In contrast to adult rat liver, which contains very small amounts of GGTP activity, the chemically induced hepatomas have also been shown to contain considerably higher GGTP activity levels (12).

Up to 4-fold elevations in serum GGTP were observed in sera from tumor-bearing rats (Table 2). The increased levels of GGTP activity observed in mammary tumors and in the sera of the tumor-bearing rats and its cell surface localization (13) suggest possible shedding of this glycoenzyme from the mammary tumors into the blood stream. The marked increase in the level of sialoglycoproteins observed in the serum of cancer-bearing animals and human beings has been attributed, at least in part, to the release of these substances from the surface of tumor cells into the rat's circulation (14,15). Indeed, if a tumor-specific isoenzyme of GGTP is released into the blood, this may provide a useful diagnostic tool for detection and treatment of human patients with mammary carcinomas.

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