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Status of the γ-Glutamyl Transpeptidase Enzyme Activity in Mouse Skin Exposed to Polyaromatic Hydrocarbons and Tumor Promotor TPA

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The enzyme γ-glutamyl transpeptidase (GGT, EC 2.3.3.2) which catalyzes the transfer of glutamyl groups of peptides to other peptides and amino acids, has been proposed as a marker of neoplasia (Fiala and Fiala 1973; Kalengayi et al. 1975). The high levels of GGT activity have been observed in a variety of neoplastic cells and tissues from several rodent species (Groscurth et al. 1977; Pitot 1980; Solt and Shklar 1982) and cursory observation suggests that GGT is a marker of cellular proliferation (Richards and Astrup 1982). The significance of this enzyme in skin neoplasms has been demonstrated by Young et al (1978).

In the present study, attempt has been made to study the status of GGT activity in mouse skin in response to some well known cutaneous carcinogenic, weakly carcinogenic and non carcinogenic polyaromatic hydrocarbons and tumour promoter, 12-O-tetradecanoyl phorbol-13-acetate (TPA),24 hours after single topical application of these compounds.

MATERIAL AND METHODS

Chemicals. 12-O-tetradecanoyl phorbol-13-acetate (TPA), benzo(a)pyrene(BaP), 7,12-dimethyl benzanthracene (DMBA), chrysene, pyrene, γ-glutamyl-p-nitroanilide hydrochloride and Tris buffer were obtained from Sigma chemical Co., St. Louis, USA. Glycylglycine was obtained from BDH England and all other chemicals of analytical grade were procured from Sisco Research Laboratories, India.

Animals and Treatment. Female, Swiss albino mice (weighing 18-20 g) were as per routine maintained on a synthetic pellet diet and water ad libitum. Animals were randomly divided into eleven groups of six animals each. Hair was shaved on the interscapular region

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over an area of 2 cm^2 with the help of an electrical hair clipper, the shaved area was treated topically with one of the following chemicals and dose level: TPA (5 µg or 10 µg), BaP (10 µg or 25 µg), DMBA (50 µg or 100 µg), Chrysene (50 µg or 100 µg) and Pyrene (25 µg or 50 µg) once only on the shaved area which was left uncovered. All the test doses were dissolved in 100 µl Acetone. Control animals were treated with an equal amount (100 µl) of acetone only.

All experimental animals were killed after 24 hours by cervical dislocation and whole skin from the treated area (about 2 cm^2) was removed immediately and weighed on Mettler balance (Model PE-160). A 5% (w/v), skin homogenate was prepared in glycerol triton buffer pH 7.0 (10% glycerol, 1% Triton X-100 and 5 mM potassium sulphate) with the help of polytron (KINEMATICA CH-6010-KRIENS-LU).

GGT activity was assayed in S-9 fraction homogenate using the method of Young et al (1978). Each mouse skin was analyzed separately. The complete assay solution (1.0 ml) contained 4.4 mM γ-glutamyl-p-nitroanilide, 40 mM glycylglycine free base, 11 mM $MgCl_2.6H_2O$ and 0.1 M Tris buffer pH 8.8. The protein content of the samples were measured according to the method of Lowry et al (1951) using bovine serum albumin as a standard.

Statistical Analysis. The data were statistically analyzed by the test described by Fischer (1950) and $P < 0.05$ was considered significant.

RESULT AND DISCUSSION

The topical application of TPA at both the dose levels led to a significant increase in the enzyme activity (Table 1). Similarly, the topical application of carcinogenic polyaromatic hydrocarbons also showed a significant increase in GGT activity (Table 2), but it was less in comparison to that of TPA treated animals, in the doses as provided here in this experiment. In contrast to TPA, BaP and DMBA treated animals, the GGT activity remains unaffected after the application of weakly carcinogenic or noncarcinogenic PAHs chrysene and pyrene at all the doses tested on mouse skin (Table 2).

GGT is a membrane bound enzyme which catalyzes the transfer of γ-glutamyl group to a wide variety of amino acid acceptors (Hanes et al 1952, Meister 1973; Meister et al 1976). Its functional significance, even in organs demonstrating high GGT activity is still

Table 1. Effect of TPA on the activity of GGT in mouse skin

Treatment and Dose	Enzyme Activity^	Percent Increase over Controls
Acetone (100 µl)	0.256 ± 0.03	-
TPA (5 µg)	0.417 ± 0.04*	62
TPA (10 µg)	0.731 ± 0.05**	185

All the values represent the mean ± SE of 6 animals.
^-nmoles-p-nitroaniline liberated/min/mg protein.
*$p < 0.05$, **$p < 0.001$

Table 2. Effect of polyaromatic hydrocarbons on the GGT activity in mouse skin

Treatment and Dose	Enzyme Activity^	Percent Increase over Control
Acetone (100 µl)	0.256 ± 0.03	-
BaP (10 µg)	0.393 ± 0.03*	53
BaP (25 µg)	0.561 ± 0.05**	119
DMBA (50 µg)	0.370 ± 0.04*	45
DMBA (100 µg)	0.407 ± 0.05*	59
Chrysene (50 µg)	0.271 ± 0.02	6
Chrysene (100 µg)	0.265 ± 0.03	4
Pyrene (25 µg)	0.293 ± 0.04	14
Pyrene (50 µg)	0.317 ± 0.04	24

All the values represent the mean ± SE of 6 animals.
^-nmoles of p-nitroaniline liberated/min/mg protein.
*$p < 0.05$; **$p < 0.01$ when compared over controls.

unknown. One proposal is that the enzyme is important in the transport of amino acids into the cells (Orlowski and Meister 1970). However, in one system studied, the active site of the enzyme was on the outer surface of the cell (Horiuchi et al 1978), a fact inconsistent with the transport function. The disparity between the high levels of GGT found in fetal and neoplastic liver compared to the low levels in the adult rat and mouse liver have made it an attractive putative marker for hepatic neoplasia (Fiala and Fiala 1973; Kalengayi et al 1975). The relationship between cell proliferation and GGT activity can also be established because the activity of GGT has been found to be localized in the lower epithelial portions of growing hair follicles (Young et al 1978) and this area has been reported to be the most active site for cell duplication in the skin (Ackerman 1975).

In the present study we demonstrated that the activity of GGT in mouse skin was significantly induced after a single application of tumour promotor TPA, a well known inducer of cell proliferation (Argyris 1980). Some carcinogenic polycyclic aromatic hydrocarbons also showed GGT inducing capability in mouse skin. However the weakly carcinogenic or noncarcinogenic PAHs chrysene and pyrene, produced no change in GGT activity. One possible explanation of the GGT inducing ability of the carcinogenic and tumour promoter compounds could be due to the previously established relationship between cell proliferation and GGT activity (Richards and Astrup 1982). From this study we conclude that measurement of GGT levels in mouse skin may be useful in evaluating carcinogenic and/or cocarcinogenic risk of xenobiotics.

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