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Modification of Genetic Damage by Dihomo-gamma-linolenic Acid

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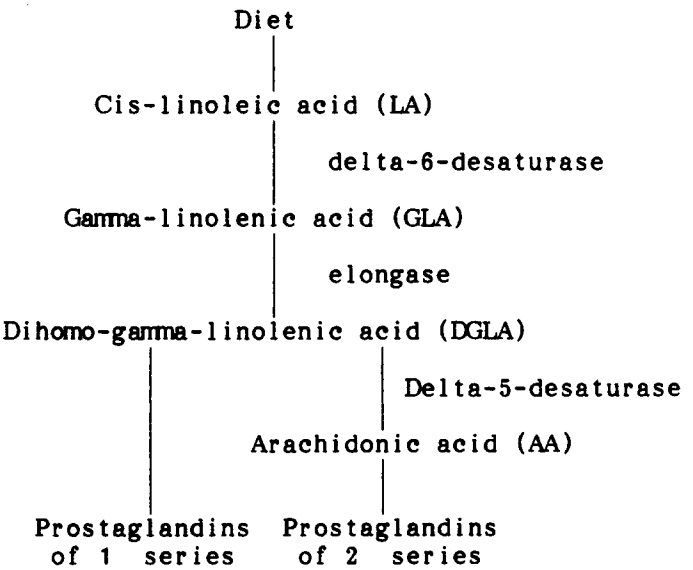
Mankind is exposed to several environmental and atmospheric pollutants such as polyaromatic hydrocarbons (PAH), pesticides, drugs etc., Benzo(a) pyrene (BP) is the prototype of PAH which occurs in smoked foods, soot and cigarette smoke (Griciute 1982) and thus a wide spread atmospheric pollutant. BP is not mutagenic per se but undergoes metabolic activation for exerting its mutagenic potential (Kliesch et al 1982). Since the total elimination of atmospheric pollutants is impractical, we have begun to identify the modifiers of mutagenicity from natural sources (Das et al 1989). The present investigation deals with the role of Dihomo-gamma-linolenic acid (DGLA), a derivative of dietary linoleic acid (Scheme-1) on genetic damage. Micronucleus test was employed to study BP induced genetic damage in vivo and chromosomal aberration tests in human lymphocyte cultures to study phorbol myristate acetate (PMA) induced chromosomal damage.

Scheme - 1



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MATERIALS AND METHODS

BP, PMA and DGLA were obtained from Sigma Chemical Co., St.Louis, USA. BP was dissolved in corn oil where as PMA and DGLA were dissolved in ethanol. For in vivo studies random bred swiss mice aged 6-7 weeks weighing 24-27g were randomly distributed to different groups of four animals each. Three different sets of experiments were performed as follows :

i.Dose response : Different concentrations of DGLA (10^{-4}M, 10^{-6}M and 10^{-8}M) were prepared fresh in saline and were administered intraperitonially. BP (75 mg/kg) dissolved in corn oil and fed orally one hour after DGLA treatment. The animals were sacrificed by cervical dislocation 24 hours after the treatment schedule and bone marrow smears were prepared as per the method of Salamone et al (1980).

ii.Time Course : 10^{-6}M concentration of DGLA was administered 24, 48, 72 and 96 hours prior to the mutagen treatmetn. Animals were sacrificed always 24 hours after BP treatment and the smears were prepared as above.

iii. Post treatment : 10^{-6}M DGLA was administered one hour after BP treatment and the animals were sacrificed 24 hours after the treatment schedule and the smears were prepared as above.

The frequency of micronuclei was screened from 1000 polychromatic erythrocytes (PCE) with the corresponding number of normochromatic erythrocytes (NCE) from each animal. The presence of micronuclei in PCE is an index of genetic damage. Further, the ratio of PCE to NCE can be used as a measure to estimate the effect on proliferative activity of bone marrow. The scoring was done blindly and the slides were decoded before statistical analysis. The results were analyzed by analysis of variance followed by Newmann-Kuel's test.

For in vitro studies, lymphocytes were cultured in RPMI 1640 medium by the method of Moorhead et al (1960). Blood was obtained from young healthy donors. PMA at 200 ng/ml was used to induce chromosomal damage and DGLA was tested at 10^{-7}M and 10^{-9}M. All the supplements were done at 48 hours of the culture. Three individual samples were cultured to minimize sample variation. One hundred well spread metaphases from each sample were scored for the presence of chromatid, isochromatid gaps and breaks and the results were analyzed by Student's "t" test.

(3H) thymidine incorporation was studied in cultured lymphocytes, the culture method was the same as that of lymphocytes except that (3H) thymidine (Sp. activity 18 mci/mmol, BARC, India) was supplemented to the culture for 16 hours. The cultures were terminated at 72 hours as usual

and the DNA was extracted and counted in a liquid scintillation counter. Student's "t" test was performed to assess the statistical significance between the groups.

RESULTS AND DISCUSSION

The incidence of micronuclei with different concentrations of DGLA alone and in combination with BP are presented in Table-1. BP increased the frequency of micronuclei (1.18%) over control (0.35%). Pretreatment with DGLA (10^{-4}M, 10^{-6}M and 10^{-8}M) inhibited BP induced micronuclei in PCE ($P < 0.05$). DGLA did not differ from control except at the highest concentration tested (10^{-4}M). The P/N ratio for all the treatments was in the range of one indicating that the cell proliferation was not affected by any of these treatments.

Table 1. Incidence of micronuclei (MN) in the bone marrow erythrocytes of Swiss albino mice treated with benzo(a) pyrene, dihomo-gamma-linolenic acid (DGLA): A dose response study.

Treatment	MN in Polychromatic erythrocytes		MN in normochromatic erythrocytes		P/N
	Cells counted / cells with MN	%	Cells counted / cells with MN	%	
Control	4000/14	0.35	3910/6	0.15	1.02
Corn oil	4000/13	0.33	3955/8	0.20	1.01
B(a)P	4000/47	1.18#	4027/12	0.30	0.99
DGLA(10^{-4}M)	4000/19	0.48#	3988/9	0.23	1.00
DGLA(10^{-6}M)	4000/16	0.40	4010/6	0.15	1.00
DGLA(10^{-8}M)	4000/16	0.40	3994/6	0.15	1.00
BP+DGLA(10^{-4}M)	4000/31	0.78*	4014/9	0.22	1.00
BP+DGLA(10^{-6}M)	4000/23	0.58*	4012/8	0.20	1.00
BP+DGLA(10^{-8}M)	4000/28	0.70*	3981/6	0.15	1.00

Compared to control, * compared to B(a) alone, ($P < 0.05$).

In the time course experiment, DGLA at 10^{-6}M was tested over different time intervals (interval between DGLA treatment and BP) to assess the longivity of the protective effect and the results are represented in Fig. 1.

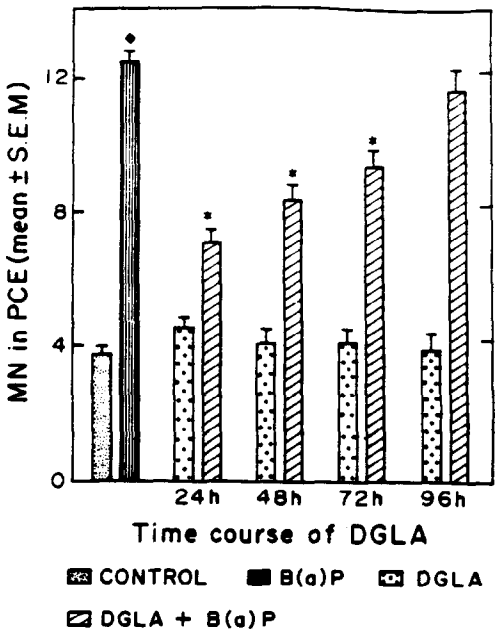

Fig. 1. Effect of DGLA at 10^{-6}M on B(a)P induced micronuclei at different time intervals.

DGLA could inhibit BP induced damage till 72 hours ($P < 0.05$), the effect was more pronounced at 24 hours and gradually decreased as the interval betweent the treatments increased. The incidence of BP induced micronuclei was also reduced with post treatment of DGLA at 10^{-6}M ($P < 0.05$) and is represented in Table-2.

Table 2. Incidence of micronuclei (MN) in the bone marrow cells of mice treated with DGLA and Benzo(a) pyrene: Post treatment.

Treatment	MN in Polychromatic erythrocytes		MN in normochromatic erythrocytes		P/N
	Cells counted / cells with MN	%	Cells counted / cells with MN	%	
Control	12000/40	0.33	11891/23	0.21	1.01
BP(75mg/Kg)	12000/159	1.33#	12031/48	0.40	0.99
DGLA(10^{-6}M)	12000/55	0.46	11796/34	0.29	1.01
BP+DGLA	12000/93	0.76*	11845/38	0.32	1.01

Compared to control, * compared to BP alone ($P < 0.05$).

In in vitro human lymphocyte cultures, PMA induced significant number of gaps (30%) and breaks (27%) whereas other chromosomal aberrations like rings, dicentrics and structurally rearranged chromosomes were negligible (1-2%).

These observations are in agreement with Emerit et al (1983). The number of breaks and total aberrations are represented separtely (Fig. 2). DGLA at both the concentrations 10^{-7}M and 10^{-9}M significantly reduced the chromosomal damage induced by PMA. The overall distribution among various types of aberrations caused by PMA was not altered by DGLA eventhough a significant reduction of gaps, breaks and total aberrations was observed. Incorporation of (3H) thymidine was monitored to check the role of PMA and DGLA on DNA synthesis. None of the treatments differ from control significantly (Table-3).

Table 3. Incorporation of (3H) thymidine in human lymphocytes in vitro with PMA at 200 ng/ml and DGLA.

Treatment	Mean counts/min X $10^3 \pm$ S.E.
Control	40.33 + 4.54
PMA	35.55 + 2.99
DGLA 10^{-7}M	42.55 + 3.98
DGLA 10^{-9}M	39.36 + 4.76
DGLA 10^{-7}M + PMA	43.40 + 1.78
DGLA 10^{-9}M + PMA	40.87 + 4.66

PMA is a potent tumor promoter in mouse skin and causes chromosomal damage through indirect action in human lymphocyte cultures in vitro (Emerit and Cerutti 1981) and primary mouse epidermal cells in culture (Dzarlieva and Fusenig 1982). It exerts its action on membrane phospholipids and releases free arachidonic acid (AA) by stimulating phospholipase A_2. Oxidation of AA by cyclooxygenase system results in the formation of 2 series of prostaglandins (PGs) and by lipoxygenase system results in the formation of leukotrienes via the hydroxy and hydroperoxy components of AA. Several carcinogens including BP get cooxidized during the formation of PGs of the 2 series (Sivarjah et al 1978; Nordenskjold et al 1984). Both AA and its products were shown to be genotoxic (Das et al 1989). AA is formed through two desaturation steps (Scheme-1) from linoleic acid (LA), a dietary n-6 polyunsaturated fatty acid. The action of delta-6-desaturate (d-6-d) on LA produces gamma-linolenic acid (GLA) which is elongated to dihomo-gamma-

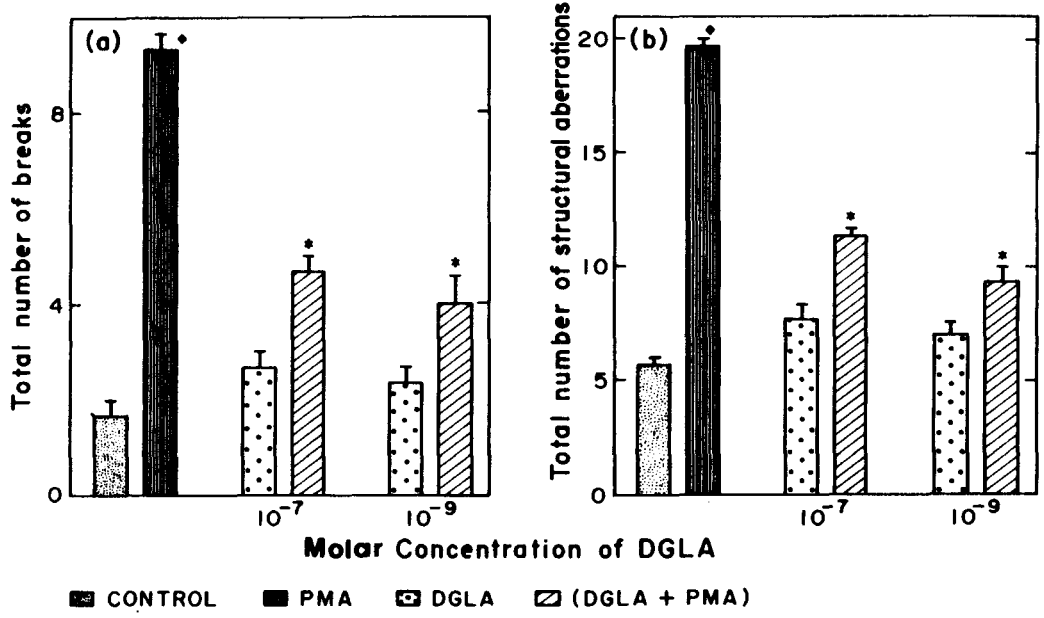

Fig. 2: Effect of DGLA on PMA induced chromosomal damage
(a) Number of breaks and (b) Total aberrations in human lymphocytes in vitro

linolenic acid (DGLA) by an enzyme elongase. DGLA is the precursor for both AA, and prostaglandins of 1 series. However, AA formation from DGLA is rate limited by delta-5-desaturase (d-5-d) and it was reported that d-5-d levels are negligible in adults (Stone et al 1979) Hayastu et al (1988) suggested that fatty acids can act as antimutagens by extracellularly trapping the mutagen in fatty acid micelles. However, the fact that the post treatment also inhibited BP enduced damage indicates that this mechanism is unlikely to account for the protective action of DGLA observed in the present study. Miller et al (1988) reported that incubation of (^{14}C) DGLA with microsomal fraction from guinea pig epidermis resulted in predominant formation of PGE_1. PGE_1 was found to be antimutagenic over a wide range of concentrations (Sridevi et al 1990) and against different mutagens like BP, Diphenyldantion and gamma-radiation (Das et al 1989). Hence it can be suggested that DGLA may be exerting its action through the formation of PGE_1.

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