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Modification of Benzo(a)pyrene Induced Chromosomal Damage in Mouse Bone Marrow by Vitamin A

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Benzo(a)pyrene (BP) is one of the major atmospheric pollutants emitted in coal furnaces, automobile exhausts etc. It exerts its mutagenic/carcinogenic effects only after metabolic activation (Kliesch et al 1982). The metabolic conversion of BP and majority of polycyclic aromatic hydrocarbons is catalysed in vivo by the microsomal mixed function oxydase system. It was reported that during the process of oxygenation of BP leading to the formation of the ultimate mutagenic product, arachidonic acid (AA) was converted to prostaglandins (PGs) of 2 series (Sivarajah et al 1978). Further, the oxygenated product of BP namely 7,8-dihydrodiol was mutagenic to Salmonella typhimurium TA98 on incubation with ram seminal vesicle microsomes in the presence of AA (Marnett et al 1978).

Several experimental studies indicated that malignant transformation could be inhibited by retinoids supressing the expression of the initiated cell (Chopra and Wilkoff 1975; Chopra and Wilkoff 1977; Merriman and Bertram 1979; Todaro et al 1978). Some other reports suggest that chemoprevention of retinoids results from interaction with a group of chemical substances which act as tumor promoters (Kensler and Mueller 1978, Weeks et al 1979). Furthermore, retinoids inhibited tumors induced in gastrointestinal tract, cervix, skin and the breast (Chu and Malmgren 1965; Davies 1967; Moon et al 1977). However, the exact mechanism of action is uncertain. It is well known that genetic damage induced by mutagens/carcinogens is implicated in predisposition to cancer. In this paper data are presented showing the modulatory effect of vitamin A on the genetic damage induced by BP in the bone marrow cells of mice.

MATERIALS AND METHODS

BP and Vitamin A (Retinol Palmitate) were obtained from Sigma Chemical Co., St. Louis, USA, and Roche Co., Bombay, India, respectively. BP was dissolved in corn oil whereas Vitamin A was dissolved in normal saline and administered orally to each mouse. Random bred Swiss mice aged 7-8 weeks weighing 24-27 g. were randomly distributed to control and treated groups as follows:

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Table 1 : Incidence of micronuclei in the bone marrow cells of mice treated with Benzo(a)pyrene and Vitamin A.

Group	No. of PCE Scored	% of MN in PCE	No. of NCE Scored	% of MN in NCE	PCE/NCE ratio
1.	6090	0.39	4929	0.12	1.2
2.	6121	0.29	5000	0.04	1.2
3.	6108	1.45*	4939	0.48	1.2
4.	6094	0.45	5111	0.21	1.2
5.	6112	0.44	6045	0.19	1.0
6.	6034	0.43	4665	0.21	1.3
7.	6076	0.32	5597	0.16	1.1
8.	6105	0.39	6048	0.19	1.0
9.	6020	0.29	4740	0.12	1.3

* $P<0.05$

PCE = polychromatic erythrocytes
NCE = normochromatic erythrocytes.
MN = micronucleus

Group 1 : Control, received normal saline.
Group 2 : Control received corn oil.
Group 3 : Animals received BP 75 mg/kg.
Group 4 : Animals received BP and Vitamin A 750 ug/kg 1 hr before BP treatment.
Group 5 : Received BP and Vitamin A 900 ug/kg 1 hr before BP treatment.
Group 6 : Received BP and Vitamin A 1500 ug/kg 1 hr before BP treatment.
Group 7 : Animals received Vitamin A 750 ug/kg only.
Group 8 : Animals received Vitamin A 900 ug/kg only.
Group 9 : Received Vitamin A 1500 ug/kg only.

Control and treated animals were sacrificed 36 h after the treatment. Preparation of bone marrow smears was carried out following the method of Schmid (1976) and stained with Giemsa and mounted in DPX. Three animals were used in each group and about 2000 polychromatic erythrocytes (PCE) and corresponding number of normochromatic erythrocytes (NCE) per animal were scored for the presence of micronuclei. The presence of micronuclei in PCE was used as an indicator of genetic damage. Further, the ratio of PCE to NCE was used to estimate to see the effect on the proliferative activity of the bone marrow cells. The scoring was done blindly and the slides were decoded before statistical analysis. The incidence of micronuclei in PCE in the treated series compared to controls was carried out by the approximate t-test as suggested by

Goldstein (1965), assuming that the occurance of micronuclei follow a Poisson distribution.

RESULTS AND DISCUSSION

The results obtained on the incidence of micronuclei in various groups of mice are presented in table. The data show an increase in the frequency of micronuclei in PCE in group 3 compared to control groups 1 and 2. Pre-treatment of Vitamin A inhibited BP induced mutations in PCE (Group 4-6), whereas Vitamin A alone (Group 7-9) did not show any increase in the number of micronuclei in PCE compared to controls. Further, there is no reduction in the ratio of PCE to NCE, suggesting that there was no effect of BP or Vitamin A on the proliferative activity of bone marrow cells (Group 3-9). The results of the present investigation clearly demonstrate that Vitamin A can prevent the induction of chromosome damage by BP in vivo.

Earlier it was reported that the metabolic activation of BP to its mutagenic/carcinogenic forms occurs through the activation of prostaglandin endoperoxide synthetase (Sivarajah et al 1978, Marnett et al 1978). During this process an excess production of PCE2 and PGF2 alpha occurs in the cells. Our earlier studies showed that gamma linolenic acid (GLA) and PGE1 inhibited the genetic damage induced by radiation and chemicals in mice (Das et al 1985a 1985b). Hence, it can be expected that the enhancement of the levels of dihomo gamma linolenic acid (DGLA) and PGE1 could bring about similar results. Vitamin A was shown to inhibit the activity of delta-5-desaturase which is essential for the conversion of DGLA to AA in cells, and this action of Vitamin A could result in an increase in the levels of DGLA (Alam et al 1984). Further, DGLA is also converted into PGE1 by prostaglandin synthetase. It is logical to speculate that the protective effect of Vitamin A observed in the present study may be due to its action on delta-5-desaturase with consequent rise in the levels of DGLA and PGE1 which have been shown to be antimutagenic in earlier studies.

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