Available online at www.sciencedirect.com
SCIENCE DIRECT
Prostaglandins, Leukotrienes and Essential Fatty Acids 74 (2006) 165–173
Prostaglandins Leukotrienes and Essential Fatty Acids
www.elsevier.com/locate/plefa

Effect of γ-linolenic acid and prostaglandins E_1 on gamma-radiation and chemical-induced genetic damage to the bone marrow cells of mice

Undurti N. Das[a,*], Kaipa P. Rao[b]

[a] *UND Life Sciences, 13800 Fairhill Road, #321, Shaker Heights, OH 44120, USA*
[b] *Department of Genetics, Osmania University, Hyderabad 500007, India*

Received 19 September 2005; accepted 7 January 2006

Abstract

The effect of γ-linolenic acid (GLA) and prostaglandin E_1 (PGE_1) on γ-radiation, diphenylhydantoin (DPH), benzo(a)pyrene (BP), and 4-α-phorbol-induced genetic damage to the bone marrow cells of mice, using the sensitive micronucleus (MN) test was investigated. PGE_1 and its precursor GLA prevented γ-radiation, DPH, BP, and 4-α-phorbol-induced genetic damage.

Keywords: Prostaglandin e1; Gamma-linolenic acid; Micronucleus test; Mutagenesis; Carcinogenesis; Polyunsaturated fatty acids; Prostaglandins

1. Introduction

A strong link exists between the location of cellular oncogenes and the chromosomal aberrations observed in various forms of cancer. It has been suggested that fragile sites on chromosomes probably predispose to chromosomal rearrangement and thus, to human neoplasia. The association between genes/oncogenes, chromosome aberrations and translocations, and heritable fragile sites in cancer has recently been reviewed [1–3].

Polycyclic aromatic hydrocarbons such as benzo(a)-pyrene (BP) are ubiquitous environmental pollutants that are capable of causing DNA damage, and thus, act as mutagenic and carcinogenic agents [4]. BP and other similar polycyclic aromatic hydrocarbons undergo intracellular oxidation that leads to the generation of reactive metabolites such as benzo(a)pyrene-dihydrodiol epoxide (BPDE). BPDE is the ultimate carcinogen that forms a covalent linkage with the exocyclic amino group of deoxyguanosine to form a bulky hydrophobic adduct [5]. Error-prone replication of adducted DNA templates during S-phase generates mutations that, in turn, activate oncogenes or inactivate tumor-suppressor genes (anti-oncogenes) leading to the development of cancer.

Cellular DNA is continuously exposed to several genotoxic insults from both endogenous and exogenous sources. Therefore, cells have evolved various mechanisms to minimize the detrimental effects of DNA damage by repairing the damage and/or allowing the cells to undergo apoptosis so that these abnormal cells are eliminated that is expected to prevent carcinogenesis. This implies that some endogenous molecules that have the ability to trigger DNA repair process are present in the cells. Identification of such endogenous molecules is important so that they could be exploited to enhance DNA repair process and/or to eliminate cells with DNA damage.

Radiation causes mutations and cause cancer by its ability to damage DNA, possibly, by the generation of free radicals, physically disrupting DNA or both [6]. It is known that radiation activates phospholipase A_2 (PLA_2) leading to the release of arachidonic acid (AA) from the cell membrane lipid pool, such that excess of

*Corresponding author. Tel.: +1 508 668 5324; fax: +1 928 833 0316.
E-mail address: undurti@hotmail.com (U.N. Das).

0952-3278/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.plefa.2006.01.002

prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ formation occurs [7]. It was also reported that PGI_2 synthesis is decreased by radiation [8,9]. In an earlier study, we showed that PGI_2 is a potent anti-mutagen [10]. This suggests that radiation-induced mutagenesis is due to decreased production of PGI_2.

BP is also known to enhance PGE_2 and $PGF_{2\alpha}$ production in MDCK cells in culture, possibly during the formation of reactive oxygen intermediates of BP [11–14]. In fact, there is evidence to suggest that many mutagens and carcinogens stimulate $PGE_2/PGF_{2\alpha}$ production during their activation to reactive metabolites [11–15]. But, it not clear whether BP can also decrease PGI_2 synthesis and release, though this is expected in view of the competition between PGE_2 and PGI_2 synthesis from the same precursor, AA.

Diphenylhydantoin (DPH), a widely used anti-epileptic drug, has been shown to cause genetic damage both in vitro and in vivo [16,17]. Studies revealed that DPH could be enzymatically bioactivated to a reactive intermediate leading to increased formation of reactive oxygen species that have the ability to damage DNA [18–20]. In addition, DPH can selectively block PGE_1 formation, and antagonize PG-actions and enhance the production of PGE_2 and $PGF_{2\alpha}$ from AA [21,22].

Phorbol esters function as tumor promoters. Phorbol ester 12-O-tetradecanoyl-phorbol 13-acetate (TPA) enhances cell transformation, modulates cell differentiation, and inhibits mixed lymphocyte reaction, and has several other biochemical effects [23]. TPA acts on cell membranes and interferes with cell–cell communication. In neutrophils, PMA causes aggregation, enzyme secretion, respiratory burst resulting in free radical generation, and protein phosphorylation [24]. PMA also causes DNA damage and enhances PG formation. On the other hand, 4-α-phorbol is considered as its inactive analogue. Thus, radiation, BP, DPH, and 4-α-phorbol all have actions on free radical generation in one way or the other, modulate PG formation, and induce DNA damage.

Previously, we showed that radiation, BP, and DPH-induced DNA damage can be modified by PGs [25–28]. In the present study, we present data concerning the effect of PGE_1 on radiation, BP, DPH, and 4-α phorbol-12, 13-didecanoate (PDD)-induced DNA damage to bone marrow cells of mice using micronucleus (MN) test. We also report the effect of γ-linolenic acid (GLA), the precursor of PGE_1, on BP-induced DNA damage to bone marrow cells of mice.

2. Materials and methods

2.1. Preparation of PGE_1, GLA, and other chemicals

BP, DPH, 4-α-phorbol, PGE_1, GLA, and corn oil were purchased from Sigma Co., USA. Swiss male mice aged 7–8 weeks and weighing 24–27 g were randomly divided into several groups depending on the experimental protocol. Stock solutions of PGE_1 and GLA were prepared initially in ethanol at a concentration of 10^{-3} M and stored at −20 °C. Working concentrations of PGE_1 and GLA were in normal saline, prepared just before use. The ethanol concentration in the final working concentrations of the solutions was never more than 0.1%, at which concentration it (ethanol) was found not to affect the bone marrow [25–28]. BP and 4-α-phorbol were dissolved in corn oil, whereas DPH was dissolved in normal saline. BP, 4-α-phorbol, and DPH were freshly prepared on the day of use. Corn oil and saline were used as vehicle controls wherever it was appropriate.

2.2. MN test

The MN test affords a procedure for the detection of aberrations involving anaphase chromosome behavior utilizing the bone marrow erythroblast [29]. The test is based on the formation of "micronuclei" from particles of chromatin material that, due to chromosome breakage or spindle dysfunction, do not migrate to the poles during anaphase and are not incorporated into the telophase nuclei of the dividing cell. Such chromatin fragment or even whole chromosomes in the case of chromosome lag, result in the formation of one or more small satellite nuclei in the cytoplasm of the daughter cells. When the treatment time with the mutagens or carcinogens is between 24 and 30 h, the majority of these micronuclei are found in the newly formed polychromatic erythrocytes (PCE).

For the purpose of detecting genetic damage in the form of chromosome disrupting capacity, the PCE have some very useful characteristics. The expulsion of the mammalian erythrocyte nucleus follows the final mitotic division by several hours, but the resulting enucleated erythrocytes retain their cytoplasmic basophilia for approximately 24 h after nuclear expulsion. In addition, the micronuclei in the cytoplasm of these cells are not expelled with the nucleus. In view of this, if the bone marrow of a test animal is examined after 24 h after treatment it is possible to discriminate between erythrocytes formed during the treatment and those formed prior to treatment. In this case, each animal could be considered to serve as its own control in as much as the frequency of micronuclei in the PCE formed during treatment may be compared with the frequency of micronuclei in the normochromatic erythrocytes (NCE) formed prior to treatment. Any mutagenic response during the 24-h time interval would result in an increase in the frequency of micronuclei only in the PCE population. Furthermore, the ratio between PCE and NCE under normal physiological conditions is ∼1 (PCE/NCE = ∼1). Any significant decrease in the