Available online at www.sciencedirect.com
SCIENCE DIRECT
Prostaglandins, Leukotrienes and Essential Fatty Acids 74 (2006) 271–282
Prostaglandins Leukotrienes and Essential Fatty Acids
www.elsevier.com/locate/plefa

Dietary γ-linolenate attenuates tumor growth in a rodent model of prostatic adenocarcinoma via suppression of elevated generation of PGE_2 and 5S-HETE

Hung Pham, Kao Vang, Vincent A. Ziboh*

Department of Dermatology TB-192, School of Medicine, University of California-Davis, Davis, CA 95616, USA

Received 30 November 2005; accepted 7 January 2006

Abstract

Prostate cancer poses considerable threat to the aging male population as it has become a leading cause of cancer death to this group. Due to the complexity of this age-related disease, the mechanism(s) and factors resulting in prostate cancer remain unclear. Reports showing an increase risk in prostatic cancer with increasing dietary fat are contrasted by other studies suggesting the beneficial effects of certain polyunsaturated fatty acid (PUFA) in the modulation of tumor development. The *n*-6 PUFA, γ-linolenic acid (GLA), has been shown to suppress tumor growth in vitro. Therefore, using the Lobund–Wistar (L–W) rat model of prostate cancer, we tested the hypothesis whether dietary supplementation of GLA could suppress tumor growth and development in vivo. Prostatic adenocarcinomas were induced in two groups of L–W rats, the experimental group (*N*-nitroso-*N*-methylurea, NMU/testosterone propionate, TP) and the GLA group (NMU/TP/GLA fed) undergoing similar treatment but fed a purified diet supplemented with GLA. Our findings revealed a decrease in prostate growth in the NMU/TP/GLA-fed group as determined by weight, tissue size, DNA content and prostate-specific antigen (tumor marker of prostate cancer). Comparison between the two groups showed a significant increase in 5*S*-hydroxyeicosatetraenoic acid and prostaglandin E_2 in the NMU/TP group. These increases paralleled the increased protein expression and activity of cyclooxygenase-2 as well as increased activity of 5-lipoxygenase. Taken together, the findings showed that intake of GLA-enriched diet does reduce prostatic cancer development in L–W rats and could serve as a non-toxic adjunct in management of human prostatic cancer.

1. Introduction

Prostate cancer poses a considerable threat to the aging male population, as it has become a leading cause of cancer death to this group [1]. This disease proceeds through two stages: a benign hyperplastic stage commonly occurring in the middle to the latter stages of life and a malignant neoplastic stage (prostate cancer) occurring widely among the westernized society [2,3]. The latter stage of prostate cancer is often characterized into two phases, an androgen dependent hyperplasia and an androgen independent neoplasia often occurring shortly after androgen ablation treatment for the prostatic enlargement. While the mechanism(s) and factors resulting in prostate cancer remain obscure, epidemiological studies have shown a positive correlation between prostate cancer mortality rates and consumption of fat, particularly polyunsaturated fatty acids (PUFAs) derived from animal fat [4,5]. These

Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; DGLA, dihomo-γ-linolenic acid; GLA, γ-linolenic acid; HETE, hydroxyeicosatetraenoic acid; LA, linoleic acid; L–W, Lobund–Wistar; LOX, lipoxygenase; NMU, *N*-nitroso-*N*-methylurea; PG, prostaglandin; PUFA, polyunsaturated fatty acid; PSA, prostate-specific antigen; TP, testosterone propionate

*Corresponding author. Tel.: +1 530 752 9765; fax: +1 530 752 9766.
E-mail address: vaziboh@ucdavis.edu (V.A. Ziboh).

0952-3278/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.plefa.2006.01.004

PUFAs, specifically arachidonic acid (AA, 20:4*n*-6), serve as precursors to a variety of mediators which influence carcinogenesis [6].

In contrast, certain dietary fats from plant sources, particularly γ-linolenic acid (GLA) derived from borage oil, have been recognized for its beneficial effects in regressing cellular proliferation and tissue hypertrophy. GLA, a member of the $n-6$ PUFA family, is synthesized from linoleic acid (LA, 18:2*n*-6) by Δ^6-desaturation (a rate-limiting step) and then rapidly elongated to dihomo-γ-linolenic acid (DGLA, 20:3*n*-6). The elongated DGLA can undergo desaturation by a Δ^5-desaturase (a rate-limiting step) to form AA (20:4*n*-6). However, the finding that DGLA not AA accumulates in vivo with GLA supplementation is of interest because GLA is capable of attenuating the downstream biosynthesis of AA metabolites and thereby exert an anti-inflammatory effect in human subjects [7,8].

Due to the complexity of this disease, establishing a representative animal model for the different stages of prostate cancer development has been a limiting factor; investigations using animal models of prostate cancer have been limited. However, the current model best representing prostate cancer other than the human disease has been a rat model of prostatic adenocarcinoma developed by Pollard [9]. This experimental animal model of prostatic adenocarcinomas exhibiting androgen dependency as in human beings with high disease spontaneity, high incidences of clinically evident induced tumors, long latency periods and the development of additional neoplasms in tissue other than the prostate was developed in the Lobund–Wistar (L–W) rats. The induction of prostatic adenocarcinomas with high incidence (70–90%) within 10 months in these rats is accomplished by an initial injection of *N*-nitroso-*N*-methylurea (NMU), serving as the initiator, followed by implantation of testosterone propionate (TP), serving as the promoter [10].

Although GLA has been reported to suppress tumor growth in vitro using androgen-primed prostatic hyperplastic and tumorigenic cells which were derived from the L–W rats [11], the efficacy of GLA-enriched diet to attenuate prostatic carcinogenesis in vivo has not been demonstrated. Because we demonstrated recently in our laboratory that pre-incubation of androgen-primed prostatic hyperplastic and tumorigenic cells with GLA and DGLA 15-lipoxygenase (15-LOX) metabolite (15S-HETrE) resulted in the suppression of the cellular growth and cyclooxygenase-2 (COX-2) overexpression/prostaglandin E_2 (PGE_2) biosynthesis, we tested the hypothesis that supplementing the diet of the L–W rat model of prostatic adenocarcinoma with GLA-enriched borage oil would suppress the in vivo elevated biosynthesis of eicosanoids as well as growth of prostatic adenocarcinoma.

2. Materials and methods

2.1. Experimental animal model and diets

Male Wistar rats (8 weeks old, 250 g) purchased from Harlan (Los Angeles, CA) were maintained in an isolated environment with a 12-h light cycle (0700–1900) and a constant temperature of 25 °C. The rats were randomly divided into three groups but individually housed in plastic cages with adequate amount of bedding material.

2.2. Preparation and assay of dietary constituents

The diets were prepared using 1 kg of AIN-93G purified rodent diet (Dyets Inc., Bethlehem, PA) mixed with 70 g of corn oil for control diet or 35 g of corn oil and 35 g of borage oil for experimental diet. These diets were prepared weekly using sterile equipment and samples were taken daily to monitor for lipid oxidation. Briefly, total lipids were extracted from 0.1 g of the prepared control or experimental diet with 1 mL of chloroform/methanol (2:1, v/v). The extracted lipids (100 μL) were transferred to a methylation tube containing 50 μg of heptadecanoate (17:0) as an internal standard (Nu-Chek-Prep, Elysian Fields, MN) and methylated with 3 mL of 6% HCl in methanol for 5 h at 75 °C. Methylated fatty acids were extracted using 2 mL of petroleum ether and centrifuged at 2500 rpm for 5 min. The petroleum ether layer was transferred to a vial, dried under nitrogen, and reconstituted with dichloromethane (250 μL). Prior to analyses of the samples, standard reference mixtures of fatty acid methyl esters from Nu-Chek-Prep (Elysian Fields, MN) were resolved by gas–liquid chromatography (GC) to determine retention times for the known fatty acids. Fatty acid methyl esters from the samples were subsequently resolved and quantified on a GC-17A (Shimadzu, Pleasanton, CA) equipped with a DB-225 fused silica capillary column (50% cyanopropylphenyl, 0.15 mm film thickness) 30 m × 0.25 mm i.d. (J&W Scientific, Rancho Cordova, CA). Operating condition was isothermal at 210 °C using helium as the carrier gas at 36 cm/s. Detection of fatty acid methyl esters was performed with a flame ionization detector set at 250 °C and quantification of fatty acid methyl esters was determined by the heptadecanoate (17:0) internal standard. Alterations in individual fatty acid molar ratio with a statistical significance of $P<0.05$ indicated the presence of lipid oxidation and resulted in the preparation of a new batch of diet.

2.3. Animal groups

The three groups consisting of 12 rats/group (36 total) were monitored daily and weighed weekly throughout