Effect of Isocaloric Low-Fat Diet on Prostate Cancer Xenograft Progression to Androgen Independence

Tung H. Ngo, R. James Barnard, Todd Anton, Chris Tran, David Elashoff, David Heber, Stephen J. Freedland, and William J. Aronson

Abstract

An isocaloric low-fat diet has been shown to slow androgen-sensitive Los Angeles Prostate Cancer-4 (LAPC-4) tumor growth in a mouse xenograft model. LAPC-4 cells were injected into male severe combined immunodeficient mice. After palpable tumors developed, the mice were divided into three groups, high-fat intact, high-fat castration, and low-fat castration. Tumor latency (18 versus 9 weeks; \( P < 0.001 \)) and mouse survival (20.8 \( \pm \) 1.3 versus 13 \( \pm \) 0.7 weeks; \( P < 0.01 \)) were significantly longer in the low-fat castration versus high-fat castration group. Reduced dietary fat intake delayed conversion from androgen-sensitive to -insensitive prostate cancer and significantly prolonged survival of severe combined immunodeficient mice bearing LAPC-4 xenografts.

Introduction

Prostate cancer growth is initially highly dependent on androgens, and androgen suppression leads to significant reduction in tumor burden in most patients. However, androgen insensitive (AI) disease inevitably develops resulting in tumor regrowth, metastasis, and eventual mortality. Presently, the biological mechanisms involved in the conversion from androgen sensitive (AS) to AI disease remain undetermined, and no effective treatments exist to prolong survival in men with AI prostate cancer.

Dietary fat intake may play a role in AI prostate cancer growth. Linoleic acid (\( \omega-6 \) polyunsaturated fatty acid) from corn oil is the predominant fatty acid in the American diet (largely in baked and fried goods). Linoleic acid has been found to exert a stimulatory effect on the growth of AS (LNCaP) and AI (PC-3) human prostate cancer cell lines (1). Moreover, animal feeding studies found increased \( \omega-6 \) dietary fat intake increased the growth of AS prostate cancer xenografts (2, 3). Membrane arachidonic acid (\( \omega-6 \)) derived from linoleic acid is converted by cyclooxygenase-2 to prostaglandin E\(_2\), which has been shown to promote prostate cancer cells growth in vitro (4, 5). Arachidonic acid is also metabolized by the lipoxygenase pathway to eicosanoids (leukotrienes and hydroxy derivatives of fatty acids) that play an important role in tumor progression and metastasis (6).

Results

Animal Husbardry, Feeding Protocol, and Los Angeles Prostate Cancer-4 (LAPC-4) Injection. Twenty-four male CB17 beige severe combined immunodeficient mice (8 weeks old) were obtained from the University of California Los Angeles Department of Laboratory Animal Medicine facility, which is accredited by the American Association for Accreditation of Laboratory Animal Care. The mice were housed 1 per cage for the maintenance of isocaloric intake between the groups. The experiments were approved by the University of California Los Angeles Chancellor’s Animal Research Committee, and animals were cared for in accordance with institutional guidelines.

The diets were prepared and sterilized (irradiated) by DYETS, Inc. (Bethlehem, PA). The high-fat diet contained 42% calories from corn oil, and the low-fat diet contained 12% calories from corn oil (Table 1). Equal caloric intake between the groups was maintained throughout the experiment by using a modified paired-feeding technique as described previously (2, 7).

After 2 weeks of the high-fat diet, 10\(^3\) LAPC-4 tumor cells in 0.1 ml of Matrigel (Collaborative Biomedical Products, Bedford, MA) were injected s.c. in the flank of all of the mice. Tumor cells were obtained from separately caged severe combined immunodeficient mice used for tumor propagation. All of the animals were maintained on a high-fat diet until they developed palpable tumors, at which time they were divided into three groups; group 1 (\( n = 4 \)) continued to receive the high-fat diet (HF) and did not undergo castration; group 2 (\( n = 10 \)) underwent castration and continued to receive the high-fat diet (HFC); and group 3 (\( n = 10 \)) underwent castration and was placed on the low-fat diet (LFC).

LAPC-4 Xenografts. The LAPC-4 cell line was a generous gift from Drs. Robert Reiter and Charles Sawyers (UCLA Departments of Urology and Medicine, Los Angeles, CA). Throughout the experiment, mice were weighed and tumors examined weekly. Tumor volumes, measured by calipers, were calculated using the formula length \( \times \) width \( \times \) height \( \times \) 0.5236.

Serum Studies and Tumor Studies. The animals were euthanized when they met institutional guidelines (ruffled fur, hunched posture, impaired ambulation, lethargy, decreased feeding, weight loss, and so forth). Serum from the brachial artery was collected at the time of sacrifice and stored at \(-80^\circ C\). Serum was also obtained from the LFC mice via the tail vein at the time the HFC mice were euthanized. Human serum prostate-specific antigen (PSA) was measured by ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX). At the time of sacrifice, the tumors were removed, weighed, and tumor dimensions measured.

Statistical Analysis. Statistical analyses (InStat Statistical Software; Graphpad, San Diego, CA) were performed by Student’s t test, Wilcoxon rank-sum, and ANOVA followed by Newman-Keuls post hoc analyses. Correlations between outcome variables were computed as the Pearson correlation coefficient. Survival curves between the different groups were compared using a log-rank survivorship analysis. Tumor latency times were calculated by modeling tumor growth as a linear latent phase followed by a linear growth phase. The latency time was determined by finding the optimal transition point between the two phases for each tumor. \( P < 0.05 \) was considered significant. Data are expressed as means \( \pm \) SE.

Materials and Methods

Received 12/8/03; accepted 12/16/03.

Grant support: NIH Specialized Programs of Research Excellence Grant P50 CA92131-01A1; CA42710, CA50151, CA100938, and PS0 AT00151-01 T. H. Ngo is currently a medical student at Western University, College of Osteopathic Medicine of the Pacific, Pomona, CA, and is supported by the American Federation for Aging Research (AFAR).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 12/8/03; accepted 12/16/03.

Grant support: NIH Specialized Programs of Research Excellence Grant P50 CA92131-01A1; CA42710, CA50151, CA100938, and PS0 AT00151-01 T. H. Ngo is currently a medical student at Western University, College of Osteopathic Medicine of the Pacific, Pomona, CA, and is supported by the American Federation for Aging Research (AFAR).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: S. J. Freedland is currently at Brady Urological Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21287.

Requests for reprints: William J. Aronson, University of California Los Angeles, Department of Urology, 66-124 Center for the Health Sciences, Los Angeles, CA 90095-1738. Phone: (310) 268-3446; Fax: (310) 268-4858; E-mail: waronson@ucla.edu.
Likewise, mouse weights were equal between the groups throughout the study (Fig. 1B). Four weeks after s.c. injection of $10^5$ LAPC4 cells, all of the mice developed palpable tumors. Tumors in the HF group grew rapidly, and at a mean of 6 weeks after randomization the mice required euthanasia (Fig. 1C). Tumor size in the HFC group stabilized for 9 weeks postcastration after which time the tumors began to regrow compared with a mean of 18 weeks of stable tumor size in the LFC group ($P < 0.001$; Fig. 1C). One LFC tumor remained in remission and did not display growth for the duration of the experiment. Postcastration survival was significantly longer in the LFC versus HFC group (20.8 ± 1.3 versus 13.0 ± 7 weeks; $P < 0.01$; Fig. 2). At the time of sacrifice the LFC group had significantly smaller tumor volumes than mice in the HFC group (0.12 ± 0.05 ml versus 0.67 ± 0.17 ml; $P < 0.01$).

Mean serum PSA levels were significantly correlated with tumor volumes for all three of the groups ($r = 0.82; P < 0.01$). Mean serum PSA in the HF group (75.2 ± 6.2 ng/ml) at the time of sacrifice (mean 6 weeks after palpable tumor) was significantly higher than in either of the two castration treated groups ($P < 0.01$; Fig. 3). Among the two groups treated with castration, at 13 weeks postcastration (the mean time of sacrifice for the HFC group), mean serum PSA levels were 70% lower in the LFC group relative to the HFC group (10.8 ± 3.8 ng/ml versus 36.5 ± 6.8 ng/ml; $P < 0.01$; Fig. 3).

Discussion

An isocaloric low-fat diet has been shown to delay AS LAPC-4 tumor growth in a mouse xenograft model (2). Whether reduced dietary fat intake can delay the progression from AS to AI is unknown. To examine this issue, we injected intact severe combined immunodeficient mice bearing human Los Angeles prostate cancer-4 prostate tumor xenografts. HF, noncastrated controls on a high-fat diet. HFC, castrated mice fed a high-fat diet. LFC, castrated mice fed a low-fat diet. $P < 0.01$, HFC versus LFC; $P < 0.001$, HF versus HFC; $P < 0.001$, HF versus LFC.

![Fig. 2. Effect of dietary fat content on survival of castrated severe combined immunodeficient mouse bearing human Los Angeles prostate cancer-4 prostate tumor xenografts. HF, noncastrated controls on a high-fat diet. HFC, castrated mice fed a high-fat diet. LFC, castrated mice fed a low-fat diet. $P < 0.01$, HFC versus LFC; $P < 0.001$, HF versus HFC; $P < 0.001$, HF versus LFC.](image)

![Fig. 3. Effect of dietary fat content on serum prostate-specific antigen (PSA). HF serum was collected at time of euthanasia (mean 6 weeks post-randomization). LFC and HFC serum was collected at time of HFC euthanasia (mean 13 weeks post-randomization). #, $P < 0.01$ LFC versus HFC; *, $P < 0.01$ HFC versus LFC; $\dagger$, $P < 0.01$ HF versus LFC. HF, noncastrated controls on a high-fat diet. HFC, castrated mice fed a high-fat diet. LFC, castrated mice fed a low-fat diet.](image)

![Fig. 1. A–C. Severe combined immunodeficient mouse caloric intake, weight, and tumor growth. HF, noncastrated controls on a high-fat diet. HFC, castrated mice fed a high-fat diet. LFC, castrated mice fed a low-fat diet. A, energy intake was measured for each mouse three times per week by subtracting the weight of uneaten food from the weight of the food placed into the feeding receptacles at the beginning of each feeding period. B, mice were weighed weekly from the start of the experiment. C, tumor volumes were measured weekly. Values are expressed as means ± SE. *, $P < 0.05$, HFC versus LFC.](image)
serum PSA levels, and prolonged survival. The current results demonstrate that in a xenograft model, a low-fat diet without caloric restriction significantly delayed progression to AI and prolonged survival.

Caloric restriction and or excess alone can impact on xenograft growth (8, 9). Thus, it is essential to distinguish effects due to caloric intake from effects due to fat intake. In the current study, due to the paired feeding protocol and the fact that mice were housed 1 per cage, caloric intake was closely monitored and was kept identical between the groups. Therefore, the current findings can be directly attributable to differences in dietary fat intake and not caloric intake.

Linoleic acid (ω-6) constitutes 60% of the fatty acids in corn oil, the dietary fat used in the current study. Linoleic acid exerts a stimulatory effect on the growth of AS (LNCaP) and AI (PC-3) prostate cancer cell lines (1). Membrane arachidonic acid (ω-6) derived from linoleic acid is converted by cyclooxygenase-2 to prostaglandin E2, which promotes in vitro prostate growth, affects cell invasion, and may play a role in metastasis (4, 5). Lowering dietary ω-6 or increasing the ω-3:ω-6 ratio may alter membrane fatty acid ratios resulting in decreased cyclooxygenase-2 expression and decreased prostaglandin E2 production (10, 11). Of interest, increased membrane ω-3:ω-6 ratios may reduce serum interleukin 6, which has been implicated in promoting the conversion from AS to AI and enhancing AI growth (11, 12). Arachidonic acid derived from linoleic acid is also metabolized by the lipoxygenase pathway to eicosanoids (leukotrienes and hydroxy derivatives of fatty acids), which have been implicated in carcinogenesis and are believed to play important roles in promotion, progression, and metastasis (6). Arachidonic acid is also metabolized via lipoxygenase-5 to 5-hydroxyeicosatetraenoic acid, which was found in higher levels in malignant prostate tissue relative to adjacent benign tissue, and has been shown to support the growth of AS and AI prostate cancer cell lines (13).

Consumption of a high-fat diet is also known to promote insulin resistance (14), and hyperinsulinemia has been associated with increased development of prostate cancer (15). Insulin enhances the production of insulin-like growth factor (IGF)-I (16), which has been shown to play a pivotal role in prostate carcinogenesis and AI growth (17, 18). Our group found previously that serum from men consuming a low-fat diet combined with exercise reduced the growth of LNCaP cells in vitro through decreased serum levels of IGF-I and increased serum IGF binding protein-I relative to serum from control untreated men (19). We also demonstrated that mice fed an isocaloric reduced ω-6 diet had reduced serum insulin levels, increased serum IGF binding protein-I levels, and decreased tumor IGF-I, which were associated with slower AS LAPC-4 xenograft growth rates, lower serum PSA levels, and lower Ki-67 tumor proliferation indices (2). In this prior experiment, the LAPC-4 tumors in the low-fat group had decreased IGF binding protein-II immunostaining relative to the high-fat group, and increased IGF binding protein-II expression may play a critical role in promoting AI growth, independent of its effect on IGF-I activity (20).

In conclusion, isocaloric dietary fat reduction delayed the conversion from AS to AI and prolonged survival in a human xenograft model. Future preclinical studies are needed to address the impact of various fatty acid compositions, which may play a role in tumor progression. The current results provide a sound basis for clinical trials evaluating the impact of dietary fat restriction in men undergoing androgen deprivation therapy for prostate cancer.

References
14. Akiyama, T., Zhao, H.,完整内容。
Effect of Isocaloric Low-Fat Diet on Prostate Cancer Xenograft Progression to Androgen Independence

Tung H. Ngo, R. James Barnard, Todd Anton, et al.

Cancer Res 2004;64:1252-1254.