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Dietary Calcium Does Not Affect Prostate Tumor Progression in LPB-Tag Transgenic Mice

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Abstract

High dietary calcium has been shown in epidemiological studies to be a risk factor for prostate cancer, and it has been postulated that this effect is secondary to calcium induced modulation of the vitamin D axis. In this study, we used LPB-Tag transgenic mice on the CD1 background to examine the impact of dietary calcium on prostate tumor progression. CD1-LPB-Tag mice predictably develop autochthonous, hormone-responsive prostate tumors by 3 months of age. Age matched transgenic and non-transgenic littermates were weaned onto high (2%) or low (0.2%) calcium diets and mice were sacrificed at 5, 7, and 9 weeks of age. The entire urogenital complex was excised, weighed, and processed for histology. There was no significant effect of dietary calcium on tumor weight or on the time course of tumor progression, as monitored using a modified Gleason grade (MGS). Serum calcium was maintained in the normal range in mice on the low and high calcium diet throughout the study. Circulating 1,25(OH)₂D₃ was elevated by low dietary calcium in 5 week old mice, but not in older animals. In summary, neither development nor progression of prostate tumors in LPB-Tag mice was accelerated by high dietary calcium.

Keywords

calcium; prostate cancer; LPB-Tag

1. Introduction

Prostate cancer is the most commonly diagnosed form of cancer in men, with an estimated 230,000 cases per year and is the second leading cause of cancer death in men in the United States, with 27,000 deaths due to the disease expected in 2006 (American Cancer Society, 2006). Early stage prostate tumors are androgen-dependent, and generally slow growing. The anti-androgen Casodex inhibits binding of testosterone to the androgen receptor (AR), thus inhibiting cell growth and promoting apoptosis. However, after prolonged treatment with anti-androgens, tumors have been shown to become androgen-independent, thus no longer responding to androgen ablation therapy. In these situations, it would be useful to have therapies available that do not require androgen-dependence. Some adjuvant therapies have been suggested for treatment of both early stage and androgen-independent tumors, including the antioxidants vitamin E and lycopene, as well as vitamin D and its analogs [1].

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The active form of vitamin D, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) is a secosteroid involved in the regulation of calcium and phosphate homeostasis in the body. The vitamin D-calcium axis is controlled by the hormones calcitonin and parathyroid hormone (PTH), which regulate the absorption of calcium by bones, as well as calcium release into the blood [2]. 1,25(OH)₂D₃ is first synthesized by conversion of 7-hydrocholesterol in the skin after sun exposure, and converted by the enzymes 25-hydroxylase in the liver and 1 α -hydroxylase in the kidney and some target tissues including prostate [3] into the active form 1,25(OH)₂D₃. The action of 1,25(OH)₂D₃ is mediated by the vitamin D receptor (VDR). 1,25(OH)₂D₃ binds to the VDR which is translocated to the nucleus where it heterodimerizes with the retinoid X receptor (RXR). The complex then binds to response elements along the genome, resulting in transcription of anti-proliferation and pro-differentiation genes, as well as genes involved in apoptosis [4,5]. *In vitro* studies have shown that 1,25(OH)₂D₃ or its non-calcemic analogues have antiproliferative and apoptotic effects in the LNCaP prostate cancer cell line [6,7]. 1,25(OH)₂D₃ is also necessary for cell differentiation in skin and other epithelial cell types [8]. Epidemiological studies have shown that low levels of circulating 25(OH)₂D₃ and 1,25(OH)₂D₃ may be a risk factor for development of prostate cancer [9–11]. Additionally, because 1,25(OH)₂D₃ is involved in regulation of calcium in the body, high dietary calcium has been suggested as a risk factor for prostate cancer, since high serum calcium levels may decrease circulating levels of 1,25(OH)₂D₃ [1].

Many *in vitro* studies have been performed on prostate cancer cells, however there has been little done *in vivo* to study incidence and progression of prostate tumors, due to the lack of a reliable model system for early stage disease. Recently a transgenic model has been developed using the SV40 large T antigen driven by the large probasin promoter which targets expression to the dorsolateral lobes of the prostate [12]. These mice develop prostate tumors starting at 5 weeks of age providing a consistent autochthonous model of human tumor progression.

Using these LPB-Tag transgenic mice, we have investigated the role of dietary calcium in prostate tumor progression in order to determine if high levels of dietary calcium accelerate tumor growth and severity of progression over a 9 week time course.

2. Materials and Methods

2.1 Animals

CD1 LPB-Tag transgenic and non-transgenic age-matched littermates were reared at the Freimann Life Science Center at the University of Notre Dame. Animals were weaned onto diets of low (0.2%) or high (2.0%) calcium and were fed food and water ad libitum. Ten transgenic animals per group were weighed and sacrificed at 5, 7, or 9 weeks of age; fifteen non-transgenic littermates per diet group were sacrificed at 9 weeks of age.

2.2 Tissue collection and processing

The urogenital complex including the dorsolateral and ventral prostate, bladder and seminal vesicles was excised and weighed at time of sacrifice. Whole prostates were fixed in 4% formalin for 12–16 h, paraffin embedded, and sectioned to 5 μ m.

2.3 Histological analysis

Paraffin sections were stained with hematoxylin and eosin and photographed under a light microscope. Tumor progression was rated on a modified Gleason scale (MGS) for mouse pathology from 0–4 based on degree of hyperplasia in the dorsolateral lobe of the prostate adapted from Shappell et al., 2004 [13] (Figure 1). Sections were divided into equal surface area plots. Two scores were taken per plot, denoting the predominant pathologies in each plot. The sums of these scores were averaged to give the total MGS for each tumor.

2.4 Serum Components

Serum was isolated from mice at time of sacrifice and stored at -80°C until time of analysis. Serum calcium was analyzed by colorimetric assay (BioAssay Systems, Hayward, CA). Circulating $1,25(\text{OH})_2\text{D}_3$ was assessed by RIA (Immunodiagnostic Systems Inc., Fountain Hills, AZ). Testosterone was analyzed by ELISA (Immutopics Int., San Clemente, CA; MP Biomedicals, Orangeburg, NJ).

3. Results

3.1 Dietary calcium levels have no effect on tumor incidence or relative size in LPB-Tag mouse

All of the LPB-Tag transgenic animals reproducibly develop prostatic neoplasias by 9 weeks of age. There was no significant difference in tumor weight between animals fed a high calcium diet and those fed a low calcium diet, and both were significantly larger than age-matched WT animals. (Figure 1)

3.2 Dietary calcium does not affect tumor progression as determined by a mouse-modified Gleason scale of hyperplasia

Tumor progression in LPB-Tag mice follows a similar progression as in early progression of human prostate tumors. Animals first develop stromal and epithelial hyperplasia, followed by low grade prostatic intraepithelial neoplasia (LGPIN) (modified Gleason score (MGS) between 3–4) and finally high grade prostatic intraepithelial neoplasia (HGPIN) (MGS 4–6). Tumors progressed at a similar rate in both high and low calcium animals, and there was no significant difference in MGS rating at any time point. (Figures 2,3)

3.3 Dietary calcium has no effect on serum component levels

3.3.1 Serum Calcium—Serum calcium levels were maintained between 12.5–13.5 mg/dL across all groups for all time points. There was no significant difference between high and low calcium diets and no significant difference between 9 week transgenic animals and WT controls. (Figure 4)

3.3.2 Serum $1,25(\text{OH})_2\text{D}_3$ —Serum calcium levels can influence circulating $1,25(\text{OH})_2\text{D}_3$ levels via the vitamin D-calcium axis. Circulating $1,25(\text{OH})_2\text{D}_3$ levels were measured by radioimmunoassay. Animals fed the low calcium diet showed high levels of circulating $1,25(\text{OH})_2\text{D}_3$ up to 400 pg/ml at 5 weeks of age, while those on the high calcium diet had significantly lower levels (less than 150 pg/ml) (Figure 4). At 7 and 9 weeks of age, $1,25(\text{OH})_2\text{D}_3$ levels decreased 7-fold or higher in both diet groups, and levels were essentially the same by 9 weeks of age averaging 2.5 pg/ml. Non-transgenic litter mates had circulating $1,25(\text{OH})_2\text{D}_3$ levels of 2–4 pg/ml at 9 weeks of age. There was no significant difference in PTH levels between animals fed the high calcium diet and those fed the low calcium diet at any time point (data not shown).

3.3.3 Serum Testosterone—While there was significant variation of serum testosterone levels within each age and treatment group, there is no significant difference in serum testosterone levels between high and low calcium groups at any time point. (Figure 4).

4. Discussion

Dietary calcium has no effect on urogenital morphology, tumor progression (MGS) or the serum components calcium, $1,25(\text{OH})_2\text{D}_3$, PTH and testosterone, in LPB-Tag transgenic mice. High calcium diet does not aggravate or accelerate tumor growth or progression. These data

agree with previous *in vivo* studies done with xenograft tumors of LNCaP cells in nude mice, where dietary calcium had no effect on tumor growth or progression [14]. However, our results contrast with several epidemiological studies in which high dietary calcium was found to be a risk factor for prostate cancer development and severity of progression [9]. This highlights the need for further study of the role of the vitamin D axis in prostate tumor progression, since it is likely that much of the effect of calcium on tumor progression can be attributed to its effects on $1,25(\text{OH})_2\text{D}_3$ levels.

Since the SV40 large T antigen is under the control of an androgen-dependent probasin promoter in the LPB-Tag model system, it is possible that serum testosterone levels may have an effect on the progression of the tumor. Additionally, it has been postulated that serum calcium levels may have an effect on testicular testosterone synthesis, potentially through modulation of cholesterol synthesis or transport, or via modulation of mitochondrial and microsomal enzymes involved in the formation of testosterone [15]. Previous studies have shown that serum calcium levels in excess of 10 mM or below 1 mM result in decreased testosterone production in the Leydig cells of the testes. In view of the significant variations in serum testosterone within the WT littermates and the LPB-Tag mice in the dietary study, it is advisable to stabilize serum testosterone levels using a slow release implant to ensure that the transgene is stimulated to an equal extent under all dietary or treatment conditions.

This study lays the groundwork for future studies using the VDR knockout mouse system [16] which requires a high calcium rescue diet to maintain serum calcium levels.

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Figure 1.

Effect of dietary calcium on tumor weight to body weight ratio (TW/BW). TW/BW ratio increases from 5–9 weeks of age and there is no significant difference between the high calcium and low calcium diets. Non-transgenic littermates at 9 weeks of age show no difference between high calcium and low calcium diets, but are significantly different from transgenic animals at 9 weeks of age. (N=10)

Figure 2. Tumor progression in low calcium (left) and high calcium (right) diets. (A–C) LPB-Tag mouse prostate tumors at 5, 7, and 9 weeks of age respectively. (D–F) Representative sections of MGS ratings corresponding to 5, 7, and 9 week old animals.

Figure 3. Gleason scale modified for mouse average ratings for low and high calcium diets from 5–9 weeks of age. Tumors show progression as determined by MGS. There is no significant difference in MGS between low and high calcium diets at any time point. (N=5)

Figure 4.

Serum levels of calcium, $1,25(\text{OH})_2\text{D}_3$, and testosterone in low calcium and high calcium diets for LPB-Tag and non-transgenic WT animals. There is no significant difference in serum calcium levels. Circulating $1,25(\text{OH})_2\text{D}_3$ levels are significantly higher in 5 week old animals on the low calcium diet ($p < 0.001$). At 7 and 9 weeks of age, serum $1,25(\text{OH})_2\text{D}_3$ levels decrease and reach less than 5 pg/ml by 9 weeks of age in both transgenic and non-transgenic WT animals. Serum testosterone levels are widely variable within all groups, but there is no significant difference between high and low calcium diets at any time point.