

Genistein Inhibits the Growth of Human-Patient BPH and Prostate Cancer in Histoculture

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BACKGROUND. There is strong epidemiological evidence that prostate disease is significantly less prevalent in the Orient, where the intake of soy products is very high, than in the United States. We therefore undertook a study of the effects of genistein, a major component of soy, on growth of human-patient benign prostatic hypertrophy (BPH) and prostate cancer tissue in three-dimensional collagen gel-supported histoculture.

METHODS. Surgical specimens of human BPH and cancer were histocultured for 5 days to study the effects of genistein on growth, as measured by inhibition of ³H-thymidine incorporation per μg protein on day 5.

RESULTS. Genistein in doses of 1.25–10 $\mu\text{g}/\text{ml}$ decreased the growth of BPH tissue in histoculture in a dose-dependent manner, with little additional effect at higher doses. Prostate cancer tissue in histoculture was similarly inhibited by these doses of genistein.

CONCLUSIONS. Genistein decreases the growth of both BPH and prostate cancer tissue in histoculture. The data suggest that genistein has potential as a therapeutic agent for BPH and prostate cancer. *Prostate* 34:75–79, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: genistein; histoculture; BPH; prostate cancer; isoflavonoid

INTRODUCTION

Prostate cancer is epidemic in the Western world. Benign prostatic hypertrophy (BPH) is likewise a very common disorder in the aging population in the Western world. By contrast, BPH and cancer of the prostate are relatively uncommon in the Orient. According to a recent survey by Gu et al. [1], prostate cancer is 20-fold less common in mainland China compared to the United States. Yet, at autopsy, microscopic (latent) prostate cancer is only slightly less frequent in mainland Chinese compared to age-matched American males [2], where its prevalence is upwards of 30% in men over age 50. These differences in BPH and prostate cancer progression are probably not genetic, since the incidence of prostate cancer and BPH increases when the Japanese migrate to the US [3]. Dietary factors are drastically different between the Oriental and Western modes of food consumption. A major dietary difference is the high intake of soybean products in the

Oriental diet compared to the West. In addition, the Oriental diet is low in fat as compared to the Western diet [4].

Genistein, which is a major ingredient of tofu, which in turn is made from the soybean, is an isoflavonoid that is found in significant levels in the blood of Orientals [5]. Peterson and Barnes [6] have shown that genistein decreases the growth of LNCaP cells, a hormone-dependent prostate cancer cell line, as well as DU-145, a hormone-independent prostate cancer cell line.

We previously established a collagen gel-matrix-supported histoculture androgen sensitivity assay for measuring the growth of androgen-dependent tissues such as prostate tissue [7–9]. We have now undertaken

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a histoculture study of the effects of genistein on prostate tissue growth in both benign and malignant disease. These studies have been done by measuring the incorporation of ^3H -thymidine into tissue during histoculture to compare tissue growth in the presence and absence of genistein.

MATERIALS AND METHODS

Clinical Material

The source of prostate tissues was either untreated surgical specimens from patients undergoing transurethral resection of the prostate (TURP) for BPH, or radical prostatectomy specimens from patients undergoing surgery for early-stage prostate cancer. In the latter instance, both BPH and cancer tissue were available from some of the same specimens.

Methods

Histoculture studies were done as previously reported, using 2-ml wells in plastic culture plates containing 6 wells per plate [7]. A detailed description of tissue preparation and subsequent sponge gel-matrix-supported histoculture has been previously published [7]. An abbreviated description follows.

Tissue Preparation

Radical prostatectomy specimens from patients with early prostate cancer were placed in minimal essential medium (MEM) solution on ice in the operating room and brought to the Department of Pathology at Mercy Hospital (San Diego, CA) or Scripps Clinic (La Jolla, CA). The pathologist weighed and measured the specimens and marked the prostate surface with green ink to aid the pathological staging of the tumor. The tissue was cut serially in its anatomical position, so that the halves of the gland above and below a midline axial cut were labeled as anterior and posterior, respectively. A sagittal cut running from distal to proximal divided the prostate into right and left sections. Approximately 0.5-cm cuts were then made, beginning at the distal urethral end of the prostate, with each section identified with a letter label. The pathologist selected representative gross specimens of cancer and BPH for our study.

Anatomical mirror-image sections of the specimens for histoculture were kept by the pathologist for determination of microscopic anatomy. Tissues obtained from TURP were carefully dissected to remove any discolored, damaged tissue from the individual chips. The tissues were then brought to the Histoculture

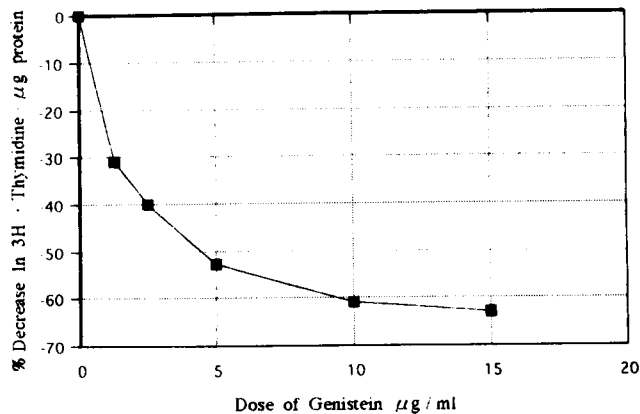


Fig. 1. Effect of genistein on BPH in histoculture, demonstrating percentage decrease in ^3H -thymidine incorporation per μg protein in human BPH tissue in histoculture with varying concentrations of genistein, beginning at 1.25 $\mu\text{g}/\text{ml}$ and extending to 15 $\mu\text{g}/\text{ml}$. Note the rather sharp decline in ^3H -thymidine incorporation occurring in the range of 1.25–10 $\mu\text{g}/\text{ml}$, with relative leveling-off thereafter in the reduction of thymidine incorporation.

Laboratory at AntiCancer, Inc., in ice-cold MEM solution.

Histoculture

BPH and cancer specimens, whether obtained from radical prostatectomy specimens or from TURP specimens, were separately and finely cut into 1-mm³ minces. The tissue cubes were planted on Gelfoam collagen-gel matrices with media containing MEM and appropriate concentrations of DHT and genistein in tissue culture wells with 2 ml of medium.

The DHT-treated histocultures were used as the untreated control and represented maximal stimulation of ^3H -thymidine incorporation into prostatic tissue. The percent reduction in ^3H -thymidine incorporation/ μg protein in the presence of varying concentrations of genistein was calculated as a percent decrease in ^3H -thymidine incorporation/ μg protein from controls as a result of the biologic effects of genistein. Histoculture, as previously described [7–9], was performed for 5 days. DHT (2×10^{-8} M) was added daily on days 2–5 of histoculture to all tissues. Genistein in concentrations ranging from 1.25 $\mu\text{g}/\text{ml}$ (4.6 μM) up to 15 $\mu\text{g}/\text{ml}$ (55.3 μM) was added on day 2 to BPH and cancer histocultures (Fig. 1).

DMSO, which was the diluent for the genistein, was also added in similar amounts to all cultures. Ethanol, which was the diluent for DHT, was added in equal amounts to all other cultures. ^3H -thymidine was added on day 5 to all cultures. Incubations were stopped 24 hr later. Tissues were kept frozen following incubation until analysis.

Measurement of ^3H -Thymidine Incorporation

Processing of tissues for measurement of ^3H -thymidine incorporation per μg of protein was done by first washing tissues to remove unbound ^3H -thymidine. Tissues were then homogenized in sucrose buffer and centrifuged. The pellet obtained was digested with benzomethonium hydroxide for measuring ^3H -thymidine counts per minute, and the supernate was analyzed for protein concentration. Results were expressed as ^3H -thymidine incorporation per μg protein for both BPH and cancer tissues histocultured with DHT, and genistein.

Assessment of Tissue Viability and Integrity Following Histoculture

To evaluate tissue for possible genistein toxicity, we measured the percentage of viable-nonviable cells in small aliquots from each histoculture plate, according to the method of Li et al. [10]. Briefly, this method used confocal microscopy, using a MRC-600 confocal imaging system (Bio-Rad, Richmond, CA) mounted on a Nikon Optiphot using a 10 \times PlanApo objective. Viable cells are selectively labeled with the dye 2',7'-bis-(2-carboxymethyl)-5 (and -6) carboxyfluorescein acetoxymethyl ester (BCECF-AM), which is activated to fluorescein by nonspecific esterases present only in living cells. Nonviable cells, whose plasma membranes are leaky, are labeled with propidium iodide (PI), a dye that enters only cells with nonintact membranes. Since the emission spectra of these two dyes are different, they can be used simultaneously on the same specimen. Both dyes are used at a concentration of 5 μM . The double-treated cultures are analyzed by fluorescence confocal microscopy within 30 min of staining.

RESULTS

Genistein Inhibits ^3H -Thymidine Incorporation in BPH and Prostate Cancer

Genistein significantly inhibited ^3H -thymidine incorporation/ μg protein in BPH tissue in a dose-response fashion, beginning at 1.25 $\mu\text{g}/\text{ml}$ and leveling off at approximately 10 $\mu\text{g}/\text{ml}$ (46 μM) (see Table I and Fig. 1). Genistein effects on ^3H -thymidine incorporation in prostate cancer histocultures were similar to those noted in BPH histocultures at similar concentrations of genistein (Table I). The fact that BPH and cancer from the same prostate in the same experiments showed similar responses to genistein suggests that the effects in many, if not all, of the cancers would be similar to that noted in BPH.

TABLE I. Effect of Genistein on BPH and Prostate Cancer: Average Percent Decrease in ^3H -Thymidine Incorporation/ μg Protein*

Genistein concentration in histoculture ($\mu\text{g}/\text{ml}$)	BPH	Prostate cancer
1.25	30% (3)	
2.5	40% (7)	28% (1)
5.0	52% (10)	37% (1)
10	60% (3)	54% (2)
15	63% (3)	55% (1)

*BPH and prostate cancer were isolated from the same radical prostatectomies in each case of cancer and put into histoculture as described. BPH tissues were also derived from TURPs. Genistein at the indicated concentrations was put into the histocultures from days 2-5. ^3H -thymidine added to the histocultures on day 5 as described in Materials and Methods. Tissues were processed to determine the amount of ^3H -thymidine incorporated into DNA, as described in Materials and Methods. Percent decrease in treated cultures was calculated with respect to untreated controls, which received 2×10^{-8} M DHT, as did the genistein-treated cultures. In parentheses, number of experiments.

Effects of Genistein on Viability and Histologic Appearance of Prostate Tissue Following Histoculture

Fluorescent confocal microscopy following two separate histoculture studies of BPH tissue indicated an average viability for DHT-treated tissue of 78% of cells compared to 5 $\mu\text{g}/\text{ml}$ genistein-treated tissue viability of 73% of cells. This strongly suggests that genistein is nontoxic to prostate tissue in histoculture.

Effects of DHT and genistein on the histology of prostate tissue following 5-day histoculture are compared to effects on preincubated tissue in Figure 2. As can be seen, there were mild changes of epithelial cell nuclear pleomorphism and dilation of acini, with sloughing of debris seen in all treated specimens following 5 days of histoculture (Fig. 2). In addition, mild and variable loss of stromal nuclei occurred in genistein 5-day histocultures. The changes noted with genistein as well as DHT appear to be very mild and are unlikely to be responsible for the highly significant decreases noted in ^3H -thymidine incorporation into histocultures incubated with genistein or with HF.

DISCUSSION

Genistein inhibits the growth of both BPH and prostate cancer in histoculture. This inhibition appears to occur in a dose-response fashion in BPH tissue beginning at 1.25 $\mu\text{g}/\text{ml}$ and continuing up to 10 $\mu\text{g}/\text{ml}$, with very little additional inhibition when higher

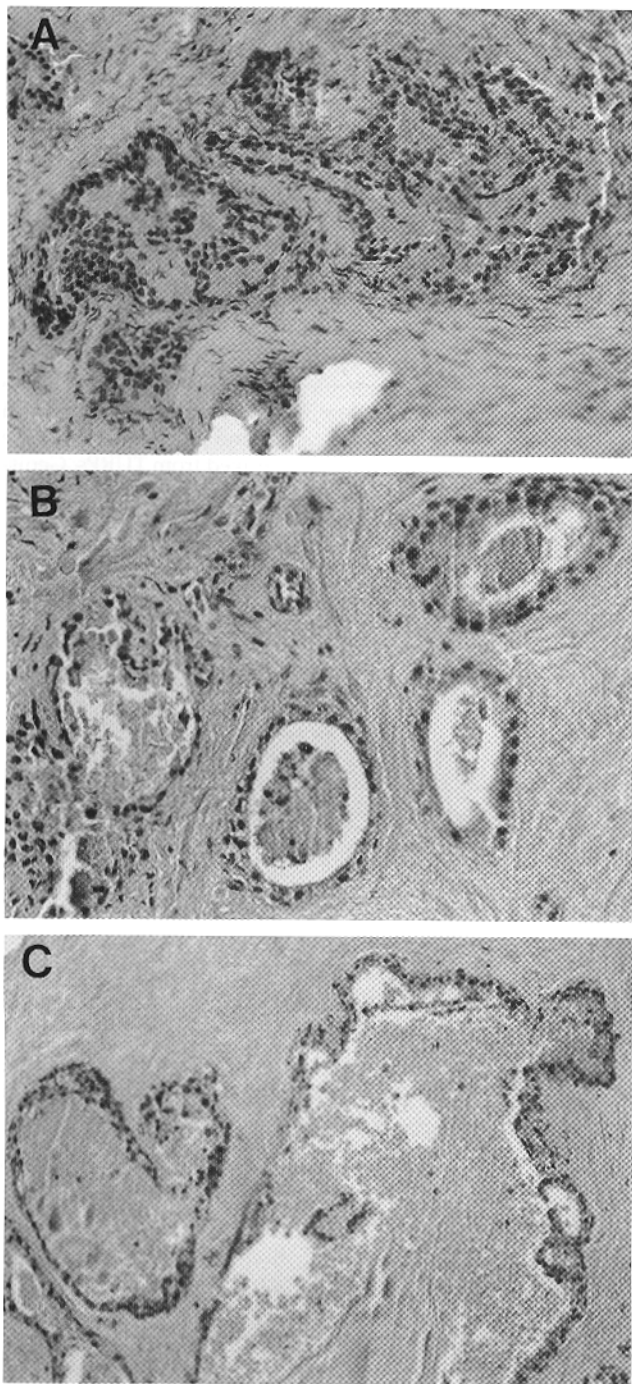


Fig. 2. Histological effects of DHT and genistein on BPH in histoculture. **A:** Microscopic appearance of BPH tissue prior to histoculture (H&E stain, $\times 200$). **B:** Microscopic appearance of BPH tissue following 5 days of histoculture with DHT at 2×10^{-8} M, added each day from days 2–5. Note mild epithelial cell nuclear pleomorphism and sloughing of debris into acini (H&E stain, $\times 200$). **C:** Microscopic appearance of BPH tissue following 5 days of histoculture with genistein (5 $\mu\text{g/ml}$), added on day 2, and DHT (2×10^{-8} M), added daily from days 2–5. Note mild nuclear pleomorphism with sloughing of debris into acini; also note mild variable decreases in stromal cell nuclei (H&E stain, $\times 100$). Note: All histocultures in b and c contain the same amounts of DMSO and ethanol.

amounts of genistein are used. Genistein similarly inhibited growth of histocultured prostate cancer tissue.

Although this study does not address the topic of mechanisms of genistein action, Peterson and Barnes [6] have indicated that genistein appears to act via multiple pathways that are not fully understood. They noted that genistein inhibits EGF-stimulated growth in hormone-dependent LNCaP and hormone-independent DU-145 cell lines, but not entirely by direct inhibition of EGF-receptor tyrosine kinase. They also demonstrated that genistein had other effects on these cell lines, including blockade of growth stimulation by high concentrations of fetal calf serum.

Since genistein was able to block the growth of both hormone-dependent LNCaP cells and hormone-independent DU-145 prostate cancer cell lines, as reported by Peterson and Barnes [6], it has the potential to be active in treating hormone-independent as well as hormone-dependent prostate cancer. Our studies in three-dimensional histoculture, which are consistent with the results reported in cell lines, are more physiologic and better predict the effectiveness of genistein *in vivo* because of maintenance of three-dimensional tissue structure, as well as the fact that patient tissues were tested. The fact that genistein is a food substance makes it unlikely that it is toxic in the range of its demonstrated growth-inhibiting effects. We plan to pursue a study of the *in vivo* efficacy and toxicity of genistein on both patient BPH and prostate cancer in prostate orthotopic-transplant mouse models [11].

CONCLUSIONS

Genistein, an isoflavonoid found in large quantities in soy products, appears to inhibit the growth of both benign and malignant prostate tissue as measured by ^3H -thymidine incorporation/ μg protein in collagen gel-matrix-supported histoculture in a dose range of 1.25 $\mu\text{g/ml}$ up to 10 $\mu\text{g/ml}$. Multiple mechanisms which have not been entirely clarified appear to account for this effect. The results suggest that genistein has potential as a therapeutic agent for BPH and prostate cancer.

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