

New Sensitive Discovery Histoculture Model for Growth-Inhibition Studies in Prostate Cancer and BPH

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BACKGROUND. A new, total-immersion three-dimensional histoculture (TIH) method was developed to evaluate growth of tissue containing a mixture of benign prostate hyperplasia (BPH) and prostate cancer in vitro.

METHODS. Efficacy of inhibitors, such as genistein, was determined by measuring ^3H -thymidine incorporation per microgram protein. Inhibitory effects obtained in TIH were compared to those in sponge-gel supported histoculture (SSH).

RESULTS. ^3H -thymidine incorporation was 2–5-fold higher in tissue cultured in TIH than in SSH. The average inhibition by genistein at a concentration of 18 μM was 73% in TIH, vs. 31% in SSH. TIH also appeared to be more sensitive, since the lowest concentration of genistein that significantly inhibited growth of BPH mixed with prostate cancer tissue was 2.3 μM , while in SSH the lowest concentration was 9.2 μM . Although the within-assay coefficient of variation (CV) was similar for both TIH and SSH, the between-assay CV was better in TIH.

CONCLUSIONS. These data suggest that TIH can be used as a discovery model for screening and evaluating inhibitors of prostate tissue growth in vitro. *Prostate 37:126–129, 1998.*

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KEY WORDS: three-dimensional tissue architecture; tumor-stromal interaction; drug sensitivity; genistein; antiandrogen; [^3H]-thymidine incorporation

INTRODUCTION

Three-dimensional histoculture has been shown to be a useful model to study cancer tissue in vitro. Histoculture enables the study of tumor cell growth, migration, invasion, and response to currently-used and new anticancer drugs [1–14]. The advantage of three-dimensional histoculture compared to monolayer culture is that structural integrity of the tissue is preserved and resembles native in vivo tissue architecture, as well as its in vivo metabolic activity.

In previous publications, we reported studies of both benign and malignant prostate tissue growth in histoculture and growth inhibition by antihormones and other growth inhibitors such as genistein [15–18]. These studies used minced tissue planted on collagen sponge-gel matrices, which allows contact of the tissue with the medium through the sponge-gel and results in cellular proliferation within the tissues, as measured by thymidine incorporation.

We report here on the development of an improved

histoculture technique for prostate tissue, in which the minced three-dimensional tissue is totally immersed without support in the medium, rather than planted on a collagen sponge-gel. We demonstrate that incorporation of [^3H]-thymidine is much higher with this modification compared to sponge-gel-supported histoculture (SSH). We have termed this new technique total-immersion histoculture (TIH). Increased diffusion into tissue of substances present in the media occurs in TIH, since rates of thymidine incorporation are almost doubled in the control specimens compared to SSH. The amount of inhibitor, such as genistein, diffusing into tissue appears to be increased in TIH, since sensitivity to inhibitors at the same concentrations as used with SSH appears to be much greater in TIH. Thus TIH appears to be a more sensitive model

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for screening and evaluating inhibitors of prostate tissue growth *in vitro*.

MATERIALS AND METHODS

Histoculture

Prostate tissue specimens were obtained from patients who underwent either radical prostatectomy or transurethral resection of the prostate (TURP). The specimens contained both benign prostate hyperplasia (BPH) and prostate cancer. The tissue was immediately placed in ice-cold MEM medium and cut into 1-mm³ pieces within 1–2 hr after the surgery. The same tissue was used to compare TIH to SSH. In the case of SSH, 50–60 mg of tissue were placed in 6-well plates, on collagen-sponge matrices (Gelfoam Sterile Sponge, Upjohn Company, Kalamazoo, MI) hydrated in supplemented minimum essential medium (MEM) as previously described [15–18]. Wells of 6-well plates were filled with 2 ml of culture medium. In the case of TIH, the pieces of tissue were placed directly into the wells filled with MEM.

Following the initial 24 hr of incubation at 37°C in a 5% CO₂ atmosphere, the medium was changed and the histocultures were treated with various agents.

In every experiment, one plate was treated with a physiological concentration of 20 nM dihydrotestosterone (DHT) alone in a total volume of 6 µl (50% ethanol) to evaluate the control growth of the tissue. All other plates were treated with both DHT and genistein (in 100% ethanol). The total volume of ethanol added to the wells was equal in all plates in each experiment and never exceeded 0.45%. For a positive inhibition control, treatment of the tissue with both DHT and the antiandrogen hydroxyflutamide (20 µM) was performed. After 4 or 5 days of histoculture, 4 µCi/ml of [³H]-thymidine were added for 24 hr. In order to stop the [³H]-thymidine incorporation, the medium was removed and the tissue was washed with ice-cold phosphate-buffered saline (PBS), pH 7.4.

[³H]-Thymidine Incorporation and Protein Assay

The pieces of histocultured tissue from one 6-well plate were pooled. Unincorporated radioactivity was removed by washing with 10 ml of saline solution on ice, until counts in 1/20 of total volume of saline decreased to less than 100 cpm. Three aliquots were made from each pool. Histocultured tissue was then homogenized in TES buffer, pH 7 (10 mM Tris, 0.05 M EDTA, 5 mM MgCl₂, 0.5 mM 2-mercaptoethanol, 1.5 mM CaCl₂, and 0.25 M sucrose). A glass homogenizer at low speed which disrupts cells without breaking the nuclei was used, such that loss of incorporated thymi-

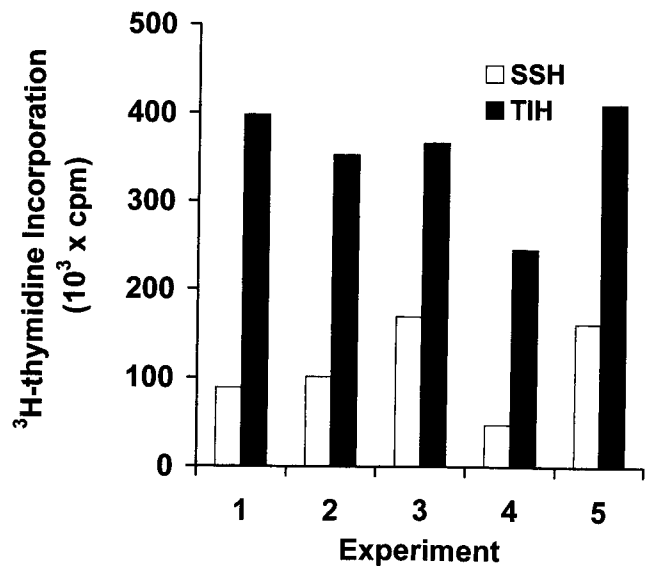


Fig. 1. Incorporation of [³H]-thymidine in mixed BPH and cancer tissue treated with DHT only, after histoculture for 5 or 6 days. [³H]-thymidine was added for the last 24 hr of histoculture. Incorporation of [³H]-thymidine in tissue using total-immersion histoculture (TIH) was higher in all five experiments (2–5-fold) compared to sponge-gels histoculture (SSH). Open bars, SSH; solid bars, TIH.

dine was avoided. The homogenate was then spun in a refrigerated centrifuge at 3,000 rpm for 30 min. The supernate was removed for protein assay. The pellet which contained nuclei was digested in benzethonium hydroxide for 60 min at 70°C. The solubilized tissue was mixed with 4 ml of scintillation liquid (CytoScint, ICN Pharmaceuticals, Inc., Costa Mesa, CA), and radioactivity was measured in a liquid scintillation counter.

Protein concentration was measured using the Bio-Rad Protein microassay (Protein Assay Dye Reagent Concentrate, catalog no. 500-0002 Bio-Rad Laboratories, Hercules, CA), according to the manufacturer's protocol [3].

The data were presented as [³H]-thymidine incorporation per microgram of protein. Inhibition of growth by genistein and hydroxyflutamide was expressed as a percentage decrease in [³H]-thymidine incorporation/µg protein by genistein and hydroxyflutamide, compared to treatment with DHT alone.

RESULTS AND DISCUSSION

We compared inhibition of growth after treatment of BPH and cancer tissue with genistein in SSH or TIH. Inhibition of growth was expressed as a percentage of control growth of the tissue treated with DHT alone. Incorporation of [³H]-thymidine dramatically increased in control histocultures by 2–5-fold in TIH compared to the same tissue in SSH (Fig. 1).

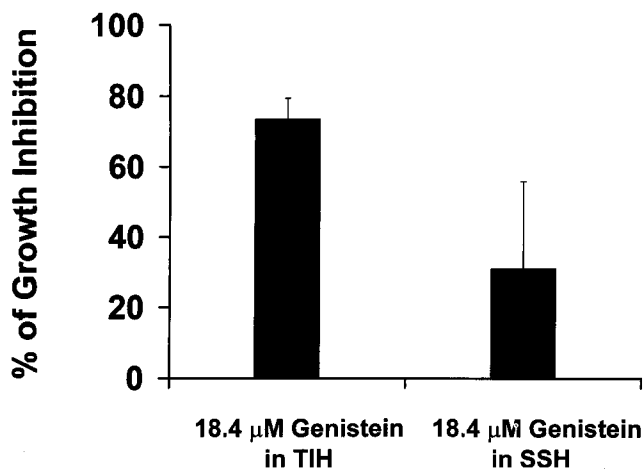


Fig. 2. Inhibition of mixed BPH and cancer tissue growth by genistein (5 μg/ml), using either TIH or SSH. Histocultures were exposed for 5 or 6 days to genistein. The inhibition was expressed as a percentage of growth of DHT-treated control histoculture, as measured by [³H]-thymidine incorporation into the tissue per microgram of protein. Error bars, standard deviation from five experiments.

We observed significantly increased growth inhibition of BPH and cancer tissue treated with 18.4 μM of genistein, using TIH compared to SSH. The average inhibition in TIH was 73% (range 67–79%), compared to a mean decrease of 31% (range 6–68%) in SSH (Fig. 2). This inhibition was statistically significant ($P < 0.05$).

To examine the sensitivity of TIH, we treated BPH and cancer tissue in TIH with decreasing concentrations of genistein. We observed 40% growth inhibition of tissue treated with 4.6 μM, and 26% inhibition with 2.3 μM, with a mean coefficient of variation (CV) of 13%. Both these values represent significant differences ($P < 0.05$). In contrast, inhibition was not significant below 9.2 μM genistein in SSH (CV in the range of 8–29%) (Fig. 3).

Inhibitory effects on [³H]-thymidine incorporation of 20 μM of hydroxyflutamide, a known antiandrogen, were compared using the same tissue in SSH and TIH. In all experiments, the incorporation of [³H]-thymidine was 3–4 times higher for TIH in control histocultures. The inhibition due to 2×10^{-5} M hydroxyflutamide was 40–60% in TIH. In SSH, inhibition by 2×10^{-5} M hydroxyflutamide was 17–18%, or in some experiments not detectable at all (Fig. 4).

Histoculture of both benign and malignant human prostate tissue was shown to be valuable in studying tissue growth and identifying inhibitors of growth in vitro [15–18]. Since prostate tissue consists of both epithelial and stromal tissue, theoretically there may be no need for additional matrix support. In our modified method, we totally immersed tissue in order to

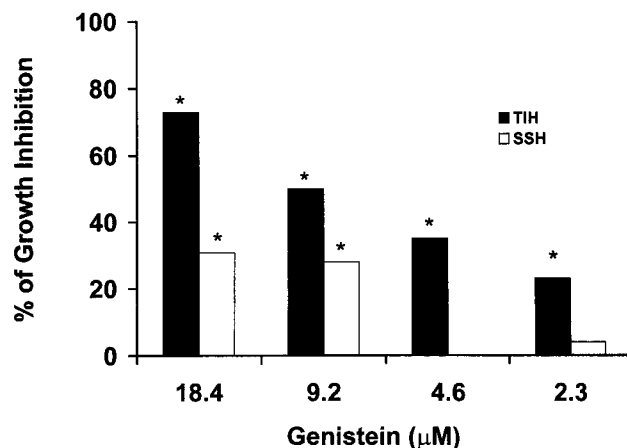


Fig. 3. Sensitivity of assays comparing TIH and SSH. The histocultured BPH and cancer tissue was treated with genistein at concentrations of 18.4, 9.2, 4.6, and 2.3 μM for 5 or 6 days. Growth was measured by [³H]-thymidine incorporation per microgram protein. Data are expressed as percentage of inhibition compared to DHT-treated control. *Significant data ($P < 0.05$).

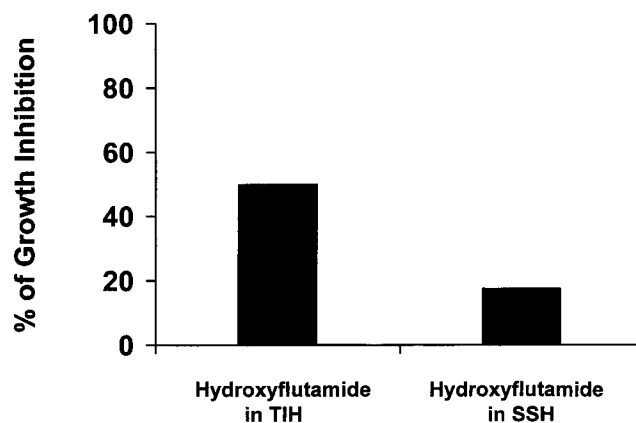


Fig. 4. Inhibition of BPH and cancer growth in TIH after treatment with the antiandrogen hydroxyflutamide, compared to inhibition of the same tissue in SSH. Inhibition was expressed as percentage of DHT-treated control.

achieve better diffusion of the compounds in the medium into tissue. As a result we observed greater inhibition of benign and malignant prostate tissue growth by genistein and hydroxyflutamide at the same concentrations previously used in collagen sponge-gel-supported tissue culture. Increased diffusion in TIH has been directly demonstrated in the case of [³H]-thymidine and can be inferred in the case of genistein and hydroxyflutamide, based on the greater sensitivity in TIH as shown in Figures 2 and 3.

Altogether, the CV for replicate samples within the same tissue for inhibition of [³H]-thymidine incorporation by genistein was similar using TIH compared to SSH (average CV 25% vs. 19%, respectively). However, the between-assay CV for percent inhibition of

[³H]-thymidine incorporation by genistein, based on five consecutive experiments done with five different prostates, was 8%, with a mean inhibition of 73% for TIH, compared to an 83% CV with a mean inhibition of 30% for SSH. Thus, the between-experiments reproducibility of TIH seems superior.

The TIH model of BPH and prostate cancer will be used for screening and evaluation of new agents effective for these diseases. Further studies will involve testing other types of cancer tissues in order to develop TIH as a general approach for studying tumor tissue growth and response to various anticancer agents.

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