# Antiproliferative and Cytotoxic Effects of Geldanamycin, Cytochalasin E, Suramin and Thiacetazone in Human Prostate Xenograft Tumor Histocultures

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*Received May 11, 1998; accepted August 15, 1998*

***Purpose.*** We have shown that the three human prostate xenograft tumors, i.e. the androgen-dependent CWR22 tumor, and the androgen-resistant CWR22R and CWR91 tumors, are comparable to patient tumors in their expression of prostate specific antigen, multidrug resistance p-glycoprotein, p53 and Bcl-2 and in their sensitivity to doxorubicin and paclitaxel. The present study used histocultures of these xenograft tumors to evaluate the antiproliferative and cytotoxic effects of several drugs (geldanamycin, cytochalasin E and thiacetazone), which have diverse action mechanisms and have shown activity against primary cultures of human prostate cancer cells. Suramin, a clinically active compound was included for comparison.

***Methods.*** The antiproliferative effect of 96 h drug treatment was measured by inhibition of DNA precursor incorporation, and the cytotoxic or cell kill effect was measured by in situ DNA end labeling of apoptotic and necrotic cells and by reduction of live cell density.

***Results.*** The rank order of molar potency was geldanamycin > cytochalasin E > suramin ≥ thiacetazone. Thiacetazone produced antiproliferation only in CWR22 tumor and had no cytotoxicity, whereas the other three drugs produced both antiproliferation and cytotoxicity in all three tumors. Geldanamycin, but not cytochalasin E and suramin, showed greater antiproliferation and cytotoxicity in tumor cells compared to normal stromal cells. The two androgen-resistant tumors were 4 to >40-fold less sensitive than the androgen-dependent tumor to drug-induced antiproliferation but were about equally or 4 to >20-fold more sensitive to drug-induced cytotoxicity. The ratios of drug concentrations that produced 50% antiproliferation to the concentrations that produced 50% cytotoxicity ranged from <0.04 to 0.3 in CWR22 tumor, but ranged from 0.3 to 2.7 in CWR22R and CWR91 tumors, indicating a shift from antiproliferation as the predominant drug effect in the androgen-dependent tumor to cytotoxicity in the androgen-resistant tumors.

***Conclusions.*** Our results indicate (a) differential drug effects in human prostate xenograft tumors with antiproliferation and cytotoxicity as the predominant drug effect in the androgen-dependent and androgen-resistant tumors, respectively, (b) that progression of tumors from androgen-dependent state to androgen-resistant state appears to be associated with a lower sensitivity to drug-induced antiproliferation and an equal or greater sensitivity to drug-induced cytotoxicity, and (c) that geldanamycin but not thiacetazone warrants further development.

**KEY WORDS:** suramin; geldanamycin; cytochalasin E; thiacetazone; pharmacodynamics.

## INTRODUCTION

The need of more effective treatments for prostate cancer has prompted significant research activity in the development of drugs for this disease. Peehl *et al.* have used primary cultures of human prostate tumors obtained via radical prostatectomy to evaluate the activity of over one thousand agents and identified several compounds with selective activity. These compounds are geldanamycin, cytochalasin E, thiacetazone, brefeldin A, jasplakinolide, cucurbitacin E, glaucarubinone, borrelidin, discorrhabdin C, and flavone derivatives (1,2). In this earlier study, which was only described in the form of meeting abstracts, the patient prostate tumors used to identify drug activity were typically derived from early stage and organ-confined disease (1,2). The activity of these compounds in androgen-resistant tumors has not been studied.

The purpose of the present study was to evaluate the activity of geldanamycin, cytochalasin E, thiacetazone, and suramin against human prostate tumors and to determine if androgen-dependent and -resistant tumors respond differently to these agents. The first three compounds were selected because of their commercial availability. Suramin, because it produces a 20 to 40% response rate in hormone refractory prostate cancer patients, was included as a reference. Geldanamycin was isolated from the culture filtrates of a streptomycetes and is the first known benzoquinoid ansamycin antibiotics (3). It has shown activity against most cancer cell lines studied and has been selected for preclinical development by the National Cancer Institute (4). By binding to the heat shock protein Hsp90 and its endoplasmic reticulum homolog GP96 which are required for the conformational maturation of nuclear hormone receptors and proto-oncogenic protein kinases, geldanamycin depletes cells of nuclear hormone receptors and the protein kinases (5–7). Thiacetazone is a ribonucleotide reductase inhibitor and inhibits the growth of human colon carcinoma cells, leukemia cells, and Ehrlich ascites carcinoma (8–10). Cytochalasin E inhibits polymerization and induces depolymerization of actin filaments (11,12). Suramin, a polysulphonated naphthylurea, has multiple functions. It blocks the binding of fibroblast growth factors to their receptors and antagonizes the mitogenic actions of epidermal growth factor, platelet-derived growth factor, insulin-like growth factor, inhibits DNA polymerase, topoisomerase II, DNA synthesis, angiogenesis, and protein kinase C, as well as alters the metabolism of phosphoinositides and glycoaminoglycan (13).

The present study was performed using three human prostate xenograft tumors, i.e. CWR22, CWR22R and CWR91. The androgen-dependent CWR22 tumor was derived from a Gleason grade 9, Stage D, prostate carcinoma with osseous metastasis. CWR22R tumor is a subline of CWR22 tumor that was established upon recurrence following androgen withdrawal. CWR22 and CWR22R represent the first pair of human androgen-

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**ABBREVIATIONS:** BrdU, bromodeoxyuridine; DMSO, dimethylsulfoxide; $IC_{50}$ and $IC_{100}$, drug concentration that produces 50% and 100% inhibition of DNA precursor incorporation; $LC_{50}$ and $LC_{100}$, drug concentration that produces 50% and 100% cell death; LI, labeling index; MEM, minimum essential medium; PSA, prostate specific antigen; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling.

0724-8741/98/1100-1760$15.00/0 

dependent and resistant xenograft tumors that are derived from the same patient tumor. CWR91 was derived separately from a Gleason grade 7, Stage C, androgen-resistant prostate carcinoma (14–16). We have shown that the three xenograft tumors encompass the majority of the heterogenous patient prostate tumors in the expression of p-glycoprotein, prostate specific antigen, p53 and Bcl-2 proteins and are comparable to patient tumors in their sensitivity to doxorubicin and paclitaxel (17).

The previous study by Peehl *et al.* on drug activity in primary prostate tumor cultures used the sulforhodamine B assay (1,2), which is an indirect measure of cell number and therefore measures the overall drug effect, i.e. combination of antiproliferation and cytotoxicity. It has been proposed that for slowly growing tumors such as prostate cancer, drug-induced cytotoxicity is more clinically relevant than antiproliferation, i.e. tumor shrinkage probably derives from elimination of tumor cells than from inhibition of tumor cell proliferation (18). The present study measured the antiproliferative and cytotoxic effects separately. Furthermore, the earlier study used monolayer primary cultures of human prostate tumor cells. The present study used tumor histocultures. The major advantages of the histoculture system over monolayer cell culture are the maintenance of 3-dimensional tissue architecture with intact stroma, cell-cell interaction, and inter- and intra-tumoral heterogeneity (19). These characteristics are important for prostate tumor growth because the interaction between tumor and stromal cells is thought to be important for epithelial growth and response to androgen stimulation (20,21). Furthermore, multilayered structure-related drug resistance is well recognized (22,23). The clinical relevance of the histoculture model has been shown by Hoffman and colleagues in retrospective and semi-prospective preclinical and clinical studies; the drug response of human tumor histocultures correlates with the sensitivity, resistance and survival of head and neck, colorectal and gastric cancer patients to treatment by mitomycin C, doxorubicin, 5-fluorouracil, or cisplatin (24–26).

## MATERIALS AND METHODS

### Chemicals and Supplies

Thiacetazone, geldanamycin, and cytochalasin E were provided by the National Cancer Institute (Bethesda, MD). BrdU was purchased from Sigma Chemical Co. (St. Louis, MO), suramin from FBA Pharmaceutical (Leverkusen, Germany), sterile pigskin collagen (Spongostan Standard) from Health Designs Industries (Rochester, NY), cefotaxime sodium from Hoechst-Roussel (Somerville, NJ), gentamicin from Solo Pak Laboratories (Franklin Park, IL), Minimum Eagle's medium (MEM), nonessential amino acid, and L-glutamine from Life Technologies, Inc. (Grand Island, NY), Matrigel from Becton Dickinson Labware (Bedford, MA), Apoptag In Situ Apoptosis Detection Kit from Oncor Inc. (Gaithersburg, MD), antibody against BrdU from BioGenex (San Ramon, CA), labeled streptavidin-biotin (LSAB) detection kit from DAKO Corp. (Carpinteria, CA). All chemicals and reagents were used as received.

### Animals and Tumors

Male athymic Nu/Nu Balb/C retired breeder mice were purchased from the National Cancer Institute, and cared for in accord with institutional guidelines. Human prostate xenografts CWR22, CWR22R and CWR91 were kindly provided by Dr. Thomas G. Pretlow at the Case Western Reserve University. The tumors were transplanted in nude mice according to the method previously described (16). Briefly, a mixture of tumor fragments and Matrigel was injected subcutaneously. Three day prior to transplantation of CWR22 tumor, the mouse was castrated and a sustained-release testosterone pellet was implanted subcutaneously. Testosterone supplement was not needed for CWR22R and CWR91 tumors. When the tumor reached about 1 g in size at about 1.5 to 2.5 months, it was resected and used for histoculture.

### Histoculture

Histoculture of tumors was performed as described previously (27), with the exception that 100% MEM was used instead of 50% MEM and 50% Dulbecco's Modified Eagle Medium. In brief, the tumors were cut to about 1 $mm^3$ pieces. Four to six tumor pieces were placed on a 1 $cm^2$ collagen gel presoaked in medium and cultured in 6-well plates in a humidified atmosphere of 95% air and 5% $CO_2$ at 37°C. The culture medium (pH 7.4) consisted of MEM supplemented with 9% heat-inactivated fetal bovine serum, 0.1 mM nonessential amino acid, 0.2 mM L-glutamine, 100 mg/ml gentamicin and 95 mg/ml cefotaxime. The histocultures were used for pharmacodynamic studies on day 3 or 4. We have shown that human prostate tumors can be maintained as histocultures for up to 56 days without significant changes in proliferative activity or secretion of prostate specific antigen (PSA) (27).

### Quantitation of PSA Secretion

Drug-induced reduction of PSA secretion in histoculture was monitored by the PSA concentrations in culture medium. PSA was measured by a sandwich-immunoassay using two antibodies against two different epitope sites on PSA. PSA measurement was performed by the Immunology Laboratory of the James Cancer Hospital (Columbus, OH).

### Pharmacodynamic Studies

Tumors were exposed for 96 h to suramin (0.01 to 10 mM), geldanamycin (0.01 to 10 μM), cytochalasin E (0.01 to 100 μM), or thiacetazone (8 to 800 μM). The earlier study which reported the activity of these compounds in primary cultures of prostate cancer cells did not provide the effective drug concentrations (1,2). In the present study, the maximum concentrations of suramin and geldanamycin represented 60–100 times the concentrations that produced a 50% reduction in the number of human prostate PC3 tumor cells grown in monolayers after 96 h treatment (unpublished results). The maximum thiacetazone concentration was near its maximal solubility in culture medium. The maximum concentration of cytochalasin E was arbitrarily selected. Drugs were first dissolved in 100% dimethyl sulfoxide (DMSO) solution and then transferred to the culture medium. The final DMSO concentration in the medium was 0.1% for cytochalasin E and 2% for other drugs. Equal concentrations of DMSO were added to controls. After drug treatment, tumors were washed three times with 5 ml of drug-free medium, each for 10 min and incubated in medium containing 40 μM BrdU for 48 h. Controls were

processed similarly, with the exception of drug treatment. Tumor tissues were fixed in formalin and embedded in paraffin. Histologic tissue slides were processed for drug activity evaluation.

Drug-induced antiproliferation was measured by inhibition of BrdU incorporation. BrdU-labeled cells were detected by an immunohistochemical method using the LSAB kit, as previously described (28). Briefly, tissue slides were dewaxed, rehydrated, boiled in citrate buffer, and then sequentially incubated with BrdU monoclonal antibody (1:250 dilution in phosphate buffered saline in 5% bovine serum albumin), linker solution, peroxidase-conjugated streptavidin, and finally with the chromogen 3,3′-diaminobenzidine and the substrate hydrogen peroxide. The slides were counter stained with hematoxylin. Mouse IgG was used as negative control. BrdU-labeled cells were scored under a microscope, and the fraction of labeled cells (LI) was determined. A typical experiment used a total of 8 to 15 tumor pieces for each drug concentration. A minimum of 200 cells per piece, or >1,600 cells, was counted per concentration. The experiments were repeated 4 to 5 times.

Drug-induced cytotoxicity was measured by two methods. The first method was to count the terminally damaged cells identified by morphological changes and by the TUNEL assay. The morphological features of apoptotic cells included chromatin condensation and margination, membrane blebbing, apoptotic bodies and cell shrinkage. Necrosis was recognized by cytoplasmic vacuolation, swelling of cells, loss of membrane integrity, and gross cytolysis. Apoptotic cells are usually scattered throughout the tumor fragment, whereas necrotic cells usually appear as groups of adjoining cells (28). The TUNEL assay labels fragmented DNA and therefore labels both apoptotic and necrotic cells. TUNEL was performed as described previously (28). The second measurement of cytotoxicity was to determine the reduction in the number of morphologically intact tumor cells that are not labeled by TUNEL per 20 counting grids.

### Pharmacodynamic Data Analysis

The sigmoidal drug concentration-dependent antiproliferative and cytotoxic effects as a function of drug concentration was analyzed using the following equation.

$$E = E_0 \cdot \left(1 - \frac{C^n}{K^n + C^n}\right) \quad (1)$$

For the analysis of antiproliferative effect, E is the LI of drug-treated tumor, $E_0$ is the LI of untreated controls. For the analysis of cytotoxic effect, E is the cell density in drug-treated tumor relative to that in untreated control ($E_0$). C is the drug concentration, K is the drug concentration at one-half $E_0$, and n is a curve shape parameter. Computer-fitting the equation to experimental data provided $IC_{50}$ and $IC_{100}$ (drug concentrations that produced 50% and 100% inhibition), and $LC_{50}$ and $LC_{100}$ (drug concentrations that produced 50% and 100% cell death).

### Statistical Analysis

Differences in mean values between groups, with standard deviations of similar magnitude, were analyzed using unpaired Student's t test, and by the Wilcoxon two-sample test when otherwise. Software for statistical analysis (NPAR1WAY and TTest procedures) was by SAS (Cary, NC).

## RESULTS

### Histocultures of Xenograft Tumors

Figure 1 shows the micrographs of histocultures of CWR22, CWR22R, and CWR91 tumors. The histocultures of all three tumors maintained the 3-dimensional structure, showed presence of epithelial tumor cells and normal stroma (fibroblasts and muscle cells). The CWR22 tumor also retained scattered acinus-like structures. The BrdU LI of the untreated controls were 32 ± 3%, 60 ± 2%, and 58 ± 5%. Our previously reported LI for patient tumor histocultures was higher than the LI of the androgen-dependent CWR22 tumor, but lower than the LI of the androgen-resistant CWR22R and CWR91 tumors. The LI in patient and xenograft tumor histocultures is higher than the proliferation index of between 1.6 to 16% found in other studies that used pulse labeling with DNA precursors or snapshot measurement of the expression of proliferation markers such as proliferation cell nuclear antigen or Ki-67 (29). This is probably because the LI of histocultures represents the cumulative LI measured over 48 h, or a 48 to 500-fold longer labeling period, compared to the short-term labeling studies (30,31).

A comparison of the untreated controls before and after culture shows no changes in morphology nor expression of PSA (data not shown), indicating the preservation of tumor morphology and functional characteristics during histoculture.

### Drug Effects in Xenograft Tumors

Figure 1 shows the micrographs of histocultures of xenograft tumors. The histocultures contained densely packed tumor cells. Hence a reduction in the density of live cells indicated the extent of drug-induced cytotoxicity. Geldanamycin, cytochalasin E, and suramin produced concentration-dependent antiproliferation and cytotoxicity in all three xenograft tumors, whereas thiacetazone at its maximal solubility produced only antiproliferation which occurred only in CWR22 tumor. Geldanamycin and cytochalasin E induced tumor cell apoptosis, whereas suramin induced necrosis (Fig. 1). In general, the rank order of molar potency in all three tumors was geldanamycin > cytochalasin E > suramin ≥ thiacetazone (Table 1). The antiproliferative and cytotoxic drug effects were observed throughout tumor histocultures, without apparent localization or preference for different regions, i.e. periphery or core, within the tumor.

### Tumor Cell Selectivity of Geldanamycin

In the three xenograft tumors, geldanamycin showed a greater effect in tumor cells compared to normal stromal cells. For example, 1 μM geldanamycin completely inhibited DNA synthesis and produced 10% cytotoxicity in tumor cells but had no effect on the stromal and muscle cells (Fig. 1). In contrast, suramin, cytochalasin E and thiacetazone produced about equal antiproliferation and/or cytotoxicity in normal and tumor cells (not shown). These results indicate that geldanamycin has the highest tumor selectivity among the four drugs.

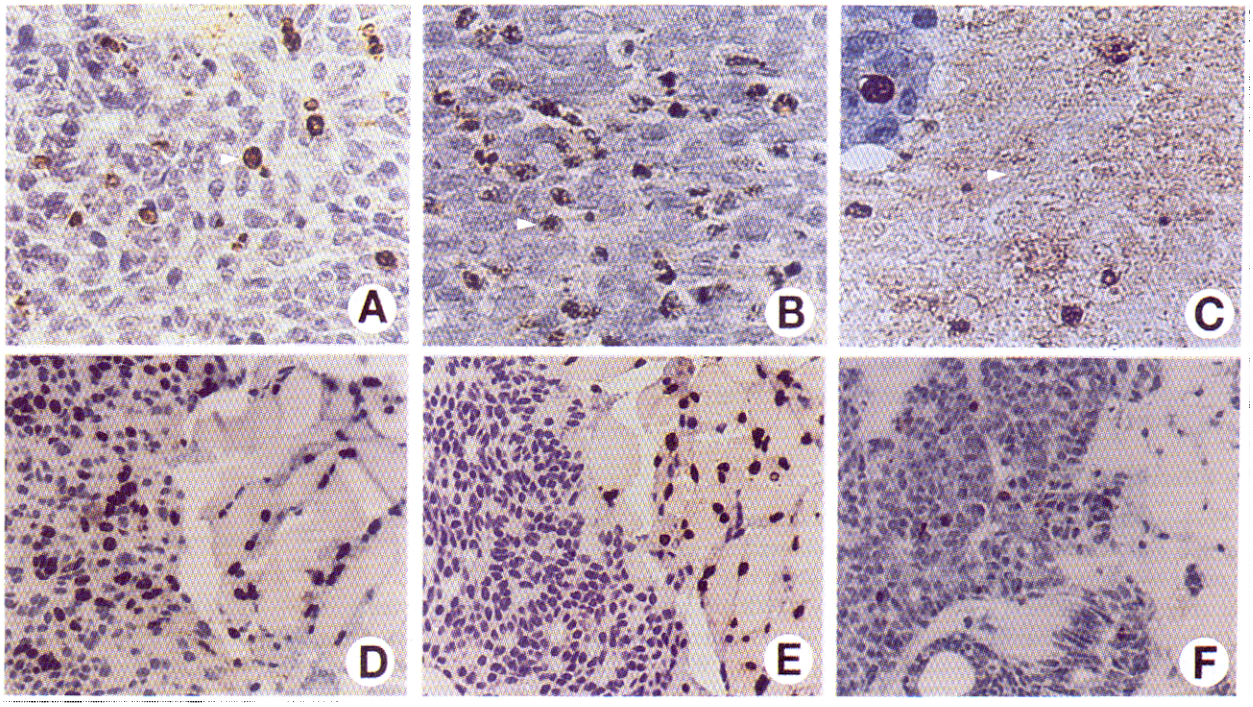


**Fig. 1.** Drug effects on CWR22, CWR22R and CWR91 tumors. Tumors were treated with drugs for 96 h. Panels A to C: Geldanamycin (1 μM in CWR22, panel A) and cytochalasin E (100 μM in CWR22R, panel B) induced apoptosis, and suramin (1,000 μM in CWR91, panel C) induced necrosis. Cells with apoptotic or necrotic morphologies are indicated by arrows. Note the scattered apoptotic cells vs the adjoining necrotic cells. Panels D to F: Tumor cell selectivity of geldanamycin in CWR22 tumor. Untreated controls showed BrdU-labeling of tumor and stromal cells (D). One μM geldanamycin completely inhibited BrdU labeling of tumor cells (E) and produced TUNEL-labeled apoptotic cells (F) but had no effect on stromal cells (E and F). Micrographs in panels A to C were obtained under 400× magnification. Micrographs in panels D to F were obtained under 200× magnification to simultaneously show the stromal and tumor cells.

**Table 1.** Effects of Suramin, Geldanamycin, Cytochalasin E, and Thiacetazone in CWR22, CWR22R, and CWR91 Tumors

| | | Antiproliferation | | | Cytotoxicity | | | |
|---|---|---|---|---|---|---|---|---|
| Drug | Tumor | $IC_{50}$ (μM) | Ratio to CWR22 | p | $LC_{50}$ (μM) | Ratio to CWR22 | p | $IC_{50}$:$LC_{50}$ ratio |
| Suramin | CWR22 | 294 ± 49 | 1 | NA | 2180 ± 420 | 1 | NA | 0.13 |
| | CWR22R | 2150 ± 690 | 7.3 | <0.01 | 3710 ± 670 | 1.7 | <0.05 | 0.7 |
| | CWR91 | 1410 ± 320 | 4.8 | <0.01 | 526 ± 106 | 0.24 | <0.01 | 2.7 |
| Geldanamycin | CWR22 | 0.3 ± 0.1 | 1 | NA | 9.8 ± 3.8 | 1 | NA | 0.03 |
| | CWR22R | 1.8 ± 0.1 | 6.0 | <0.01 | 5.4 ± 1.0 | 0.55 | <0.05 | 0.33 |
| | CWR91 | 1.3 ± 0.3 | 4.3 | <0.01 | 1.1 ± 0.3 | 0.11 | <0.01 | 1.2 |
| Cytochalasin E | CWR22 | 3.7 ± 1.3 | 1 | NA | >100 | 1 | NA | <0.04 |
| | CWR22R | 29 ± 7.6 | 7.8 | <0.01 | >100 | NA | NA | <0.29 |
| | CWR91 | 9.2 ± 4.9 | 2.5 | <0.01 | 4.8 ± 0.4 | <0.05 | <0.01 | 1.9 |
| Thiacetazone | CWR22 | 213 ± 54 | 1 | NA | >800 | 1 | NA | <0.27 |
| | CWR22R | >800 | >3.8 | <0.01 | >800 | NA | NA | NA |
| | CWR91 | >800 | >3.8 | <0.01 | >800 | NA | NA | NA |

*Note*: Tumors were treated with drugs for 96 h. $IC_{50}$ and $LC_{50}$ are the drug concentrations that produced 50% antiproliferative and cytotoxic effects, respectively. Data are mean ± SD of 4 to 5 experiments with 2 to 3 replicates per experiment. The tumor LI of untreated controls were 32 ± 3%, 60 ± 2%, and 58 ± 6%, for CWR22, CWR22R, and CWR91 tumors, respectively. P: level of significance, comparing to CWR22 tumor. NA: not applicable.

### Drug Effects in CWR22 Tumor

Figures 2 and 3 show the concentration-dependent antiproliferative and cytotoxic drug effects in CWR22 tumor. All four drugs were able to induce 100% antiproliferation. The cytotoxic effects of the four drugs were considerably lower than their antiproliferative effects. For example, the cytotoxicity of thiacetazone was not detectable at a concentration that is twice its $IC_{100}$, the cytotoxicity of cytochalasin E was only 10% at a concentration that was equal to its $IC_{100}$, and geldanamycin and suramin did not produce cytotoxicity at concentrations that were equal to their $IC_{100}$. Consistent with these findings, the ratios of $IC_{50}$ to $LC_{50}$ values were far less than one (Table 1). These data indicate antiproliferation as the predominant drug effect in CWR22 tumor.

### Drug Effects in CWR22R Tumor

For antiproliferation, the activity of geldanamycin, cytochalasin E and suramin in CWR22R tumor followed the same rank order but were ~7 fold less potent compared to CWR22 tumor (Fig. 2 and Table 1). For cytotoxicity, CWR22R was more sensitive to geldanamycin, about equally sensitive to cytochalasin E, but less sensitive to suramin compared to CWR22 tumor (Fig. 3 and Table 1). As in CWR22 tumors, the antiproliferative effects of geldanamycin and suramin were greater than their cytotoxic effects, resulting in $IC_{50}$:$LC_{50}$ ratios of <1.0.

### Drug Effects in CWR91 Tumor

In CWR91 tumor, geldanamycin, cytochalasin E, and suramin produced a significantly lower antiproliferative effect compared to CWR22 tumor but significantly more cytotoxicity compared to CWR22 and CWR22R tumors. The $LC_{50}$ values for geldanamycin, cytochalasin E and suramin were lower than their $IC_{50}$ values, indicating cytotoxicity as the predominant drug effect in CWR91 tumor (Figs. 2 and 3, and Table 1).

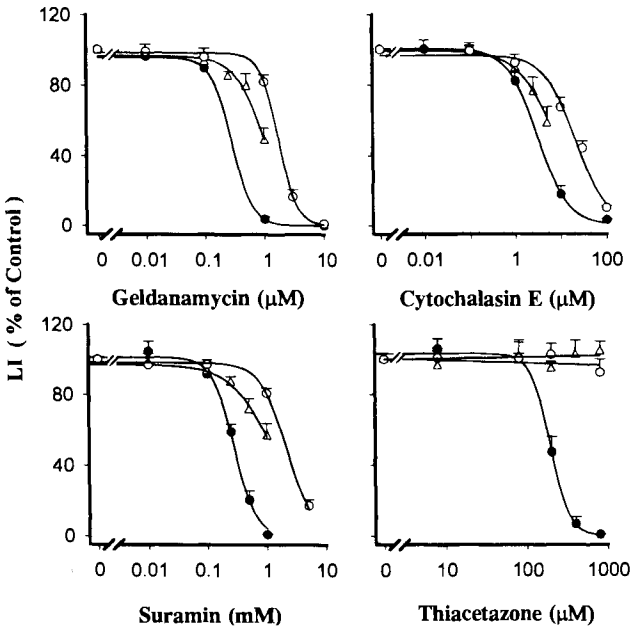


**Fig. 2.** Concentration-effect relationship of drug-induced antiproliferation. Tumors were treated with drugs for 96 h. Antiproliferative effect was measured by inhibition of 48 h cumulative BrdU incorporation. Closed circles, CWR22; open circles, CWR22R; open triangle, CWR91 tumors. Data are mean ± SD of 8–15 observations per concentration in a representative tumor. Lines are computer-fitted concentration-effect curves according to equation 1. For CWR91 tumors, the antiproliferative effect of >1 μM geldanamycin, >50 μM cytochalasin E, and >1 mM suramin could not be measured because of the 100% cell kill at these drug concentrations.

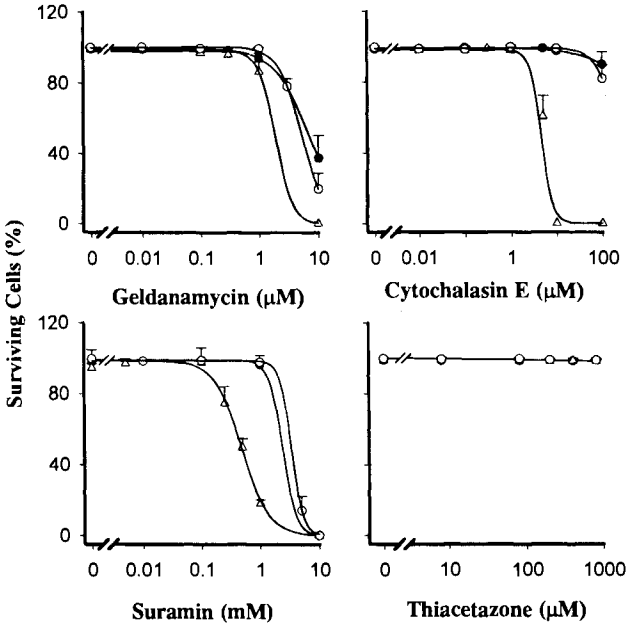


**Fig. 3.** Concentration-effect relationship of drug-induced cytotoxicity. Tumors were treated with drugs for 96 h. Cytotoxic effects were evaluated by the reduction in the live tumor cell numbers per 20 grids. Live cells were identified as cells with intact morphology and were not labeled by TUNEL. Closed circles, CWR22; open circles, CWR22R; open triangle, CWR91 tumors. Data are mean ± SD of 8–15 observations per concentration in a representative tumor. Lines are computer-fitted concentration-effect curves according to equation 1.

## DISCUSSION

Results of the present study show that geldanamycin, cytochalasin E and suramin produced antiproliferation and cytotoxicity in androgen-dependent and -resistant human prostate xenograft tumors, whereas thiacetazone produced only antiproliferation which occurred only in the androgen-dependent tumor. Our finding that thiacetazone has limited activity in the androgen-independent tumor histocultures and no cytotoxicity in the androgen-independent tumor histocultures is opposite to the previous finding that thiacetazone has significant activity in primary culture of human prostate tumor cells (1,2). This may be due to the sbustantial differences in the two model systems (see Introduction).

Our results indicate that the predominant drug effect was antiproliferation in the androgen-dependent CWR22 tumor and cytotoxicity in the androgen-resistant CWR91 tumor, and that the progression of tumor from androgen-dependent state to androgen-resistant state generally resulted in a lower sensitivity to drug-induced antiproliferation but a higher sensitivity to drug-induced cytotoxicity. The latter is consistent with our finding in these xenograft tumors treated with doxorubicin and paclitaxel (17). Very little is known on the chemosensitivity of the androgen-dependent prostate tumors, in part because hormone deprivation instead of chemotherapy has been the

traditional treatment used to treat this disease. Likewise, very little is known on the chemosensitivity of the androgen-independent tumors. Hence, our finding is noteworthy and supports the use of chemotherapy to eliminate the small subset of androgen-independent tumor cells early in the course of the disease rather than after androgen ablation therapy has failed. Collectively, these findings indicate a shift from antiproliferation as the predominant drug effect in androgen-dependent tumors to cytotoxicity in androgen-independent tumors.

The characterization of histocultures of the three tumors used in the present study is described in our previous publication (17). The results show that there are no systematic differences in the expression of several of the genes known to be related to drug effects (i.e. *mdr1*, tumor suppressor gene *p53*, and antiapoptotic gene *bcl-2*) between androgen-dependent and -independent tumors; semi-quantitative measurement of the corresponding protein products of these genes in the three tumors shows low expression of *mdr1* and *bcl-2* in all three tumors and that the *p53* expression of the androgen-dependent CWR22 is between the other two androgen-independent tumors (17). Elucidation of the relationship between the expression of these genes and drug effect is of interest, but would require a larger number of xenograft models displaying different levels of gene expression than the models that are currently available.

The drug potency in the three xenograft tumors follows a rank order of geldanamycin > cytochalasin E > suramin ≥ thiacetazone. Because drug-induced antiproliferation and cell death in the hisocultures occurred randomly throughout the tumor mass (which suggests no restriction in drug penetration), we propose that variability in drug penetration in the histocultures is not a likely cause of the different activity of the four drugs. Extrapolation of the results in histocultures to project the rank order of the clinical potency of these four drugs, one must take into consideration the clinical achievable concentrations, i.e. 300 μg/ml or 210 μM for suramin (13,32) and unknown for the other three drugs, as well as the different drug penetration into solid tumors under *in vitro* and *in vivo* conditions. Finally, our results also indicate that geldanamycin has the highest tumor-to-stromal selectivity. Geldanamycin has been shown to produce a greater cytotoxicity in the less differentiated neuroectodermal tumor cells compared to fibroblasts and highly differentiated neuroma cells (33). This tumor-to-normal cell selectivity may result in therapeutic selectivity and is therefore of interest. The high tumor selectivity of geldanamycin in the three xenograft tumors and its significant cytotoxicity in CWR91 tumor warrant further development of this drug for the treatment of prostate cancer, particularly for androgen-resistant cancer. Conversely, the limited activity of thiacetazone in CWR22R and CWR91 tumors and its lack of cytotoxic effect in androgen-dependent and -independent tumors suggests that this agent has limited use.

## ACKNOWLEDGMENTS

The authors thank Dr. Thomas Pretlow for providing the CWR22, CWR22R and CWR91 tumors. We thank Mike Lembach for technical assistance. This work was supported in part by an award from the CaPCure Foundation, a grant from Bristol Myers Squibb Co., and the OSUCCC Cancer Center Support Grant P30 CA16058.

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