Effects of Curcumin on LNCaP Prostate Cancer Cells

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ABSTRACT

Dietary phytochemicals have come under increasing scrutiny as new chemotherapeutic drugs. Curcumin has been used as an anti-inflammatory agent, an antioxidant and investigated as a cancer chemoprevention agent. It has been recently reported that the phytochemical curcumin is able to induce micronucleation in MCF-7 breast cancer cells by perturbing mitotic spindle organization. However, it is not known whether this effect of curcumin occurs in other types of transformed cells. The purpose of this research is to test whether curcumin can also disrupt the mitotic spindle organization in a prostate cancer cell line. Observations were made using immunolabeling methods, viability assays and time-lapse movies of living curcumin treated cultures. LNCaP cells treated with 10-20 µM curcumin exhibited monopolar spindles and a higher percentage of mitotic cells than control cells. Cell counts showed that total number of cells decreased significantly with curcumin treatments, suggesting that M-phase was abnormal. Cells treated with 10-20 µM curcumin rounded up during mitosis as did the control cells, however, the outcomes varied, and included normal division, prolonged M-phase, and irregular departure from M-phase after long delays. Although curcumin resulted in monopolar spindle formation, similar to that reported for MCF-7 breast cancer cells, very few micronucleated cells were apparent after curcumin treatment. These experiments demonstrate that curcumin is able to disrupt mitosis in a number of transformed cell types, but that the end result varies depending on the specific cell line. The ability of curcumin to interfere with cancer cell division may be useful in the further development of this phytochemical as an anti-cancer agent.

Introduction

About 189,000 men will be diagnosed in the United States with prostate cancer this year and of those diagnosed, 30,200 will die of the disease [1]. Prostate cancer is affecting more males today, given its late life occurrence and the increasing average life span. Currently, enduring
therapies for prostate cancer are lacking due to toxic side effects and the emergence of resistant clones of malignant cells [2,3].

Natural products in the diet, such as plant phytochemicals, have received increasing attention as chemopreventive agents because of their antioxidative, anti-inflammatory activities and low toxicity [4]. Examples include, biochanin A, a major isoflavone present in red clover and indole-3-carbinol, which both inhibit prostate cancer cell growth by inducing cell cycle arrest and apoptosis [5,6].

Curcumin, a natural pigment of the spice turmeric, is a phytochemical used world wide for different treatments. India has used curcumin for hundreds of years as an anti-inflammatory agent and as a remedy for fevers, wounds, pain, ulcers and even the common cold [7,8]. Curcumin has shown chemopreventive activity in colon cancer [9]. It also shows anticancer activity, demonstrating its possible use as a chemotherapeutic agent [10, 11]. Curcumin inhibits cell proliferation by disrupting the signaling pathways in prostate cancer cells, and some cells treated with curcumin undergo apoptosis [7]. Interestingly, there is conflicting data regarding the role of curcumin in genetic instability. Wistar rat cells treated with curcumin display a reduced incidence of chromosomal aberration following treatment with cyclophosphamide [12]. On the other hand, curcumin increases the frequency of chromosomal aberration in Chinese hamster ovary cells [13].

Little is known concerning the effects of curcumin on cellular organization, however one study has revealed that curcumin can cause both, multinucleation and M-phase arrest, and can disrupt mitotic spindle organization [14]. The purpose of this research is to determine if curcumin causes structural changes in the mitotic spindles of other hormone dependent cancer cell lines. For this purpose, LNCaP prostate cancer cells were treated with curcumin, fixed and labeled for immunofluorescence. Proliferation rates and mitotic rates were studied via viability assays, and cellular division was monitored with time-lapse movies.

Materials and Methods

Cell Culture and Drug Treatments. LNCaP prostate cancer cells (American Type Culture Collection, Manassas, VA) were maintained in 90% Dulbecco’s minimal essential media (DMEM) and 10% fetal calf serum (FBS) (HyClone Inc., Logan, UT). Media was replaced every 4-5 days, and cells were passaged using 0.05% trypsin and 0.5 mM EDTA in phosphate-buffered saline (PBS). To determine effects of curcumin on cell proliferation, cells were seeded at 2X10^4 cells/ml and grown for two days directly on six well tissue culture plates. Each well was treated for 24 and 72 hours with 0, 10 or 20 µM of curcumin (Sigma Chemical Co., St. Louis,
Mo) from a 100X stock solution prepared fresh from a 0.2 M solution in dimethylsulfoxide (DMSO). Cells were suspended with trypsin/EDTA and counted with a hemocytometer using 0.05% Typan Blue.

**Immunofluorescence.** LNCaP cells were seeded onto coverslips at 2\times 10^4 cells/ml and treated with 0, 5, 10, 20, 40, or 80 µM curcumin for 12, 24, 48, 72, 96 or 120 hours. Coverslips were removed from media and fixed in cold methanol at specified time points. Cells were rehydrated in PBST and blocked for 1 hour at 37°C in a 5% milk solution in PBST. They were double labeled with two primary antibodies, NuMA and E-7 for an hour each. NuMA targets the proteins in the centosomes during mitosis, while E-7 labels tubulin found in the microtubules. Coverslips were then washed 3 times in PBST for 10 minutes and labeled with a mixture of goat-anti-mouse/sb conjugate to Texas red, goat-anti-rabbit conjugate to fluorescein, and 10 µg/ml Hoechst 33258 (Sigma) to counterstain DNA. Cells were photographed, with Nikon Optiphot epifluorescence microscope, for cell counts and qualitative analysis.

**Time-lapse movies.** 25 cm³ flasks were seeded at 2\times 10^4 cells/ml and grown for two days. Flasks were then tightly capped to prevent lose of CO₂, and placed on the pre-warmed stage of an olympus XXX microscope and imaged using a XXX camera and NIH image software. They were then drugged with 0, 10 and 20 µM curcumin and filmed for 23 hours.

**Results**

A total count of living and dead cells treated with 10 µM curcumin for 24 hours were 24.5% of the control total cell count, while the 20 µM was 22.5%. At 72 hours the percentages were 27% and 23% respectively [Figure 1]. Treated cultures had low number of dead cells.

Cells treated with 0, 10, and 20 µM of curcumin were counted for percent mitosis [Figure 2]. At 18 hours, control cells had a mitotic rate of 2.5%, 10 µM and 20 µM had mitotic rates of 8.4% and 3.8% respectively. At 24 hours the mitotic rate decreased to 6.4 in the 10 µM and increased to 19.3% in the 20 µM, but at 72 hours both the 10 µM and 20 µM decreased significantly to 2.4 and 0.7%. The rate of cells in telophase was less than 1% in all treatments.

Hoechst stain revealed the condensed mitotic DNA did differ between treated and non-treated cells (Figure 3.1, 3.4 and 3.7). The treated cells had irregular condensed spheres of DNA, while the control cells had one evenly condensed sphere.
The E-7 targeted tubulin in the mitotic spindles, revealing that bipolar spindles were extremely straight, while monopolar cells were straight or wavy (Figure 3.6 and 3.9). In the control cells there was no development of monopolar spindles. The 10 µM treated cells had about half monopolar and half bipolar spindles, whereas the 20 µM treated cells had about 80 to 98% monopolar spindles.

NuMA labeling showed that control mitotic cells had tightly condensed centrosomes (Figure 3.2). In contrast, the 10 and 20 µM treated cells had centrosomes that were elongated or fractured (Figure 3.5 and 3.8).

Observations from the immunolabeled slides revealed significant cell death from the 40-80 µM curcumin treatments. In the 20 µM treatment at 72 hours more cells appeared to be apoptotic, exhibiting the characteristic fragmented nuclei. Also in the 20 µM treatment at 72 hours, a few multinucleated cells were seen.

From the time-lapse videos it is apparent that control cells rapidly divide after rounding up. However, cells drugged with 10 or 20 µM of curcumin display different outcomes. Early in the drugging some cells round up and divide into two, but many round up and stay rounded up as the time of drug exposure increased. At 20 µM many more cells rounded up and stayed round than seen in the 10 µM. Only a low percentage of cells would round up and eventually flatten back down with time. The division of a control cell occurs with a 15 minute time frame, whereas the 20 µM treated cells stayed rounded up for over 20 hours (Figure 4.1 and 4.2)

**Discussion**

The fewer number of cells in curcumin treated cultures, observed by the typan blue counts, indicates that cell death is occurring, the cell cycle is being halted, or a combination of the two. The low number of telophase cells suggests that monopolar cells are not getting past anaphase, but are arrested in the G2-M phase, or are dying before they get to telophase. Examination of the drugged cultures suggests that there is an arrest given the low number of dead cells.

The higher mitotic cell count in 10 µM and 20 µM at 18 and 24 hours suggests that the cells are either arrested in mitosis or that this concentration of curcumin induces premature entry into mitosis. The idea that curcumin induces premature entry into mitosis seems unlikely given no increase in mitotic rates at earlier time points.

Immunofluorescent labeling showed that LNCaP cells treated with curcumin produced largely monopolar mitotic spindles, whereas, control cells produced bipolar spindles. At higher concentrations and longer
treatment times the mitotic spindles looked more disorganized. The centrosomes were unusual given their singular and often disorganized nature versus the control cells with their condensed duplex centrosomes.

Not all cells are affected in the same way, which is demonstrated by the three different outcomes in the time-lapse movies. It is possible that different degrees of spindle disruption might be correlated with whether the cell remains rounded up or eventually flattens back down. The multinucleated cells might be the cells that round up and flatten back down again, suggesting a failed division. Since there were some treated cells in the time-lapse movies that did round up and divide normally, these could be the cells seen in the immunolabeled slides that have bipolar spindles. Another observation from the time-lapse movies is that there is not a large increase in mitotic cells, suggesting a cell cycle arrest instead of premature entry of mitosis.

LNCaP prostate cancer cells treated with curcumin developed multinucleated cells that resembled MCF-7 breast cancer cells treated with curcumin [14], however, the multinucleated cells seen in the MCF-7 cells were often in the 20-30% range, where the LNCaP cells never exceed 1-2%. The lower number of multinucleated LNCaP cells would suggest that the abnormal monopolar cells are leaving mitosis in a different manner than the MCF-7 cells, either by surviving with a single nucleus with twice the DNA, or that the cells are entering apoptosis instead. Given only a few apoptotic cells in the 20 µM curcumin at 72 hours, it is more likely that the cells are leaving M-phase with twice the amount of DNA in one nucleus.

All of the experiments show that curcumin disrupts the mitotic spindle organization in LNCaP prostate cancer cells. Along with the research on MCF-7 breast cancer cells [14] it is evident that curcumin has an effect on hormone dependent breast and prostate cancer cells.

These results suggest that curcumin holds promise as a chemotherapeutic drug because of its ability to disrupt mitotic spindle organization, and thereby inhibit cell growth. Interestingly, this effect has now been observed in two types of hormone dependent cancer cells. It will be important to determine whether curcumin exhibit similar effects against other types of cancers as well, and whether it also perturbs cell division in normal, untransformed cell types.

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[Figure 1]

Proliferation rates were obtained by cell counts of trypsinized cells at 24 and 72 hours. The treatments were compared to the total cell count of the control cells.
[Figure 2]

Mitotic rates were obtained from cell counts of the immunolabeled slides at 18, 24 and 72 hours.
[Figure 3]

Immunofluorescent labeled slides of control and curcumin treated LNCaP cells to reveal cellular disorganization. (1) control, Hoechst stain. (2) control, NuMA labeled. (3) control, E-7 antibody. (4) 10 µM, Hoechst. (5) 10 µM, NuMA labeled. (6) 10 µM, E-7 antibody. (7) 20 µM, Hoechst. (8) 20 µM, NuMA labeled. (9) 20 µM, E-7 antibody.
[Figure 4.1 and 4.2]

Time-lapse movie frames taken at 37°C. (1) The two frames, from a control treatment, were photographed at 7.5 and 7.75 hours, respectively. (2) These three frames were of 20 μM treatments at 5, 12.3, and 24 hours. The cells were filmed for one hour without curcumin, then were drugged for a 23 hour period.
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Works Cited


