Curcumin: A new radio-sensitizer of squamous cell carcinoma cells

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PURPOSE: Curcumin, a potential chemopreventive agent, was found to inhibit cancer cells in S/G2M phases of the cell cycle, when radiation is more effective. The purpose of the current study was to investigate whether curcumin can sensitize squamous cell carcinoma (SCC) cells to the ionizing effects of irradiation.

METHODS: Curcumin (3.5 µM) was added for 48 hours to an SCC cell line prior to irradiation. Cell growth (counts) and colony-formation (colony-genic assay) were examined after radiation.

RESULTS: Incubation with curcumin only (3.75 µM) for 48 hours did not decrease the number of cells or the ability to form colonies in the absence of radiation. However, in plates that were exposed to 1-5 Gy of radiation, cell counts dropped significantly if pretreated with curcumin with a maximal effect at 2.5 Gy (where the cell counts dropped from 1240 to 1017, P < 0.001). The colonyogenic assay revealed a significant decrease in the ability to form colonies following pretreatment with curcumin in all radiation doses (P < 0.05).


Curcumin is the major phenolic antioxidant found in the spice turmeric. It has long been used as the yellow spice in Indian food and as a naturally occurring medicine for the treatment of inflammatory diseases.1 Curcumin has been shown to inhibit human myeloid leukemia cells2 and epidermoid carcinoma cells.3 Curcumin was also shown to inhibit the formation of chemically induced tumors in animal models (skin, gastrointestinal, mammary gland).4-8 The mechanism of action is not fully understood and is attributed to some of its properties such as its antioxidant activity,1 inhibition of protein kinase,9 inhibition of induced proto-oncogenes,10 and inhibition of mutagen binding to cellular DNA receptors.11 Recently, curcumin was shown to induce apoptosis in several human cancer cell lines but not in normal foreskin fibroblast cells.12 In previous studies, we found that curcumin blocks squamous cell carcinoma (SCC) cells at G2/S phases of cell cycle.13 This is in agreement with Chen et al14 who recently reported that curcumin caused a cell cycle arrest at the S, G2/M phases. Most chemopreventive/chemotherapy drugs block cell cycle in the G1 phase of the cell cycle (green tea15 for example). Holy16 reported interference in the mitotic spindle structure in breast cancer cells caused by curcumin that is similar to the alterations in spindle produced by the taxols, suggesting inhibition of the cell cycle at the G2/M phases of the cell cycle.

Radio sensitization has been studied extensively with various chemotherapy drugs (Cisplatinum/5FU/taxols) prior to irradiation or as concomitant treatment for patients with head and neck SCC. The side effects of these drugs, however, prevent their routine use for all irradiated patients and thus the chemotherapy-radiation protocols are currently used only as a part of clinical trials for organ preservation or for patients with unresectable cancers.

Radiation is known to have a more effective cytotoxic effect on cells that are at the G2 and mitosis phases more than to cells at other phases of cell cycle.17 It seems reasonable that pretreating cells with curcumin could result in radio-sensitization secondary to this specific cell cycle inhibition in a similar mechanism caused by the taxols, a family of potent radio-sensitizing agents. The purpose of the current study was to investigate the effect of curcumin as a potential radio-sensitizer of SCC cells in vitro.

MATERIALS AND METHODS

Cell Culture

The SCC cell line SCC2 is derived from an SCC of the oral cavity and is routinely maintained in modified eagles medium (MEM) with 10% fetal calf
serum (FCS) supplemented with antibiotics, multivitamins, glutamate, and amino acids. Cells were trypsinized using commercially available trypsin (0.125% trypsin-2mM EDTA). Radiation was delivered using a cobalt source, and the radiation doses ranged between 0-5 Gy. All experiments were performed under sterile conditions. Curcumin (Sigma) was dissolved in ethanol and stored as a 0.01M stock in -20°C. All manipulations with curcumin were performed under subdued light.

**Cell Counts**

A single cell suspension of cells at the log phase of their growth (100K cells) was plated on 24 petri dishes and allowed to attach over night. The medium was then changed to one containing 3.75 μM curcumin in 12 plates and regular media in the control plates (n = 12). After 48 hours of incubation, the plates were taken to the radiation center and irradiated at different doses of radiation. Immediately after the radiation treatment, the plates were brought on ice to the laboratory, trypsinized and replated. After 3 days, the cells were trypsinized off each plate and counted. Every count was repeated 3 times and averaged. Each group of 3 plates that received similar treatments (0, 1, 2.5, 5 Gy of radiation with and without curcumin) were plated on a petri dish, resulting in 24 plates for every experiment. The counts of each 3 plates were averaged and a standard deviation calculated.

**Colonogenic Assay**

In this part of the experiment, cells were treated with curcumin and radiation treatments similar to the previous section. After the radiation the cells were plated on small petri dishes with girds (Comig) at very low concentrations (0.1-1K) and left in the incubator. After 2 to 3 weeks, the plates were fixed with formalin and stained with methylene blue (enough stain to cover the entire plate). All colonies larger than 50 cells were counted. The number of colonies from plates with or without prior curcumin treatment was compared. Every 3 plates received similar treatment and were averaged and a standard deviation was calculated. The results of both parts of the study were analyzed using a paired t test.

**RESULTS**

A dose response curve was first plotted with various concentrations of curcumin (Fig 1). At a concentration of 3.75 μM, curcumin did not decrease the number of cells significantly and that concentration was chosen for the experiments.

**Cell Counts**

No major decrease in cell counts was noticed after treatment with curcumin alone (3.75 μM/48 hours) compared to control plates (Table 1). However, a significant decrease in cell number was found in plates that were treated with curcumin before irradiation compared to irradiation alone. As shown in Table 1, that was found true for all radiation doses but was more prominent after 2.5 Gy of radiation (P <0.001). Looking at cell counts as percentage of the control (Fig 2, A), there is a noticeable shift of the counts downward when pretreated with curcumin suggesting a more potent effect of radiotherapy on cells that were pretreated with curcumin. Since the P values were calculated as paired t test, the trend in the various experiments was toward radiosensitization. The effect of curcumin is shown in a graphic way (Fig 3) for cells treated with 2.5 Gy radiation, and there was a decrease in cell number after pretreatment with curcumin in all but 1 experiment, strengthening the significance of our results.

**Colonogenic Assay**

Colony counting also revealed a significant decrease in the ability of the treated cells to form colonies if these cells were treated with curcumin before the radiation compared to cells that were only treated with radiation or curcumin alone (Table 2). Colony formation as a percentage of control showed again a shift of the graph downward indicating a significant decrease in the ability of cells to form colonies if they were exposed to curcumin before the radiation compared to controls (Fig 2, B). The results were significant on a paired t test (P <0.05) and again looking at each experiment (at 2.5 Gy) re-
revealed a decrease in colony formation in all but one experiment (Fig 3, B).

**DISCUSSION**

Curcumin, a major antioxidant, was previously considered to protect cells against the ionizing effects of radiation by inhibiting the formation of free radicals and thereby reducing cell damage. In this study, curcumin caused a decrease in cell counts that was not observed after radiation alone. Results are shown as a percentage of the control for every radiation dose. A significant decrease in cell counts was observed when pretreated with curcumin compared to radiation alone.

Curcumin's effect on colony formation was also observed. A significant decrease in the number of colonies was noticed when pretreated with curcumin compared to radiation alone. In all but one experiment, there was a decrease in the number of colonies after curcumin pretreatment compared to the controls.

**Table 1.** The effect of curcumin on irradiated SCC cells: cell counts ($n = 7$)

<table>
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<tr>
<th>Radiation (Gy)</th>
<th>Control</th>
<th>SD</th>
<th>Curcumin</th>
<th>SD</th>
<th>$P$ value</th>
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<td>1492</td>
<td>995</td>
<td>1292</td>
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**Fig 2.** A, Effect of pretreatment with curcumin on cell counts is shown in relation to the radiation dose. Curcumin caused a decrease in cell counts that was not observed after radiation alone. B, Effect of pretreatment with curcumin on colony formation capability is shown in relation to the radiation dose. A significant decrease in the number of colonies when pretreated with curcumin compared to radiation alone is noticed.

**Fig 3.** A, Cells treated with 2.5 Gy radiation pretreated with curcumin vs control is shown (7 experiments). In all but 1 experiment curcumin caused radio-sensitization with a decrease in the number of cells counted. B, Colony formation capability is shown in relation to curcumin treatment for each experiment (5 experiments). In all but 1, there was a decrease in the number of colonies after curcumin pretreatment compared to the controls.
Curcumin was shown to prevent DNA damage and late effects of radiation on mice. However, more recently curcumin was found to act as a radio-sensitizer and potentiate the effect of gamma-radiation on hamster ovary cells and potentially prevent radio-resistance to irradiation in mice. In one study, the effect of curcumin before irradiation was investigated by studying quercetin-induced DNA damage, lipid peroxidation, and protein degradation. Curcumin did not inhibit DNA damage and protein degradation caused by radiation, and in an environment that was high in copper or iron it even stimulated quercetin-induced DNA damage and lipid peroxidation. Sahu and Washington concluded that the effect of curcumin on irradiated cells may be 2-fold: at times curcumin serves as an antioxidant and under certain conditions it may serve as a pro-oxidant and stimulate radiation damage. We based our hypothesis on previous results where we treated SCC cells with curcumin and found a S/G2M cell cycle arrest. In agreement with our previous results, Chen et al have also found that curcumin caused a cell cycle arrest in colon carcinoma cells at the S/G2M phases and Holy has shown that curcumin disrupts mitosis by interfering with the microtubule structures.

In the current study curcumin exhibited radio-sensitizing effects on squamous carcinoma cells suggesting that arrest at the S/G2M phases of the cell cycle may have had a radio-sensitizing effect on these cells in a way that resembles the action of the taxols, a family of potent radio-sensitizing agents. Our data suggest that curcumin did not cause a radio-sensitizing effect only by direct cell death. In the colonogenic assay, only cells that were able to replicate and form a colony of at least 50 cells were counted. The importance of that assay is thus in making the results more reliable by examining not only the immediate effects of radiation on cell viability but also late effects of radiation causing inhibition of the cellular proliferative capability. This may be caused by channeling cells into an apoptotic pathway and thus preventing them from replicating and forming colonies, an effect that would not be appreciated on cell growth where cells are counted a few days after radiation and apoptosis occurring at a later time may be overlooked. This is in agreement with the results reported by Chen et al for colon carcinoma cells and in other human cancer cell lines where curcumin induced apoptosis and cell death.

Curcumin may act as a radio-sensitizer of SCC cells and further studies need to confirm these preliminary results in an in vivo model.

REFERENCES


Table 2. The effect of curcumin on irradiated SCC cells: Colony formation (n = 5)

<table>
<thead>
<tr>
<th>Radiation (Gy)</th>
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<th>SD</th>
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