The Inhibition of the Estrogenic Effects of Pesticides and Environmental Chemicals by Curcumin and Isoflavonoids

Surendra P. Verma, Barry R. Goldin, and Peck S. Lin

1Department of Family Medicine and Community Health, Tufts University School of Medicine, Boston, MA 02111 USA; 2Department of Radiation Oncology, Medical College of Virginia, Richmond, VA 23298 USA

Many environmental chemicals and pesticides have been shown to be estrogenic and have been found to stimulate the growth of estrogen receptor-positive (ER-positive) human breast cancer cells. Since it is difficult to avoid human exposure to environmental estrogens, a potentially important area of research is the development of dietary strategies to prevent the stimulated growth of breast tumors by environmental estrogens. In this context, the inhibitory action of curcumin and a combination of curcumin and isoflavonoids were studied in ER-positive human breast cancer cells (MCF-7 and T47D) and ER-negative MDA-MB-231 cells induced by the pesticide p,p′-DDT and the environmental pollutants 4-nonylphenol and 4-octylphenol. The median inhibitory concentration (IC₅₀) for curcumin in T47D cells was 10 μM when measured at either a 48-hr or a 6-day incubation time. The IC₅₀ value for curcumin was within the 8–10 μM range for inhibiting the growth of T47D cells induced by a 10-μM concentration of each of 4-nonylphenol, 4-octylphenol, and p,p′-DDT. The IC₅₀ for curcumin in MCF-7 cells induced by 10 μM of either p,p′-DDT, 4-octylphenol, or 4-nonylphenol were 9, 39, and >50 μM, respectively. A combination of curcumin and isoflavonoids was able to inhibit the induced growth of ER-positive cells up to 95%. For MDA-MB-231 cells, the IC₅₀ for curcumin was 17 μM, which was reduced to 11 μM in the presence of 25 μM genistein. Curcumin and genistein induce drastic changes in the morphological shape of both ER-positive and ER-negative cells. Data presented here indicate that a mixture of curcumin and isoflavonoids is the most potent inhibitor against the growth of breast human tumor cells. These data suggest that combinations of natural plant compounds may have preventive and therapeutic applications against the growth of breast tumors induced by environmental estrogens.

Key words: curcumin, estrogenic chemicals, human breast cancer cells, isoflavonoids, pesticides.

Environ Health Perspect 106:807–812 (1998). [Online 13 November 1998]
http://ehpnet1.niehs.nih.gov/docs/1998/106p807-812verma/abstract.html

Curcumin has also been shown to have anti-stimulatory properties toward human immunodeficiency virus (HIV) type I by inhibiting HIV-1 integrase and protease (23). The anti-inflammatory and antioxidant properties of curcumin have been known for a long time (24). Most of the above studies on animal and cell models suggest curcumin, also found in many plant products other than turmeric, is an antiproliferative agent. Its effects on the growth of breast cancer cells are less well studied, and epidemiological or clinical studies of the risk for breast cancer in populations consuming curcumin in their daily diets have not been well documented.

Another class of plant-derived dietary products, isoflavonoids, have been shown to have many beneficial clinical and antitumorogenic properties (25–27). In addition to the beneficial properties, in vitro data have indicated that certain dietary compounds such as genistein and some metabolites of isoflavonoids, at concentrations below 10 μM, can enhance the growth of MCF-7 cells (28–30). At higher concentrations (>100 μM), isoflavonoids have been shown to cause nonbeneficial cytotoxicity or sublethal injury (29). The effect of low or high levels of isoflavonoids would yield undesirable results. Considering these limitations, studies of the effect of the combination of curcumin and isoflavonoids were initiated in our laboratory, where concentrations of individual compounds could be controlled to achieve the maximum beneficial effects against the growth of breast cells (31).

We previously reported that the combination of curcumin and genistein is the most sensitive inhibitor against estrogenic pesticide-induced growth of ER-positive MCF-7 human breast cancer cells (31). Since the inhibitory potency of a single compound is limited and higher concentrations of inhibitory compounds may cause toxicity to normal cells or induce sublethal effects that may cause cellular transformation or tumor stimulation, the use of a combination of natural compounds with different mechanisms of action constitutes a

Address correspondence to S.P. Verma, Department of Family Medicine and Community Health, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111 USA.

These studies were supported by NIH grant RO1 CA54349 (to B.R.G.).

Received 9 June 1998; accepted 28 July 1998.
reasonable approach for preventing the harmful effects of environmental estrogens.

In this study, the ER-positive (MCF-7 and T47D) and ER-negative (MDA-MB-231) cell lines were used as models for human breast cancer. In this paper, we describe the inhibitory and cell killing effects of curcumin and curcumin plus isoflavonoids on the growth of these cells and on the growth of ER-positive cells induced by the estrogenic environmental chemicals 4,4'-DDT, 4-NP, and 4-OP. These data support the conclusion that a combination of curcumin and isoflavonoids is the most effective means for preventing the growth of both ER-positive and ER-negative cells.

Materials and Methods

Chemicals. Stock solutions of 4,4'-DDT from Ultra Scientific, Kinston, Rhode Island; 4-NP and 4-OP from Aldrich Chemical Company, Milwaukee, Wisconsin; 17β-estradiol and curcumin from Sigma Chemical Company, St. Louis, Missouri; and genistein, daidzein, biochanin A, foronononolinetin, and equol from Indofine, Somerville, New Jersey, were prepared by dissolving the compounds in DMSO. All stock solutions of compounds were diluted with phenol red-free tissue culture media supplemented with 5% dextrane-coated charcoal-treated serum (DCC; Sigma Chemical Co.) and 100 units/ml of penicillin/streptomycin to an appropriate concentration (test media). The control media did not contain more than 0.5% DMSO.

Cell culture and growth procedure. Estrogen receptor-positive MCF-7 and T47D and ER-negative MDA-MB-231 human breast cancer cells were grown in phenol red RPMI 1640 media (Gibco BRL, Grand Island, NY) supplemented with 5% fetal calf serum (FCS) and 5 ml 10,000 U/ml of penicillin/streptomycin in an incubator maintained at 5% CO2 95% air and 100% humidity at 37°C.

Prior to each experiment with MCF-7 and T47D cells, we replaced phenol red maintenance media with phenol red-free media for 24 hr. The phenol red-free media was then replaced with phenol red-free RPMI 1640 media supplemented with 5% DCC-stripped bovine serum for 48 hr. Cells were detached by adding 2–3 ml 2.5% trypsin solution. After 15 min, cells were harvested and washed with Ca-Mg free phosphate buffered saline (PBS). Washed cells were suspended in phenol red-free RPMI 1640 media supplemented with 5% DCC and antibiotics and were gently agitated by passing up and down in a pipet to get a single cell suspension. Cells were counted by a Coulter Counter. Cells (2–3 x 10^9/ml) were plated in a 12-well culture plate (2 ml/well) and were allowed to attach for 48 hr.

After 48 hr, media was removed and replaced by test media (prepared as above) containing the appropriate concentrations

\[
\text{Cells / chemical} = \frac{\text{Concentration (µM)}}{\text{Percent}}
\]

| Cells/chemical | Concentration (µM) | Percent |
|----------------|-------------------|---------|
| MCF-7 cells   | 17β-estradiol 0.005 | 100     |
|               | 4-NP 5           | 100     |
|               | 4-OP 5           | 93      |
|               | a,p'-DDT 10      | 93      |
| T47D cells    | 17β-estradiol 0.005 | 100     |
|               | 4-NP 5           | 79      |
|               | 4-OP 5           | 76      |
|               | a,p'-DDT 10      | 83      |

*Calculated induced growth of MCF-7 and T47D cells by 5 mM 17β-estradiol has been normalized to 100%. The rest of the values are normalized to 100% growth of 17β-estradiol. Average values of three to four separate experiments are presented.

Figure 1. Effect of curcumin concentration on the growth of T47D cells. See Materials and Methods for details. Cell growth was measured after 48 hr and on day 6 using the tetrazolium (MTT) assay. Growth of cells in control medium without curcumin was normalized to 100%.

Figure 2. Effect of curcumin concentration on the percent growth of MCF-7 cells induced by 4-nonylphenol (4-NP, 10 µM), 4-octylphenol (4-OP, 10 µM), and a,p'-DDT (10 µM). See Materials and Methods for details. Cell growth was measured on day 6 using the tetrazolium (MTT) assay. Induced cell growth was normalized to 100% in each case.

Table 1. Effect of 17β-estradiol, 4-nonylphenol (4-NP), 4-octylphenol (4-OP), and a,p'-DDT on the percent proliferation of MCF-7 and T47D cells

| Chemicals (concentration) | Percent proliferation |
|---------------------------|-----------------------|
| MCF-7 cells               |                       |
| 4-NP (10 µM)              | 100                   |
| 4-NP (10 µM) + Cur (10 µM) + Gen (25 µM) | 48                   |
| 4-NP (10 µM) + Cur (10 µM) + Eq (10 µM) | 62                   |
| 4-NP (10 µM) + Cur (25 µM) + Eq (25 µM) | 18                   |
| 4-NP (10 µM) + Cur (10 µM) + Gen (15 µM) + Bio (15 µM) + For (15 µM) | 25                   |
| 4-NP (10 µM) + TAM (1 µM) | 81                   |
| 4-NP (10 µM) + TAM (2.5 µM) | 79                   |
| 4-NP (10 µM) + TAM (10 µM) | 74                   |
| 4-NP (10 µM) + TAM (2.5 µM) + Cur (10 µM) | 47                   |
| 4-NP (10 µM) + TAM (2.5 µM) + CUR (60 µM) | 24                   |
| 4-NP (10 µM) + TAM (2.5 µM) + CUR (75 µM) | 17                   |
| 4-OP (10 µM)              | 100                   |
| 4-OP (10 µM) + Cur (10 µM) + Gen (50 µM) | 21                   |
| 4-OP (10 µM) + Cur (50 µM) + Gen (50 µM) | 6                   |
| a,p'-DDT (10 µM)           | 100                   |
| a,p'-DDT (10 µM) + Cur (25 µM) + Bio (25 µM) | 20                   |
| a,p'-DDT (10 µM) + Cur (10 µM) + Eq (10 µM) | 42                   |
| a,p'-DDT (10 µM) + Cur (25 µM) + Eq (25 µM) | 11                   |
| a,p'-DDT (10 µM) + Cur (25 µM) + For (25 µM) | 20                   |
| T47D cells                |                       |
| a,p'-DDT (10 µM)           | 100                   |
| Cur (15 µM) + Gen (10 µM) | 14                   |
| Cur (15 µM) + Gen (25 µM) | 15                   |
| Cur (15 µM) + Gen (50 µM) | 5                    |

Abbreviations: Cur, curcumin; Gen, genistein; Eq, equol; Bio, biochanin A; For, foronononolinetin; Tam, tamoxifen citrate. The induced cell growth by 10 µM concentrations of 4-NP, 4-OP and a,p'-DDT has been normalized to 100% growth for each compound.
of environmental chemicals, curcumin, and/or isoflavones. Control cells were incubated in phenol red-free media containing 5% DCC and an equivalent amount of the solvent without the test material. The solvent concentration did not exceed 1%, and this concentration does not appreciably alter the cell growth. The cell growth was estimated on day 6 using the tetrazolium assay (MTT), as reported previously (31). The absorbance at 540 nm was used as a measure of cell density of live cells. The percent proliferation was calculated using the following equation:

Percent proliferation =

\[
\frac{\text{Cell Density with Test Compound}}{\text{Cell Density of Control}} \times 100
\]

Estrogen receptor-negative MDA-MB-231 cells (2 × 10^5 in each well) were plated in 12-well plates and were allowed to attach for 24 hr. We replaced the media with the media containing appropriate concentrations of curcumin and isoflavones. Cell densities were estimated on day 4 using the MTT assay. Growth of control and treated cells was calculated using the above equation, and data were normalized to 100%.

In order to see the time-dependent inhibition, cells plated in 12-well plates were treated with phenol red-free media containing varying concentrations of curcumin for 4 hr. Media containing curcumin was removed. After washing cells with PBS, curcumin and phenol red-free complete medium was added to each well and cells were allowed to grow in the incubator for 5 days.

**Cell morphology.** For morphological studies, MDA-MB-231 and MCF-7 cells were plated in tissue culture dishes containing glass coverslips. After reaching confluence, cells were treated with curcumin and genistein as mentioned above and were incubated for additional 48 hr. Treated cells were washed with PBS, fixed with methanol, stained with Giems, and photographed.

MDA-MB-231 control and curcumin-treated cells (48 hr) were also stained by the terminal deoxynucleotidyl transferase-mediated nick labeling (TUNEL) reaction for determining apoptosis using the Boehringer Mannheim kit and kit protocol (Boehringer Mannheim, Indianapolis, IN). Cells were permeabilized and stained with propidium iodide (PI). Apoptotic cells were quantitated using a Zeiss fluorescence microscope (Zeiss, Thornwood, NY).

**Results**

**Effect of Curcumin on the Growth of T47D Cells**

The T47D cells used in these studies respond to 17β-estradiol (i.e., ER-positive). The effect of curcumin on the growth of these cells as a function of the concentration and time of incubation is shown in Figure 1. The cell growth in 2% FCS has been normalized to 100% for data shown in Figure 1. The cell growth is inhibited as a function of curcumin concentration when measured on day 6 while the inhibition levels off at approximately 40% when measured after 48 hr (Fig. 1). However, the concentration of curcumin required to inhibit the growth of T47D cells by 50% (IC50) is approximately the same (10 μM) when measured after 48 hr or on day 6.

**Effect of Curcumin on Induced Proliferation of ER-positive Cells**

**MCF-7 cells.** It has been reported that the pesticide o,p'-DDT and the environmental chemicals 4-NP and 4-OP have estrogenic properties. These compounds can induce the proliferation of MCF-7 cells (Table 1) (32). 4-NP was found to be more potent at inducing growth at the 5 μM concentration. Higher concentrations (>10 μM) of 4-NP and 4-OP are found to be toxic to MCF-7 cells.

The effect of curcumin on the proliferation of MCF-7 cells induced by o,p'-DDT, 4-NP, and 4-OP is shown in Figure 2. For the sake of calculating the IC50 values for individual antagonistic compounds, the percent growth of cells induced by environmental chemicals has been normalized to 100%. The curcumin IC50 for inhibition of growth induced by o,p'-DDT, 4-OP, and 4-NP are 9, 39, and >50 μM, respectively. Curcumin appears to be a potent inhibitor of o,p'-DDT-induced cell proliferation and a less effective inhibitor for 4-NP-induced proliferation. At 50 μM of curcumin, the cell growth induced by o,p'-DDT is reduced to 12% of that noted in the absence of curcumin.

Table 2 shows the inhibitory effects of various combinations of curcumin and isoflavonoids on the growth of MCF-7 cells induced by environmental pollutants. The induced growth by pollutants has been normalized to 100%. The percent growth in the presence of the combination of curcumin and isoflavonoids was then calculated with respect to this normalized value. Data presented in Table 2 show a synergistic inhibitory effect in the presence of the combination of curcumin and isoflavonoids. As shown in Table 2, the 4-NP-induced cell growth is reduced to 48% (52% inhibition) in the presence of a mixture of curcumin (10 μM) and genistein (25 μM). A mixture of curcumin and equol at 25 μM each reduces the growth of MCF-7 cells induced by 4-NP and o,p'-DDT to 18% and 11%, respectively. Tamoxifen, a well-studied antiestrogen can reduce the growth of MCF-7 cells induced by 4-NP to 70–80%, while the mixtures of tamoxifen (2.5 μM) and curcumin (10–75 μM) can substantially reduce the induced growth (Table 2). The combination of curcumin and genistein at 50 μM each is able to reduce the growth of MCF-7 cells induced by 10 μM 4-OP to 6% (94% inhibition). 

**T47D cells.** The clone of T47D cells used here is estrogen responsive and shows induced proliferation in the presence of 17β-estradiol. The growth of T47D cells in the presence of 5 nM 17β-estradiol was normalized to 100%; relative normalized values for the induced percent proliferation by o,p'-DDT, 4-NP, and 4-OP are 83, 79, and 76%, respectively. In Figure 3, the induced growth by individual environmental chemicals has been normalized to 100% and each data point represents the percent growth inhibition as a function of curcumin concentration. The IC50 values of curcumin are within the 8–10 μM range for inhibiting the induced growth by 10 μM concentrations of 4-NP, 4-OP, and o,p'-DDT. Unlike the inhibitory effects seen in MCF-7 cells, curcumin seems to be an equally effective growth inhibitor of T47D cells in the presence of each of these growth inducers.

The combination of curcumin and isoflavonoids is more potent in T47D cells induced by environmental pollutants (Table 1). Curcumin alone, at the concentration of 15 μM, could reduce the growth of T47D cells induced by o,p'-DDT (10 μM) to approximately 35% (Fig. 3), while a mixture of curcumin (15 μM) and genistein (10 and 25 μM) is able to reduce the induced growth to about 15% (Table 1).

**Effect of Curcumin on the Growth of ER-negative MDA-MB-231 Cells**

Estrogen receptor-negative MDA-MB-231 cells were used to determine whether
curcumin inhibits cell proliferation by an estrogen receptor-independent pathway. Curcumin treatment at concentrations of 10, 15, and 25 μM reduced cell growth to 70, 57, and 31%, respectively. Under similar experimental conditions, genistein (10–50 μM) was not found to inhibit the growth of MDA-MB-231 cells after treatment for 4 hr. The cell growth of MDA-MB-231 cells as a function of concentration of curcumin and a mixture of curcumin and genistein, when present during the growth period of 5 days, is shown in Figure 4. Curcumin was found to be cytotoxic for these cells. The IC_{50} of curcumin is 17 μM (Fig. 4). In the presence of 25 μM genistein, the IC_{50} of curcumin is reduced to 11 μM (Fig. 4). The IC_{50} value for genistein in the presence of 10 μM of curcumin is about 70 μM.

**Cell Morphology**

Figure 5A and Figure 6A show the normal shape of MDA-MB-231 and MCF-7 cells, respectively, growing in culture at 37°C. When these cells were treated with curcumin (20 μM) for 48 hr, we observed a profound change in the shape of MDA-MB-231 (Fig. 5B) and MCF-7 cells (Fig. 6B). MCF-7 cells in phenol red-free media and 5% DCC serum responded to curcumin plus genistein in a similar manner as MDA-MB-231 cells in standard medium treated with curcumin (data not shown).

The TUNEL labeling for apoptosis demonstrated that curcumin treatment increased apoptosis, but the absolute number was small. Apoptosis data showed a 10% increase in the apoptotic death of cells when measured after 48 hr of treatment with 10 μM of curcumin.

**Discussion**

The data presented in this paper demonstrate several major points: 1) curcumin can inhibit the growth of human breast cancer cells independent of the expression of estrogen receptors; 2) curcumin more effectively inhibits the growth of ER-negative cells in comparison to ER-positive cells when cells are growing in normal media; 3)
Curcumin can inhibit the growth of both ER-positive MCF-7 and T47D cells induced by estrogenic environmental chemicals and pesticides; 4) the combination of curcumin and genistein is the most potent inhibitor against the growth of both ER-positive and ER-negative breast cancer cells; 5) tamoxifen, a well studied antiestrogen, could only reduce the growth of MCF-7 cells induced by 4-NP to about 70–80%, while a combination of tamoxifen and curcumin can further reduce the induced growth; and 6) the inhibitory effects of curcumin and isoflavonoids are synergistic and may involve separate pathways of action.

The exact mechanism of action of curcumin for inhibiting the growth of tumor cells induced by estrogenic environmental chemicals (ER-positive cells) or ER-negative cells growing in the normal tissue culture media has not been fully explored. Many biological and medicinal properties of curcumin are now well recognized. The inhibitory action of curcumin against the growth of tumor cells should be associated with some of its biological properties. The anti-inflammatory properties of curcumin have been known for a long time. Curcumin has been shown to influence the activities of enzymes such as cyclooxygenase (COX), lipoproteinase (LOX), phospholipase A2 (PLA2), and phospholipase C-gamma-1 (PLCg1) (12,13). Several metabolites of COX and LOX are involved in growth signaling (33–35). Increased activities of PLA2 and PLCg1 have been observed in breast tumors (36). PLCg1 may produce diacyl glycerol and inositol 1,4,5-triphosphate; these signaling compounds can stimulate protein kinase C, which has been implicated in modulating cell growth. Curcumin may be inhibiting the growth of both ER-positive and ER-negative cells by directly modulating the activities of COX, LOX, PLA2, and PLCg1 enzymes, including the activity of protein kinase C, as has been reported in colon tumors (12,13).

Other feasible mechanisms of action of curcumin could involve suppression of the activities of protein kinases, (e.g., kinase C and tyrosine kinase (18,20)), many types of transcription factors (such as hydrogen peroxide, tumor necrosis factor, NF-kB) (21), and proto-oncogenes (22,23).

The cell morphology data presented in this paper show that cells become rounded after 48 hr of treatment with curcumin or curcumin plus genistein. These data suggest the possibility of a disruption in cell matrix and a reduction in cytoplasmic contents of curcumin and curcumin plus genistein-treated cells. These initial structural disruptions could direct cells to initiate the apoptotic or programmed cell death process. There is some indication by TUNEL data (10% increase in apoptosis) that this process may actually be triggered. However, more data are needed for determining the exact mechanism by which curcumin inhibits the induced growth of breast tumor cells.

Genistein is well recognized for its inhibition of various kinds of protein kinases. There is a possibility that genistein inhibits cell growth by its direct action on protein kinases (e.g., protein kinase C, tyrosine kinase, MAP-kinase). Protein kinases are involved in the phosphorylation of estrogen and progesterone receptors. Phosphorylated receptors are involved in activating transcription factors. Since curcumin and genistein have been shown to have a synergistic effect against the growth of both ER-positive and ER-negative cells, the action of these compounds may be through separate pathways. The concentration and time-dependent growth inhibition data suggest that curcumin and isoflavonoids may not be competing for the same target in order to block the growth. The exact mechanism of action of these compounds remains to be elucidated.

These results support the concept that a combination of natural chemopreventive nutrients is more potent than individual compounds against suppressing the growth of breast tumors induced either by environmental chemicals or other carcinogens (31). Also, the in vitro data presented in this paper constitute the framework for further studies in animal models and clinical trials. Data on natural plant compounds may further be helpful in developing new therapeutic agents.

References and Notes

1. Perera FP. Environmental and cancer: who are susceptible? Science 278:1068–1073 (1997).
2. Estrogens in the environment, III: global health implications. Environ Health Perspect 103(suppl 7) (1995).
3. Felic F, Ricci AJ, Wolff MS, Godbold J, Deckers P. Pesticides and polychlorinated biphenyl residues in human breast tissues and their relation to breast cancer. Arch Environ Health 47:145–146 (1992).
4. Wolff MS, Toniole PG, Lee EW, Rivera M, Rubin D. Blood levels of organochlorine residues and risk of breast cancer. J Natl Cancer Inst 85:658–652 (1993).
5. Dewarly E, Davis AR, Avotte P, Sauer H, Morin J, Brison J. High organochlorine body burden in women with estrogen receptor positive breast cancer. J Natl Cancer Inst 86:232–234 (1994).
6. Katzenellenbogen BS, Montano MM, Le Goff P, Schodin DJ, Kraus W, Bhardwaj B, Fujimoto NJ. Antiestrogens: mechanisms and actions in target cells. Steroid Biochem Mol Biol 53:387–393 (1992).
7. Gottardis MM, Tan W, VC. Development of tamoxifen-stimulated growth of MCF-7 tumor in athymic mice after long-term antiestrogen administration. Cancer Res 48:5163–5167 (1988).
8. de Jong PC, van de Ven J, Nortier WHR, Maitimu-Smeale I, Donker TH, Thijssen JHH, Sleis PHTJ, Blankenstein RA. Inhibition of breast cancer tissue aromatase activity and estrogen concentrations by the third-generation aromatase inhibitor vorozole. Cancer Res 57:2109–2111 (1997).
9. Huang MT, Lon Y-R, Ma W, Newmark HL, Reuhl KR, Conney AH. Inhibitory effects of dietary curcumin on forestomach, duodenal and colon carcinogenesis. Cancer Res 54:5841–5847 (1994).
10. Huang MT, Ma W, Lu Y-P, Chang RL, Fisher C, Manchand FS, Newmark HL, Conney AH. Effect of curcumin, dimethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin on 12-0-tetradecanoylphorbol-13-acetate-induced tumor promotion. Carcinogenesis 16:2493–2497 (1995).
11. Rao CV, Sim B, Reddy BS. Inhibition by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. Carcinogenesis 14:2219–2225 (1993).
12. Rao CV, Rivenson A, Sim B, Reddy BS. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. Cancer Res 55:259–266 (1995).
13. Samaha HS, Kelloff GR, Stevie V, Rao CV, Reddy BS. Modulation of apoptosis by sulindac, curcumin, phenylethyl-3-methyl caffeate, and 6-phenethyl isothiocyanate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. Cancer Res 57:1301–1307 (1997).
14. Stoner GD, Mukhtar H. Polyphenols as cancer chemopreventive agents. J Cell Biochem Suppl 22:163–180 (1995).
15. Singletary K, MacDonald C, Wallig M, Fisher C. Inhibition of 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumors and DMBA-DNA adduct formation by curcumin. Cancer Lett 102:137–141 (1996).
16. Sacks PG, Kaffath A, Al-Rawi M, Dennerlage AJ, Schantz SP. Anti proliferative effects of curcumin and (1-epigallocatechin-3-gallate (EGCG) on normal and premalignant human oral epithelial cells [abstract]. Proc Am Assoc Cancer Res 36:281 (1995).
17. Korutla L, Cheung JY, Mendelsohn J, Kumar R. Inhibition of ligand-induced activation of epithelial growth factor receptor tyrosine phosphorylation by curcumin. Carcinogenesis 16:1741–1745 (1995).
18. Chan MM. Inhibition of tumor necrosis factor by curcumin, a phytochemical. Biochem Pharmacol 26:1515–1519 (1995).
19. Liu YY, Lin SJ, Lin JK. Inhibitory effects of curcumin on protein kinase C activity induced by 12-0-tetradecanoyl-phorbol-13-acetate in NIH 3T3 cells. Carcinogenesis 14:857–861 (1993).
20. Singh S, Aggarwal BB. Activation of transcription factor NF-kB is suppressed by curcumin (diferuloylmethane). J Biol Chem 270:24955–25000 (1995).
21. Kakkar SS, Roy D. Curcumin inhibits TPA induced expression of c-fos, c-jun and c-myc proto-oncogene messenger RNAs in mouse skin. J Invest Dermatol 100:89–90 (1993).
22. Huang TS, Lee SC, Lin JK. Suppression of c-jun/AP-1 activation by an inhibitor of tumor promotion in mouse fibroblast cells. Proc Natl Acad Sci USA 88:5292–5296 (1991).
23. Mazumder A, Wang S, Neamarti N, Nicklaus M, Sundar S, Chen J, Milane GW, Rice W, Burke TR Jr, Pommier Y. Antitumoroviral agents as inhibitors of both human immunodeficiency virus type 1 integrase and protease. J Med Chem 33:2492–2498 (1996).
24. Ruby A, Kuttan G, Babu K, Rajasakharan K, Kuttan R. Anti-tumor and antioxidant activity of natural curcuminoids. Cancer Lett 74:79–83 (1993).
25. Adlercreutz H. Phytoestrogens: epidemiology and a possible role in cancer protection. Environ Health Perspect 103(suppl 7):103–112 (1996).
26. Fotitis T, Pepper MS, Akta N, Breit S, Rasku S, Adlercreutz H, Wihalle K, Montano R, Schweiger L, Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis. Cancer Res 57:2916–2921 (1997).
27. Adlercreutz H, Goldin BR, Borbach SL, Hockerstedt KAV, Watanabe S, Hamalainen EK, Markkanen MH, Makela T, Wahala KM, Hase TA, Fotitis T. Soybean phytoestrogen intake and cancer risk. J Nutr 125:767–770 (1995).
28. Verma SP, Goldin BR. The effect of soy-derived isoflavonoids toward the induced growth of MCF-7 cells by estrogenic environmental chemicals. Nutr Cancer 30:232–239 (1998).
29. So FV, Guthrie N, Chambers AF, Carroll KK. Inhibition of proliferation of estrogen-positive MCF-7 human breast cancer cells by flavonoids in the presence and absence of excess estrogen. Cancer Lett 112:127–133 (1997).
30. Balabhadrapathruni S, Thomas T, Thomas TJ. Estrogenic and antiestrogenic actions of genistein in human breast cancer cell growth mediated through the polyamine pathway [abstract]. Proc Am Assoc Cancer Res 38:207 (1997).
31. Verma SP, Salamone E, Goldin BR. Curcumin and genistein, plant natural products, show synergistic inhibitory effects on the growth of human breast cancer MCF-7 cells induced by estrogenic pesticides. Biochem Biophys Res Commun 233:892–896 (1997).
32. Soto AM, Justicia H, Wray JW, Sonnenschein C. p-Nonyl-phenol, an estrogenic xenobiotic released from “modified” polystyrene. Environ Health Perspect 92:167–173 (1991).
33. Smith WL. Prostanoid biosynthesis and mechanisms of action. Am J Physiol 263:F181–F191 (1992).
34. Timer J, Chen YO, Liu B, Bazar R, Taylor JD, Honn KV. The lipoxygenase metabolite 12(S)-HETE promotes alphaβ3 integrin-mediated tumor cell spreading on fibronectin. Int J Cancer 52:594–603 (1992).
35. Dennis AE, Rhee SG, Billah MM, Hannun YA. A role of phospholipases in generating lipid second messengers in signal transduction. FASEB J 5:2068–2077 (1991).
36. Arteaga CL, Johnson MD, Todderud G, Cofey RJ, Carpenter O, Page DL. Elevated content of the tyrosine kinase substrate phospholipase Cα1 in primary human breast carcinomas. Proc Natl Acad Sci USA 88:10435–10439 (1991).