CURCUMIN AND TURMERIC ATTENUATE ARSENIC-INDUCED ANGIOGENESIS IN OVO

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Trivalent arsenic [As(III)] is currently approved by the FDA for the treatment of chronic and acute leukemias. However, As(III) has also demonstrated damaging effects on human health, including development of cardiovascular disease, diabetes, and cancer. Furthermore, As(III) is a potent angiogenic agent. In this context, curcumin, an active ingredient in the dietary agent turmeric, has demonstrated potent antiproliferative, anti-inflammatory, and antiangiogenic properties. In this report, we have shown that both curcumin and turmeric inhibit expression of vascular endothelial growth factor in HCT-116 human colon cancer cells exposed to As(III). Further, in the chicken chorioallantoic membrane assay model, treatment with low As(III) concentrations results in extensive increase in blood vessel density, which, however, is reduced in the presence of curcumin or turmeric. Collectively, the findings reported here strongly suggest that turmeric and curcumin can dramatically attenuate the process of angiogenesis induced by low As(III) concentrations.

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Curcumin, the active ingredient in turmeric, traditionally has been used in the Indian subcontinent and in Southeast Asia to treat throat ulcers, inflammation, skin wounds, and cancer. Further, laboratory studies have demonstrated the antiproliferative, anti-inflammatory, and antiangiogenic properties of curcumin. In addition, current clinical trials of curcumin in humans assess its role in the prevention or treatment of several types of cancer, rheumatoid arthritis, irritable bowel syndrome, and Alzheimer’s disease.

Arsenic compounds have been used since ancient times to treat medical conditions, but at the same time, these compounds also have been associated with a range of detrimental effects on human health, including increased incidence of ischemic heart disease, arrhythmias, hypertension, and peripheral vascular disease. Although the mechanism(s) for these vascular effects remains unknown, it has been demonstrated that some effects are dose-related. Angiogenic effects of arsenic also have been shown to be dose-dependent, with lower doses being angiogenic and higher doses antiangiogenic.

Angiogenesis, the process of generating new blood vessels, is a critical step in cancer growth and metastasis, and increased tumor angiogenesis has been associated with an increased incidence of distant metastasis. Therefore, the characterization of natural and synthetic agents that can inhibit angiogenesis is of great clinical and public interest.

In the present study, we have demonstrated the ability of the plant-derived products, turmeric and curcumin, to attenuate the production of a major angiogenic factor, vascular endothelial growth factor (VEGF), in trivalent arsenic [As(III)]-treated human colon cancer cells in culture. Further, we have demonstrated that turmeric and curcumin inhibit angiogenesis induced by low As(III) concentrations in the chick chorioallantoic membrane (CAM) model.
MATERIALS AND METHODS

Reagents and Cells

Curcumin powder and turmeric powder were purchased from LKT Laboratories, Inc (St Paul, Minnesota) and Sabinsa Corporation (Piscataway, New Jersey), respectively. All reagents used in routine molecular biology experimentation were purchased from Sigma-Aldrich (St Louis, Missouri). HCT-116 human colon cancer cells (American Type Culture Collection, Manassas, Virginia) were grown in Dulbecco's modified Eagle medium, supplemented with 10% heat-inactivated fetal bovine serum and standard antibiotics (Sigma-Aldrich) in a 37°C-humidified incubator containing 5% CO2.

VEGF Enzyme-linked Immunosorbent Assay

HCT-116 cells were exposed to 0.1 μM As(III) in absence or presence of 30 μM curcumin or 50 mg/mL turmeric for 72 hours with serum-free media used for the last 24 hours of the experiment. Supernatants were subjected to enzyme-linked immunosorbent assay (ELISA) analysis using the QuantiGlo Chemiluminescent ELISA Human VEGF kit from R&D Systems (Minneapolis, Minnesota) according to instructions provided by the manufacturer. The plate was read on a BioTek Synergy HT luminometer (BioTek Instruments, Winooski, Vermont). Results were quantified in relative light units after considering the control value as 100%.

Chick CAM Assay of Angiogenesis

The chick CAM assay is a standard assay for testing antiangiogenic agents. In this assay, purified growth factors are added locally to the highly vascularized CAM to induce angiogenesis. Inhibitors are then added to the same localized area of the membrane and, after a desired incubation period, the blood vessel density/branching of the treated area of the CAM is determined. The CAM assay used in these studies was performed according to the modified version of Brooks.

RESULTS

Curcumin Inhibits As(III)-induced VEGF Expression

We initially examined whether addition of curcumin or turmeric extract to As(III)-treated cells would affect expression of VEGF, a primary angiogenic factor in colon cancer cells. It was found that the addition of curcumin to As(III) cells treated for 72 hours resulted in a significant decrease in soluble VEGF amounts, as compared to As(III) exposure alone, to levels approaching those in untreated cells (Figure 1). Further, addition of turmeric resulted in a lesser decrease in VEGF amounts in As(III)-treated cells.

Curcumin Inhibit As(III)-induced Angiogenesis

As previously shown, addition of either 200 ng/mL VEGF or 0.1 μM As(III) for 48 hours resulted in a significant stimulation of angiogenesis in the CAM assay as demonstrated by an extensive increase in blood vessel density (Figure 2). However, presence of curcumin extensively inhibited angiogenesis induced by the presence of VEGF in CAM (Figure 2). The addition of either curcumin or turmeric along with As(III) resulted in a significant decrease in blood vessel density, as compared to addition of As(III) alone to levels approaching those of untreated CAMs. The results are shown upon direct visualization of microscopy images and measuring the relative angiogenic index (Figure 2).

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) to compare the means of all groups. The Dunnett’s multiple comparison test was used to compare the treatments vs the control groups and unpaired two-tailed Student’s t-test was used to compare treatment groups to one another. Difference was considered significant at P<.05. All analyses were performed with the aid of Prism 5.0 software (Graphpad Software, San Diego, California).
inducing VEGF production in HCT-116 cells and angiogenesis in the CAM model. We also have determined that low concentrations of As(III) can protect IκB-modulated TNF-α induction of apoptosis in HCT-116 cells in vitro in agreement with a published report and a manuscript in preparation.

IKB is a regulatory protein that inhibits degradation of the nuclear factor-KB (NF-κB) that has been implicated in the proliferation of various tumor cells, angiogenesis, therapeutic resistance during tumor development, progression, and metastasis of carcinomas. It should be noted that in the experiments described in this report, the HCT-116 cells were treated with As(III) concentrations several-fold lower than the cytotoxic As(III) concentrations of near 100 μM (manuscript in preparation).

In conclusion, the experimental findings described in this report demonstrate that turmeric and curcumin attenuate or completely prevent low As(III) concentrations from inducing angiogenesis in the CAM model and that curcumin reduces VEGF production in HCT-116 cells. In addition to being used in cooking, turmeric and related spices are actively promoted for their suggested antiinflammatory and anticancer activities and are available in powder or tablet form from a number of suppliers worldwide. However, we caution that these commercially available products may contain traces or low levels of arsenic, as we detected using high-performance liquid chromatography-hydride generation-inductively coupled plasma mass spectrometry (HPLC-HG-ICPMS) analysis (unpublished data) in agreement with a recent report that some commercial preparations of herbal medicines contain detectable toxic metals such as arsenic. Further studies are needed to investigate the functional activities of different turmeric and related spice preparations, as well as to identify contaminants that may be health hazards.

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**REFERENCES**
