Inhibition of Epidermal Growth Factor-induced Cell Transformation and Activator Protein 1 Activation by [6]-Gingerol

Ann M. Bode, Wei-Ya Ma, Young-Joon Surh, and Zigang Dong

Abstract

Many spices, including plants of the ginger family, possess anticarcinogenic activity. However, the molecular mechanisms by which they exert their antitumorigenic effects are unknown. Activator protein 1 (AP-1) has a critical role in tumor promotion, and blocking of tumor promoter-induced activation of AP-1 inhibits neoplastic transformation. Epidermal growth factor induces cell transformation and AP-1 activity. The purpose of this study was to investigate the effect of two structurally related compounds of the ginger family, [6]-gingerol and [6]-paradol, on EGF-induced cell transformation and AP-1 activation. Our results provide the first evidence that both block EGF-induced cell transformation but act by different mechanisms.

Introduction

The identification of plant-derived compounds or phytochemicals having the capacity to interfere with carcinogenic processes has been receiving increased interest. Many herbs and spices are known to possess an array of biochemical and pharmacological activities including antioxidant and anti-inflammatory properties that are believed to contribute to their anticarcinogenic and antimutagenic activities. Plants of the ginger (Zingiber officinale Roscoe, Zingiberaceae) family, one of the most heavily consumed dietary substances in the world (1), have been shown to inhibit tumor promotion in mouse skin (2). The oleoresin from the root of ginger contains [6]-gingerol, the major pharmacologically active component (Fig. 1A; Ref. 1), and lesser amounts of a structurally related vaniloid, [6]-paradol (Fig. 1B). At least two recent studies suggest that these compounds suppress proliferation of human cancer cells through the induction of apoptosis (3, 4). However, very little is known regarding the molecular mechanisms by which they may exert their antitumorigenic effects. Previously, through the comparison of promotion sensitive (P

Materials and Methods

Chemicals. [6]-Gingerol (98% purity verified by TLC) was from BioMol Research Laboratories, Inc. (Plymouth Meeting, PA). [6]-Paradol was purified through column chromatography and 99% purity verified by TLC (3). The DePsipher kit was from R&D Systems, Inc. (Minneapolis, MN). FBS, gentamicin, t-glutamine, and Eagle’s MEM were from BioWhittaker (Walkersville, MD); luciferase assay substrate was from Promega Corp. (Madison, WI); nonphospho- and phospho-specific mitogen-activated protein kinase antibodies against phosphorylated sites of p38 kinase and ERKs were from New England Biolabs, and EGF was from Collaborative Research (Bedford, MA).

Anchorage-independent Transformation Assay. The effect of [6]-gingerol and [6]-paradol on EGF-induced cell transformation was investigated. JB6 cells (1 × 10^4) were exposed to EGF (20 ng/ml) with or without [6]-gingerol or [6]-paradol in 1 ml of 0.33% basal medium Eagle agar containing 10% FBS over 3.5 ml of 0.5% basal medium Eagle agar containing 10% FBS. The cultures were maintained in a 37°C, 5% CO2 incubator for 14 days, and the cell colonies were scored as described previously (5, 7, 8).

Assay of AP-1 Activity. Confluent monolayers of AP-1 luciferase reporter plasmid stably transfected mouse epidermal JB6 P

Results and Discussion

[6]-Gingerol and [6]-Paradol Inhibit EGF-induced Cell Transformation. The mouse epidermal JB6 cell system is a well-developed model for studying tumor promotion and was therefore used as a cell
[6]-GINGEROL INHIBITION OF CELL TRANSFORMATION AND AP-1

Culture paragon to test the antitumor promoting effect of [6]-gingerol and [6]-paradol. Previous studies examining the chemopreventive effects of ginger extracts focused mainly on 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion. In particular, investigators showed recently that [6]-gingerol inhibited 12-O-tetradecanoylphorbol-13-acetate-induced skin tumor promotion in addition to inhibiting epidermal ornithine decarboxylase activity in ICR mice (11). To our knowledge, no other information is available regarding the effects of [6]-gingerol or [6]-paradol on EGF-induced cell transformation.

As reported previously, EGF induces 1000–2000/cm² transformed colonies in soft agar, and in the present study, we found that [6]-gingerol significantly \( P < 0.001 \); Fig. 1A) blocked EGF-induced cell transformation in a concentration-dependent manner. The concentration range for effective inhibition was from 50 to 200 \( \mu M \), for which no cytotoxic effects were observed by either trypan blue exclusion or Live/Dead Viability/Cytotoxicity assessment (Molecular Probes, Eugene, OR; data not shown). On the other hand, lower concentrations of the structural analogue, [6]-paradol, effectively blocked cell transformation (Fig. 1B). Although cell transformation was not totally blocked at 25 \( \mu M \), 50 \( \mu M \) paradol resulted in almost complete cell death, as indicated by the Live/Dead assessment assay (data not shown) and the DePsipher Assay (Fig. 4).

[6]-Gingerol Inhibits EGF-induced AP-1 Activity. AP-1 activation was shown previously to be required for neoplastic transformation in JB6 cells. To test whether the repression of transformation by [6]-gingerol involves the inhibition of AP-1 activity, stably transfected JB6 cells with AP-1 reporters were used. Results indicated that EGF stimulated AP-1 transactivation activity significantly above the untreated control value \( P < 0.0007 \); Fig. 2A), and treatment with

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**Fig. 1.** Inhibition of EGF-induced transformation by [6]-gingerol and [6]-paradol. Both [6]-gingerol (A) and [6]-paradol (B) are phenolic compounds differing only by the presence of a hydroxyl group. JB6 P⁺ cells were exposed simultaneously to EGF with or without [6]-gingerol or [6]-paradol in 0.33% agar for 14 days and scored for colonies at the end of the experiment. Results are expressed as the mean of three independent experiments; bars, SE.

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**Fig. 2.** Inhibition of AP-1 activity of JB6 P⁺ cells by [6]-gingerol. Stable AP-1 luciferase transfecteds C341 cells were exposed to EGF with or without [6]-gingerol (A) or [6]-paradol (B) for 24 h. Results are the means of four samples from a representative experiment that was repeated at least three times; bars, SE.

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**Fig. 3.** Inhibition of EGF-induced DNA-binding activity by [6]-gingerol. JB6 cells were treated with [6]-gingerol as indicated. Sequence-specific AP-1 binding activity was determined by gel-shift analysis using a 32P-labeled oligonucleotide containing the AP-1 binding site as described in “Materials and Methods.” Arrow, position of specific AP-1 DNA binding activity. A 100-fold excess of unlabeled probe confirmed specificity of DNA binding (data not shown).
[6]-gingerol resulted in a dose-dependent inhibition of activity (P < 0.007) to a level close to the untreated control value (Fig. 2A). In contrast, [6]-paradol had no effect on EGF-induced AP-1 transactivation (Fig. 2B).

[6]-Gingerol Has No Effect on EGF-induced ERKs or p38 Kinase Phosphorylation. The EGF receptor is a transmembrane protein with intrinsic tyrosine kinase activity (12), and activation of the receptor results in phosphorylation and activation of a number of substrates, including ERKs and p38 kinases (13, 14), leading to activation of AP-1 (15). The influence of [6]-gingerol on EGF-induced phosphorylation of ERKs and p38 kinases was investigated, and results indicated that [6]-gingerol had no effect on EGF-induced phosphorylation of ERKs or p38 kinase (data not shown), suggesting that other mechanisms must be involved.

[6]-Gingerol Inhibits EGF-induced Sequence-specific AP-1 DNA Binding. To further study the molecular basis of [6]-gingerol on the inhibition of AP-1 transactivation, AP-1 DNA binding activity was analyzed by gel shift assay. As shown in Fig. 3, [6]-gingerol inhibited EGF-induced AP-1 DNA binding activity in a concentration-dependent manner. The concentration range effective for inhibition of EGF-induced AP-1 DNA binding was similar to that required for inhibition of AP-1 transactivation activity. Thus, inhibition of EGF-induced AP-1 transactivation by [6]-gingerol is, at least in part, attributable to the inhibition of AP-1 DNA binding activity.

[6]-Paradol Induces Cell Death in Cl41 Cells. At concentrations up to 25 μM, [6]-paradol induced apoptosis in JB6 cells (Fig. 4), but concentrations of 50 μM or greater resulted in apparent necrotic cell death (6). Therefore, [6]-paradol appears to exert its primary inhibitory effect on cell transformation through the induction of apoptosis. On the other hand, at least 300 μM [6]-gingerol was required to induce apoptosis (Fig. 4). At least one study has shown that in human promyelocytic leukemia (HL-60) cells, [6]-gingerol acts by inducing apoptosis (3). The concentration of [6]-gingerol used to induce apoptosis in that study was 300 μM, agreeing well with the concentration used in the present study to induce apoptosis but higher than that required to totally inhibit cell transformation. Thus, in these studies, apoptosis does not appear to be a significant mechanism in the inhibitory effect of [6]-gingerol on cell transformation.

Our results provide the first evidence that [6]-gingerol and [6]-paradol block EGF-induced cell transformation and although closely related structurally, act through different mechanisms. [6]-Gingerol inhibited EGF-induced AP-1 transactivation by blocking...
EGF-induced AP-1 DNA binding activity in a concentration-dependent manner, and in contrast, [6]-paradol had no effect on AP-1 activation. Therefore, in exerting their antitumorigenic effects, [6]-gingerol appears to act through inhibition of AP-1 activation, whereas [6]-paradol appears to act through induction of apoptosis.

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