SHORT COMMUNICATION

Relation of structure of curcumin analogs to their potencies as inducers of Phase 2 detoxification enzymes

Albena T. Dinkova-Kostova and Paul Talalay

Department of Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

To whom correspondence should be addressed
Email: ptalalay@welchlink.welch.jhu.edu

A series of naturally occurring as well as synthetic structural analogs of the dietary constituent curcumin were examined in order to elucidate which portions of the molecule are critical for the ability to induce Phase 2 detoxification enzymes in murine hepatoma cells, and hence to assess the chemoprotective potential of these compounds. Two groups of compounds were studied: classical Michael reaction acceptors such as curcumin and related β-diketones such as dibenzoylemethane which lack direct Michael reactivity. The presence of two structural elements was found to be required for high inducer potency: (i) hydroxyl groups at ortho-position on the aromatic rings and (ii) the β-diketone functionality. All curcuminoids elevate the specific activity of quinone reductase in both wild type and mutant cells defective in either the aryl hydrocarbon (Ah) receptor or cytochrome P4501A1 activity. This indicates that neither binding to this receptor, nor metabolic activation by P4501A1 are required for the signaling process originating from this family of electrophiles and ultimately resulting in Phase 2 enzyme induction.

Introduction

Curcumin 1 (diferuloylmethane) is a component of turmeric, a yellow spice extracted from the rhizome of the East Indian herb Curcuma longa L. (Zingiberaceae), that is widely used as a food flavoring and coloring agent (e.g. in curry). Extracts of the plant rich in curcumin usually also contain lesser quantities of demethoxycurcumin 2 and bisdemethoxycurcumin 3. Curcumin has a long history of medicinal use in India and Southeast Asia for a wide variety of medical conditions (1) and has been shown in experimental studies to have anti-inflammatory properties, to prevent tumorigenesis and mutagenesis, to block carcinogen-DNA adduct formation, and to inhibit angiogenesis (2–5). Interest in curcumin and its promising cancer-protective potential is growing, especially since it does not appear to be significantly toxic (1). Plans for its clinical development as an anticancer agent are in progress (6).

Several possibilities have been raised regarding the potential mechanisms of the observed antitumor effects of curcumin, and among these its antioxidant and anti-inflammatory properties have received major attention. Curcumin scavenges active oxygen species including superoxide, hydroxyl radical, and nitric oxide (7,8). It decreases the 12- O-tetradecanoylphorbol-13-acetate (TPA)-induced expression of c-jun, c-fos and c-myc proto-oncogenes (9), and suppresses activation of nuclear factor-κB (NF-κB) (10). Curcumin represents a candidate for a natural chemoprotective agent, since it also elevates the activities of Phase 2 detoxification enzymes of xenobiotic metabolism, such as glutathione transferases (11) and NAD(P)H: quinone reductase (QR) (5), while inhibiting procarcinogen activating Phase 1 enzymes, such as cytochrome P4501A1 (12).

In the light of compelling evidence that the coordinate induction of Phase 2 enzymes [e.g. glutathione transferases, NAD(P)H:QR, glucuronosyltransferases, epoxide hydrolase] is a critical and sufficient condition for protection against toxicity and carcinogenicity (13,14), the present study was designed to define which structural features of the curcumin molecule contribute to its ability to serve as an inducer of Phase 2 enzymes. For this purpose, we employed the Prochaska test (15), which provides a highly quantitative bioassay that measures quinone reductase activity (a prototype Phase 2 enzyme) in Hepa1c1c7 murine hepatoma cells grown in 96-well microtiter plates. Potencies of inducers are customarily expressed as concentration required to double the quinone reductase specific activity (CD value). The response of this cell line to a variety of inducers mimics the response of many rodent tissues. Furthermore, the ability to predict very accurately the competence or incompetence of a given compound to raise tissue levels of Phase 2 enzymes and ultimately to protect against toxicity and carcinogenicity is a recognized merit of this assay system.

With use of animal models, as well as the microtiter plate assay, it was established unambiguously in this laboratory that Michael reaction acceptors (olefins or acetylenes conjugated to electron-withdrawing groups) constitute a major class of Phase 2 enzyme inducers, and their inducer potency was closely correlated with their reactivities with nucleophiles in the Michael reaction (16). Not surprisingly, curcumin 1[1,7-bis-(3-methoxy-4-hydroxyphenyl)-1,6-heptadiene-3,5-dione], which contains two Michael reaction acceptor functionalities in its molecule, induced quinone reductase with a CD value of 7.3 μM (Figures 1 and 2).

The initial goals of our experiments were to establish the contribution of individual portions of the curcumin molecule to its inducer potency. Thus, vanillin 7 (3-methoxy-4-hydroxybenzaldehyde) was chosen as the first model compound in order to determine whether an aromatic ring system possessing one methoxyl and one hydroxyl substituent, but no Michael reaction acceptor functionality, could induce quinone reductase. As expected, vanillin 7 was inactive (Figure 3). Next, ferulic acid 8, which contains an olefin conjugated to a carboxyl group (weakly electron withdrawing) was tested. In this case as well, no inducer activity was found. However, its methyl ester 9, a better Michael reaction acceptor, was a definite although weak inducer (CD = 83 μM). Removal of the methoxyl substituent, as in methyl p-coumarate 10 (CD = 83 μM) or methyl m-coumarate 11 (CD = 83 μM), did not affect the inducer potency. In sharp contrast, the methyl ester
Fig. 1. Structures of curcuminoids tested in this study and their inducer potencies (CD values) in the QR assay in Hepa1c1c7 murine hepatoma cells.

Fig. 2. Induction of QR as a function of concentration by curcumin in murine hepatoma cells Hepa 1c1c7 (●) and its mutants Bp/c1 (○) and c1 (▲).

Fig. 3. Structures of vanillin and cinnamic acid derivatives used as model compounds and their inducer potencies (CD values) in the QR assay in Hepa1c1c7 murine hepatoma cells.

Fig. 4. Structures of diketone model compounds and their inducer potencies (CD values) in the QR assay in Hepa1c1c7 murine hepatoma cells.

Fig. 4 Structures of diketone model compounds and their inducer potencies (CD values) in the QR assay in Hepa1c1c7 murine hepatoma cells.
induced mammary tumors in the rat (28) and the mouse (29). Importantly, in the same experimental models, 1% dietary curcumin 1 was without effect, implying large differences in their bioavailability and/or mechanism(s) of action.

The next question was whether other types of compounds containing the tautomeric keto-enol function would be inducers. To this end, four 2,4-pentanedione derivatives were tested, namely 2,4-pentanediene 16; 1-benzoylacetone 17; 1,1,1-trifluoro-2,4-butanedione 18 and 1,1,1,5,5,5-hexafluoro-2,4-pentanediene 19. All were either very weakly effective or inactive as inducers (Figure 4). Taken together, these findings established that neither the presence of a keto-enol functionality nor of the aromatic ring system is sufficient alone to provide inducer activity; both must be present to confer this biological activity.

Since it was concluded previously that the only substituent on the aromatic ring with significant impact on the inducer potency was a hydroxyl group at the ortho-position with respect to the Michael reaction acceptor portion of the molecule (30 and this study), it was important to examine the biological effect of such a substituent on a curcumin analog. For this purpose, salicylcurcuminoid 6 [1,7-bis-(2-hydroxyphenyl)-1,6-heptadiene-3,5-dione] was synthesized according to the method of Dinesh Babu and Rajasekharan (31). The crystalline product had the appropriate 1H NMR spectrum (DMSO-d6, 300 MHz) δ (p.p.m.): 6.16 (1H, s, C4-H), 6.91 (2H, d, J=16 Hz, C2-H + C6-H), 6.95–7.00 (4H, m, Ar-H), 7.29 (2H, m, Ar-H), 7.68 (2H, d, Ar-H), 7.92 (2H, d, J=16 Hz, C1-H + C7-H), 10.3 (2H, s, Ar-OH). When tested as a QR inducer, it was shown to have a CD value of 0.3–0.4 µM (Figures 1 and 5), and consequently was the most potent inducer in the curcuminoid series identified by us. Importantly, no cytotoxicity falls into the monofunctional or bifunctional category, and which of these systems (if not both) is involved in the mechanism of induction of QR by a particular stimulant.

When induction of QR by curcumin was compared in Hepa1c1c7 murine hepatoma cells and the two aforementioned mutants that lack a functional Ah receptor or cytochrome P450A1 gene product, the inducer potencies of the curcuminoids tested here were similar (Figure 2) with the following CD values: curcumin, 8.9 and 10.9 µM; demethoxycurcumin, 12.5 and 14.3 µM; bisdemethoxycurcin, 19.1 and 21.7 µM; and salicylcurcuminoid, 0.8 and 3.1 µM, respectively. In the same experiment, β-naphthoflavone, a very potent synthetic bifunctional inducer had a CD of 15 nM in the wild-type Hepa1c1c7 cells, but was inactive as an inducer in both mutants. Curcumin would accordingly be classified as a monofunctional inducer. However, a recent study has indicated that curcumin can also be a Phase 1 enzyme inducer. It binds to the Ah receptor, activates the transcription of the cytochrome P450A1 gene, and elevates its enzyme activity (12). Curcumin is therefore a bifunctional inducer, but differs from ‘conventional’ bifunctional inducers (such as polycyclic aromatic hydrocarbons, β-naphthoflavone and azo dyes), in that induction of Phase 2 enzymes proceeds by transcription-signaling mechanisms that are independent of the Ah receptor or its gene targets. The existence of such mechanisms has been suggested earlier (32,33), and curcumin provides a clear-cut example.

In conclusion, curcumin and a number of naturally occurring and synthetic analogs are Phase 2 enzyme inducers, as demonstrated by their ability to elevate the enzyme activity of QR in murine hepatoma cells. It is reasonable to assume that Phase 2 enzyme induction plays a significant role in the chemoprotective and antioxidant activities of these curcuminoids. The introduction of aromatic o-hydroxyl groups into the curcuminoid skeleton raises the inducer potency >30-fold. The activities of these compounds are generally attributable to their Michael reaction acceptor centers. The finding that dibenzoylmethane, a β-diketone that is not a classical Michael reaction acceptor, is also a potent inducer, focuses attention on the activities of QR, can be used as a highly reliable tool to predict the in vivo tumor-preventive properties of various natural and synthetic products.
on the fact that the β-diketone moiety of curcuminoids may also play a significant role in their biological activities.

Acknowledgements

We thank Professor Gary H.Posner and M.Cristina White for advice on the synthesis of salicylcurcuminoid, and Jack Arbiser and Mou-Tuan Huang for the generous gift of pure samples of curcumin, demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin. This work was supported by a program-project (PO1 CA 44530) from the National Cancer Institute, Department of Health and Human Services, and by a postdoctoral fellowship (to A.D.-K.) from the Cancer Research Foundation of America.

References


Received November 2, 1998; revised January 22, 1999; accepted January 25, 1999.