A Review of Antioxidant Polyphenol Curcumin and its Role in Detoxification

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Abstract: Curcumin (diferuloylmethane), a yellow colouring agent present in the rhizome of Curcuma longa Linn (Zingiberaceae), has been reported to possess anti-inflammatory, antioxidant and anticarcinogenic activities. Despite such significant anti-tumor efficacies and bio-safety profiles of curcumin, its poor systemic bioavailability originating from sub-optimal absorption, rapid metabolism and fast systemic elimination, is turning out to be one of the major impeding factors retarding its clinical success. Curcumin exerts its chemoprotective and chemopreventive effects via multiple mechanisms. It has been reported to induce expression of the antioxidant enzymes in various cell lines. Curcumin significantly increased expression of Nrf2 as well as phase II detoxifying and antioxidant enzymes. The redox-sensitive transcription factor, nuclear factor erythroid 2 p45 (NF-E2)-related factor (Nrf2) plays a key role in regulating induction of phase II detoxifying or antioxidant enzymes. Thus, activation of Nrf2 is considered to be an important molecular target of many chemopreventive and chemoprotective agents. This review summarizes the molecular basis of chemoprevention and cytoprotection afforded by curcumin with emphasis on its ability to modulate Nrf2-mediated cellular events. In this review, we describe both antioxidant activity and effects of curcumin on biotransformation enzymes involved in carcinogen metabolism.

Key words: Curcumin, metabolism, Nrf2, phase II detoxifying enzymes.

Introduction

Turmeric is a spice stemming from the rhizomes belonging to a ginger family (Zingiberaceae) component called Curcuma longa. Described as horizontal underground stems with shoots and leaves, rhizomes are notable for their vibrant yellow colour (Figure 1). This colour is largely derived from fat-soluble polyphenolic pigments called curcuminoids).

The yellow pigment segregated from the rhizomes of Curcuma longa is termed curcumin, or turmeric, as it is more commonly called¹, ². The distinctive yellow colour of turmeric stems from the natural phenol curcuminoid³. The structure of curcumin was established in the year 1910 and it was originally isolated almost two hundred years ago ⁴. The main constituent of turmeric (a spice) is curcumin, which is similar to aspirin (a renowned carcinogenesis prevention chemical) in its chemical make-up ⁵. The dietary phytochemical curcumin
(chemical structure shown in Fig. 1) has a long history of medicinal use in India and Southeast Asia for a wide variety of medical conditions. The safe consumption of curcumin is easily confirmed by the fact that for hundreds of years it has frequently been part of the diet of people in a number of countries. Curcumin was reported to have antitumor, antioxidant, antiarthritic and anti-inflammatory properties.

Figure 1: *Curcuma longa* Plant and chemical structure of curcumin

Chemistry of curcumin

Curcumin, a hydrophobic natural product, comprises two phenolic rings. Each ring is replaced with methoxy ether functionality in the ortho-position and attached to each other by an aliphatic unsaturated heptene linker in the para-position with an α, β diketonic functionality on carbon-3 and -5. The electrophilic α, β-unsaturated carbonyl groups are capable of reacting with a nucleophile such as glutathione. Results from a number of studies suggest that the diketone functionality is able to go through reversible tautomerization between enolic- and ketonic-forms.

Tautomerization of curcumin takes place in a pH-dependent mode. In this situation the bis-keto form prevails in acidic and neutral solutions while the enol form prevails in alkaline solutions. While in the bis-keto form, the performance of carbon-4 of the heptene linker as a potent contributor of protons has been noted while the enol form operates chiefly as a contributor of electrons, a chemical activity responsible for the antioxidant qualities of curcumin.

Commercial curcumin characteristically comprises three main curcuminoids: curcumin (~77%), demethoxycurcumin (~17%) and bis-demethoxycurcumin (~3%). Curcumin is radiant yellow in colour at pH 2.5 to 7 and evolves to red at pH ≥7. Whether in solid or soluble form, curcumin goes through photo degradation upon exposure to light. Curcumin has a melting point of 183°C, a molecular formula of C21H20O6, and a molecular weight of 368.37 g/mol. It is not as soluble in water as in organic solvents such as dimethyl sulfoxide, ethanol, methanol and acetone.

Curcumin exists in enolic and β-diketone forms. While it remains stable at acidic pH, instability sets in at neutral and basic pH as it breaks down into ferulic acid and feruloylmethane. The presence of curcumin in a solution is for the most part in the form of an enolic and this fact significantly influences its capacity for radical scavenging. The enolic tautomer of curcumin is deemed the major contributor to its bioactivities as well as its photophysical and photochemical features. The stability level of curcumin is low and degradation occurs when it is exposed to adverse physiological situations. Trans-6-(4′-hydroxy-3′ methoxyphenyl)-2, 4-
dioxo-5-hexenal, ferulic acid, feruloyl methane and vanillin have been identified as the end-products of degradation. Unlike the original compound, the metabolic derivatives of curcumin were found to be lacking in biological activity\footnote{21-23}.

Even so, degradative products like ferulic acid and vanillin are considered to possess anti-oxidant activity qualities\footnote{24}. While the hydrophobic nature of curcumin renders it unstable in an aqueous solution\footnote{7}, the degradative products of curcumin boasts a higher aqueous solubility than the original compound itself.

Bioavailability, pharmacokinetics and metabolism of curcumin

Curcumin may surpass other bioactive components with regard to potential, but because of its low bioavailability its usefulness and attainable blood levels associated to its consumption is laced with doubt\footnote{25,26}. HPLC with ultraviolet or mass spectrometry detectors have been utilised in studies on the metabolism and pharmacokinetics of curcumin\footnote{27-30}.

The metabolization of curcumin in humans and rodents can be achieved through either conjugation or reduction pathways. Orally administered curcumin goes through conjugation with curcumin-glucuronide and sulphates as the end result, while curcumin administered intraperitoneally, or systemically, goes through reduction to produce tetrahydrocurcumin, hexahydrocurcumin and octahydrocurcumin\footnote{31,32}.

The many studies conducted to assess the post-administration level of curcumin have established that other than negligible traces spotted in serum or tissue, the presence of curcumin or its metabolites was generally non-existent\footnote{33-36}.

The tissue dissemination of curcumin plays a significant role in its pharmacological activities and improved dissemination was noted with the intraperitoneal (i.p) administration of curcumin. The maximum levels in mice were recorded as 73±20, 200±23, 9.1±1.1, 16±3, 8.4±6.0, 78±03 and 2.9± 0.4 nmol/g in the liver, intestinal mucosa, heart, lungs, muscle, kidneys and brain respectively after an intraperitoneal (i.p.) injection of 100 mg/kg of curcumin\footnote{37}. It was observed that the level of curcumin deteriorated at a rapid pace and in 3 to 6 hours after administration it was no longer quantifiable\footnote{37}.

The antioxidant activity and effects of curcumin on biotransformation enzymes involved in carcinogen metabolism

While curcumin is a robust antioxidant in vitro and in vivo\footnote{8,38,39}, it is inclined to encourage the formation of pro-oxidants (ROS and GSSG) through a method that is dependent on its concentration.

As the pace of ROS formation runs parallel to that of cytotoxicity, it is possible that the proapoptotic effect of curcumin is attributed to pro-oxidant activity. According to recent studies, the removal of the cytotoxic effect by exogenous GSH was for the purpose of scavenging for oxidants\footnote{38,40}. It has been established that the excellent antioxidant activity of curcumin is more intense towards the enol isomer than the keto form\footnote{41}. It was found that by combining it with ascorbic acid, the antioxidant and antifungal properties of curcumin is boosted\footnote{42}. Among the degradation products of curcumin, ferulic acid and vanillin are well-documented antioxidants\footnote{43-46}.

Linoleate, a polyunsaturated fatty acid that can be oxidized to form a fatty acid radical, has been observed to be harnessed by curcumin to inhibit lipid peroxidation. Curcumin, performing as a chain-breaking antioxidant at the 3’ location, activates an intramolecular Diels-Alder reaction culminating in lipid radical neutralization\footnote{47}.

The scavenging and trapping procedures of curcumin are many and complicating in nature\footnote{48}. The capability of curcuminoids to perform these tasks can be attributed to their chain-breaking anti-oxidant activity from hydrogen atoms, presumably a contribution from the phenol (OH) groups\footnote{49}. As observed in studies involving the use of animal models, curcumin offers protection against oxidative agents for the brain, liver, lungs, kidneys and heart. Some examples are: the inhibition by curcumin of ROS generation in activated rat peritoneal macrophages\footnote{50} and lipid peroxidation in the brain homogenates and liver microsomes of rats\footnote{51}; and the provision of protection by curcumin for male Wistar albino rats against acetaminophen-induced nephrotoxicity
by reducing the level of malondialdehyde and raising the activity levels of GSH, GSH peroxidase, catalase and superoxide dismutase activities in renal tissue.

By increasing the activities of GSH S-transferase, superoxide dismutase and catalase, curcumin can defend the male rat liver, testis, brain, kidneys and lungs against sodium-arsenite-induced oxidative injury and reduce oxidative DNA and protein injury in the heart of a diabetes-afflicted rat.

The defensive properties of curcumin seem to be mediated by its capacity for direct scavenging of free radicals or indirect scavenging through the up-regulating of endogenous cellular antioxidant devices which include the initiation of cytoprotective Nrf2-induced target genes.

Nrf2 actually performs the role of a transcriptional activator of the antioxidant responsive element (ARE)-mediated gene expression. This is inclusive of phase II detoxification and antioxidant stress enzymes such as hemeoxygenase-1, glutathione peroxidase, modulatory subunit of gamma-glutamyl-cysteine ligase, which is engaged in glutathione synthesis, and NAD(P)H:quinoneoxidoreductase 1. Thus, curcumin modulation of these gene outcomes could possibly be a contributing factor to its antioxidant and cytoprotective roles in normal cells and this includes its neuroprotective activity.

In in vivo studies, the enhancement in the expression of detoxifying enzymes including GST, glutathione reductase, NQO1, epoxide hydrolase and HO-1 in the liver, small intestine, and kidney tissues of mice was attributed to the influence of curcumin.

It has been established through a microarray analysis, that curcumin induces GSTP1 expression in MCF-7 (a human breast cancer cell line) and in the liver of mice fed a curcumin-inclusive diet. A study by revealed that curcumin could bring about the expression of human glutathione S-transferase P1 (GSTP1). The level of GSTP1 mRNA in HepG2 cells was substantially raised with a curcumin treatment of 20µM. In the nuclear extracts of HepG2 cells treated with curcumin, a rise in the sum of the binding complex was detected through a gel mobility shift assay involving an oligonucleotide with GSTP1 ARE. These findings indicate that ARE is the main sequence involved in the curcumin-influenced transactivation of the GSTP1 gene.

Through the retina-derived cell lines (661W and ARPE-19), pre-treatment of curcumin can deter cell destruction caused by H$_2$O$_2$ through the up-regulation of HO-1 and thioredoxin. Curcumin induction of HO-1 has been seen to take place through Nrf2 in porcine renal epithelial cells, human monocytes and also in wild-type mouse embryo fibroblasts.

In renal epithelial cells, expression of Nrf-2 through stimulation by curcumin occurs in an approach that is influenced by concentration as well as time and correlates with a substantial rise in HO-1 protein expression and hemeoxygenase activity. By enforcing the inactivation of the Nrf-2 Keap1 complex, curcumin stimulates HO-1 gene activity which culminates in the enhanced binding of Nrf-2 to the resident HO-1 ARE.

(Farombi et al. 2008) discovered that a 200 mg/kg oral administration of curcumin for four successive days resulted not only in protection from hepatic damage caused by dimethylnitrosamine (DMN), but also more than tripled the induction Of HO-1 protein expression and activity in the liver of rats. The administration of curcumin led to a boost in the nuclear translocation and ARE binding of Nrf2. Taken as a whole, the information gathered indicates that through the ARE-driven induction of HO-1 expression, curcumin assumes a defensive role (at least partially) against hepatotoxicity brought about by DMN.

Curcumin has also been seen to raise the levels of the significant antioxidant, glutathione. A low dose administration of curcumin elicited the dual adaptive response of an instant enhancement in GSH and the knack for producing an increased amount glutathione. The effects of curcumin can be attributed to its affinity for inducing Messenger RNA (mRNA) of the GSH biosynthetic genes Gclc and Gclm.

Gastrointestinal glutathione peroxidase has been proposed as a defence against hydrogen peroxide absorption and was also acclaimed for its role in the management of inflammation and malignant tumours. The up-regulation of the selenoprotein gastrointestinal glutathione by curcumin through the activation of the Nrf-2/Keap1 system opens the door to its anti-inflammatory and anti-carcinogenic effects (Banning et al. 2005). Also, the influence of curcumin on the expression of detoxifying enzymes like NQO1, GST, AR and HO-1 sets off a xenobiotic reaction in the cells.
Treatment by curcumin resulted in an enhancement in the nuclear build-up of Nrf2, Nrf2-ARE binding and heightened activities in phase II detoxifying enzymes including glutathione S-transferase and NAD(P)H:quinone oxidoreductase-1 (NQO1) in the liver and lungs of mice susceptible to the effects of the carcinogen known as benz- \( \alpha \)-pyrene (B \( \alpha \)P)\(^{74}\). In contrast to curcumin, tetrahydrocurcumin did not bring about haem oxygenase-1 (HO-1) expression and Nrf2 nuclear translocation in RAW264.7 macrophages \(^{75}\).

A study by \(^{76}\) scrutinized the capability of curcumin to induce transcription factor Nrf2 in a neurodegenerative model involving quinolinic acid in rats. The outcomes of this study are in line with the perception that neuroprotection brought about by curcumin is linked to its capacity for activating the Nrf2 cytoprotective pathway and enhancing the total superoxide dismutase and glutathione peroxidise activities.

Curcumin fed to pregnant mice could in fact raise the hepatic levels of GST and acid-soluble sulfhydryl of trans lactationally exposed F1 pups \(^{77}\). In a study employing curcumin to ward off cataractogenesis in rat lenses, the initiation of GST activity was proposed as the defensive procedure against lipid peroxidation \(^{78}\).

Curcumin is an effective generator of cytoprotective enzymes in cultured cell models. In spite of its modest ability to impede UGT activities, curcumin still brought about the expression of UGT 1A1 and 1A6 in Caco-2 cells \(^{79}\).

Bovine aortic endothelial cells treated with curcumin were observed to undergo an increase in HO-1 messengicbonuleic acid (mRNA), protein, and cell protection from oxidative stress. While curcumin induced HO-1 and GST P1 in normal lung fibroblasts, it did not do so in scleroderma lung fibroblasts and this may be a contributory factor to the discriminative induction of apoptosis in scleroderma lung fibroblasts \(^{80}\).

Curcumin also brought about HO-1, NQO1, and GST in astrocytes and hippocampal neurons and also shielded cells from oxidative injuries \(^{81}\). Hepa1c1c7 murine hepatoma cells were the subjects in a study on the structure-activity association of curcumin and its analogues as NQO1 inducers \(^{82}\).

On the other hand, the \( \beta \)-diketone moiety is also a significant functional group in the induction of NQO1 owing to the domination of the keto-enoltautomerism. Generally, when it involves nucleophilic groups such as thios, the activity of the inducer corresponds to the reactivity. The structure–activity relationship in the induction of NQO1 has also been verified in the induction of HO-1 and the protection from oxidative injuries \(^{83}\).

Curcumin has been observed to briefly activate PKC, and PKC activation is a requirement in the curcumin-induced expression of HO-1, GST, and GCLM \(^{69, 84}\). Through the stimulation of upstream kinases by phosphorylation or by oxidizing the cysteine thiol of Keap1, the administration of curcumin induced Nrf2-ARE/EpRE (electrophile response element) signalling (Farombi et al. 2008). As curcumin assumes two \( \alpha \), \( \beta \)-unsaturated carbonyl moieties, it is able to take on the role of a Michael reaction acceptor. It is thus conceivable that the attendance of these electrophilic carbonyl moieties can act together with a critical cysteine thiol of Keap1 to decrease its affinity for Nrf2 and liberate it for nuclear translocation (Farombi et al. 2008).

Also, through the stimulation of the upstream kinases which can phosphorylate Nrf2 at serine and/or threonine residues, curcumin may set off Nrf2-ARE signalling. It is on record that curcumin activates the ARE-mediated antioxidant defence gene expression in human monocytes through Protein Kinase C Delta (PKCd) upstream of p38MAPK and Nrf2 \(^{69}\). Curcumin induced the expression of HO-1 in mouse \( \beta \) cells through a pathway that involved the transcription factor Nrf2 and PI3K/Akt-mediated signalling pathway \(^{85}\). Additionally, curcumin is known to up-regulate aldose reductase expression through Nrf2 with a PI3K/Akt-dependent approach \(^{86}\).

Conclusion

Curcumin, a phytochemical agent in the spice turmeric, has received increasing attention for its anticancer, anti-inflammatory and antioxidant properties. It seems that prooxidant or antioxidant properties of curcumin depend on its concentration. Thus, curcumin cannot only be considered purely as antioxidants, since under certain reaction conditions they can also display prooxidant activity. Induction of phase-2 detoxifying or antioxidant genes represents an important cellular defence in response to oxidative and electrophilic insults. Nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) plays a crucial role in regulating phase-2 detoxifying/antioxidant gene induction. Information derived from these studies suggests that curcumin induces phase II detoxifying enzymes concerned with the detoxification of carcinogens.
All of these findings reinforce the idea that curcumin could be considered as a potent natural compound both for prevention and treatment of multifactorial disease such as cancer.

References


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