Determining whether curcumin degradation/ condensation is actually bioactivation (Review)

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Abstract. Curcumin has been shown to exert therapeutic or protective effects against a variety of diseases, such as cancer, pulmonary diseases, neurological, liver, metabolic, autoimmune, cardiovascular diseases and numerous other chronic ailments. Over 116 clinical studies on curcumin in humans were registered with the US National Institutes of Health in 2015. However, it is mystifying how curcumin can be so effective in the treatment of many diseases since it has very low water solubility and bioavailability. Furthermore, curcumin is not stable under various conditions; its degradation or condensation into different bioactive compounds may be responsible for its biological activities rather than curcumin itself. In this review, we provide evidence of curcumin degradation and condensation into different compounds which have or may have health benefits themselves. Literature reviews strongly suggest that these molecules contribute to the observed health benefits, rather than curcumin itself.

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1. Introduction

Curcumin is the principal constituent of turmeric i.e., the ground rhizomes of *Curcuma longa*, which contains two other curcuminoids: desmethoxycurcumin and bis-desmethoxycurcumin (1). Turmeric is widely used as a spice mostly in Asian countries. However, it is also used to treat acne, psoriasis, dermatitis and rash. It should be stressed that traditionally, turmeric was suspended in whole milk or buttermilk that dissolved it in fat fractions and/or stabilized curcumin (2). Over the past few decades, preclinical and clinical studies have revealed that curcumin is active against variety of diseases, such as cancer and pulmonary diseases, as well as neurological, liver, metabolic, autoimmune and cardiovascular diseases, and numerous other chronic ailments (3-6). Over 116 clinical studies on curcumin in humans were registered with the US National Institutes of Health in 2015 encomapssing a number of conditions, such as cancer, cognitive disorders, gastrointestinal diseases and psychiatric conditions. In humans, the administration of curcumin at up to 12 g per day has not been found to exert any toxic effects (7-10).

One of the puzzling questions is how curcumin can be so effective in the treatment of diseases, since it has a very low water solubility and bioavailability. For example, the oral dose of 8 g/day in humans translates to low nanogram levels of circulating curcumin in plasma (only 22-41 ng/ml) (11,12). Moreover, curcumin is not stable under various conditions, such as aqueous phosphate buffer or serum-free medium at 37˚C, degrading to the bioactive compounds, including ferulic acid, feruloylmethane and vanillin, which may be responsible for its biological activities rather than curcumin itself (12,13).

In view of the very low bioavailability of curcumin as observed in clinical studies, the role of the degradation or condensation products should be taken into consideration when evaluating the activity of curcumin in various diseases.

2. Physico-chemical properties of curcumin

Curcumin (IUPAC name: (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is, practically insoluble

in water at a neutral and lower pH, but is soluble in acetone, dichloromethane, methanol, ethanol, alkali and oils. The water solubility of curcumin may be increased by its incorporation into various surfactants, such as sodium dodecyl sulfate, polysaccharides, polyethylene glycol and cyclodextrins, as well as others (13,14). In addition, in aqueous solutions and at an alkaline pH, the acidic phenol group in curcumin dissociates its hydrogen, forming the phenolate ion(s) that render the solubility of curcumin in water somewhat possible (15-18). Curcumin is a natural polyphenol that is responsible for the yellow color of turmeric and exhibits *keto-enol* tautomerism (Fig. 1). The *enol* form is more energetically stable in the solid phase and, depending on the solvent, up to 95% can be in the *enol* form (1). Three reactive functional groups, namely diketone moiety and two phenolic groups determine the activity of curcumin. The biologically important chemical reactions of curcumin are the following: the hydrogen donation leading to oxidation, reversible and irreversible nucleophilic addition (Michael reaction), hydrolysis, degradation and enzymatic reactions (19).

In a previous review article, Agrawal and Mishra analyzed studies (years 1815-2009) on curcumin and 728 curcumin analogs (20). This very large group of compounds was tested for pharmacological properties and mostly on anticancer activity on different cell lines. Some analogs have been shown to exhibit antioxidant, anti-mutagenic and anti-HIV activities (21), as well as anti-angiogenic anti-malaria and anti-tuberculosis activities (7) or anti-inflammatory activities [cyclooxygenases (COX) inhibitors]. Based on a literature search, the authors concluded the following (Fig. 2): the anticancer properties of curcuminoids depend on the presence of OH groups in the phenolic ring (entries 4 and 4'). These groups are an electron donor to free radicals. The methoxy group at position 3 and 3' increases the antioxidant properties of curcuminoids; substitution in the 2 and 2' positions increases all activities than the unsubstituted analogs; cyclization in the central part of the compound and the introduction of heteroatoms (oxygen and nitrogen) leads to the formation of compounds with enhanced antitumor and anti-angiogenic activities; attaching solubilizing groups to the OH group in position 4 and 4' is responsible for the cytotoxicity of curcuminoids; the elimination of one of the methoxy group reveals the effect of tuberculosis (7); conversion of methoxy groups to hydroxyl increases the anti-HIV activity (21).

3. Alkaline degradation and autoxidation of curcumin

Wang *et al* (13) incubated curcumin in 0.1 M phosphate buffer, pH 7.2 at 37˚C, and found that 90% was degraded in 30 min. Trans-6-(4-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexenal, vanillin, ferulic acid and feruloyl methane (Fig. 3A-D) were identified as degradation products (13). This is a plausible explanation of the biological activity of curcumin, since the degradation products have better aqueous solubility as reflected by their respective logP values: 1.42 for ferulic acid and 1.09 for vanillin, lower than the *keto* and *enol* form of curcumin, which are respectively 2.56 and 2.17 (12). Moreover, it has been reported that ferulic acid inhibits COX-1 and -2 and suppresses the activation of nuclear factor-κB (NF-κB), which are known to be important targets in the prevention of cancer development (12,22,23). Vanillin as well can inhibit COX-2 gene expression and NF-κB activation (12,24).

Shen and Ji (12), in a comprehensive review of curcumin degradation, described the curcumin-mediated inhibition of xanthine oxidase that is involved in the pathogenesis of many diseases. The authors described molecular modeling, demonstrating that all degradation products can enter into the binding pocket of an enzyme. Surprisingly, curcumin itself failed to efficiently fit within the binding pocket of xanthine oxidase and only entered the binding pocket with low binding affinity (12). This is consistent with the experimental findings that the degradation products (ferulic acid, vanillin, ferulic acid and feruloyl methane), rather than curcumin itself can inhibit xanthine oxidase (12,25,26).

In a previous study, Gordon and Schneider demonstrated that the cleavage of the heptadienedione chain, resulting in vanillin, ferulic acid and feruloylmethane as products, was not the prevailing degradation reaction (27). Rather, they proposed that the degradation of curcumin is a spontaneous autoxidation, free radical-driven incorporation of oxygen and that the major product of this process is a bicyclopentadione (15,27). It has been reported that different product profiles of curcumin autoxidation reactions are dependent on time. In reactions between 20-45 min, the chromatograms exhibit peaks, indicating spiroepoxide and vinylether as major products (Fig. 3E-G), and dihydroxy, ketohydroxy and hemiketal cyclopentadiones as minor products. Degradation between 30 min and 4 h also produces the bicyclopentadiones as major products and, several unidentified chemicals. When autoxidation is longer than 4 h, bicyclopentadione is detected as well (28,29).

Naturally occurring polyphenols have been shown to act with topoisomerase II, increasing the levels of topoisomerase II-mediated DNA cleavage. Topoisomerase poisons are used in anticancer and antibacterial therapies. Thus, Ketron *et al* (30) investigated whether curcumin, its structurally related degradation products (vanillin, ferulic acid and feruloylmethane) and its oxidative metabolites exert any effects on the DNA cleavage of human topoisomerase IIα and IIβ. Curcumin, bicyclopentadione, vanillin, ferulic acid and feruloylmethane were shown to have no effect on DNA cleavage. However, intermediates of the curcumin oxidation pathway increased the level of DNA cleavage by both enzymes ~4-5-fold. Moreover, under conditions that promote oxidation, curcumin enhanced topoisomerase II-mediated DNA cleavage even further (30).

Gordon *et al* (28) also demonstrated that the product of curcumin oxidation, a stable spiroepoxide, was able to poison recombinant human topoisomerase IIα and that this process was significantly increased in the presence of potassium ferricyanide, indicating that oxidative conversion was needed to achieve full DNA cleavage activity. They concluded that oxidative metabolites may be responsible for the biological effects of curcumin (28).

4. Photodegradation of curcumin

It is common knowledge that turmeric stains can be removed by exposure to sunlight. This is due to the fact that curcumin absorbs strongly in the visible wavelength range, making it predisposed to degradation and modification in daylight and artificial lighting. The photodegradation of curcumin takes place in solid state, as well as in different organic solvents (14,18,31-34). However, the composition, degradation kinetics and the relative

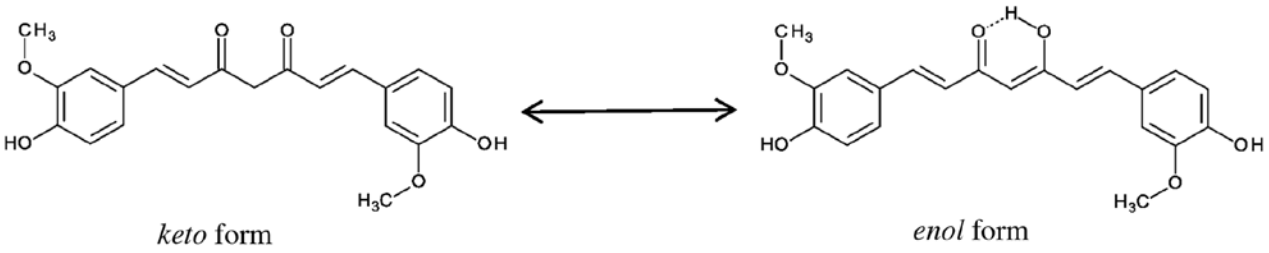

Figure 1. Curcumin coexists in *keto* and *enol* forms; the *enol* form is the dominant type.

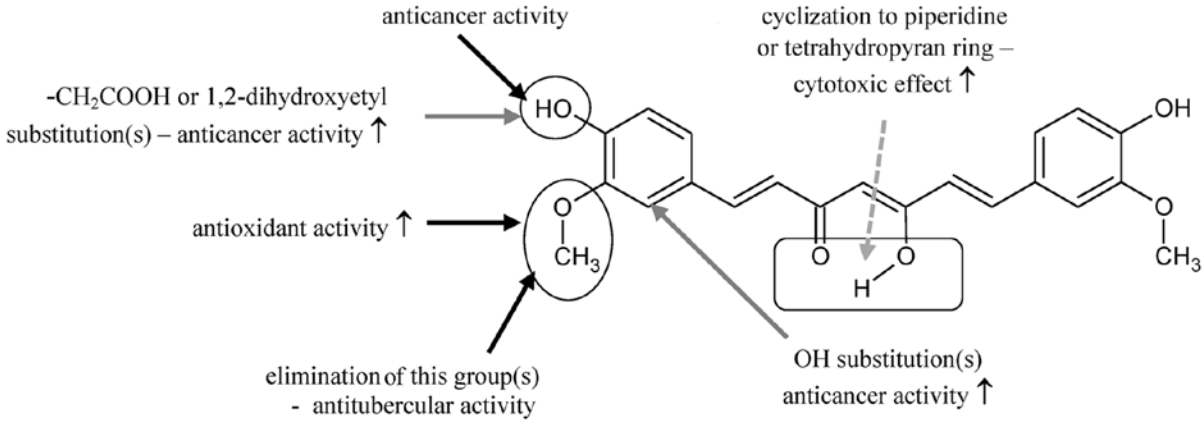

Figure 2. Structure-activity relationships of curcumin analogs.

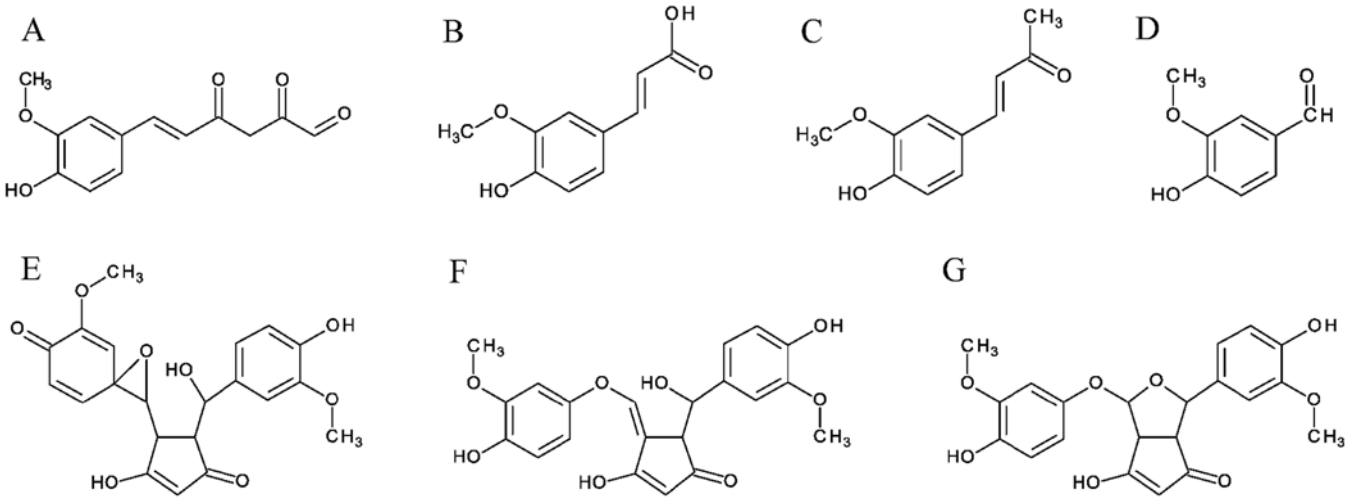

Figure 3. Degradation of curcumin to: (A) trans-6-(4-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexenal; (B) ferulic acid; (C) feruloyl methane; (D) vanilin (13). (E) spiroepoxide; (F) vinylether; (G) bicyclopentadione (28,91).

abundance of the degradation products differ, depending on the physical state of the compound and the conditions.

Previously, the photochemical degradation of solid state curcumin exposed to sunlight for 120 h yielded vanillin (34%), ferulic aldehyde (0.5%), ferulic acid (0.5%), vanillic acid (0.5%) and three unidentified compounds. The photodegradation of dissolved curcumin depends on the solvent and wavelength. When curcumin was dissolved in isopropanol and irradiated for 4 h at 400-510 nm, then similar products as in the case of light-irradiated crystalline curcumin were observed, such us vanillin, vanillic acid and ferulic acid, in addition to aldehyde 4-vinylguaiacol (34).

Exposure to visible light inflicts more degradation than UV light; the irradiation of curcumin in 254-nm in methanol has been shown to produce three unspecified degradation products, whereas irradiation with daylight produces five unspecified degradation chemicals products (31). The exposure of curcumin to visible light is solvent-dependent. Irradiation with light (400-750-nm) for 4 h was shown to be associated with cyclization at one of the o-methoxyphenyl groups, producing 7-hydroxy-1-[(2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]-6-methoxy-naphthalen-2(1H)-one in isopropanol, methanol and chloroform, but not in acetonitrile and ethyl acetate (32,34). Galer and Šket irradiated acetonitrile solution of curcumin by

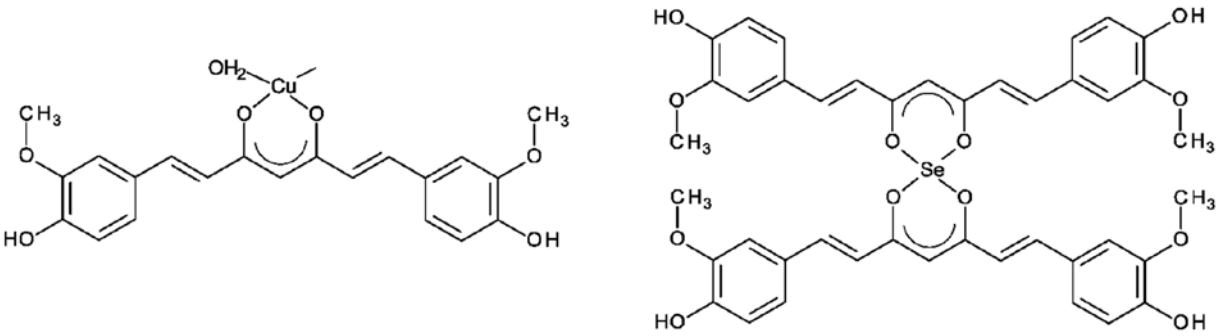

Figure 4. Proposed structure of 1:1 metal (Cu, Mg and Zn) curcumin complexes and 1:2 selenium complex (50).

light (350 nm) and found that 90% of all formed products included 3,5-dimethoxybenzaldehyde, 3,5-dimethoxybenzoic acid and Z and E isomers of 3-(3,5-dimethoxyphenyl)propenoic acid (35).

It has also been reported that the photodegradation of curcumin involves the formation of the excited states and generation of singlet oxygen that is responsible for the photobiological and photodynamic activity of curcumin (19,33). Thus, it was postulated that the degradation of curcumin following photoexcitation must proceed though the triplet excited state of curcumin (19). Curcumin is photoactivated by blue light (420-480 nm) that has limited tissue penetration. That property makes curcumin an ideal surface antibacterial agent for oral or skin disinfection, particularly for antibiotic-resistant bacterial strains as it does not affect healthy tissue (36-38).

An interesting observation was previously made when studying the interaction of curcumin with lipoxygenase (LOX) by a single-crystal X-ray analysis, showing the complex Enz:Fe-O-O-R with the curcumin degradation product instead, identified as 4-hydroperoxy-2-methoxyphenol bound to the enzyme's iron cofactor. Irradiation by X-ray is known to produce free radicals, but curcumin itself is stable under such conditions. LOX is a very good biocatalyst stimulating many reactions, neither of which would lead to the observed product. Thus, it was obvious that the X-ray radiation, LOX and curcumin properties together were responsible for the curcumin transformation to this peroxide, which converts it into 2-methoxycyclohexa-2,5-diene-1,4-dione (39). While the enzyme in that experiment was of plant origin, humans do have six LOXs, four of which (5-, 12S-, 12R-LOX and eLOX-3, [for comparison see (40)] have a highly similar structure of the enzymatic active site. X-rays radiation used for curative purposes in humans often causes severe side-effects, including inflammatory responses caused by various eicosanoids produced by oxygenases: COX and LOX and cytochrome P450 (CYP450). It may be worth exploring whether and how curcumin may be utilized during radiation therapy to improve the treatment outcomes and the comfort of patients.

5. Curcumin complexes with metals

Curcumin can form complexes with transition metals to protect against degradation in the treatment of Alzheimer's disease (41,42). Several curcumin complexes with metals (Cu, Mn, V, Ga and In) have been synthesized and evaluated for their biological activity (43-49). However, all these metallocomplexes have been synthetized under high temperature conditions, reflux at 100˚C in the presence of different organic solvents for 3 h. Zebib *et al* (50) synthesized curcumin complexes with divalent ions of Zn^{2+}, Cu^{2+}, Mg^{2+} and Se^{2+}, in glycerol/water solution and room temperature (50) (Fig. 4). They found that all complexes were stable in water at pH 6.5 up to 30 h at 37˚C. All complexes rapidly decomposed by demetallization at acidic pH 2 and greatly decreased at higher pH 10. At pH 7.0, in phosphate buffer, curcumin was degraded after 1 h, while <5% of complexes were degraded. The authors estimated that the stability of curcumin metal complexes at pH 7 was ~20-fold greater than that of curcumin alone (50).

John *et al* (49) synthetized four synthetic curcuminoids and their Cu^{2+} metallocomplexes. Using L929 mouse fibrosarcoma cells, they found that the concentration required for the 50% inhibition of cell growth was ~10 μg/ml for curcuminoids, but only 1 μg/ml for their copper counterparts. Moreover, they observed a significant reduction ($p<0.001$) in tumor volume in mice treated with copper chelates of curcuminoids (49). Mei *et al* (51) investigated the anti-ulcerogenic effects of a Zn-curcumin chelate in mice. Treatment with Zn-curcumin reduced gastric lesions in a dose-dependent manner (12, 24 and 48 mg/kg) in comparison with the control group (51). In a different study from the same group, the effects of Zn-curcumin on hemorheological alterations, oxidative stress and liver injury in a rat model of acute alcoholism were investigated. They found that the oral dose of Zn-chelated curcumin prevented the alcohol-induced increase in malondialdehyde (MDA) levels in serum and the reduction in glutathione levels and superoxide dismutase (SOD) activity. Furthermore the Zn-curcumin complex inhibited ethanol-induced liver injury. In addition, this curcumin derivative reduced the alcohol-induced elevation of blood viscosity, plasma viscosity, erythrocyte aggregation index and hematocrit. In all of these experiments Zn-curcumin was found to be more effective than curcumin (52).

In another study, Refat (53) synthesized curcumin complexes with Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) and tested the antibacterial and antifungal activity. Only the cobalt [Co(II)]-curcumin complex exhibited antibacterial activity against three bacterial strains (*Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) (53).

6. Interaction of enzymes with curcumin

Curcumin interacts with very large number of proteins, such us albumin (54), Ca^{2+}-ATPase of the sarcoplasmic

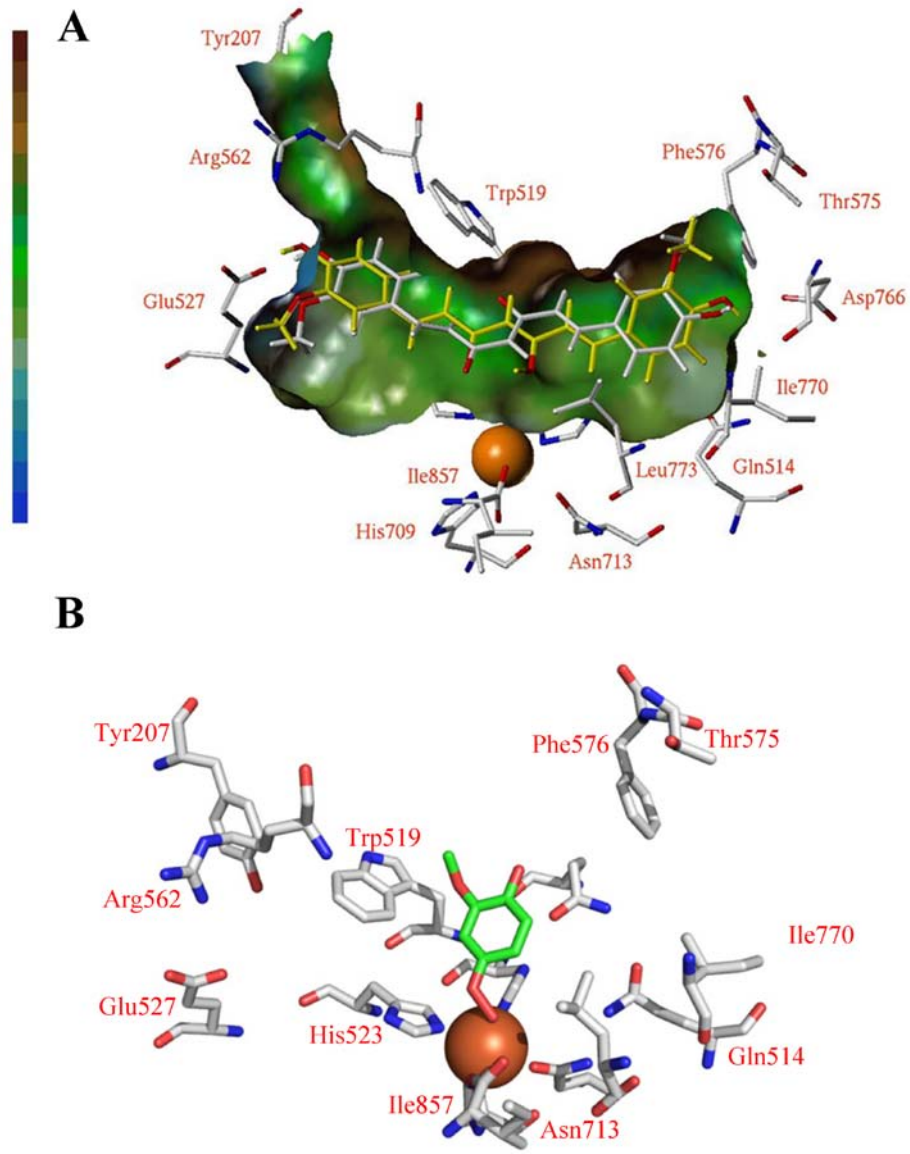

Figure 5. Based on molecular modeling (92,93), curcumin can fit into the soybean lipoxygenases-3 (LOX-3) lipoxygnase central cavity: (A) gray, *keto* form, yellow, *enol* form. Surface shown as: brown according to the lipophilic properties; blue, hydrophilic properties of the residues lining the cavity. Iron is indicated by an orange sphere, and selected residues are shown as stick models to illustrate curcumin's positioning. (B) Lipoxygnase central cavity molecule with the observed photodegradation product (carbon in green, oxygen in red) near the iron atom (in orange). Reprinted with permission from and based on structure (39,60). Permission to reprint part A granted by the editor.

reticulum (55,56), Ca^{2+}-dependent protein kinase (CDPK) (57), COX-2 (58,59), LOX (39,60), LOX-5 (61), pp60c-src tyrosine kinase (62,63), PKC (63), xanthine oxidase (64,65) and many others (66). Dr Duke's Phytochemical and Ethnobotanical Database (https://phytochem.nal.usda.gov/phytochem/search) provides a long list of curcumin anti- and pro-health properties. It is known as an inhibitor of the oxygenases 5- and 12-LOX, COX-2 and CYP450, but an inducer of lipase which is up in the arachidonic acid pathway. It can inhibit protein kinase C (PKC), protein tyrosine kinase (PTK), IKB-kinase and IKK involved in NF-κB signaling pathway, tumor necrosis factor (TNF), topoisomerase I and II, vascular endothelial growth factor (VEGF), sortase A and ornithine decarboxylase. It promotes maltase and sucroses, and glutathione synthetase activity, while it suppresses amyloid β activity. This list, probably far from complete, shows that curcumin has a very broad range of activities and that its impact would depend on dose and environment.

Computational molecular modeling methods have shown that curcumin can bind into the central active pocket of soybean LOX-3 lipoxygnase (39). However, the solved X-ray structure of LOX shows the photodegradation product of curcumin (Fig. 5). That was based on the size, volume and position of the unoccupied (Fo-Fc) difference map (39,60).

7. Changes in the body

Interestingly, curcumin has is metabolized differently in the mammalian body depending on the type of administration (oral, intravenous or intraperitoneal). Curcumin administered orally undergoes glucuronidation and sulfation to the glucuronide of hexahydrocurcumin (67). However, when administered intravenously or intraperitoneally, it undergoes a reduction that leads to the formation of tetrahydrocurcumin and hexahydrocurcumin, the two major metabolites of curcumin in body fluids, organs

and cells (68), and two minor metabolites: octahydrocurcumin and dihydrocurcumin. Curcumin is poorly absorbed from the gastrointestinal tract after oral intake, with extremely low concentrations being detected only in bile, urine (67,69) and blood plasma (70). Furthermore, in available tissue samples obtained during surgery from patients administered high doses of curcumin, either no curcumin, or very low concentrations of curcumin conjugates were detected (71). Despite the lower bioavailability, in clinical investigations, curcumin has been shown to have therapeutic potential in various human diseases. The main activity of curcumin seems to be connected with its modulatory activity of cell signaling pathways and transcription factors, such as NF-κB, activator protein 1 (AP-1) and mitogen-activated protein kinase (MAPK) (72), and its suppressive effects on the expression of inflammatory cytokines, such as interleukin-6 (IL-6), IL-1β, TNF-α, matrix metalloproteinase (MMP)-2 and MMP-9 (73).

Periodontitis, as an imbalance between biofilm and immune host reaction, is an opportunistic and chronic infection in which the above-mentioned cell signaling pathways regulating the expression of inflammatory mediators have become promising therapeutic targets (74). The oral cavity can be easily used to observe the reactions of tissue to curcumin *in vitro* and *in vivo*. In many animal studies, the antioxidant (75) and anti-inflammatory (76), as well as the angiogenic and wound-healing effects of curcumin, by increasing the number of fibroblasts and promoting collagen synthesis (77), or by modulating urokinase plasminogen activator (uPA) expression (78), have been demonstrated. Interestingly, curcumin does not prevent alveolar bone resorption *in vivo* in rats (76), but inhibits RANKL-induced osteoclastogenesis *in vitro* (79). Guimarães *et al* also observed the lack of inhibition of bone resorption in an experimental model of lipopolysacharide (LPS)-induced periodontal disease (76), which is surprising, keeping in mind that curcumin was shown to significantly inhibit *Porphyromonas gingivalis* LPS-induced TNF-α and IL-1β production (80). Curcumin has also exhibited exhibited bactericidal activity against *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum* and *capnocytophaga* at a minimal inhibitory concentration (MIC) of 1 mg/ml by the local application into periodontal pockets in humans (81). The results of the benzoyl-DL-arginine-naphthylamide (BANA) test, which verifyied the presence of *Tannerella forsythia*, *Treponema denticola*, *Porphyromonas gingivalis* and *capnocytophaga* species, was similar for 1% curcumin solution and 0.2% chlorhexidine gluconate in subgingival irrigation. The only difference was the earlier re-colonization by periopathogens places treated with curcumin (82). Curcumin exhibits a local activity in the chemopreventive therapy of premalignant lesions for oral cancer. In an *in vitro* model consisting of primary cultures of normal epithelial cells, cell lines derived from dysplastic leukoplakia and squamous cell carcinoma cells, curcumin was equally effective for all cell types tested and blocked the cells in the S/G_2M phase of the cell cycle (83). The local effectiveness of curcumin was higher in combination with(-)-epigallocatechin-3-gallate (EGCG) (83). This suggests some limitations of curcumin even in local treatment.

Numerous approaches have been undertaken to improve the poor solubility, rapid metabolic disposition and the lack of systemic bioavailability of curcumin. One of the possibilities is the use of curcumin metabolites or chemically modified curcumin. Unlike curcumin, tetrahydrocurcumin is stable in phosphate buffer and in saline at various pH values and is easy absorbed through the gastrointestinal tract (84). An *in vitro* investigation revealed that treatment with tetrahydrocurcumin reduced fibrosarcoma cell (HT1080) adhesion to the extracellular matrix and laminin, and the secretion of MMPs (MMP-2 and MMP-9) and uPA (85). It has also been shown to affect the migration and proliferation of gingival fibroblast cells (86). Chemically modified curcumin with a carbonyl substituent at the C-4 position exhibits better solubility, serum albumin-binding activity and enhanced zinc-binding characteristics (87). 4-Metoxycarbonyl curcumin, administered orally, was shown to inhibit MMP-9 and MMP-13 activity more effectively than curcumin (by 2-7-fold). It also demonstrates greater therapeutic activity, based on its inhibitory effect on MMPs and pro-inflammatory mediators [TNF-α, IL-1β, monocyte chemoattractant protein-1 (MCP-1), IL-6 and prostaglandin (PGE)-2] (87). In another study, phenylamino carbonyl curcumin exerted inhibitory effects on MMPs in rats with LPS-induced periodontitis; however, unlike curcumin, it significantly reduced alveolar bone loss (88). The bioavailability of curcumin extremely was shown to increase when combined with piperine (20 mg/kg) in rats and humans with no side-effects (89). Promising results were also obtained in a recent study which used mucoadhesive nanoparticles loaded with curcumin as a new approach with which to deliver curcumin for the local treatment of oral cancer (90).

8. Conclusion

Clearly curcumin exhibits a variety of health benefits; however, proof that the curcumin molecule itself is responsible for these effects seems to be illusive. This is due to the poor bioavailability, instability and strong reactivity of curcumin. An additional complicating factor is the fact that curcumin metabolizes differently depending on the type of administration. The ability of curcumin to undergo degradation or condensation strongly suggests that research should also focus on the health benefits of these molecules rather than only on curcumin itself.

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