

Curcumin as an ROS Scavenger in Amyotrophic Lateral Sclerosis

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ABSTRACT | Oxidative stress, a deleterious process resulting from an imbalance between pro-oxidants and anti-oxidative defenses, plays a key role in several neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS). Curcumin has been studied for its anti-inflammatory, anti-cancer, and antioxidant effects. Curcuminoids exhibit a protective effect by accelerating antioxidant defense mechanisms and attenuating mitochondrial dysfunction. As a result of epidemiological, clinical, and animal studies, several molecular mechanisms, such as the activation of Nrf2 pathway and the decrease of aberrant proteins aggregation, are emerging to account for the multiple biological effects of curcumin and provide a basis for its potential use in the treatment of ALS. This review focuses on oxidative damage, with particular reference to ALS pathogenesis, and antioxidant defense mechanisms to limit such damage, and summarizes the most interesting in vitro and in vivo studies on the effects of curcumin as an antioxidant and its implications in ALS.

KEYWORDS | Amyotrophic lateral sclerosis; Oxidative stress; Antioxidants; Autophagy; Curcumin

ABBREVIATIONS | 3-NT, 3-nitrotyrosine; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; AD, Alzheimer disease; ALS, amyotrophic lateral sclerosis; AMPK, AMP-activated kinase; AOPP, advanced oxidation protein product; CAT, catalase; CSF, cerebrospinal fluid; CuII(atm), diacetyl-bis(4-methylthiosemicarbazono) copperII; DMC, Dimethoxy curcumin; FRAP, ferric reducing antioxidant power; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced form of glutathione; GST, glutathione S-transferase; HNE, 4-hydroxy-2-nonenal; HO-1, heme oxygenase-1; MDA, malondialdehyde; mTOR, mammalian target of rapamycin; NMJ, neuromuscular junctions; NQO1, NAD(P)H:quinone oxidoreductase 1; Nrf2, nuclear transcription factor erythroid-2-related factor 2; ORAC, oxygen radical absorbance capacity; ROS, reactive oxygen species; SOD, superoxide dismutase; TDP-43, TAR DNA-binding protein; TEAC, trolox equivalent antioxidant capacity

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1. INTRODUCTION

Reactive species include reactive oxygen species (ROS) and reactive nitrogen species (RNS); among them, the most important ones are superoxide ($O_2^{\cdot-}$), hydroxyl radical (OH^{\cdot}), hydrogen peroxide (H_2O_2), nitric oxide (NO) and peroxynitrite ($ONOO^{\cdot}$) [1]. Moderate levels of ROS/RNS function as signals to promote cell proliferation, regulation, and survival, whereas increased levels of ROS/RNS can induce cell death [2].

The deleterious process resulting from an imbalance between ROS/RNS and efficiency of cell antioxidant defense system, provoking damages to cellular macromolecules, is known as oxidative/nitrosative stress [2, 3]. An uncontrolled ROS accumulation may be an important factor that leads to structural and functional mitochondrial damage, such as complex I inhibition, giving rise to increased ROS [4]. Mitochondrial alterations in bioenergetic, dynamic, and trafficking patterns as well as oxidative stress play a key role in several neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS) [5].

ALS is a severe neurodegenerative disease that is characterized by progressive loss of the upper and lower motor neurons at the spinal or bulbar level [6, 7]. The most common form of ALS is sporadic (sALS, 90–95%) which has no obvious genetically inherited component. The remaining 5–10% of the cases are familial-type ALS due to their associated genetically dominant inheritance factor [8]. ALS pathology is still not completely understood. Many pathways have been suggested to play a role in the disease pathogenesis including genetic factors, defects in mRNA processing, protein aggregation, glutamate excitotoxicity, as well as mitochondrial dysfunction and oxidative stress [7].

Currently there is no effective treatment for ALS. Riluzole, the only approved compound for the disease, has been shown to slow disease progression; nevertheless, the survival of patients was prolonged

by only 2–3 months, and death due to respiratory failure occurred within 3–5 years of the diagnosis [9]. Strategies and agents which enhance mitochondrial bioenergetics and reverse oxidative stress could be potentially useful as therapeutics in this pathology [10]. Although data are limited, both pre-clinical and clinical studies with antioxidant supplements (e.g., catechins, co-enzyme Q_{10} , ibedenone, resveratrol, vitamin A) have been proposed to be potentially beneficial for ALS patients [11]. In the past years, a particular interest has been devoted to studying the effects of curcumin, a natural compound of vegetal origin, on neurodegenerative diseases [10]. In this manuscript, we will focus on oxidative stress and ROS production and analyze the effects of curcumin as an antioxidant compound in limiting oxidative damage in ALS.

2. OXIDATIVE STRESS IN ALS

The most commonly known genetic mutations for ALS are localized in Cu,Zn superoxide dismutase (SOD1) gene. SOD1 catalyzes the conversion of $O_2^{\cdot-}$ to H_2O_2 , which is then decomposed to water, and its functional loss can lead to increased levels of ROS [12]. These mutations structurally weaken SOD, which indirectly decreases its affinity for Zn. The loss of Zn profoundly alters the redox properties of the enzyme and makes SOD toxic to motor neurons. Rather than acting as a scavenger of $O_2^{\cdot-}$, Zn-deficient SOD can steal electrons from cellular antioxidants and transfer these electrons to oxygen to produce $O_2^{\cdot-}$ [13].

Mutated SOD1 can form cytotoxic protein aggregates that lead to loss of the enzymatic function and decrease of free radical scavenging activity. The remaining wild-type SOD1 could become target of oxidative modification, dissociating itself from dimers to monomers and further forming aggregates with toxic properties of mutant forms of SOD1 [12]. Guareschi et al. [14] show, in sALS patients, that wild-

type SOD1 is post-translationally modified and iper-oxidized, and that through this oxidation, modified SOD1 acquires toxic properties similar to those induced by disease-causing genetic mutations in patient-derived cells. Furthermore, the iper-oxidation of SOD1 is specific, not generalized to all ALS patients, but occurring only in a subset of patients with bulbar onset (iperOxSOD1-sALS). In iperOxSOD1-sALS, highly oxidized SOD1 is present at baseline and, in contrast to normally oxidized SOD1, is not sensitive to further oxidation. However, this iperOxSOD1 requires further oxidative stress to acquire fully toxic characteristics leading to mitochondrial damage.

In some mutant SOD1 mouse models, oxidative stress seems to originate from distal muscles before the disease onset [15]. Oxidative stress affects the presynaptic transmitter releasing machinery. Increased ROS levels inhibit neuromuscular junctions (NMJ) function in spite of already elevated level of oxidative stress, suggesting that oxidative damage could start in the peripheral tissue and proceed retrogradely to the neurons [16]. Pollari et al. [17] suggest that early damage to the NMJ in ALS is due to intra-terminal dysregulation of nerve terminals without essential changes in their morphology. In ALS mouse models, nerve terminals are sensitive to ROS and oxidative stress, which along with compromised mitochondria and increased intracellular Ca^{2+} amplifies the presynaptic decline in NMJ. In the later stage of ALS, the main damage results from an excessive accumulation of toxic ROS. Thus, oxidative stress promotes tissue damage by exacerbating and interacting with the other pathological events, such as inflammation (that is an additional source of ROS), and absence of neuroprotective trophic factors, that promotes motor neuron degeneration.

Altered oxidative stress biomarkers have been repeatedly found in sALS patients, which may indicate that oxidative stress is relevant in the pathogenesis of this disease [18]. Bogdanov et al. [19] demonstrated that oxidative damage to DNA, evaluated by levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the plasma, urine, and cerebrospinal fluid (CSF), was significantly elevated in the ALS group as compared to control subjects. Plasma and urine 8-OHdG levels were increased significantly with the disease progression and correlated with disease severity. Analogously, in the spinal cords of sALS patients, the levels of protein carbonyl groups were increased

[20], but plasma protein carbonyl levels surprisingly did not differ between sALS patients and control subjects [21]. Another possible marker for oxidative alteration of proteins is an increase in the levels of nitrotyrosine residues. Increased levels of 3-nitrotyrosine (3-NT) and 3-nitro-4-hydroxyphenylacetic acid in the lumbar and thoracic spinal cord of ALS patients were found by Beal and colleagues [22]. Increased 3-NT immunoreactivity was observed in the motor neurons of both sporadic and familial ALS patients. Furthermore, the concentration of 3-NT and the 3-NT/tyrosine ratio in patients with sALS were approximately seven times those of controls [23]. Also raised levels of advanced oxidation protein products (AOPPs) were detected in the plasma [24, 25] and CSF of sALS patients [24]. The AOPP assay is a method to determine oxidant-mediated protein damage, based on a spectrophotometric assay, and it is regarded as an easily measurable marker of oxidative stress [25].

Several markers of lipid peroxidation have been found increased in ALS [26]. For example, the lipid peroxidation product 4-hydroxy-2-nonenal (HNE) was elevated in the CSF and serum of sALS patients compared to normal controls [21]. In another study, HNE levels were significantly elevated in the sera and spinal fluid of sALS patients compared with control populations and positively correlated with extent of disease but not with rate of progression [27]. Various oxidative stressors cause insolubilization, aberrant intracellular localization, and phosphorylation of TAR DNA-binding protein 43 (TDP-43), which resemble the features of TDP-43 in the pathology of sALS [28]. TDP-43 is an RNA-binding protein normally localized to the nucleus. Missense mutations in the TDP-43 gene have been reported to be causative in fALS. In aberrant inclusions, TDP-43 is phosphorylated and re-distributes from the nucleus to the cytosol. Alterations of TDP-43 are thought to be involved in the pathogenesis of sALS [29]. The results obtained by Kabuta and coworkers [30] suggest that HNE is one of the key mediators of oxidative stress, involved in the TDP-43 pathology observed in sALS, and that elevation of HNE could be a risk factor for ALS. HNE treatment of cells causes insolubilization, phosphorylation, and partial cytosolic localization of TDP-43. In the above cited study, HNE-modified cytosolic TDP-43 was diffusely localized and HNE-induced insolubilization of TDP-43 was retained even 24 h after removal of

HNE, indicating that insolubilization of TDP-43 by HNE is a persistent change. Cysteine residues of TDP-43 are responsible for HNE-induced insolubilization of TDP-43.

The levels of urinary F_2 -isoprostanes, derived from free radical-mediated peroxidation of arachidonic acid, have been found to be significantly increased by an immunoassay technique in a cross-sectional pilot study of 50 sALS patients compared to 46 healthy control subjects [21]. In addition, plasma thiobarbituric acid-reactive substances levels, another biomarker that reflects the reactive species-induced peroxynitrite, which can induce lipid peroxidation, have been found increased (21% higher) in ALS patients compared to controls [31].

3. ANTIOXIDANT DEFENSE MECHANISMS IN ALS

The cellular antioxidant system is composed of antioxidant enzymes and other non-enzymatic compounds that have the ability to maintain the balance between pro- and antioxidant agents, thereby limiting oxidative stress. The main antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and thioredoxin reductase (TrxR) [6].

In humans, three forms of SOD are recognized: cytosolic Cu,ZnSOD, mitochondrial MnSOD, and extracellular SOD. The SOD enzymes catalyze the dismutation of $O_2^{\cdot -}$ to H_2O_2 and O_2 . In turn, H_2O_2 can be dismuted by CAT or reduced by GPx. GR catalyzes the reduction of glutathione disulfide (GSSG) to the reduced form of glutathione (GSH), which is a critical molecule in protecting against oxidative stress and maintaining the reducing environment of the cell [32, 33]. GST catalyzes the conjugation reaction of xenobiotics and endogenous or exogenous electrophilic compounds with GSH. TrxR catalyzes the NADPH-dependent reduction of the oxidized form of the redox protein thioredoxin (Trx) [6].

Besides enzymatic antioxidants, there are several non-enzymatic antioxidants like GSH, proteins (e.g., ferritin, transferrin, ceruloplasmin, albumin, and metallothionein) [6], and low molecular weight scavengers, like uric acid, coenzyme Q, and lipoic acid. Antioxidants like vitamins A, E, and C, flavonoids,

and carotenoids are considered to be the main exogenous antioxidants [34]. In a study conducted by Babu et al. [35], the erythrocyte GSH levels, and GR and CAT activities were found to be significantly lower in sALS patients with respect to healthy controls, though SOD and GPx enzyme activities were found unchanged compared to healthy controls. Similarly, Baillet and collaborators [36] did not find difference in the levels of SOD and GPx in sALS patients versus controls. In another study, instead, GR activity was higher in sALS and fALS patients than in the control group, though CAT activity was decreased in both patients groups [37]. Przedborski et al. [38] have found, via comparing 31 sALS, 18 fALS, and 24 controls, that mean Cu,ZnSOD activity was reduced in eight fALS patients with mutations in Cu,ZnSOD gene, but was normal in patients with both fALS without Cu,ZnSOD mutations and sALS. GPx activity was significantly reduced only in sALS patients treated with insulin-like growth factor I. CAT activity was not different in sporadic and familial ALS patients versus controls.

Due to its antioxidant effects, higher concentrations of uric acid might protect against the development of neurodegenerative diseases and modulate their natural history. Reduced levels of uric acid were found in certain neurodegenerative diseases and correlated with more rapid disease progression. Serum levels of uric acid in ALS patients were decreased with respect to the control group, suggesting that this could be the outcome of an oxidative stress-related process in the central nervous system and possibly in other tissues as well. This decrease is the result of the direct reaction between uric acid and oxidizing agents with the metabolism of uric acid [39]. The levels of ferric ion reducing antioxidant power (FRAP), another antioxidant marker, were found decreased both in the CSF [24] and in plasma [25] of sALS patients versus controls, while the levels of plasma thiol groups did not differ between the two groups [25]. The FRAP assay offers a putative index of antioxidants, or reducing potential of biological fluids, while thiol groups are mainly responsible for antioxidative effects of plasma proteins [24]. Thiols are extraordinarily efficient antioxidants in the cells, reacting with and neutralizing the damage induced by free radicals [40].

Some studies demonstrated multiple and pronounced therapeutic effects of diacetyl-bis(4-methylthiosemicarbazonato) copper(II) [CuII(atsm)]

on clinical and pathological progression in a mouse model of ALS [41–43]. The beneficial effect of CuII(atm) is likely due to its peroxynitrite scavenging ability, which reduces tissue accumulation of this oxidant. CuII(atm) possesses an SOD1-like antioxidant activity, decreasing lipid peroxidation and radical-mediated tissue damage. In vitro the compound inhibits the action of peroxynitrite on SOD1 and subsequent nitration of cellular proteins [41].

In SOD1 G93A mice, oral administration of CuII(atm) (30 mg/kg) in the presymptomatic phase (at 140 days) delayed disease onset and extended survival by 14%, whereas treatment of symptomatic mice (at 200 days) still prolonged survival by 10%. CuII(atm) treatment markedly reduced levels of 3-NT when compared with vehicle-treated SOD1 G93A mice, whereas administration of CuII(atm) to wild-type mice did not affect 3-NT levels [41]. Subsequently, both McAllum et al. [42] and Roberts et al. [43] have analyzed the therapeutic effects of CuII(atm) in the SOD1 G37R mouse model. Oral treatment increased the concentration of mutant SOD1 in ALS model mice, but paradoxically improved locomotor function and survival of the mice [43]. Administration of CuII(atm) (10, 30, and 60 mg/kg) delayed the progressive locomotor decline and increased survival in the SOD1-G37R mice in a dose-dependent manner. The highest dose tested improved survival by 26% [42]. CuII(atm) therefore represents a potential new class of neuroprotective agents targeting multiple major disease pathways of motor neurons with therapeutic potential for ALS, though clinical trials in ALS patients, up to date, have not been conducted.

Thus, the antioxidant therapy is important in scavenging free radicals and ROS, and preventing neuronal degeneration in post-oxidative stress scenario [44], although the studies on this topic are inconclusive (for a detailed review see [45–47]). The critical points for most of the clinical trials of antioxidants and other therapies in ALS are the lack of standardization of the study design, effect size, and reliable outcome measures. There has been a recent move to coordinate collaborative studies, among several research groups, to foster the understanding of disease mechanisms and the development of effective therapies. The future role of the antioxidant therapy will be based on the identification of the sources and targets of increased oxidative stress in ALS. It will also depend on a practical and sequential approach to the

study of these therapies being adopted by those involved in ALS clinical research [27].

4. AUTOPHAGY, OXIDATIVE STRESS, AND ALS

When the antioxidant protective systems are overcome by oxidative stress, it leads to the accumulation of oxidatively modified macromolecules and damaged cellular organelles. Autophagy is an effective pathway to eliminate these potentially toxic byproducts and protects cells from oxidative damage by removing oxidatively damaged endoplasmic reticulum, mitochondria, and peroxisomes as well as aggregated proteins, facilitating cell survival during exposure to energy or oxygen deprivation [48].

Mitophagy is one of the most investigated autophagic pathways, through which damaged mitochondria are degraded. The mitochondrial ROS homeostasis plays an important role in the autophagy [49]. Mitochondrial electron transport chain inhibitors, such as rotenone, can induce ROS-mediated autophagy and autophagic cell death. In a study conducted by Chen et al. [50], the authors indicate that autophagy is selectively mediated by $O_2^{\cdot-}$, and that exogenous H_2O_2 is effectively converted to intracellular $O_2^{\cdot-}$, leading to induction of autophagy. Upregulation of autophagy can be achieved by inhibiting SOD to elevate $O_2^{\cdot-}$ levels, and SOD2 overexpression reduces autophagy, supporting the involvement of $O_2^{\cdot-}$ in autophagy induction. Activation of downstream antioxidant enzymes that catalyze H_2O_2 to H_2O , such as CAT and GPx, can also reduce autophagy levels.

Autophagy in neurons is a protective mechanism that slows the advance of neurodegenerative disorders, and that its inhibition is associated with neurodegeneration. Perhaps related to this, autophagy also appears to play an important role in synaptic growth and plasticity [51]. Moreover, neuroinflammation is a common accompaniment to neurodegenerative diseases. Inflammatory mediators (NO and ROS) have pleiotropic effects, one of which is their diversion or disruption of autophagic functions. The influx of Ca^{2+} , acting via ROS, activates caspases and calpains with consequent reduction of neuronal autophagy. Deficits in autophagy in differentiated cells such as neurons can have a profoundly negative impact by, for example, provoking aggregation or misfolding of

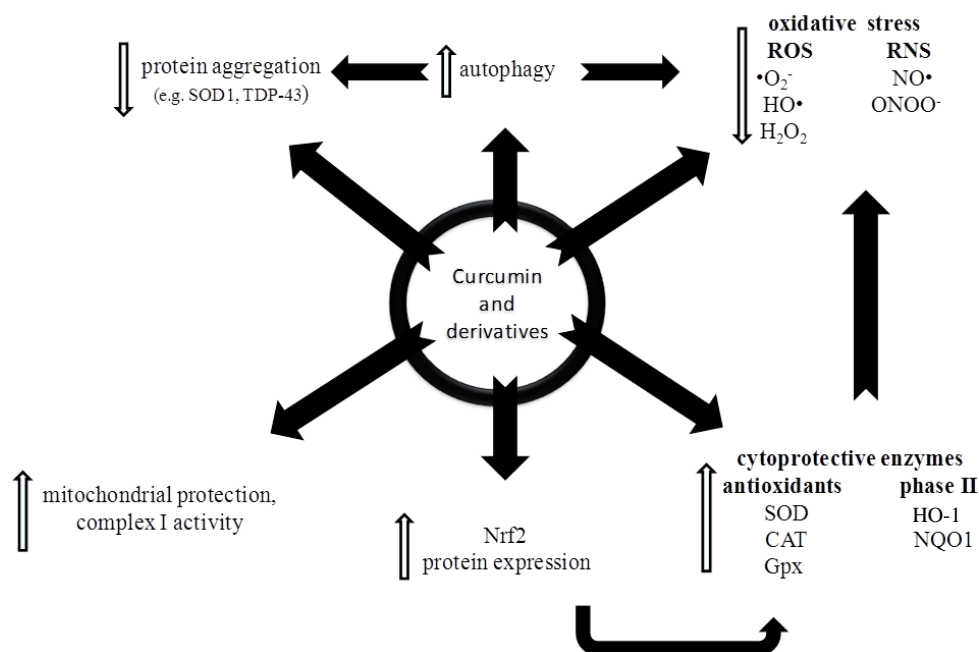


FIGURE 1. Biological effects of curcumin. Curcumin and its derivatives attenuate the ROS production and stimulate antioxidant production, thereby protecting against oxidative damage. Curcumin increases mitochondrial activity and Nrf2 expression leading to increased expression of cytoprotective proteins, thereby limiting the dangerous effects of free radicals. Also autophagy and protein aggregation are regulated by curcuminoids that contribute to the limitation of oxidative stress.

proteins, with consequent neurotoxicity [51]. In fact, autophagy is the main route to eliminate misfolded and long-lived proteins; defective autophagic pathways or alterations in autophagy-related genes are present in various neurodegenerative disorders, such as ALS [52]. For example, genetic screening in ALS identifies mutations in proteins involved in the regulation of autophagy, including SQSTM1 and UBQLN2 [53]. Elimination of SOD1 and TDP-43 protein aggregates, through autophagy induction, results in the suppression of apoptosis and neuronal loss and slows down the progression of ALS [54]. Animal studies in neurodegenerative diseases using autophagy up-regulators have also shown positive results [55]. For example, rapamycin is frequently used to increase autophagy by inhibiting the phosphorylation of the mammalian target of rapamycin (mTOR). Nevertheless, while increasing autophagy, rapamycin does not improve survival of mutant SOD1 mice. Also, this substance is used as a potent

immunosuppressant as it inhibits the activation of T-cells. In fact, rapamycin moderately increased the survival of ALS mice deficient of mature lymphocytes, suggesting that rapamycin could suppress protective immune responses while enhancing protective autophagy reactions during the ALS disease process [56].

Castillo and coworkers [53] found that administration of trehalose, a disaccharide that induces mTOR-independent autophagy, to mutant SOD1 transgenic mice, significantly prolonged life span and attenuated the progression of the disease. The protective effects of trehalose were associated with increased autophagy levels in motor neurons and decreased accumulation of SOD1 aggregates, and an enhanced motor neuron survival.

Consequently, substantial attention is being paid to the molecular mechanisms by which autophagy limits neurodegenerative diseases, to the role of autophagy in early stages of disease pathogenesis and to the

development of methods to upregulate neuronal autophagy for therapeutic benefits [51]. The induction of autophagy, which is correlated with enhanced cell survival and decreased apoptosis, might be a potential mechanism underlying the protective effects of some antioxidants, such as curcumin, against oxidative stress [57].

5. CURCUMIN AS AN ROS SCAVENGER

Turmeric, a rhizome of *Curcuma longa*, is a well-known culinary herb with high medicinal value, and widely used in the traditional medicine system of South Asia and China [58]. The main curcuminoids are curcumin, demethoxycurcumin, and bisdemethoxycurcumin [48, 59]; among these, curcumin (a polyphenolic molecule) is the most active component extracted from *C. longa* [60]. Curcumin seems to show anti-inflammatory, anti-carcinogenic, and antioxidant properties [61]. Additionally, it has been suggested that curcumin can confer protection in neurological and neurodegenerative disorders [62], such as ALS [10]. Regarding the anti-oxidant properties, it has been demonstrated that curcumin is an appropriate compound to prevent or attenuate remarkably the production of ROS and to neutralize harmful free radicals [63] (**Figure 1**).

Curcumin is composed of two monomers of ferulic acid and the presence of the phenolic, β -diketone, as well as the methoxy groups contributes to its free radical-scavenging activity. However, it has been postulated that the free radical-scavenging function of curcumin mainly derives from its phenolic groups [59]. The highest radical-scavenging activity has been observed because phenolic $-\text{OH}$ is the most preferable group for the proton loss from the one-electron oxidized species. As the resultant phenoxyl radical is stabilized by delocalization of electrons, the ability of curcumin to scavenge the oxidizing free radicals is greatly increased. The stabilized radicals can undergo further loss of the second hydrogen atom from the second phenolic $-\text{OH}$ group, producing a diradical (**Figure 2**). This diradical may be converted into stable products like quinones or undergo cleavage to produce smaller phenols like ferulic acid [64]. The ferulic acid (monomer) has been studied for its antioxidant properties. In this regard, according to the trolox equivalent antioxidant capacity (TEAC), FRAP, and oxygen radical absorbance ca-

pacity (ORAC) assays, the free radical-scavenging property of curcumin is lower than that of its monomer [59]. These characteristics suggest that curcumin may be a suitable and potential candidate for maintaining or improving the redox balance. Both in vitro and in vivo investigations suggest potential antioxidant effects of curcumin. In vitro studies on keratinocytes and fibroblasts treated with curcumin showed optimal protection against H_2O_2 -induced cytotoxicity (tested by MTT cell viability assay). However, it was also found that curcumin at a high concentration of 25 $\mu\text{g/ml}$ was cytotoxic to keratinocytes [65]. For example, Scharstuhl and co-workers [66] showed that curcumin at 25 $\mu\text{g/ml}$ significantly increased ROS formation, resulting in fibroblast apoptosis.

In animal models, in which oxidative stress was induced by homocysteine administration, curcumin treatment at both low and high doses (5 and 50 mg/kg) inhibited lipid peroxidation with decreased levels of malondialdehyde (MDA) and $\text{O}_2^{\cdot-}$ in the hippocampi of homocysteine-treated animals [67]. Also, treatment of mice with curcumin (90 mg/kg for three consecutive days) significantly reduced the increase of ROS and protein carbonyl levels as a function of age, compared to the controls [68]. In rats treated with formaldehyde, a common environmental contaminant that causes oxidative DNA damage in cells by increasing the production of ROS, the levels of MDA (serum) and 8-OHdG (brain tissue and urine) were increased and the total antioxidant capacity (TAC) levels were reduced with respect to the control group (treated with saline). After treatment with curcumin, the levels of MDA and of whole brain and urinary 8-OHdG were significantly reduced, while the TAC was significantly increased [69].

The potential health benefits of curcumin are possibly limited because of its relatively low bioavailability, and this may have limited clinical trials [10]. Low serum and tissue levels of curcumin irrespective of the route of administration, as well as rapid metabolism and elimination are major factors curtailing curcumin bioavailability [70]. However, by virtue of its lipophilicity, curcumin is able to permeate the blood-brain barrier and may, therefore, reach brain tissue in biologically effective concentrations [59]. To improve the bioavailability of curcumin, numerous approaches have been undertaken, including the use of (1) adjuvants like piperine that interfere with

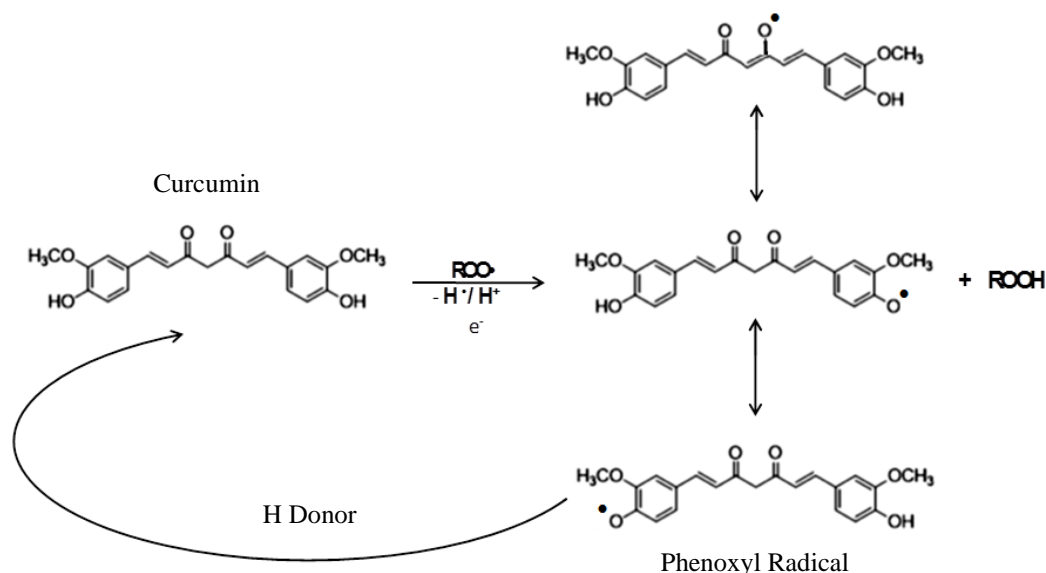


FIGURE 2. Sites of attack of free radicals in curcumin and stabilization of the phenoxyl radical intermediate. See text (Section 5) for more description. ROO and ROOH denote peroxy radical and organic hydroperoxide, respectively.

glucuronidation; (2) liposomal curcumin; (3) curcumin nanoparticles; (4) curcumin phospholipid complex; and (5) structural analogues of curcumin [70]. In human studies, after curcumin treatment, the levels of xanthine oxidase, $O_2^{\bullet-}$, lipid peroxides, and myeloperoxidase were decreased and the levels of SOD, CAT, GPx, and GST activities were significantly increased [71]. Morabito et al. [3] showed that treatment of erythrocytes with 10 μ M curcumin prevented thiol groups from oxidation provoked by *N*-ethylmaleimide or pH 6.5 modified solutions, protecting erythrocytes from oxidative stress events at the level of cell membrane transport. Further studies [72, 73] showed that curcumin was several times more potent than vitamin E as a free radical scavenger, and could protect the brain from lipid peroxidation and scavenge NO radicals. Therefore, it was thought that curcumin might protect neuronal cells from oxidative stress-induced damage [74].

5.1. Curcumin as an Inducer of Nrf2

Curcumin induces endogenous antioxidant defenses through gene regulatory mechanisms. It induces the expression of cytoprotective proteins, including

SOD, CAT, GR, heme oxygenase-1 (HO-1), and NAD(P)H:quinone oxidoreductase 1 (NQO1) through the activation of the nuclear transcription factor erythroid-2-related factor 2 (Nrf2) [75]. Under redox homeostasis conditions, Kelch-like ECH-associated protein-1 (Keap1) binds to Nrf2, inhibiting its translocation to the nucleus and promoting its degradation by the ubiquitin proteasome pathway. When stress stimuli are sensed, or in response to an inducer (such as curcumin), the Keap1/Nrf2 complex is disrupted allowing the translocation of Nrf2 into the nucleus where it forms a heterodimer with small Maf proteins and binds to antioxidant response element (ARE), thus leading to the antioxidant genes transcription [76]. Curcumin acts by irreversibly binding to Keap1 (a cysteine-rich protein with thiol groups) modifying its specific reactive cysteine residues. The modification of cysteine residues in Keap1 results in an overall conformational change that promotes the Nrf2 dissociation and its nuclear translocation, to finally target gene expression of enzymes with antioxidant activity [77, 78]. The oxidation of a polyphenol such as curcumin to the quinone form appears to be critical to its ability to activate Nrf2 through Keap1 conjugation. It is possible that when

polyphenols are oxidized by reacting with free radicals, the resulting electrophilic quinones are involved in signal transduction pathways to upregulate cellular antioxidant systems via Nrf2 activation (the so-called “paradoxical effect of antioxidants”) [78].

It has been shown that curcumin activates the transcription factor Nrf2; in cerebral cells obtained from mouse treated with ionic irradiation, curcumin consistently increases the Nrf2 protein levels and induces Nrf2 downstream target genes, NQO1 and HO-1 [75]. Mouse embryonic fibroblast cell line (NIH3T3) treated with curcumin showed a significant dose-dependent increase in HO-1 mRNA and protein levels compared to untreated cells. Conversely, the exposure to feluric acid did not induce HO-1 mRNA and protein levels, suggesting that curcumin, but not its monomer, exerts the cellular antioxidant effects [59]. Wu et al. [76] observed that cell lines derived from rat cortical neurons treated with curcumin showed decrease of cell injury, a prominent increase of Nrf2 protein expression, and an increased accumulation of Nrf2 into the nucleus. Similar results were obtained from a recent study [79], in which the authors showed protective role of curcumin against oxidative stress in rat hepatic stellate cells (HSCs) by upregulating Nrf2 nuclear translocation.

5.2. Curcumin and Autophagy

It is known that curcumin is able to trigger autophagy [57] and, probably, also mitophagy [80]. Han and coworkers [57] have found that curcumin can induce a beneficial autophagic process to protect the endothelial cells. Recently, Gu et al. [81] demonstrated that nicotinate-curcumin, a curcumin derivative, restored autophagy flux and thus decreased foam cell formation in ox-LDL-treated THP-1 cells likely via inhibition of the PI3K/Akt/mTOR pathway, which in turn negatively regulates the autophagy pathway.

A curcumin analog, termed compound C1, was found to potently activate the transcription factor EB (TFEB), a master regulator of autophagy, and to promote TFEB-mediated autophagy and lysosome biogenesis without inhibiting mTOR activity [82]. Aoki et al. [83] have found that curcumin efficiently inhibited growth of U87-MG and U373-MG human malignant glioma cells in vitro and in vivo by inducing non-apoptotic autophagic cell death. MDA-MB-231 breast cancer cells, treated with curcumin (25 μ M for 12 h) showed increased AMP-activated ki-

nase (AMPK) phosphorylation suggesting that AMPK was activated by curcumin. It is well known that AMPK can activate autophagy, and inactivation of AMPK by its specific inhibitor or by target shRNA-mediated silencing attenuated curcumin-activated autophagy [84].

The exact role of curcumin as an inducer of mitophagy is still unclear. In this context, only one study was carried out, demonstrating that the combination of ultrasound (1 h) and curcumin (10 μ M, 6 h) treatment in nasopharyngeal carcinoma CNE2 cells resulted in severe swelling and degradation of damaged mitochondria. The authors have shown that this combined treatment could initiate and extend the mitophagy pathway though the effect of curcumin alone was not investigated. Thus, additional studies need to elucidate the involvement of curcumin in mitophagy [85].

5.3. Clinical Studies

Although clinical studies on curcumin are limited, curcumin has been claimed as a therapeutic option for the treatment of several diseases like cardiovascular disorders, diabetes, arthritis, cancer, and neurodegenerative diseases [64]. In a therapeutic trial conducted on volunteer subjects, it was found that oral administration of 0.5 g/day of curcumin for 1 week decreased total serum cholesterol and increased high-density lipoprotein (HDL) cholesterol [86]. Chuengsamarn et al. [87], in a randomized, double-blind, placebo-controlled clinical trial, have found that curcumin intervention (6 capsules/day for 9 months; each capsule has curcuminoid content of 250 mg) was able to substantially prevent type 2 diabetes mellitus development in the prediabetic population. In addition, the curcumin treatment appeared to improve overall function of β -cells, with very minor adverse effects. Patients with mild or moderate rheumatoid arthritis (RA) and treated for 8 weeks with 500 mg of curcumin, showed a higher percentage of improvement in Disease Activity Score (DAS) and American College of Rheumatology (ACR) criteria for reduction in tenderness and swelling of joint scores, two indicators of the physical functioning in RA [88].

Regarding the antitumor properties of curcumin, several clinical trials in cancer patients have been performed. Some of them have described the anticancer potential of curcumin [89, 90] and many of

them have investigated the capacity of curcumin as adjuvant in anticancer therapy or reducing adverse effects associated with the treatment. For example, the results obtained by Belcaro and coworkers [91] showed that curcumin (100 mg for 4 months) might alleviate the burden of side effects associated to chemo- and radiotherapy. Similar results have been found by other investigators (NCT01917890; clinicaltrials.gov) suggesting that the use of various preparations of curcumin as supportive agent for cancer treatment is well worth a systematic investigation in larger scale clinical trials. Curcumin could also be a therapeutic option for the treatment of neurodegenerative disorders [64]. Because of its anti-inflammatory and antioxidant properties, curcumin has been tested in Alzheimer disease (AD). AD is characterized by the deposition of extracellular β amyloid ($A\beta$) plaques and intracellular neurofibrillary tangles containing accumulation of hyperphosphorylated tau protein. Curcumin is a highly lipophilic compound and crosses the blood-brain barrier where it can bind to the plaques and prevent the $A\beta$ peptide aggregation. In vitro studies have shown that curcumin inhibits the production of $A\beta$ and can reduce $A\beta$ -induced toxicity [92]. For example, Zhang et al. [93] treated mouse primary cortical neurons with different concentrations of curcumin (1–20 μ M) for 24 h and found that both $A\beta$ 40 and $A\beta$ 42 levels significantly decreased compared with controls. Curcumin (1–30 μ M) was also shown to attenuate the production of $A\beta$ -induced radical species in neuronal cell cultures. Up to date, only few clinical trials have been published on the use of curcumin in AD patients. Baum et al. [94] randomized 34 AD patients to receive either curcumin at two different doses (1 g/day or 4 g/day) or placebo for six months. The authors did not observe any significant difference in Mini-Mental State Examination (MMSE) scores and in plasma $A\beta$ 40 levels between curcumin and placebo groups. Ringman and colleagues [95] recruited 36 patients with mild-to-moderate AD in a randomized, double-blind, placebo-controlled study (24 weeks) evaluating the efficacy of two dosages of curcumin (2 and 4 g/day) with an open-label extension for 48 weeks in which patients who received placebo were randomly assigned to 2 or 4 g/day of curcumin, while patients on treatment continued with the same dose assigned at baseline. The results showed no significant differences in MMSE scores and in the levels of plasma or cerebrospinal fluid $A\beta$ 40/ $A\beta$ 42 or tau, between pla-

cebo and intervention groups. These studies have not been particularly successful, probably because the low bioavailability of curcumin markedly reduces its potential to reach the brain at sufficient concentrations to provide benefits. Interestingly, there are several ongoing clinical trials evaluating the efficacy of curcumin in AD or minimal cognitive impairment (MCI). One study (NCT01811381; clinicaltrials.gov) will evaluate the effect of curcumin (800 mg/day for 12 months) and yoga in MCI patients, and a phase II study (NCT01001637; clinicaltrials.gov) will compare curcumin (4 or 6 g/day for 60 days) and placebo treatment in AD patients. Another trial (NCT01383161; clinicaltrials.gov) will investigate the effect of curcumin (90 mg, twice daily, for 18 months) in 132 subjects with memory complaints.

5.4. Use of Curcumin in ALS

To date, there are no in vivo studies and only few limited in vitro studies that have been performed to test the efficacy of curcumin and its derivatives to counteract ALS pathogenic mechanisms. The rationale for the use of turmeric in ALS derives from the observation that, in addition to reducing cellular ROS production, curcumin prevents toxicity associated with pathological protein aggregation, including the ALS-associated TDP-43 aggregates. It is well documented that mitochondrial dysfunction is important in triggering cell death in TDP-43-associated ALS. Dimethoxy curcumin (DMC), an analog of curcumin, could be potentially useful for neurodegenerative diseases associated with mutated TDP-43. In fact, DMC (15 μ M for 3 day) improved mitochondrial transmembrane potential, complex I activity, and mitochondrial morphology in NSC-34 cells transfected with mutated TDP-43 [96]. Moreover, DMC could increase autophagy and TDP-43 degradation and upregulate Nrf2 and phase II enzymes, such as HO-1, thereby protecting against oxidative stress and cell injury [96].

Another analog of curcumin, monocarbonyl dimethoxycurcumin C (Compound C), was tested in NSC-34 cells transfected with mutant TDP-43. Duan and coworkers [97] found that Compound C (10 μ M for 24 h) is a promising agent for the protection of the neurons in the hippocampus against the toxicity of pathological TDP-43. In fact, it reduced the level of oxidative stress caused by mutant TDP-43, as well as induced the expression, in the hippocampus, of

HO-1 which is involved in the heme catabolism, iron ion homeostasis, and in the process of anti-oxidative stress. The activity of HO-1 can be stimulated by its substrate heme and by various nonheme substances, such as oxidative stress, hypoxia, and antioxidant compounds. Thus, Compound C, which degrades TDP-43 fragment and increases the antioxidant ability by upregulating HO-1, is a promising agent for protecting against TDP-43 proteinopathy [97].

Recently, Bahatia and colleagues [61] performed a study to test the role of curcumin (300 μ M) as a potential inhibitor of SOD1 aggregation and fibrillation in human monocytic THP-1 cells. The authors also examined the effect of curcumin on the amyloidogenic pathway of aggregation of reduced SOD1 (dithiothreitol-treated SOD1) to understand the mechanism of its action. The results suggested that curcumin modulated the early aggregation steps of dithiothreitol-treated SOD1, leading to the formation of smaller, disordered, and non-fibrillar aggregates with reduced toxicity. In particular, curcumin treatment inhibited the growth of amyloid fibrils of SOD1 and led to the formation of unstructured aggregates of SOD1 [61].

We have recently performed, on matched groups of sALS patients, a double-blind cross-over study to evaluate the effects of 6 months of oral supplementation with Brainoil (Aliveda srl, Fauglia, Pisa, Italy), a dietary supplement containing curcumin (1 sachet/day dose; curcumin 1000 mg), on clinical indices of muscle strength and quality of life in these patients, and on laboratory parameters of oxidative stress, namely AOPPs, FRAP, and total thiol groups. The study is still in progress and only partial results are available. While the use of curcumin does not seem to affect the oxidative stress markers analyzed, after 6 months of treatment a slowing of clinical worsening, evaluated with the Medical Research Council Scale and the ALS-Functional Rating Scale-revised, is noticed (data unpublished). The preliminary results also demonstrate that the administration at this dose of the compound does not cause any adverse effects.

6. CONCLUSIONS

Curcumin and its derivatives exhibit several beneficial effects and appear to have a significant potential in the treatment of diseases that occur as a result of

oxidative stress, such as ALS. Thus, these compounds could have a good potential in clinical practice and should be further exploited to develop novel drugs. ALS is a neurodegenerative disease that has been demonstrated to exhibit a complex multifactorial pathogenesis involving unclear interactions between genetic susceptibility and environmental risk factors. Strong evidence, in fact, supports the involvement of oxidative stress in ALS pathogenesis. Although attempts in humans to reverse the clinical progression of the disease with antioxidant agents have been generally negative, a number of clinical trials with antioxidants and inducers of cytoprotective enzymes are in progress. In this context, curcumin has been demonstrated to exert its protective effects by acting either as a direct (free radical scavenger) or an indirect (cytoprotective) antioxidant, and therefore, may be considered as a good candidate for further studies in ALS patients. However, to use curcumin in a safe and effective manner against neurodegenerative diseases and other oxidative stress associated disorders, some critical factors should be considered. In fact, to date, human clinical studies with curcumin have revealed limited effects most likely because of curcumin's relatively low solubility and bioavailability.

The bioavailability of curcumin can be improved with piperine, nanoparticles, and liposomes. It is also important to consider that "antioxidants" *in vivo* are either pro-oxidants or electrophiles and such properties are typically associated with toxic substances. Therefore, the ideal balance between protective (antioxidant) and deleterious (pro-oxidant) effects of curcumin requires to be elucidated in order to determine its therapeutic potential. Finally, the main implications for the future of translational research in this field will come from an improved understanding of the role of oxidative stress in neuronal degeneration to clarify if oxidative stress is really involved in the pathogenesis of ALS or is merely a related epiphenomenon.

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