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Review

Curcumin and the cellular stress response in free radical-related diseases

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Free radicals play a main pathogenic role in several human diseases such as neurodegenerative disorders, diabetes, and cancer. Although there has been progress in treatment of these diseases, the development of important side effects may complicate the therapeutic course. Curcumin, a well known spice commonly used in India to make foods colored and flavored, is also used in traditional medicine to treat mild or moderate human diseases. In the recent years, a growing body of literature has unraveled the antioxidant, anticarcinogenic, and antinfectious activity of curcumin based on the ability of this compound to regulate a number of cellular signal transduction pathways. These promising data obtained *in vitro* are now being translated to the clinic and more than ten clinical trials are currently ongoing worldwide. This review outlines the biological activities of curcumin and discusses its potential use in the prevention and treatment of human diseases.

Keywords: Curcumin / Free radicals / Heat shock proteins / Heme oxygenase / Neurodegenerative disorders

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1 Introduction

Curcumin (1,7-bis(4-hydroxy 3-methoxy phenyl)-1,6-heptadiene-3,5-dione) (Fig. 1) is a phenolic compound extracted from the rhizome of *Curcuma longa* L. (family Zingiberaceae) and it is commonly used in the Asian continent, especially in India, as a spice to make food colored and flavored. Furthermore, traditional Indian medicine has considered curcumin a drug effective on several disorders including anorexia, coryza, cough, hepatic diseases, and sinusitis [1, 2]. Recently, several studies have substantiated and provided scientific evidence regarding the potential prophylactic or therapeutic use of curcumin, unraveling the anti-inflammatory, anticarcinogenic, and anti-infectious activities of this compound [3–6]. In the field of neuroprotection, especially exciting are the findings of Cole and his colleagues who demonstrated that curcumin inhibits formation of amyloid β oligomers and fibrils at submicromolar concentrations, crosses the blood–brain barrier, and reduces amyloid levels and plaque burden in a mouse model of Alzheimer's disease (AD) [7].

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Abbreviations: AD, Alzheimer's disease; **APP,** amyloid precursor protein; **ARE,** antioxidant response element; **BM,** bone marrow; **BR,** bilirubin; **BV,** biliverdin; **GST,** glutathione *S*-transferase; **HIF-1,** hypoxia-inducible factor-1; **HO-1,** heme oxygenase-1; **Hsp70,** heat shock protein70; **IkB,** inhibitory kappa B; **Keap1,** Kelch-like ECH-associated protein 1; **MPTP,** 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; **NFkB,** nuclear factor kB; **NO,** nitric oxide; **NOS,** nitric oxide synthase; **Nrf2,** nuclear factor-erythroid 2-related factor 2; **PD,** Parkinson's disease; **RNS,** reactive nitrogen species; **ROS,** reactive oxygen species; **THC,** tetrahydrocurcumin; **Trx,** thioredoxin; **TrxR,** thioredoxin reductase; **UPR,** unfolded protein response; **UPS,** ubiquitin–proteasome system

2 Pharmacokinetics of curcumin

Curcumin is quite stable at acidic pH and upon ingestion almost 40–80% of this compound remains in the gastroin-

testinal tract [1]. However, curcumin undergoes a marked first-pass metabolism which limits its systemic bioavailability (~60%) as demonstrated in humans and rodents [8–10]. Interestingly, in order to increase its bioavailability, the co-administration of curcumin with piperine or its complexation with phospholipids to form a curcumin–phospholipid complex have been proposed [8, 11, 12]. Preclinical studies have shown that administration of 1 g/kg of curcumin to the rat allows the polyphenol to reach plasma concentrations around 0.5 μg/mL; on the other hand, patients affected by malignant or premalignant conditions of the bladder, skin, cervix, stomach, or oral mucosa, treated with doses of curcumin in the range of 0.5–8 g/day for 3 months had a plasma concentration of this compound of 1.75 ± 0.8 μM [8, 13]. In the rat, the volume of distribution of curcumin is around 190 L suggesting that this polyphenol may accumulate in many organs including colorectal tissue, liver, and brain [8, 11, 14]. Studies in rodents and humans demonstrated that, after oral dosing, curcumin is conjugated to curcumin glucuronide and curcumin sulfate as well as reduced into dihydrocurcumin (DHC), tetrahydrocurcumin (THC), hexahydrocurcumin, octahydrocurcumin, and hexahydrocurcuminol [1, 15, 16]; curcumin, DHC, and THC can be further converted in monoglucuronide conjugates [15, 17]. These metabolic changes seem to occur not only in the liver, the main organ deputed to biotransformation, but also in the intestinal tract [1, 16]. Interestingly, the metabolism of curcumin generates products such as THC which retains anti-inflammatory activity comparable to that of the parental compound [1, 16]. In rodents and humans curcumin inhibits cytochrome P450 enzymes, glutathione *S*-transferase (GST) and UDP-glucuronosyltransferases, therefore the ingestion of this spice may alter the metabolism of drugs thus increasing their plasma concentrations and initiating potential toxic effects [18–21]. In the rat, curcumin is mainly excreted into the bile and eliminated in the feces, only a small amount is excreted in the urine [9, 10] with a half-life of elimination of ~1.5 h [11]. The urinary elimination of curcumin and its metabolites seems to increase if curcumin is administered at large doses (*e.g.*, 3.6 g/day for up to 4 months) [8, 22]. With regard to the toxicity profile of curcumin, studies in rodents and primates have shown that doses of up to 3.5 g/kg body weight administered for up to 3 months were well tolerated by the animals [8]. In humans, curcumin at doses ranging from 2.1 to 8 g/day for up to 3 months did not originate any toxic effects [13, 23]. However, patients affected by advanced colorectal cancer treated with curcumin (3.6 g/day) developed diarrhea whereas a dose of 0.9 g/day was associated with nausea, which resolved spontaneously. In the same patients, blood test abnormalities related to curcumin administration were a rise in serum alkaline phosphatase and lactate dehydrogenase, but the possibility that they resulted from the progression of cancer rather than curcumin toxicity can not be excluded [8, 22].

3 Pharmacodynamics of curcumin

Early studies have shown that curcumin and related products such as THC, have a strong antioxidant activity. In fact, these compounds have been shown to reduce free radical- or copper-induced lipid peroxidation in several experimental systems [24–26]. Furthermore, structure–activity studies clearly demonstrated the importance of the β-diketone moiety and especially the phenolic hydroxyl group, for the antioxidant activity of curcumin and its analogues [24, 27]. Very recently, many papers have appeared in the literature demonstrating that curcumin and its metabolites affect numerous intracellular systems such as transcription factor nuclear factor kB (NFkB), inducible nitric oxide synthase (iNOS), hypoxia-inducible factor-1 (HIF-1), nuclear factor-erythroid 2-related factor 2 (Nrf2), and members of the vitagene family (*e.g.*, heat shock protein70 (Hsp70), heme oxygenase-1 (HO-1), thioredoxin (Trx)). This complex array of interactions is in agreement with the well-known ability of curcumin to serve not only as an antioxidant but also as anti-inflammatory and anticarcinogenic molecule.

Reyes-Gordillo *et al*. [28], in an elegant paper, have shown that curcumin reduced the CCl4-induced liver toxicity in the rat; in particular, curcumin reduced the CCl4-related increase in proinflammatory cytokines and blocked the nuclear translocation of NFkB [28]. Similarly, curcumin prevented the dinitrochlorobenzene-induced colitis in the rat by downregulating both NFkB and iNOS [29]. In lung epithelial cells, curcumin exerted anticarcinogenic activity and prevented the cigarette smoke-induced NFkB activation through the inhibition of inhibitory kappa B (IkB)α kinase activation, IkBα phosphorylation, and degradation [30]. The inhibition of the NFkB activation was paralleled by the suppression of many NFkB-related genes, including cyclin D1, cyclooxygenase-2, and matrix metalloproteinase-9 (MMP-9) [30]. Comparable results have been found in a macrophage cell line (RAW 264.7) challenged with bacterial endotoxin. In these cells, curcumin and its reduced metabolites blocked the activation of NFkB, and the downstream activation of iNOS, *via* inhibition of the IkB kinases 1 and 2, thus providing further evidence about the importance of the effects on NFkB in the anti-inflammatory and anticarcinogenic activity of this phenolic compound [31]. Through interaction with NFkB, curcumin exerts protective function also in the regulation of T-cell-mediated immunity. In fact, overexpression of NFkB in T-cells confers protection against tumor-induced apoptosis, whereas when NFkB is inhibited, the cell becomes much more vulnerable and undergoes apoptosis [32]. By so doing, NFkB plays an important role in the regulation of T-cell apoptosis and the related thymic atrophy which occurs during carcinogenesis. In this experimental model, curcumin prevented the tumor-induced apoptosis and the following thymic atrophy by restoring the activity of NFkB [32].

Another transcription factor involved in the anticarcinogenic effect of curcumin is HIF-1. HIF-1 is composed of two proteins, HIF-1α and the aryl hydrocarbon receptor nuclear translocator (ARNT) and plays a major role in the development of hypoxic tumors [33]. Curcumin has been demonstrated to inactivate HIF-1 in several cell lines and this effect has been related to its ability to promote ARNT degradation [33]. As a consequence of HIF-1 inactivation, several proteins downstream to HIF-1 were downregulated, such as erythropoietin and the vascular endothelial growth factor (VEGF) [33].

Particularly interesting is the interaction of curcumin with the vitagene system. The term vitagenes refers to an integrated network of protective mechanisms involved in preserving cellular homeostasis during stress conditions, which are under control of redox-sensitive genes and related signaling pathways that result in increased expression of specific genes, such as those responding to antioxidant compounds [34 – 36]. The vitagene family is composed of the Hsps HO-1, Hsp70 and by the Trx/thioredoxin reductase (TrxR) system [34 – 36]. HO-1, also referred to as Hsp32, degrades heme, which is toxic if produced in excess, into ferrous iron, carbon monoxide, and biliverdin (BV); BV is the precursor of bilirubin (BR), a linear tetrapyrrole which has been shown to effectively counteract oxidative and nitrosative stress due to its ability to interact with reactive oxygen species (ROS), nitric oxide (NO), and reactive nitrogen species (RNS) [34, 35, 37 – 40]. Hsp70 is a functional chaperone and acts by inhibiting key effectors of the apoptotic machinery [34, 37]. Finally, Trx is responsible for the reduction of protein disulfide bonds whereas TrxR serves to maintain Trx in a reduced form [34]. Very recently, curcumin was shown to have cytoprotective effects by interacting with all members of the vitagene family. In particular, curcumin increased the expression of HO-1 in human cardiac myoblasts, hepatocytes, monocytes, and endothelial cells [41 – 44], rat neurons and astrocytes [45] as well as porcine endothelial cells [46]. In several rodents and human cells, the curcumin-induced HO-1 overexpression was correlated with production of mitochondrial ROS, activation of transcription factors Nrf2 and NFkB, induction of mytogen activated protein kinase (MAPK) p38, and inhibition of phosphatase activity [44, 47, 48]. Moreover, curcumin upregulated Hsp70 in human colorectal carcinoma cells, proximal tubule cells [49 – 52], and rat glioma cells [53]. Quite different is the effect of curcumin on TrxR, as it has been shown that curcumin irreversibly inhibits TrxR activity. As a consequence, there was increased NADPH oxidase activity, which in turn, produced an abundance of ROS [54]. This latter paradoxical effect may explain, at least in part, the cancer chemopreventive activity of curcumin [54].

Having two Michael acceptor functionalities on its molecule (Fig. 1), curcumin and its structural analogues induce the gene expression of a battery of cytoprotective proteins

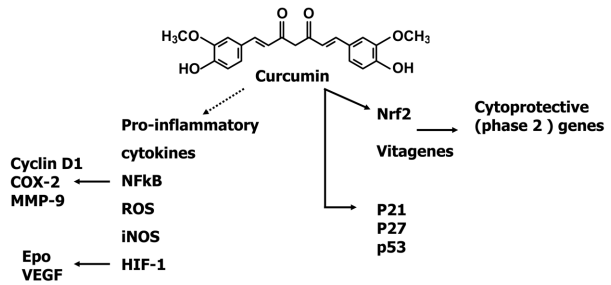

Figure 1. Schematic diagram showing the main molecular targets of curcumin. Curcumin inhibits pathways that could lead to neurodegeneration and upregulates cytoprotective (phase 2) enzymes and vitagenes thus counteracting free radical-induced damage and exerting a neuroprotective role. By modulating the activation of various transcription factors, curcumin regulates the expression of inflammatory enzymes, cytokines, adhesion molecules, and cell survival proteins. Curcumin also downregulates cyclin D1, COX-2, and MMP-9, and upregulates p21, p27, and p53. Straight arrows, stimulation; dashed arrows, inhibition.

in a process known as "the phase 2 response" [55] or the "electrophile counterattack response" [56]. The designation "phase 2" historically comes from the fact that many of these proteins are involved in the second step of the metabolism of xenobiotics, *e.g.*, the diverse families of glutathione transferases and UDP-glucuronosyltransferases [57]. However, the understanding of the common molecular regulation of the basal and inducible levels of their gene expression has placed many other proteins, *e.g.*, HO-1 and γ-glutamylcysteine synthetase, in this category of cytoprotective proteins [58, 59]. There are three essential cellular components in the general scheme of induction of the phase 2 response (Fig. 2): (i) the antioxidant response elements (AREs), (ii) transcription factor Nrf2, and (iii) the sensor for inducers Kelch-like ECH-associated protein 1 (Keap1). The AREs represent single or multiple copies of upstream regulatory sequences present on all genes encoding for phase 2 cytoprotective proteins [60, 61]. Binding to the AREs is Nrf2, a 66-kDa transcription factor [62], the principal transcription factor that determines the levels of expression of these cytoprotective genes [63]. Nrf2 is a cap ‚n'-collar (CNC) transcription factor that has a highly conserved basic region-leucine zipper (bZIP) domain [64]. In order to bind to the AREs, Nrf2 has to form a heterodimer first with a small Maf protein [65]. Binding of the dimeric complex so formed to the ARE and subsequent recruitment of the general transcriptional machinery ultimately results in the activation of transcription of ARE-dependent genes and correlates with protection against a wide array of electrophiles and oxidants [66 – 68]. A great body of genetic evidence utilizing *nrf2*-knockout mice reveled that Nrf2 serves as a master regulator of the ARE-driven cellular defense systems against electrophiles and oxidants. Indeed, *nrf2*-knockout mice have low and uninducible levels of glutathione and phase 2 proteins compared to otherwise geneti-

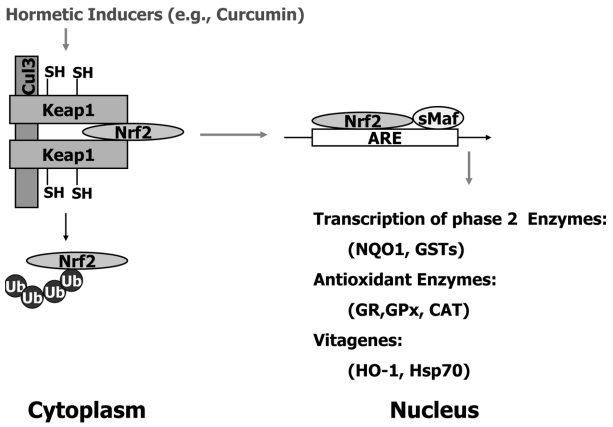

Figure 2. Model for induction of phase 2 cytoprotective genes. Nrf2 is a transcription factor responsible for the induction of several genes related to the cellular stress response, including phase 2 enzymes, such as GST and NAD(P)H:quinone oxidoreductase 1 (NQO1); antioxidants, such as glutathione reductase (GR), glutathione peroxidase (GPx), and catalase; and vitagenes such as Heme oxygenase-1 (HO-1) and Hsp70. At basal conditions the cysteine rich sensor metalloprotein Keap1 binds and targets transcription factor Nrf2 for ubiquitination and proteasomal degradation *via* association with the Cullin 3 (Cul3)-based E3 ubiquitin – ligase complex. Inducers, by promoting mild oxidative stress (hormesis) react and chemically modify specific highly reactive cysteine residues of the sensor Keap1. Consequently, Keap1 loses its ability to repress transcription factor Nrf2. This leads to increased stabilization of Nrf2, its nuclear translocation, binding to the ARE (in heterodimeric combinations with members of the small Maf family of transcription factors), and ultimately transcriptional activation of phase 2 cytoprotective genes.

cally identical wild-type mice and are much more sensitive to toxic challenges of many different types [66 – 68].

The entry of Michael acceptors like curcumin into the cell is "sensed" by the cysteine-rich sensor protein Keap1 [69], a dimeric cytosolic repressor protein that binds and targets Nrf2 for ubiquitination and subsequent proteasomal degradation *via* association with Cullin 3 to form an E3 ubiquitin – ligase complex [70]. Inducers chemically modify specific highly reactive cysteine residues of Keap1 [71, 72]. Direct sulfhydryl-addition reaction of the structurally related to curcumin synthetic bis(2-hydroxybenzylidene)acetone with purified recombinant murine Keap1 has been observed spectroscopically [71]. Such reaction with the cysteine sulfhydryls of Keap1 leads to loss of its ability to repress Nrf2 which then undergoes nuclear translocation and activates the transcription of ARE-dependent genes [66 – 73]. Murine Keap1 has 25 cysteine residues (its human homologue has 27) and amino acid replacements of C273 and C288, either individually or in combination, result in loss of the repressor function of Keap1 and constitutive expression of ARE-dependent genes [73].

Notably, the gene expression of HO-1, Trx, and TrxR can be upregulated in a manner dependent on Nrf2 and ARE. To our knowledge, there are no reports of a similar type of regulation of the third member of the vitagene family, Hsp70. However, curcumin as well as several other compounds (*e.g.*, hydrogen peroxide [74], the cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, and the vicinal dithiol reagent phenylarsine oxide [75, 76]), all of which can react with sulfhydryl groups, have been reported to increase the protein levels of Hsp70. Indeed, the transcriptional activation of both Nrf2 and heat shock factor 1 (HSF1), the major activator of Hsp70 gene expression, depend on cysteine modification, in one case within the Nrf2 regulator Keap1 [72, 76], and in the other, within HSF1 [77].

4 Curcumin and neurodegenerative disorders

Neurodegenerative disorders, such as AD and Parkinson's disease (PD), belong to the family of the protein conformational diseases (PCD) and affect a large portion of our aging population [78]. In general, PCD are conditions that arise from the dysfunctional aggregation of proteins in non native conformations. It is known that the β-conformation in proteins is particularly susceptible to perturbations in the quality control system and that ROS play an important role in the development and/or pathogenetic progression of aging and neurodegenerative diseases [79 – 81]. Chaperones can rescue misfolded proteins by breaking up aggregates and assisting the refolding process [80, 82]. Proteins that cannot be rescued by refolding can be delivered to the proteasome by chaperones to be recycled [82, 59]. If the cell is not able to eliminate misfolded proteins multiple metabolic derangements resulting in the excessive production of ROS and RNS occur [83]. The ability of a cell to deal with oxidative and nitrosative stress requires functional chaperones, antioxidant production, protein degradation, and a cascade of intracellular events collectively known as unfolded protein response (UPR), a form of cell stress response [84, 85]. As the cell's quality control system becomes overwhelmed (see below), conformational changes occur to amyloid polypeptide intermediates, generating stable oligomers with an antiparallel crossed β-pleated sheet structure that eventually accumulate as space-occupying lesions within neurons [81]. Although it is clear why mutant proteins form amyloid, it is hard to rationalize why a wild-type protein adopts a native conformation in most individuals, but it misfolds in a minority of others, in what should be a common extracellular environment. This discrepancy suggests that other events most likely trigger misfolding processes in sporadic amyloid disease. One possibility is that an abnormal metabolite, generated only in some individuals, covalently modifies the protein or peptide and causes it to misfold. Candidate metabolites are suggested by the recently recognized links between AD and atherosclerosis, in which known

chronic inflammatory metabolites, may play a critical pathogenic role. If this holds true, then new targets are disclosed for a prevention strategy brought about through nutritional antioxidants.

AD is characterized by a subtly impaired cognitive function or a disturbance of behavior. With time there is a gradual memory loss and disorientation which eventually progress into dementia. Although, most cases are sporadic, 5–10% or more are familial [86]. Families that have an autosomal dominant pattern for AD constitute about 13% of early cases and less than 0.01% of the total number of patients. Molecular analysis of families with early onset AD has made it possible to identify mutations in three different genes that are responsible for the disease: the gene encoding for the amyloid precursor protein (APP) peptide, and the presenilin 1 (PSEN1) and presenilin 2 (PSEN2) genes. Yet, these genes are involved in less than 5% of the total number of cases of AD [86]. Gross examination of the brain in AD shows a variable degree of cortical atrophy with narrowed gyri and widened *sulci* most apparent in the frontal, parietal, and temporal lobes. Microscopically, the features include neurofibrillary tangles, neurite (senile) plaques, the central core of which is amyloid-β peptide (Aβ) derived from the transmembrane APP, amyloid angiopathy, granulovacuolar degeneration, and Hirano bodies. Importantly, all of these changes are present in the brains of nondemented older individuals but to a much lesser extent [87, 88].

Recent studies have suggested that neuronal death in AD could arise from dysfunction of the ER. Proteins in the ER require an efficient system of molecular chaperones whose role is to assure their proper folding and to prevent accumulation of unfolded proteins. The response of cells to the accumulation of unfolded proteins in the ER is termed UPR. UPR is a functional mechanism by which cells attempt to protect themselves against ER stress, resulting from the accumulation of the unfolded/misfolded proteins. Inhibition of protein glycosylation, perturbation of calcium homeostasis, and reduction of disulfide bonds provoke accumulation of unfolded protein in the ER, and are called ER stress. Normal cells respond to ER stress by increasing transcription of genes encoding ER-resident chaperones such as GRP78/BiP, to facilitate protein folding or by suppressing the mRNA translation to synthesize proteins. These systems are termed the UPR. Familial AD-linked PSEN1 (PS1) mutation downregulates the UPR and leads to vulnerability to ER stress [89]. Given that AD involves accumulation of aggregates of two different proteins, the potential involvement of the UPR and ER dysfunction has been suggested to lead to cell death. In actively dividing cells, activation of the UPR is accompanied by decreased cell cycle protein expression and an arrest in the G1 phase of the cell cycle [90]. It has been shown that amyloidogenic proteins can give rise to amyloid fibrils *in vitro* when a segment of one of its β-sheets undergoes a conformational change, exposing an Hsp70 binding site. While normal proteins are rapidly oxidized and subsequently secreted, mutated proteins remain in the reduced state. Most of these protein molecules are dislocated out of the ER into the cytosol, where they are ubiquitinylated and degraded by proteasomes. A parallel pathway for molecules that are not degraded is condensation into perinuclear aggresomes that are surrounded by vimentin- or tubulin-containing intermediate filaments and are dependent upon intact microtubules. Inhibition of proteasome activity shifts the balance toward aggresome formation. Intracellular aggregation is decreased and targeting to proteasomes improved by overexpression of the cytosolic chaperone Hsp70. Importantly, transduction into the cell of an Hsp70 target peptide, derived from the mutated protein sequence, also reduces aggresome formation and increases amyloid degradation. These results demonstrate that amyloidogenic proteins can aggregate intracellularly despite the common presentation of extracellular aggregates, and that a similar molecular surface mediates both *in vitro* fibril formation and *in vivo* aggregation. Furthermore, rationally designed peptides can be used to suppress this aggregation and may provide a feasible therapeutic approach [91]. Aggresomes are associated with many neurodegenerative disorders, including AD, PD, and polyglutamine disorders such as Huntington's disease. These inclusions commonly contain ubiquitylated proteins. The stage at which these proteins are ubiquitylated remains unclear. A malfunction of the ubiquitin–proteasome system (UPS) may be associated with their formation. Conversely, it may reflect an unsuccessful attempt by the cell to remove them. The 26S proteasome system is involved in eliminating ubiquitinated misfolded/unfolded proteins, and its inhibition results in cellular accumulation of protein aggregates. Accordingly, proteasome dysfunction in AD neurons may play a role in the accumulation of misfolded, potentially cytotoxic proteins and may be induced by increased intracellular AbetaPP/Abeta. Moreover, a role of microglia recruited from bone marrow (BM) into the CNS during the progression of AD has been considered and emerging evidence suggests that infiltration of BM-derived monocytic cells into the brain contributes to the development of microglial reaction in AD, where APP enhances BM-derived macrophage-mediated clearance of Abeta [92].

Several lines of evidence support a fundamental role for calcium, ROS, and RNS secondary to ER, oxidative and nitrosative stress, respectively, in the pathogenesis of AD [34, 87, 93]. In particular, neurofibrillary Aβ has been shown to generate both superoxide anion and α-carbon-centered radicals; in addition the upregulation of iNOS increases the formation of NO and RNS [94]. Finally, Ab increases intracellular calcium levels *via* both the ER ryanodine and inositol 1,4,5-trisphosphate receptors thus inducing ER stress which culminates with the apoptotic death of neuronal cells [95, 96]. All these prooxidant pathways activated by Aβ contribute to the massive destruction

of brain cells in AD. As mentioned above, antioxidant enzymes such as HO-1 and TrxR along with the chaperone Hsp70, are well known intracellular antioxidant systems which contribute to the "UPR" and guarantee an important cytoprotective effect against free radical-mediated injury in AD.

The first evidence of a protective role of curcumin in the onset of AD derived from epidemiological studies. Ganguli *et al*. [97] demonstrated that Indian population, who have a curcumin-enriched diet, has a reduced prevalence of AD compared to United States. Following this initial observation, many basic studies were conducted and the neuroprotective role of curcumin was corroborated. *In vitro* studies have shown that curcumin protects neuron-like PC12 cells from β-amyloid toxicity and, interestingly, the polyphenol displayed a neuroprotective effect greater than a well known antioxidant such as α-tocopherol [98]. By using an Alzheimer transgenic APPSw mouse model (Tg2576), Lim *et al*. [99] have shown that dietary curcumin suppressed inflammation and oxidative damage in the brain of these mice. More recently, Garcia-Alloza *et al*. [14] in transgenic APPswe/PS1dE9 mice demonstrated that curcumin, given intravenously for 7 days, crosses the blood–brain barrier, binds to β-amyloid deposits in the brain and accelerates their rate of clearance. These latter results are in good agreement with previous findings which demonstrated that curcumin disaggregates and inhibits β-amyloid aggregation at submicromolar concentrations *in vitro*, and more importantly, reduces amyloid levels and plaque burden *in vivo* in Tg2576 mice [100, 101]. Curcumin has an important impact on ER stress biology, as its apoptosis-induced effect is associated with its ability to cause ER stress. Removing two double bonds in curcumin, which was speculated to form Michael adducts with thiols in secretory proteins, resulted in a loss of the ability of curcumin to induce apoptosis as well as ER stress. Inhibition of caspase-4 activity by z-LEVD-FMK, blockage of survival molecules such as CAAT/enhancer binding protein homologous protein (CHOP) expression by small interfering RNA, and treatment with salubrinal, an ER inhibitor, significantly reduced curcumin-induced apoptosis [102]. In addition to this, impairment of UPS has been demonstrated to mediate curcumin proapoptotic effects. Curcumin disrupts UPS function by directly inhibiting the enzyme activity of the proteasome's 20S core catalytic component. Like other proteasome inhibitors, curcumin exposure induces neurite outgrowth and the stress response, as evident from the induction of various cytosolic and ER chaperones as well as induction of transcription factor CHOP/GADD153. The direct inhibition of proteasome activity also causes an increase in half-life of IkBα that ultimately leads to the downregulation of NF-kappaB activation. These results suggest that curcumin-induced proteasomal malfunction might be linked with both antiproliferative and anti-inflammatory activities [103].

PD, whose cardinal features include tremor, slowness of movement, stiffness, and poor balance, is attributed to a profound deficit in dopamine that follows the loss of dopaminergic neurons in the *substantia nigra pars compacta* and dopaminergic nerve terminals in the *striatum* [34, 104]. Although the mechanisms of PD are still uncertain, a large amount of experimental evidence implicates oxidative and nitrosative stress as one of the crucial factors in the pathogenesis of PD [105, 106]. Considerable insights into the pathogenesis of PD, indeed, have been achieved by use of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is commonly used to induce an experiment model of PD [105, 107]. Excessive free radical formation or antioxidant deficiency and the resulting oxidative stress are all mechanisms involved in MPTP neurotoxicity [108]. Rajeswari [109] has shown that curcumin protects rat brain from MPTP-induced neurotoxicity by virtue of its scavenger activity. On the other hand, curcumin has been shown to protect PC12 cells from MPP^+ (the active metabolite of MPTP) by inducing the anti-apoptotic protein bcl-2, preventing the dissipation of mitochondrial membrane potential and reducing the intracellular iNOS levels [3]. The importance of mitochondria in the neuroprotective effect of curcumin has been also stressed by Mythri *et al*. [110] who demonstrated that curcumin prevents the formation of peroxynitrite which is responsible for the complex I damage which is a common feature in PD.

Transient forebrain ischemia is a common cause of stroke and occurs in people suffering from cardiovascular diseases [111]. As a consequence of ischemia and the following reperfusion, a cascade of events such as increased calcium release, the overexpression of cycloxygenase-2 (COX-2) and iNOS both of which are important free-radical generators and trigger neuronal cell death in selected brain areas including the hippocampal cornu ammonis 1 (CA1) [35, 38, 111, 112]. Curcumin exerted a neuroprotective effect in rats who underwent ischemia/reperfusion injury and this effect has been related to the direct scavenger effect of curcumin as well as to a curcumin-induced interference with the apoptotic machinery, increase in antioxidant molecules (reduced glutathione (GSH)) and enzymes (catalase, superoxide dismutase) [111, 113, 114].

The mechanism(s) underlying these neuroprotective effects of curcumin are still debated. Although it is clear that the free-radical scavenging activity of curcumin plays a main role in its antioxidant activity, new lines of evidence propose that curcumin is neuroprotective because of its ability to induce vitagenes (Figs. 2 and 3). As mentioned above, curcumin induced HO-1 in rat neurons and astrocytes and, consequently the heme-degrading activity of HO-1 increased [45]. Keeping in mind that through the HO activity heme is degraded into BV which is then reduced into BR by biliverdin reductase (BVR) and that BR is a very efficient scavenger of ROS, NO, and RNS [38, 39, 115–117], it is possible to conclude that the strong antioxidant

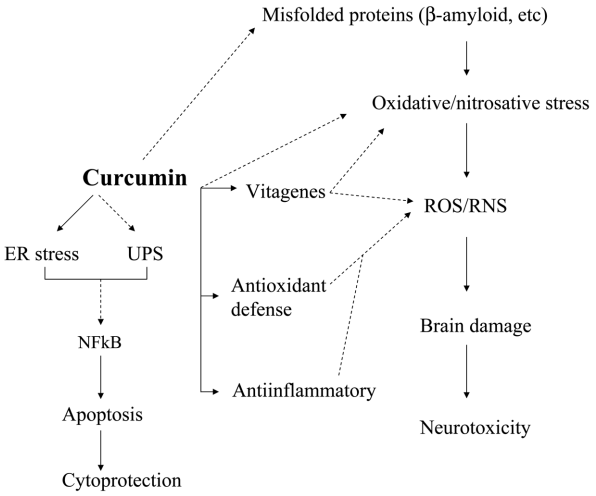

Figure 3. Curcumin and cellular stress response mediated by vitagenes. Under conditions of oxidative/nitrosative stress, there is an increased formation of ROS or RNS. They play a key role in the pathogenesis of free radical-induced diseases, such as neurodegenerative disorders. Vitagenes (HO-1 and Hsp70) contribute to counteract the ROS/RNS-mediated neurotoxic insult, thus initiating a neuroprotective response. In addition, curcumin by inducing ER stress, disrupting UPS and downregulating NF-kappaB activation exerts antiproliferative and anti-inflammatory activities resulting in cytoprotection. Straight arrows, stimulation; dashed arrows, inhibition.

and anti-inflammatory activities of curcumin could be mediated by the induction of HO-1.

5 Curcumin and diabetes

Compelling evidence has been provided that both insulin dependent and noninsulin dependent diabetic patients are under conditions of oxidative stress and that the complications of diabetes mellitus could be partially mediated by oxidative stress [118, 119]. Several mechanisms seem to be involved in the genesis of oxidative stress in diabetic patients, including glucose autoxidation, protein glycation, as well as the formation of advanced-glycation end-products [119, 120].

Recent findings have shown that curcumin can reduce the degree of systemic oxidative stress as well as retinal inflammation and diabetic nephropathy in streptozocin-treated rats, an animal model for diabetes [121 – 123]. The protective effect of curcumin and THC in diabetes and its complications seems to be due to the inhibition of proinflammatory cytokines (IL-1β) and growth factors (VEGF), to the blockade of the NFkB signaling and the increasing activity of chaperone molecules [122, 124]. Remarkably, the cytoprotective effect of curcumin in diabetic complications could be related to the interaction of this spice with the vitagene system. In fact, type 2 diabetic patients with or without nephropathy have an increased level of both HO-1 and Hsp70 in lymphocytes and this is considered as an attempt of the immune system to react to oxidative insults [125]. The possibility that curcumin may further upregulate HO-1 and Hsp70 in diabetic patients thus contributing to counteract prooxidant conditions allows to hypothesize a potential role of curcumin in the prevention of diabetes and its complications.

6 Curcumin and other oxidative stress-related conditions

Oxidative stress plays an important role in the pathogenesis of lung and liver diseases, both of which recognize oxidative stress as a main pathogenetic factor.

The pharmacological therapy of both asthma and chronic obstructive pulmonary disease (COPD) is based on the administration of corticosteroids. Unfortunately, in some cases, these drugs are ineffective and this is often due to the development of steroid resistance. Histone deacetylase is involved in the mechanism of action of corticosteroid and its activity is often reduced in case of steroid insensitivity [126]. Curcumin has been shown to restore histone deacetylase activity and therefore its use can be hypothesized in the treatment of lung disease unresponsive to corticosteroids [126]. However, the concomitant administration of curcumin with systemic corticosteroids should be discouraged because curcumin may inhibit cytochrome P450 and UDP-glucuronosyltransferases (see above), two enzymatic routes through which corticosteroids are metabolized.

Curcumin reduces both thioacetamide- and endotoxin-induced liver dysfunction in rodents and this effect is attributed to the inhibition of the expression of proinflammatory cytokines (tumor necrosis factor-α and IL-1β), transcription factors (NFkB), and enzymes (iNOS) [127 – 129].

The long term use of some drugs is associated with the development of organ toxicity often due to increased oxidative stress. Curcumin and THC reduced the kidney toxicity of chloroquine, gentamicin, and cyclosporin A in the rat [2, 130, 131]. Furthermore, curcumin reduced the indomethacin-induced intestinal damage in the rat and the ritonavir-related vascular dysfunction in porcine coronary arteries [132, 133]. All these reports agree that the protective effects of curcumin were due to the well-known ability of this compound to increase both the enzymatic and nonenzymatic intracellular antioxidant molecules.

Recent evidence highlighted the potential use of curcumin in the treatment of cancer [134]. In animal models, curcumin demonstrated an anticancer activity against skin, colon, lung, and gastrointestinal tumors [134]. Notably, a phase II clinical trial on patients affected by advanced pancreatic cancer and treated with 8 g of curcumin *per* os for 2 months has shown a biologic activity of this spice in the therapy of pancreatic cancer [134].

7 Conclusions and perspectives

As highlighted in this review, the cytoprotective role of curcumin in several experimental systems is well established. Commercial grade curcumin contains 10 – 20% curcuminoids, desmethoxycurcumin, and bisdesmethoxycurcumin and they are as effective as pure curcumin. Based on a number of clinical studies in carcinogenesis, a daily oral dose of 3.6 g curcumin has been efficacious for colorectal cancer and advocates its advancement into Phase II clinical studies. In addition to the anticancer effects, curcumin has been effective against a variety of disease conditions in both *in vitro* and *in vivo* preclinical studies [135]. Interestingly, nanodelivery biotechnology, using primarily composed phospholipids coated with prostate membrane specific antigen specific antibodies, has been developed to enhance targeted delivery of curcumin as an anticancer agent showing that treatment of human prostate cancer cell lines with liposomal curcumin resulted in at least 70 – 80% inhibition of cellular proliferation at ten-fold lower doses compared to free curcumin [136].

Although curcumin has been described in Ayurveda, as a treatment for inflammatory diseases and is referred by different names in different cultures, extensive research over the last half century has revealed several important functions, as it binds to a variety of proteins and inhibits the activity of various kinases. By modulating the activation of various transcription factors, curcumin regulates the expression of inflammatory enzymes, cytokines, adhesion molecules, and cell survival proteins. Curcumin also downregulates cyclin D1, cyclin E, and MDM2; and upregulates p21, p27, and p53. Various preclinical cell culture and animal studies suggest that curcumin has potential as an antiproliferative, anti-invasive, and anti-angiogenic agent; as a mediator of chemoresistance and radioresistance; as a chemopreventive agent; and as a therapeutic agent in wound healing, diabetes, AD, PD, cardiovascular disease, pulmonary disease, and arthritis. Pilot phase I clinical trials have shown curcumin to be safe even when consumed at a daily dose of 12 g for 3 months. Other clinical trials suggest a potential therapeutic role for curcumin in diseases such as familial adenomatous polyposis, inflammatory bowel disease, ulcerative colitis, colon cancer, pancreatic cancer, hypercholesteremia, atherosclerosis, pancreatitis, psoriasis, chronic anterior uveitis, and arthritis. Thus, curcumin is emerging into the clinic and may prove to be "Curecumin" [137]. However, the potential use of dietary or supplemental curcumin in the treatment of human pathologies remains to be refined. Some concern derive from the pharmacokinetics of curcumin and in particular its poor bioavailability and metabolic fate [8, 138]. Also, no conclusive data are available regarding the concentrations of curcumin in the nervous system as well as the molecular targets of nanomolar concentrations of curcumin, which are most likely the concentrations attained *in vivo* after regular dietary intake. In this regard, the possible effects on vitagenes [34, 139] and related signal transduction pathways which ultimately can provide cytoprotective and chemopreventive effects open new targeted strategies for its clinical use. Once better pharmacodynamics will be achieved then the inhibitory effects on cytochrome P450 enzymes, GST and UDP-glucuronosyltransferases elicited by curcumin should be taken into consideration because many drugs are metabolised by these enzymes in the liver and gastrointestinal tract and therefore their inhibition could increase the plasma concentrations potentiating toxic effects. Keeping all this in mind, it is possible to conclude that clinical research on curcumin and its potential use in human diseases needs to be expanded and therapeutic use of curcumin in several human oxidant stress pathologies has to be considered a promising strategy.

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