Vitagenes, dietary antioxidants and neuroprotection in neurodegenerative diseases

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1. ABSTRACT

The ability of a cell to counteract stressful conditions, known as cellular stress response, requires the activation of pro-survival pathways and the production of molecules with anti-oxidant, anti-apoptotic or pro-apoptotic activities. Among the cellular pathways conferring protection against oxidative stress, a key role is played by vitagenes, which include heat shock proteins (Hsps) heme oxygenase-1 and Hsp70, as well as the thioredoxin/thioredoxin reductase system. Heat shock response contributes to establish a cytoprotective state in a wide variety of human diseases, including inflammation, cancer, aging and neurodegenerative disorders. Given the broad cytoprotective properties of the heat shock response there is now strong interest in discovering and developing pharmacological agents capable of inducing stress responses. Dietary antioxidants, such as curcumin, L-carnitine/acetyl-L-carnitine and carnosine have recently been demonstrated in vitro to be neuroprotective through the activation of hormetic pathways, including vitagenes. In the present review we discuss the importance of vitagenes in the cellular stress response and analyse, from a pharmacological point of view, the potential use of dietary antioxidants in the treatment of neurodegenerative disorders in humans.

2. INTRODUCTION

It is well established that living cells are constantly challenged by conditions which cause acute or chronic stress. The brain has a large potential oxidative capacity but a limited ability to counteract oxidative stress (1-3). Within the cell, reactive oxygen species (ROS) are physiologically present at minimal concentration as by-products of aerobic metabolism as well as second messengers in many signal transduction pathways and, in normal conditions, there is a steady-state balance between pro-oxidants and antioxidants which is necessary to ensure optimal efficiency of antioxidant defenses (4-7). However, when the rate of free radical generation exceeds the capacity of antioxidant defenses, oxidative stress ensues with consequential severe damage to DNA, protein and lipid (8-10). Oxidative stress has been implicated in mechanisms leading to neuronal cell injury in various pathological states of the brain, including neurodegenerative disorders such as Alzheimer’s disease (AD) (11-15). Recently the term “nitrosative stress” has been used to indicate the cellular damage elicited by nitric oxide and its congeners peroxynitrite, N₂O₃, nitroxy1 anion and nitrosourea (all can be indicated as reactive nitrogen species or RNS) (16-18).

From a molecular point of view, the cell is able to fight against oxidant stress using many resources, including vitamins (A, C and E), bioactive molecules (glutathione, thioredoxin, flavonoids), enzymes (heat shock proteins, superoxide dismutase, catalase, glutathione peroxidases, thioredoxin reductase, etc) and redox sensitive protein transcriptional factors (AP-1, NFκB, Nrf-2, HSF, etc). The heat shock proteins (Hsps) are one of the most studied defense systems active against cellular damage.
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In this chapter we describe the recent discoveries about the biochemical changes occurring in the central nervous system (CNS) when brain cells are challenged to activate an integrated network of protective mechanisms which are under control of genes called vitagenes. During times of chronic oxidative insult, the key role played by the heat shock response, particularly the heme oxygenase-1 (Hsp32) and Hsp70 pathways, as potential target for nutritional interventions are also discussed. Although the notion that stress proteins are neuroprotective is broadly accepted, still much work needs to be done in order to associate neuroprotection with specific pattern of stress responses. Emerging evidence underscores the high potential of the Hsp system as target for new neuroprotective strategies, especially those aimed at minimizing deleterious consequences associated to oxidative stress, such as in neurodegenerative disorders and brain aging. We review here also the evidence for the role of some polyphenols and acetyl carnitine in modulating redox-dependent mechanisms leading to up-regulation of vitagenes in brain, and hence potentiate brain stress tolerance.

3. THE VITAGENE FAMILY

The term vitagene refers to a group of genes which are strictly involved in preserving cellular homeostasis during stressful conditions. The vitagene family is actually composed of the heat shock proteins (Hsp) Hsp32, Hsp70 and by the thioredoxin system (1,19,20). Among these genes heme oxygenase-1 (HO-1), also known as Hsp32, is receiving considerable attention because of its major role in countering both oxidative and nitrosative stress. In fact, HO-1 induction is one of the earlier events in the cell response to stress. Heme oxygenase-1 exerts protective role, by degrading the intracellular levels of pro-oxidant heme and by producing biliverdin, the precursor of bilirubin (BR), this latter being an endogenous molecule with powerful antioxidant and antinitrosative features (20-24).

3.1. Heme oxygenase-1

The mechanisms responsible for neuronal death are not completely elucidated, even if many studies suggest that ROS are primarily involved in the genesis of neurodegenerative disorders (11,12,25-27). Due to its strong antioxidant properties and wide distribution within the CNS HO-1 has been proposed as a key enzyme in the prevention of brain damage (24,28,29). Panahian et al., using transgenic mice over-expressing HO-1 in neurons, demonstrated the neuroprotective effect of this enzyme in an experimental model of ischemic brain damage (30). The neuroprotective effects of over-expressed HO-1 has been attributed to several factors such as an increased level of both cGMP and bcl-2 in neurons, the inactivation of the pro-apoptotic transcription factor p53, an increase in both antioxidant sources and in the iron sequestering protein, ferritin. Particularly interesting is the role played by HO-1 in AD, a neurodegenerative disorder which involves a chronic inflammatory response characterized by oxidative brain injury and β-amyloid associated pathology. Significant increases in the levels of HO-1 have been observed in AD brains in association with neurofibrillary tangles and also HO-1 mRNA was found increased in AD neocortex and cerebral vessels (31,32). HO-1 increase was not only in association with neurofibrillary tangles, but also co-localized with senile plaques and glial fibrillary acidic protein-positive astrocytes in AD brains (33). It is plausible that the dramatic increase in HO-1 in AD may be a direct response to an increase in free heme concentrations, associated with neurodegeneration, and can be considered as an attempt of brain cells to convert the highly toxic heme into the antioxidants carbon monoxide (CO) and BR. The protective role played by HO-1 and its products in AD prompted investigators to propose natural substances, which are able to increase HO-1 levels in vitro, as potential drugs for the treatment of AD. In this light, several in vitro studies have been focused on polyphenolic compounds contained in some herbs and spices, e.g. curcumin (34-36). Curcumin (Figure 2) is the active anti-oxidant principle in Curcuma longa, a colouring agent and food additive commonly used in Indian culinary preparations. This polyphenolic substance has the potential to inhibit lipid peroxidation and to effectively intercept and neutralize ROS and RNS (37). In addition, curcumin has been shown to significantly increase HO-1 in astrocytes and vascular endothelial cells (34,35). This latter effect on HO-1 can explain, at least in part, the anti-oxidant properties of curcumin, in particular keeping in mind that HO-1-derived BR has the ability to scavenge both ROS and RNS (22,23,38,39). An epidemiological study suggested that curcumin, as one of the most prevalent nutritional and medicinal compounds used by the Indian population, is responsible for the reduced (4-4 fold) prevalence of AD in India compared to United States (40). Based on these findings, Lim and colleagues have provided evidence that dietary curcumin given to an Alzheimer transgenic APPSw mouse model (Tg2576) for 6 months resulted in a suppression of indices of inflammation and oxidative damage in the brain of these mice (41). Furthermore, in a human neuroblastoma cell line it has recently been shown that curcumin inhibits NFkB activation, efficiently preventing neuronal cell death (37).

Although it is generally agreed that HO-1 over-expression is a common feature during oxidative stress, recent papers demonstrated that HO-1 can be repressed following oxidant conditions. In particular human and rodent cells exposed to oxidative stress conditions showed a marked HO-1 repression (42-46). The importance of HO-1 repression has been corroborated by the discovery of Bach1/Bach2 as heme-regulated transcription factors for HO-1 gene (47). In fact, Bach1 is broadly expressed in mice and human tissues and, in human cells, it is induced by the same stimuli which are able to repress HO-1 gene (43,48,49). The reason why the cell should react to an oxidant stress by repressing HO-1 gene is strictly related to the maintenance of a good metabolic balance during stressful conditions. The current hypothesis suggests that HO-1 repression is useful for the cell because (i) decreases the energy costs necessary for heme degradation, (ii) reduces the accumulation of CO and BR which can become toxic if produced in excess and (iii) increases the intracellular content of heme necessary for the preservation of vital functions such as respiration and defense (49).
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Carbon monoxide (CO) is the gaseous products of HO and it has been found to play a role in several biological phenomena, including hippocampal long-term potentiation, non-adrenergic non-cholinergic gastrointestinal relaxation and vasodilatation, and is currently regarded as a neuromodulator in the peripheral and central nervous system (1,2,5,11,12). Evidence from in vitro and in vivo studies suggests that the HO-CO pathway is involved in the modulation of the neuroendocrine mechanism of stress. Thus, increased CO generation is clearly associated with the inhibition of K⁺-stimulated arginine vasopressin (AVP) and oxytocin release from rat hypothalamic explants, whereas the inhibition of HO activity significantly potentiates the LPS-induced increase in AVP circulating levels while reducing the hypothalamic content of this neuropeptide (53-55). With regards to corticotropin-releasing hormone (CRH), the effects of CO on the release of this hormone are contradictory, since increases in CO generation induced by two HO substrates, hematin and hemin, were associated with reduced or enhanced CRH release respectively, in two different in vitro models (56,57). As far as the intracellular mechanism(s) by which CO exerts its biological functions, it is generally agreed that this gas activates the cytosolic form of guanylyl cyclase (sGC) which in turn increases intracellular cGMP levels (24,29). However during the last ten years many studies arose in literature demonstrating that CO signals through the activation of alternative intracellular signal transduction pathways. Studies from our laboratory have suggested that the activation of another hemoprotein, cyclooxygenase (COX), plays a significant role in CO signaling in the rat hypothalamus. In these studies we demonstrated that hemin, the precursor of CO via HO, dose-dependently increases prostaglandin (PG) E2 (PGE2) production from rat hypothalamus in vitro and this effect is specifically due to CO because it is counteracted by the HO inhibitor Sn-mesoporphyrin-IX and oxyhemoglobin, the latter being a well known scavenger for CO (52,58). The direct evidence about the stimulatory role of CO on PGs production was obtained incubating hypothalami directly in CO saturated solutions and measuring significantly increased PGE2 levels with respect to control tissue (59). Recently Jaggar and coll. have demonstrated that exogenous or endogenously produced CO dilates cerebral arterioles by directly activating large-conductance Ca²⁺-activated K⁺ (KCa) channels primarily by increasing the coupling ratio and amplitude relationship between Ca²⁺ sparks and KCa channels (60,61). Although CO is a potent and effective activator of KCa channels, the gas does not dilate arterioles in the absence of Ca²⁺ sparks. Therefore, CO appears to act by priming KCa channels for activation by Ca²⁺ sparks, and this ultimately leads to arteriole dilation via membrane hyperpolarization (61). Finally, Otterbein and coll. have shown that in organs and tissues different from brain, exogenous CO exerts anti-inflammatory and anti-apoptotic effects dependent on the modulation of the p38 MAPK-signaling pathway (62). By virtue of these effects, CO confers protection in oxidative lung injury models, and perhaps plays a role in HO-1 mediated tissue protection (63).

3.2. Heat shock protein 70

The 70 kDa family of stress proteins is one of the most extensively studied. Included in this family are Hsc70 (heat shock cognate, the constitutive form), Hsp70 (the inducible form, also referred to as Hsp72) and GRP-75 (a constitutively expressed glucose-regulated protein found in the endoplasmatic reticulum) (20,21). Only recently, the availability of transgenic animals and gene transfer allowed us to over-express the gene encoding for Hsp70, thus demonstrating that overproduction of this protein leads to protection in several different models of nervous system injury (64,65). Following focal cerebral ischemia, Hsp70 mRNA is synthesized in most ischemic cells except in areas of very low blood flow, due to scarce ATP levels. Hsp70 proteins are produced mainly in endothelial cells, in the core of infarcts in the cells that are most resistant to ischemia, in glial cells at the edges of infarcts and in neurons outside the areas of infarction (66). It has been suggested that this neuronal expression of Hsp70 outside an infarct can be used to define the ischemic penumbra, which means the zone of protein denaturation in the ischemic areas (66).

As mentioned above, Hsps are induced in many neurodegenerative disorders mainly in the view of its cytoprotective function. Hsp72 was overexpressed in post-mortem cortical tissue of AD patients and an increase in Hsp70 mRNA was found in cerebellum hippocampus and cortex of AD patients during the agonal phase of the disease (67-69). Recently Kakimura et al., demonstrated that Hsp70 induces IL-6 and TNF-α in microglial cells and this event is associated with an increased phagocytosis and clearance of Aβ peptides (70) (Figure 1). The same authors hypothesize that Hsps could activate microglial cells through Nfkb and p-38 MAPK-dependent pathways (70) (Figure 1).

A large body of evidence now suggest a correlation between mechanisms of nitrosative stress and Hsp induction. We have demonstrated in astroglial cell cultures that cytokine-induced nitrosative stress is associated with an increased synthesis of Hsp70 stress proteins. The molecular mechanisms regulating the NO-induced activation of heat-shock signal seems to involve cellular oxidant/antioxidant balance, mainly represented by the glutathione status and the antioxidant enzymes (71,72).

3.3. Thioredoxin/Thioredoxin reductase

The thioredoxin (Trx) system, originally identified in Escherichia coli, in 1964, as a hydrogen donor for ribonucleotide reductase required for DNA synthesis, plays a key role in cell function by limiting oxidative stress directly via antioxidant effects and indirectly by protein-protein interactions (73). It is well established that, in mammals, cellular redox regulation of many processes is provided by the cooperation between the Trx and glutathione systems (74). In fact, thioredoxin and reduced glutathione (GSH) systems are involved in a variety of redox-dependent pathways such as supplying reducing equivalents for ribonucleotide reductase, and peptide methionine sulfoxide reductase, the latter being involved in antioxidant defense and regulation of the cellular redox state (75). Therefore, Trx and GSH form a powerful system controlling redox regulation of gene expression, signal transduction, cell proliferation, protection against oxidative stress, anti-apoptotic functions, growth factor and...
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cocytokine effects, as well as regulation of the redox state of the extracellular environment (76). The promoter of the Trx gene contains a series of stress-responsive elements, various transcription factor binding sites, such as SP1, AP-1, NFkB, and the antioxidant-response element (ARE) (77-79). Importantly, induction of thioredoxin reductase and glutathione has been demonstrated to occur in parallel with other ARE-dependent phase 2 cytoprotective genes in several experimental systems, e.g., in cortical astrocytes (80), in human hepatoma cells (81) and in human keratinocytes (82). Similarly to induction of HO-1 gene expression, the ARE-mediated Trx-1 induction involves transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2) (83).

Importantly, it has been reported that Trx is constitutively present as a surface-associated sulphhydril protein in plasma membrane of a wide range of cells (84).