Amino Acids (2003) 25: 437–444
DOI 10.1007/s00726-003-0048-2

Amino Acids
Printed in Austria

Redox regulation of heat shock protein expression in aging and neurodegenerative disorders associated with oxidative stress: A nutritional approach

Review Article

V. Calabrese[1], **G. Scapagnini**[1], **C. Colombrita**[1], **A. Ravagna**[1], **G. Pennisi**[2], **A. M. Giuffrida Stella**[1], **F. Galli**[3], and **D. A. Butterfield**[4]

[1] Department of Chemistry, Section of Biochemistry and Molecular Biology, Faculty of Medicine, University of Catania, Catania, Italy
[2] Department of Neurological Sciences, Faculty of Medicine, University of Catania, Catania, Italy
[3] Section of Clinical Biochemistry, DI.M.I., University of Perugia, Perugia, Italy
[4] Department of Chemistry, Center of Membrane Sciences, and Sanders-Brown Center on Aging, University of Kentucky, Lexington, Kentucky, U.S.A.

Received September 10, 2003
Accepted September 21, 2003
Published online November 7, 2003;

Summary. Oxidative stress has been implicated in mechanisms leading to neuronal cell injury in various pathological states of the brain. Alzheimer's disease (AD) is a progressive disorder with cognitive and memory decline, speech loss, personality changes and synapse loss. Many approaches have been undertaken to understand AD, but the heterogeneity of the etiologic factors makes it difficult to define the clinically most important factor determining the onset and progression of the disease. However, increasing evidence indicates that factors such as oxidative stress and disturbed protein metabolism and their interaction in a vicious cycle are central to AD pathogenesis.

Brains of AD patients undergo many changes, such as disruption of protein synthesis and degradation, classically associated with the heat shock response, which is one form of stress response. Heat shock proteins are proteins serving as molecular chaperones involved in the protection of cells from various forms of stress.

Recently, the involvement of the heme oxygenase (HO) pathway in antidegenerative mechanisms operating in AD has received considerable attention, as it has been demonstrated that the expression of HO is closely related to that of amyloid precursor protein (APP). HO induction occurs together with the induction of other HSPs during various physiopathological conditions. The vasoactive molecule carbon monoxide and the potent antioxidant bilirubin, products of HO-catalyzed reaction, represent a protective system potentially active against brain oxidative injury. Given the broad cytoprotective properties of the heat shock response there is now strong interest in discovering and developing pharmacological agents capable of inducing the heat shock response.

Increasing interest has been focused on identifying dietary compounds that can inhibit, retard or reverse the multi-stage pathophysiological events underlying AD pathology. Alzheimer's disease, in fact, involves a chronic inflammatory response associated with both brain injury and β-amyloid associated pathology. All of the above evidence suggests that stimulation of various repair pathways by mild stress has significant effects on delaying the onset of various age-associated alterations in cells, tissues and organisms. Spice and herbs contain phenolic substances with potent antioxidative and chemopreventive properties, and it is generally assumed that the phenol moiety is responsible for the antioxidant activity. In particular, curcumin, a powerful antioxidant derived from the curry spice turmeric, has emerged as a strong inducer of the heat shock response. In light of this finding, curcumin supplementation has been recently considered as an alternative, nutritional approach to reduce oxidative damage and amyloid pathology associated with AD. Here we review the importance of the heme oxygenase pathway in brain stress tolerance and its significance as an antidegenerative mechanism potentially important in AD pathogenesis. These findings have offered new perspectives in medicine and pharmacology, as molecules inducing this defense mechanism appear to be possible candidates for novel cytoprotective strategies. In particular, manipulation of endogenous cellular defense mechanisms such as the heat shock response, through nutritional antioxidants or pharmacological compounds, represents an innovative approach to therapeutic intervention in diseases causing tissue damage, such as neurodegeneration. Consistent with this notion, maintenance or recovery of the activity of vitagenes, such as the HO gene, conceivably may delay the aging process and decrease the occurrence of age-related neurodegenerative diseases.

Keywords: Oxidative stress – AD pathogenesis – Heme oxygenase pathway – Vitamin E – Vitamin C – Micronutrients – Antioxidants

The heat shock pathway of cell stress tolerance

Living cells are continually challenged by conditions which cause acute or chronic stress. To adapt to environmental

changes and survive different types of injuries, eukaryotic cells have evolved networks of different responses which detect and control diverse forms of stress. One of these responses, known as the heat shock response, has attracted a great deal of attention as a universal fundamental mechanism necessary for cell survival under a wide variety of toxic conditions. In mammalian cells heat shock protein (HSP) synthesis is induced not only after hyperthermia, but also following alterations in the intracellular redox environment, exposure to heavy metals, amino acid analogs or cytotoxic drugs. While prolonged exposure to conditions of extreme stress is harmful and can lead to cell death, induction of HSP synthesis can result in stress tolerance and cytoprotection against stress-induced molecular damage. Furthermore, transient exposure to elevated temperatures has a cross-protective effect against sustained, normally lethal exposures to other pathogenic stimuli. Hence, the heat shock response contributes to establish a cytoprotective state in a variety of metabolic disturbances and injuries, including stroke, epilepsy, cell and tissue trauma, neurodegenerative disease and aging (Mattson et al., 2002; Calabrese et al., 2001a). This has opened new perspectives in medicine and pharmacology, as molecules activating this defense mechanism appear as possible candidates for novel cytoprotective strategies (Calabrese et al., 1998, 2001b, 2002a; Wheller et al., 2003; Misseri et al., 2003; Ropeleski et al., 2003; Goodman and Blank, 2002). In mammalian cells the induction of the heat shock response requires the activation and translocation to the nucleus of one or more heat shock transcription factors which control the expression of a specific set of genes encoding cytoprotective heat shock proteins. Some of the known HSPs include ubiquitin, HSP10, HSP27, HSP32 (or HO-1), HSP47, HSP60, HSC70, HSP70 (or HSP72), HSP90 and HSP100/105. Most of the proteins are named according to their molecular weight:

HSP70. The 70 Da 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 GRP75 (a constitutively expressed glucose-regulated protein found in the endoplasmic *reticulum*). After a variety of central nervous system (CNS) insults, HSP70 is synthesized at high levels and is present in the cytosol, nucleus and endoplasmic reticulum). Denaturated proteins are thought to serve as a stimulus for induction. These denaturated proteins activate heat shock factors (HSFs) within the cytosol by dissociating other HSPs that are normally bound to HSF (Calabrese et al., 2001a). Freed HSF is phosphorylated and forms trimers, which enter the nucleus and bind to heat shock elements (HSE) within the promoters of different heat shock genes leading to transcription and synthesis of HSPs. After heat shock, for instance, the synthesis of HSP70 increases to a point where it becomes the most abundant single protein in a cell. Once synthesized, HSP70 binds to denaturated proteins in an ATP-dependent manner. The N-terminal end contains an ATP-binding domain, whereas the C-terminal region contains a substrate-binding domain. Heat shock proteins serve as chaperones that bind to other proteins and regulate their conformation, regulate the protein movement across membranes or through organelles, or regulate the availability of a receptor or activity of an enzyme.

In the nervous system HSPs are induced in a variety of pathological conditions, including cerebral ischemia, neurodegenerative disorders, epilepsy and trauma. Expression of the gene encoding HSPs has been found in various cell populations within the nervous system, including neurons, glia and endothelial cells (Kelly and Yenari, 2002a). HSPs consist of both stress-inducible and constitutive family members. Whether stress proteins are neuroprotective has been the subject of much debate, as it has been speculated that these proteins might be merely an ephiphenomenon unrelated to cell survival. Only recently, however, with the availability of transgenic animals and gene transfer, has it become possible to overexpress the gene encoding HSP70 to test directly the hypothesis that stress proteins protect cells from injury, and it has been demonstrated that overproduction of HSP70 leads to protection in several different models of nervous system injury (Kelly et al., 2002b; Yenari, 2002). Following focal cerebral ischemia, mRNA encoding HSP70 is synthesized in most ischemic cells except in areas of very low blood flow, because of limited ATP levels. HSP70 proteins is 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. It has been suggested that this neuronal expression of HSP70 outside an infarct can be used to define the ischemic penumbras, the zone of protein denaturation in the ischemic areas (Hata et al., 1998). A number of in vitro studies show that both heat shock and HSP overproduction protect CNS cells against both necrosis and apoptosis. Mild heat shock protects neurons against glutamate-mediated toxicity and protects astrocytes against injury produced by lethal acidosis (Narasimham et al., 1996). Transfection of cultured astrocytes with the gene for HSP70 protects them from

ischemia or glucose deprivation (Fink et al., 1997). HSP70 has been demonstrated to inhibit caspase-3 activation caused by ceramide, and also affect JUN kinase and p38-kinase activation (Mosser et al., 1997). In addition, HSP70 binds to and modulates the function of BAG-1, the bcl-2 binding protein (McLaughlin et al., 2003), thus modulating some type of apoptosis-related cell death.

A large body of evidence now suggests a correlation between mechanisms of oxidative and/or nitrosative stress and HSP induction. Current opinion hold also the possibility that the heat shock response can exert its protective effects through inhibition of NFkB signaling pathway (Calabrese et al., 2001a). We have demonstrated in astroglial cell cultures that cytokine-induced nitrosative stress is associated with an increased synthesis of HSP70 stress proteins. Increase in HSP70 protein expression was also found after treatment of cells with the NO generating compound sodium nitroprusside (SNP), thus suggesting a role for NO in inducing HSP70 proteins. The molecular mechanisms regulating the NO-induced activation of a heat shock signal seems to involve cellular oxidant/antioxidant balance, mainly represented by the glutathione status and the antioxidant enzymes (McLaughlin et al., 2003; Calabrese et al., 2000b, 2000c).

Ubiquitin is one of the smallest HSPs and is expressed throughout brain in response to ischemia. It is involved in targeting and chaperoning of proteins degraded in proteasomes, which include NFKB, cyclins, HSFs, hypoxia-inducible factor, some apoptosis-related proteins, tumor necrosis factor and erythropoietin receptors (Mayer, 2003).

HSP27 is synthesized mainly in astrocytes in response to ischemic situations or to kainic acid administration. It chaperones cytoskeletal proteins, such as intermediate filaments, actin or glial fibrillary acidic protein following stress in astrocytes. It also protects against Fas-Apo-1, staurosporine, TNF and etoposide-induced apoptotic cell death as well as H_2O_2-induced necrosis (Bechtold and Brown, 2003; Valentim et al., 2003). *HSP47* is synthesized mainly in microglia following cerebral ischemia and subarachnoid hemorrhage (Turner et al., 1999).

HSP60, glucose-regulated protein 75 (GRP75) and HSP10 chaperone proteins within mitochondria. GRP75 and GRP78, also called oxygen-regulated proteins (ORPs) are produced by low levels of oxygen and glucose. These protect brain cells against ischemia and seizures *in vivo*, after viral-induced overexpression (Izaki et al., 2001).

HSP32 or heme oxygenase is the rate-limiting enzyme in the production of bilirubin. There are three isoforms of heme oxygenase: HO-1 or inducible isoform, HO-2 or constitutive isoform and the recently discovered HO-3 (Sahlas et al., 2002; Goldbaum and Richter-Landsberg, 2001; Schipper et al., 1998; Schipper, 2000; Calabrese et al., 2002b; Scapagnini et al., 2002a).

HO as target for neuroprotective strategies in AD pathology

Heme oxygenase is the rate-limiting enzyme in the production of bilirubin. It catalyzes the degradation of heme in a multistep, energy-requiring system. The reaction catalyzed by HO is the α-specific oxidative cleavage of the heme molecule to form equimolar amounts of biliverdin and carbon monoxide (CO). The iron released by HO-1 is bound by ferritin, perhaps via a HO-1 chaperone function (Nath et al., 2000). The redox environment of cytosol and mitochondria is critical to cellular performance and protection against oxidative stress (Drake et al., 2003). Increasing evidence suggests that the HO-1 gene is redox regulated and contains in its promoter region the antioxidant responsive element (ARE), similar to other antioxidant enzymes. Since the expression of heat shock proteins is closely related to that of amyloid precursor protein (APP), heat-shock proteins have been studied in brains of patients with Alzheimer's disease. Significant increases in the levels of HO-1 have been observed in AD brains in association with neurofibrillary tangles (Takeda et al., 2000), and HO-1 mRNA was found to be increased in AD neocortex and cerebral vessels (Premkumar et al., 1995). 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 (Takeda et al., 2000). It is conceivable that the dramatic increase in HO-1 in AD may be a direct response to increased free heme associated with neurodegeneration and an attempt to convert the highly damaging heme into the antioxidants biliverdin and bilirubin. Heme oxygenase-1 is rapidly upregulated by oxidative and nitrosative stresses, as well as by glutathione depletion. All these findings have introduced new perspectives in medicine and pharmacology, as molecules activating this defense mechanism appear to be possible candidates for novel cytoprotective strategies (Butterfield et al., 2002).

HO and the therapeutic potential of nutritional antioxidants in AD. Recently, considerable attention has been focused on identifying dietary and medicinal phytochemicals that can inhibit, retard or reverse the multi-stage pathophysiological events underlying AD pathology (Butterfield et al., 2001, 2002a; Calabrese et al., 2000a; Butterfield, 2003). Spice and herbs contain phenolic

substances with potent antioxidative and chemopreventive properties (Scapagnini et al., 2002b). The active antioxidant principle in *Curcuma longa*, a colouring agent and food additive used in Indian culinary preparations, has been identified as curcumin (diferuloylmethane). Due to the presence in its structure of two electrophilic α, β-unsaturated carbonyl groups which, by virtue of Michael reaction, can react with nucleophiles such as glutathione, curcumin has the potential to inhibit lipid peroxidation and effectively to intercept and neutralize reactive oxygen and NO-based free radicals (Butterfield and Lauderback, 2002c). This agent is a potent inhibitor of tumor initiation *in vivo* and possesses antiproliferative activities against tumor cells *in vitro* (Hayes and McMahon, 2001). Recent epidemiological studies (Ganguli et al., 2000), have raised the possibility that this molecule, as one of the most prevalent nutritional and medicinal compounds used by the Indian population, is responsible for the significantly reduced (4.4-fold) prevalence of AD in India compared to United States. Based on these findings, Lim and colleagues (2000) provided compelling evidence that dietary curcumin given to transgenic APPSw mouse model (Tg2576) for 6 months resulted in a suppression of indices of inflammation and oxidative damage in the brain of this murine model of AD. Furthermore, in a human neuroblastoma cell line it has recently been shown that curcumin inhibits NFkB activation, effectively preventing neuronal cell death Butterfield et al., 2002). Remarkably, recent evidence has demonstrated that curcumin is a potent inducer of HO-1 in vascular endothelial cells (Motterlini et al., 2000a, 2000b). We have also recently demonstrated in astroglial cells the role of caffeic acid phenylethyl ester (CAPE), an active component of propolis, as a novel HO-1 inducer (Scapagnini et al., 2002b). The similarity of CAPE to curcumin is striking because CAPE is also a Michael reaction acceptor, endowed with anti-inflammatory, antioxidant and anticancer effects (Butterfield et al., 2002c). These agents all appear capable of transcriptionally activating a gene battery that includes antioxidant enzymes and heme oxygenase (Dinkova-Kostova et al., 2001). Gene induction occurs through the antioxidant responsive element (ARE) (Hayes and McMahon, 2001). Thus, increased expression of genes regulated by the ARE in cells of the central nervous system may provide protection against oxidative stress.

Vitamin E and AD. Alzheimer's disease (AD) is characterised pathologically by deposition of amyloid β-peptide (Aβ) in senile (neuritic) plaques, the presence of neurofibrillary tangles, and synapse loss (Katzman and Saitoh, 1991). Oxidative damage in AD brain is extensive, and Aβ and its sequelae may be associated with this oxidative stress (Butterfield et al., 2001, 2002b). Aβ causes oxidative damage to and neurotoxicity of neurons (Varadarajan et al., 2000). Vitamin E blocks these effects *in vitro* (Butterfield et al., 1999, 2002b; Yatin et al., 2000). Plasma from AD and control subjects is low in vitamins E and C, and the level of lipid peroxidation is reportedly inversely correlated with the level of vitamin E (Bourdel-Marchasson et al., 2001). There is a dearth of information about dietary antioxidants on development and progression of neurodegenerative disorders, especially Alzheimer's disease (Halliwell, 2003). Still, trials of vitamin E have been conducted. In one trial, AD patients received 400 IU of vitamin E and 1.0 g of vitamin C daily for one month (Kontush et al., 2001). Plasma and CSF levels of both vitamins E and C were elevated in AD patients relative to baseline, and this increased CSF level of vitamin E was correlated to decreased susceptibility of lipoproteins to *in vitro* oxidation. A combined approach using the antioxidants vitamins E and C may protect the CNS from oxidative insult, which may reflect the relative stability of the tocopheryl radical and the presence of a reducing agent such as vitamin C to regenerate vitamin E from this radical. Another study of vitamin E and vitamin C supplementation reported that high doses of vitamin E and C supplements may lower the progression of AD symptoms and are protective against vascular dementia (Butterfield et al., 2002a). However, a more recent study suggested that dietary sources rich in vitamin E and C significantly lowered the risk of developing AD relative to supplements alone (Engelhart et al., 2002). One of the largest vitamin E trials in AD is the placebo-controlled, clinical trial of high dose vitamin E (2000 IU per day) in moderate AD patients carried out by the Alzheimer's Disease Cooperative Study (Sano et al., 1997). These large doses of vitamin E, that appeared to pose little risk over an approximately two-year period, were reported to slow the progression of AD. Similar trials involving early AD and mild cognitive impairment subjects are on-going (Grundam, 2000).

However, almost the totality of the vitamin E-based intervention trials are based exclusively on the supplementation of the alpha form of this vitamin while other homologues such as the γ-tocopherol are completely ignored. Emerging evidence on the metabolic fate and biological functions of vitamin E homologues suggests that this could represent a major bias in vitamin E-based intervention trials aimed to prevent oxidative and nitrosative stress in inflammatory and chronic-degenerative disorders (Jiang et al., 2001; Brigelius-Flohé et al., 2002).

The physiologic regulation of vitamin E metabolism make α-tocopherol to be mainly retained and delivered to lipoproteins and tissues while non-alpha vitamers (essentially γ-tocopherols that is abundantly introduced with the diet in western countries) undergo preferential metabolic processing to carboxyethyl hydroxychroman metabolites (CEHC) by shortening of the phytyl chain (Brigelius-Flohé et al., 2002). This metabolic setting results in high plasma ratios of alpha to gamma tocopherol and sustained hepatic degradation of γ-tocopherol to γ-CEHC so that this is the main metabolic product of vitamin E in biological fluids and can be used to mirror the intake of its parental vitamer (Galli et al, 2002; Radosavac et al., 2002; Morinobu et al., 2003). In the brain tissue homogenate the α- to γ-tocopherol ratio is the same than in plasma (Williamson et al., 2002), but in other tissues such as fat and skin the gamma form predominates. This metabolism is also responsible for the lowering effect that a sustained intake of α-tocopherol exerts on the blood levels γ-tocopherol (Brigelius-Flohé et al., 2002; Morton et al., 2002).

These aspects of vitamin E metabolism are particularly important in the light of the recent findings that demonstrated the role of γ-tocopherol as scavenger of reactive nitrogen species (Jiang et al., 2001, 2002; Appenroth et al., 2001), and the contribution that CEHC may give to the antioxidant and anti-inflammatory properties of vitamin E (Betancor-Fernandez et al., 2002; Jiang et al., 2000; Galli et al., 2003). As a consequence, both the gamma homologue of tocopherol and CEHC might play biological roles of relevance in the context of neuroprotection during the different stages of AD. In detail, other than an efficient hydroperoxyl radical scavenger and chain breaker, the gamma configuration of the chroman ring has got the exclusive property to directly scavenge nitric oxide-derived species such as peroxynitrite by the formation of a corresponding 5′-nitro-γ-cromanoxyl derivative (Jiang et al., 2001, 2002; Williamson et al., 2002; Appenroth et al., 2001). Again, the gamma configuration of the vitamin E and particularly the γ-CEHC was reported to inhibit both *in vitro* and *in vivo* the activity of the pro-inflammatory and pro-oxidant enzyme COX 2 (Jiang et al., 2000, 2002). These specific functions of γ-tocopherol and γ-CEHC may thus lead to prevent oxidative and nitrative injury to proteins and lipids in degenerating tissues. Recently, Williamson et al. (2002) have demonstrated the accumulation of 5′-nitro-γ-tocopherol other than 3′-nitro-Tyr in the AD brain and confirmed the superiority of γ-tocopherol vs. α-tocopherol in preventing the peroxynitrite-dependent inactivation of rat brain α-ketoglutarate dehydrogenase as a biomarker of nitrative damage in the AD brain. Gamma tocopherol shows also other important non-antioxidant based functions such as the regulation of cell cycle and apoptotic signalling in proliferating cells (Galli et al., 2003).

Although further studies are required to establish whether the vitamin E could represent an useful tool in the prevention and therapy of neurodegenerative diseases and particularly AD, it is obvious that present intervention studies should be re-examined in the context of these pieces of information. In fact, the disregarded role of γ-tocopherol in neuroprotection and antioxidant defence of brain tissues could explain some actually incongruous findings such as the absence of positive effects of supplementing administered as synthetic α-tocopherol opposed to a better outcome obtained in patients to which a diet rich in vitamin E (with all the homologues represented) was administered (Engelhart et al., 2002). At the same time, also the role of CEHC as highly deliverable surrogates of vitamin E could deserve more attention and may explain the positive outcome obtained with large dosages of vitamin E (Sano et al., 1997).

A risk factor for AD is the presence of allele 4 of apolipoprotein E (apoE) (Roses et al., 1996). Synaptosomes from apoE knock-out mice, containing no gene for apoE, show increased susceptibility to oxidative stress induced by Aβ (Lauderback et al., 2001), while synaptosomes from knock-in mice containing human apoE4 with no mouse background show significantly increased Aβ-induced oxidative stress compared to synaptosomes from human apoE2 or apoE3 knock-in mice (Lauderback et al., 2002). Thus, apoE may serve an antioxidant function, but apoE4 may be less able than apoE2 or apoE3 to do so (Lauderback et al., 2002). This notion was tested using 1-month old control and apoE deficient mice. Both received dietary vitamin E for 12 months. Vitamin E-fed animals had better behavioural outcomes of spatial motor activity and decreased levels of lipid peroxidation relative to apoE deficient mice fed a normal diet (Veinbergs et al., 2000). The sum of these studies suggests a decreased risk for and diminished oxidative stress in Alzheimer's disease in persons taking high dose dietary, or perhaps supplemental, vitamin E (and vitamin C to regenerate vitamin E from the tocopherol radical). If proven to be true by additional studies, vitamin E or newer analogues of this antioxidant may prove beneficial in this dementing disorder associated with oxidative stress (Calabrese et al., 2001a; Scapagnini et al., 2002b; Butterfield et al., 2002a).

Conceivably, dietary supplementation with vitamin E or with polyphenolic agents, such as curcumin and its

derivatives, can forestall the development of AD, consistent with a major ''metabolic'' component to this disorder. Nutritional biochemical research is providing optimism that this devastating brain disorder of aging may be significantly delayed and/or modulated.

Acknowledgements

This work was supported, in part, by a grant from the Wellcome Trust (V.C.) and COFIN 2001 (VC, A.M.G.) and by grants from the National Institute of Health (D.A.B.).

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Authors' address: Prof. Vittorio Calabrese, Section of Biochemistry and Molecular Biology, Department of Chemistry, Faculty of Medicine, University of Catania, Viale Andrea Doria 6, 95100 Catania, Italy,
Fax: 0039-095-580138, E-mail: calabres@mbox.unict.it