

Review Article

Reactive oxygen species and antioxidants: implications for clinical nutrition

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ABSTRACT: Background: Oxidative stress is an important pathogenetic mechanism in several diseases, and antioxidant supplementation may reduce morbidity and mortality. The aim of this review is to focus on the role of oxidative stress in the pathogenesis and clinical outcome of critical illness, cancer, and neurological disorders and to evaluate the efficacy of antioxidant supplementation on morbidity and mortality in these conditions.

Methods: We reviewed the literature on reactive oxygen species and antioxidants in critically ill, neurological, and neoplastic patients.

Results and conclusions: Clinical and experimental evidence demonstrates that oxidative stress and/or antioxidant deficiency contribute to morbidity and mortality in critically ill, neurological, and cancer patients. Nutritional and pharmacological approaches to modulate oxidative stress are beneficial in improving clinical outcome in critically ill (especially respiratory distress syndrome) patients. In contrast, antioxidant supplementation does not substantially reduce the risk of cancer in clinical trials and paradoxically beta carotene has been shown to increase cancer risk in heavy smokers. Increased reactive oxygen species production may be exploited to induce apoptosis and to cure selected types of cancer. In dementia, modulation of oxidative stress by vitamin E administration has failed to provide clinical benefits; other oral antioxidants (lipoic acid and n-acetylcysteine) are currently under investigation. (*Nutritional Therapy & Metabolism* 2009; 27: 62-72)

KEY WORDS: Antioxidants, Artificial nutrition, Cancer, Critically ill, Neurological, Oxidative stress

INTRODUCTION

Oxidative stress is a feature of several pathological conditions and is implicated in their pathogenesis and progression. In particular, growing evidence suggests that reactive oxygen species (ROS) and reduced antioxidants strongly contribute to morbidity and mortality in critically ill, cancer, and neurological patients (1). Administration of antioxidants counteracts increased oxidative stress, prevents reduced antioxidant levels, and therefore may impact the clinical course and improve outcome.

This article, while reviewing the pathophysiology of ROS and antioxidants addresses a key question: can

supplementation with antioxidants reduce all-cause mortality and improve outcome in critically ill, cancer, and neurological patients?

PHYSIOLOGY OF ROS AND ANTIOXIDANTS

The reduction of the diatomic molecules of oxygen under aerobic conditions results in the generation of different oxygen metabolites, collectively known as reactive oxygen species or ROS (2). Superoxide anion (O₂⁻) is considered the primary ROS and can further interact with other molecules to generate "secondary ROS" or with nitric oxide (NO) to produce some of the reactive

nitrogen species (RNS) (2). ROS are generated by intracellular and extracellular sources and can be targeted by specific antioxidants. Superoxide anion is produced by oxygen and by electrons leaked in the mitochondrial respiratory chain during resting as well as during normal metabolic activity. In addition to mitochondrial sources of ROS, other important sources are represented by membrane-bound and cytosolic enzymes. The membrane leukocyte enzymes nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and myeloperoxidase represent important first-line defenses against microbial invasions. The former, initially characterized in phagocytes, has later been described in all types of vascular cells and plays an important role in hypertension and atherosclerosis (3). Cytosolic sources of ROS include the cytochrome P450 monooxygenase, peroxidases, nitric oxide synthase (NOS), and the xanthine oxidoreductase system.

ROS elicit a wide spectrum of responses, which depend upon the magnitude of their levels, the cell type, and the duration of the exposure. While ROS are predominantly implicated in causing cell damage, they also play a major physiological role in several aspects of intracellular signaling and regulation. It has been clearly demonstrated that ROS interfere with the expression of a number of genes and signal transduction pathways by regulating phosphorylation and dephosphorylation of enzymes and transcription factors (4). Typically, low levels of ROS, particularly hydrogen peroxide, are mitogenic and promote cell proliferation, while intermediate levels result in either temporary or permanent growth arrest, such as replicative senescence. Very high concentrations of ROS ultimately cause cell death via either apoptotic or necrotic mechanisms (5).

Cells have developed efficient protective mechanisms against accumulation of ROS, including enzymatic and nonenzymatic antioxidants. An antioxidant is a compound that scavenges ROS, is itself transformed into a rather inert radical, and thereby terminates a radical-driven chain reaction (2). Antioxidants can be classified into endogenous and exogenous. A list is provided in Table I. In addition to preventing cell damage by quenching excess oxidative stress, evidence is now emerging that some dietary antioxidants (especially vitamin E) exert a variety of biological functions, such as modulation of cell signaling and of gene expression by mechanisms other than antioxidant (6). High doses of selected antioxidants have however been associated with possible prooxidant effects resulting in cell damage (7).

Many techniques have been developed to detect ROS and antioxidants in biological samples, which differ for sensitivity, specificity, and availability (8). Ideally, a valid biomarker of oxidative stress should be (a) a

stable and major product of oxidative damage, directly implicated in the onset and/or progression of disease and sensitive to specific antioxidant treatments; (b) accessible in a target compartment where it is present at concentrations high enough to quantitatively reflect the oxidative modifications with low interperson variability; (c) specific and free of confounding factors from dietary intake; and (d) measurable by an assay that is noninvasive, easy to perform, sensitive, specific, and reproducible.

The usefulness of biomarkers of oxidative damage lies in the ability to provide early indication of disease and/or its progression, as well as to assess therapy efficacy. Global assessment of oxidative stress involves evaluation of prooxidant and antioxidant status. ROS are, by definition, highly reactive molecules with a short half-life; this feature hampers the direct assessment of their levels in biological samples. From a methodological standpoint, the only technique for direct detection of ROS is electron spin resonance (ESR) (9), which combined with the spin trapping method is the golden standard for the assessment of ROS in biological samples. Unfortunately, ESR is a complex technique with limited availability, which is not routinely used. In addition, it does not provide information on antioxidant status. Thus, so-called fingerprinting techniques have been developed to assess oxidative stress. According to this concept, the presence of ROS is commonly detected by specific end products resulting from their interaction with biological macromolecules, such as DNA, proteins, and lipids (Tab. II).

With regard to the assessment of antioxidant status, many methods are currently available to evaluate both extracellular and intracellular antioxidants (Tab. III). However, while indirect methods to measure antioxidant reserve are felt to assess only partly total antioxidant capacity, they do not reflect the most important antioxidants, i.e., the endogenous antioxidant enzymes. Therefore direct measurement of antioxidant enzymes represents a more accurate estimate of antioxidative status.

TABLE I - ENDOGENOUS AND EXOGENOUS ANTIOXIDANTS

Endogenous	Exogenous
Proteins	Vitamin E (α , γ -tocopherol)
Superoxide dismutases	Vitamin C (ascorbic acid)
Glutathione peroxidase (GPX)	Vitamin A
Catalase	Carotenoids (β -carotene, lycopene)
Uric Acid	Flavonoids and other polyphenols
Glutathione (GSH)	Metallothionein
	Coenzyme Q10
	Xanthophylls
	Herbals (theaflavin, <i>Ginkgo biloba</i> , etc)

REDOX IMBALANCE IN DISEASE STATES

Oxidative stress has been implicated in cancer and neurodegenerative disorders and has been demonstrated to play a relevant role in critically ill patients. In this part of the review, pathophysiological mechanisms of redox imbalance in these conditions will be discussed along with the rationale for the use of antioxidants.

Critical illness and redox imbalance

ROS production is increased in critically ill patients (23), and different mechanisms account for it. In infections, microbicidal activity of activated neutrophils and macrophages involves ROS generation. In addition, when stimulated during sepsis, endothelial NOS is uncoupled resulting in reduced NO and increased $O_2^{\cdot-}$ production, contributing to systemic oxidative stress (Fig. 1) (24). This could be the consequence of reduced plasma levels of the precursor L-arginine during sepsis (25). In ischemia/reperfusion, as well as in cerebral and myocardial infarction, or during cardiac and vascular surgery or transplantation, activation of the xanthine oxidase enzyme results in increased ROS production. Iron and copper ions are released from necrotic and apoptotic cells, leading to enhanced ROS generation. Energy requirement is increased during critical illness, resulting

in the activation of the mitochondrial respiratory chain activity. Although actively respiring mitochondria do not double basal superoxide production, this is increased in active compared with resting mitochondria, further contributing to systemic oxidative stress.

Another important source of ROS production in critical illness is hyperglycemia. Hyperglycemia, which develops during critical illness also in patients who were not diabetic previously, reflects an adaptive development of insulin resistance (26). There is evidence that sustained hyperglycemia (>110 mg/dL) induces oxida-

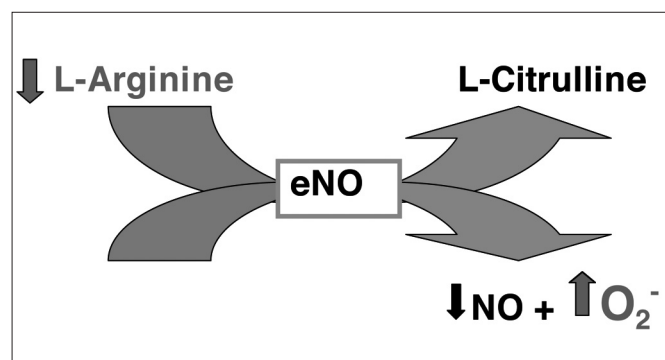


Fig. 1 - Endothelial nitric oxide synthase (eNOS) pathway during sepsis. NO = nitric oxide.

TABLE II - ESTABLISHED AND EMERGING MODALITIES FOR MEASURING OXIDATIVE STRESS IN BIOLOGICAL SAMPLES

Oxidative status (reference)	Measure	Sensitivity and specificity	Reproducibility	Availability
Pox-Act (10)	Lipid peroxidation	Good	Good	Wide
TBARS (MDA) (11)	Lipid peroxidation	Fair	Fair to good	Wide
Lipid peroxidation degradation products (LPO) (12)	Lipid peroxidation	Very good	Good	Moderate
F2-Isoprostanes (13)	Arachidonic acid peroxidation	Very Good	Good	Wide
8-Hydroxy-2'-deoxyguanosine (8-OHdG) (14)	DNA oxidation	Good	Very good	Moderate
Dityrosine and tyrosine oxidation products (15)	Protein oxidation	Very Good	Good	Limited
ESR (\pm spin trap) (9)	Oxygen free radicals	Excellent	Excellent	Limited

ESR = electron spin resonance; MDA = Malonyldialdehyde; TBARS = Thiobarbituric acid reactive substances.

TABLE III - ESTABLISHED AND EMERGING MODALITIES FOR MEASURING ANTIOXIDANT STATUS IN BIOLOGICAL SAMPLES

Antioxidant status (reference)	Measure	Sensitivity and specificity	Reproducibility	Availability
Oxy Adsorbent test, FRAP test, TEAC I-III, TRAP assay, DPPH, DMDP assay (16-19)	Antioxidant reserve	Fair	Fair to Good	Wide
PCL test (18)	Antioxidant Reserve	Good	Good	Wide
Single antioxidant measurement (GSH, SOD, catalase, vitamins C and E, uric acid, selenium, flavonoids) (19-23)	Single antioxidant	Good	Good	Variable

GSH = glutathione; SOD = superoxide dismutase.

tive stress at the level of endothelial cells, macrophages, and monocytes, in the beta cells of pancreas, and in the liver mitochondria (27). Mitochondria are an important source of ROS in cells during hyperglycemia as a result of imperfectly coupled electron transport (28). In addition, overproduction of superoxide by the mitochondria is responsible for activation of the protein kinase C, and polyol and hexosamine pathways (29), which further contribute to ROS overproduction and to the development of clinical complications. Hyperglycemia in critical illness predisposes patients to many of the typical intensive care complications – sepsis, excess inflammation, critical illness, polyneuropathy, and multiple organ failure – prolonged intensive care dependence, and death (30). As a matter of fact, the risk of mortality or significant morbidity is high among critically ill patients who are treated in the intensive care unit (ICU) for >3 days.

In addition to increased ROS generation, critically ill patients are characterized by decreased antioxidant capacity (31, 32). Activities of endogenous scavenger enzymes, such as superoxide dismutases (SODs), catalase, and glutathione peroxidase, are reduced. Plasma and tissue levels of tracer elements, cofactors of antioxidant enzymes (zinc, selenium, manganese, copper), antioxidant vitamins (ascorbic acid, tocopherols and tocotrienols, beta carotene, and other carotenoids), and free thiols (cysteine, lycopene, lutein, etc) are greatly decreased as a result of both systemic oxidative stress and inflammation. Hyperglycemia-induced overproduction of ROS in the mitochondria is associated with accelerated reduction of coenzyme Q10 and concomitant depletion of glutathione (33). The resulting loss of antioxidant equivalents results in enhanced sensitivity to oxidative stress associated with intracellular ROS. These opposite changes of prooxidant and antioxidant factors result in redox imbalance.

Increased ROS production results in direct cell/tissue damage through oxidation of structural proteins, enzymes, and DNA, as well as through peroxidation of membrane lipids. In addition, redox imbalance activates stress kinases and nuclear transcription factor kappa B, resulting in increased expression of acute phase mediators. Redox imbalance stimulates the production of NO in endothelial cells promoting vasorelaxation and permeability (34). Finally, redox imbalance perpetuates and amplifies the inflammatory response. Given the relevance of oxidative stress in the pathogenesis of many ICU syndromes and diseases, including sepsis/systemic inflammatory response syndrome (SIRS), acute respiratory distress syndrome (ARDS), and multiple organ failure, reducing oxidative stress by administration of antioxidants may reverse these processes (35).

Cancer and oxidative stress: implications for genetic instability and drug resistance

Growing evidence suggests that oxidative stress is increased in cancer cells and contributes to genetic instability and drug resistance. Enhanced ROS generation has been demonstrated in cancer cells as a result of the ability of oncogenes *c-myc* and *c-ras* to induce ROS production (36, 37). The accumulation of ROS-mediated reaction products in cancer cells has been confirmed by the presence of oxidative products in plasma and urine (38). SOD and other antioxidant enzymes (catalase and glutathione-S-transferase) are overexpressed in cancer cells (39). The overexpression of antioxidant enzymes has been interpreted as a compensatory response to increased oxidative stress. In cancer cells, ROS promote DNA damage and instability and contribute to DNA mutations. ROS have been shown to modulate the ability of thioredoxin (TRX) to interact and inhibit apoptosis signaling kinase-1, a MAP3K family member involved in the signaling pathway from the TNF receptor to the stress-activated protein kinase, thus stimulating cell proliferation (40). Gene mutations can be promoted by free radicals through ROS-mediated DNA damage, largely due to base pair mismatching during DNA replication across the ROS-modified base or nucleotide insertions, particularly in mitochondrial DNA (41, 42). T cells cultured in the absence of survival factors accumulate ROS, up-regulate the expression of BCL-2–interacting mediator of death and inducible nitric oxide synthase, and undergo apoptosis, which could be inhibited by antioxidants (43).

In addition, ROS are able to inflict a direct, severe cellular damage by decreasing the mitochondrial membrane potential, impairing the mitochondrial respiratory chain, and finally depleting adenosine triphosphate (ATP) (44). Primary leukemia cells from chronic lymphatic leukemia patients refractory to chemotherapy with fludarabine/cyclophosphamide are characterized by a higher frequency of mitochondrial mutations and higher levels of intracellular ROS, although these leukemia cells are sensitive to 2-methoxyestradiol, a novel anticancer agent that causes ROS accumulation by inhibiting SODs (45). The exact relationship between mitochondrial DNA mutations, ROS generation, and drug sensitivity still remains elusive, and it is likely to be dependent on the nature of the mutations and the mechanism of action of the anticancer drugs involved.

Neurological disorders and oxidative stress

Neuronal cells are notable for their level of oxygen utilization and ATP synthesis, resulting in a distinct sus-

ceptibility to oxidative stress. Neurons are most susceptible to direct oxidative injury by ROS and RNS, resulting in neuronal cell death (46). In addition, ROS and RNS can also indirectly contribute to tissue damage by activating a number of cellular pathways leading to a glia-mediated inflammatory response (particularly astrocytes and microglia) at the sites of injury, which also causes secondary neuronal damage. The immune mediators (e.g., NO and ROS, proinflammatory cytokines, and chemokines) released by activated glial cells are currently considered to be candidate neurotoxins (47). Thus, ROS and RNS generated extracellularly and intracellularly initiate and promote neurodegeneration in the central nervous system (48). Oxidative stress is a feature and is implicated in several neurological and neurodegenerative diseases (Tab. IV) (46, 49). Although the cause of each disease is different, when oxidative stress is implicated, it can affect cellular function by the following basic mechanisms: (a) glial cell activation, (b) neuronal apoptosis or programmed cell death, (c) protein misfolding resulting in proteosomal malfunction, and (d) mitochondrial dysfunction. These processes, variably contributing to different pathological processes, constitute the basis of oxidative-stress-related neurological disorders.

IMPLICATIONS FOR THERAPY

In the final part of this review, by reviewing the clinical evidence, the impact of modulating oxidative stress by nutritional and pharmacological approaches in critically ill, neoplastic, and neurological patients will be addressed.

Critical illness and antioxidant therapy

Enteral nutrition is increasingly becoming the standard of care for critically ill patients, with the goal of providing nutritional support that prevents nutritional deficiencies and reduces morbidity, avoiding the complications associated with parenteral feeding (50). Supplementation with antioxidants has been shown to improve surrogate markers of antioxidant capacity and outcome in critically ill patients affected by ARDS. In this condition, an enteral diet containing elevated antioxidants, eicosapentaenoic acid, and gamma-linolenic acid significantly reduces pulmonary inflammation, increases oxygenation, and improves clinical outcomes (51-54). In other acute illnesses – brain injury, burns, pancreatitis, SIRS, or trauma – data are not conclusive. Some recent meta-analyses have shown a statistically significant reduction in mortality but not in infectious

TABLE IV - NEURODEGENERATIVE DISORDERS ASSOCIATED WITH OXIDATIVE STRESS

Alzheimer's disease
Amyotrophic lateral sclerosis
Demyelinating diseases
Diabetic polyneuropathy
Down syndrome
Friedreich's ataxia
HIV neuropathy
Huntington's disease
Multiple system atrophy
Parkinson's disease
Prion disease
Stroke-ischemic reperfusion injury
Tardive dyskinesia
Traumatic brain injury

complications associated with the provision of selenium alone or in combination only by the intravenous route (55-57). Available data do not give a definitive answer regarding antioxidant combinations. Many successful trials have combined selenium with vitamins A, C, and E and zinc. Multivitamin supplementation alone was not associated with benefits in outcomes (55). The timing of administration is important. Antioxidant therapy in established diseases has not been effective (55). Ideal therapy should start early after admission to the ICU. There is not enough data to define the appropriate amount of antioxidant nutrients necessary to reduce oxidative damage in any specific disease condition, and the optimal doses have not been definitively determined. Reduced mortality in critically ill patients following selenium administration by the enteral route was observed in the presence of doses that were 5-20 times the recommended parenteral nutrition intakes (300–1,000 µg/day) (55). The mechanisms by which antioxidants improve outcome in critical illness are unknown. At present, there is no evidence to support the contention that any enteral formulas enriched with antioxidants may improve outcome by modulating oxidative stress. Alternative mechanisms by which antioxidants are beneficial in selected groups of acute patients include by blunting inflammation, thus acting as immunonutrients (58).

Another strategy to modulate oxidative stress in critically ill patients is to prevent hyperglycemia. To this aim, intensive insulin therapy targeted at maintaining blood glucose levels below 110 mg/dL has been proven to significantly reduce mortality and morbidity in surgical nondiabetic ICU patients (59, 60), while blood glucose >150 mg/dL was associated with increased mortality (61). In medical ICU patients, intensive insulin therapy aiming at maintaining blood glucose levels below

110 mg/dL has been shown to reduce morbidity but not mortality (62). However, so far no evidence is available to define whether this effect is related to the normalization of glucose levels, to the antiinflammatory action of insulin, and/or to the antioxidant effect of insulin (63).

In contrast to that for the critically ill, the evidence for the beneficial effects of antioxidant supplementation in patients receiving long-term artificial nutrition for chronic diseases is less consistent. One reason for this discrepancy may be that the presence of oxidative stress in patients on long-term home parenteral nutrition has not been univocally demonstrated. Enhanced lipid peroxidation as a result of lack of vitamin E has been suggested by some studies (64, 65), but not by others (66). Another study, while showing increased oxidative stress, failed to demonstrate oxidative damage in these patients (67). Abnormalities of antioxidant status, especially glutathione peroxidase, as a consequence of low plasma selenium levels have been more consistently reported (64-66); however, their clinical significance in relation to deficiency or toxicity states is not always clear. As a matter of fact, in most studies there was no clinical evidence of toxicity or deficiency, and serious clinical consequences have not been reported (67, 68). At present, there is not enough evidence suggesting that oxidative stress results in oxidative damage in patients on long-term artificial nutrition, and thus supplemental antioxidants in addition to the daily requirement (Tab. V) are not recommended.

Cancer and therapeutical modulation of oxidative stress

The increased oxidative stress of cancer cells is a feature that can be exploited therapeutically both in primary prevention and in chemotherapy.

The possibility of reducing the risk of cancer by administering antioxidants has been addressed in randomized placebo-controlled trials. The results of these studies are conflicting, failing to provide clear evidence of beneficial effects, sometimes suggesting paradoxically an increased incidence of cancer (71, 72) or a positive effect restricted to selected groups of patients (males with reduced baseline levels of antioxidants and low risk of cancer, receiving selenium supplements) (73). Therefore, so far there is insufficient clinical evidence from randomized trials for or against the use of antioxidant vitamins (especially vitamins A, C, and E) for the prevention of cancer in the general population. Selenium might prevent cancer in males, but further studies are required to confirm this finding. If patients choose to take antioxidant supplements (at the dosages recommended in the Dietary Reference Intake), they should be

reminded that antioxidant vitamins do not replace fruit and vegetables in the diet and that some vitamins (i.e., vitamin A) may have harmful effects at high doses. In contrast, there is good evidence against the use of beta carotene supplements alone or in combination, especially in selected groups of patients. In heavy smokers, large doses of beta carotene supplementation were associated with higher incidence of lung cancer and increased all-cause mortality, and therefore its administration should be discouraged (74).

While lowering oxidative stress has been explored as a strategy to prevent the incidence of neoplastic diseases, enhancing ROS production represents a promising therapeutic option to cure neoplastic patients. There is evidence that many chemotherapeutic agents and ionizing radiation exert their killing effect by enhancing ROS production, leading to irreversible cell injury, and that overproduction of ROS in cancer cells may exhaust the capacity of SOD and other adaptive antioxidant defences (Tab. VI). With this view, antioxidant inhibitors and/or ROS-generating compounds have been used to trigger apoptosis in cancer cells. This concept was supported by recent studies demonstrating exquisite sensitivity to SOD-inhibiting chemotherapies in cancers with high cellular levels of oxidative stress (45). Thus, in the near future it will be possible to select cancers that will be suitable candidates for treatment with ROS-generating agents. The ideal candidates would be the cancers that exhibit increased oxidative stress, with high levels of cellular ROS and a low antioxidant capacity. Such features may characterize relapsed and chemotherapy-refractory cancers, which are likely to carry mitochondrial mutations due to possible prior therapy with DNA-damaging agents, or to have a high rate of ROS generation due to continuous oncogenic stimulation. This therapeutic strategy is promising in a variety of clinical settings and should be considered particularly in the salvage situation of relapsing or primarily nonresponding cancers. However, caution should be applied in using ROS-generating agents, since ROS are involved in im-

TABLE V - DAILY REQUIREMENT FOR ANTIOXIDANT VITAMINS AND MICROELEMENTS COMMONLY SUPPLIED IN LONG-TERM PARENTERAL AND ENTERAL NUTRITION

	Enteral	Parenteral
Folic Acid (mg)	400	400
Vitamin A (mg)	900 (770-2,100)	1,000
Vitamin C (mg)	90 (100-150)	100
Vitamin E (mg)	15 (18-36)	10
Selenium (μ g)	55 (69-129)	20-60

Sources (69, 70).

portant roles in toxic adverse effects such as cisplatin-induced nephrotoxicity, ototoxicity, and chemoradiation therapy-associated lung damage (87).

Neurological diseases and antioxidants

Experimental evidence shows that oxidative stress is an early event in the pathogenesis of some neurological disorders and thus modulation of oxidative stress by antioxidant strategies is neuroprotective (88-90). Neuroprotective antioxidants act either by inhibiting ROS formation, scavenging ROS, or reducing ROS-induced cellular damage. In addition to scavenging ROS, some antioxidants (i.e., vitamin E) have been shown to suppress the expression of genes induced by proinflammatory cytokines and other mediators released by glial cells (91). Based on these observations, clinical trials have been conducted to assess the effects of vitamin E on the cognitive impairment of dementia. The results of these studies have unexpectedly shown little or no efficacy of vitamin E at dosages of 2,000 IU/day (92, 93). In addition, treatment with vitamin E may increase mortality in a dose-dependent fashion (94) and the rate of heart failure in patients with diabetes mellitus and/or vascular disease (95), and may worsen coagulation defects in patients with vitamin K deficiency. Based on these findings, vitamin E is no longer recommended for the treatment of cognitive impairment in dementia, and if used, special caution is recommended, as dosage should be limited to 400 IU/day or less.

One important issue regarding vitamin E and other

oral antioxidants is intestinal absorbance and tissue uptake. Once ingested, oral antioxidants are partly metabolized in the gut, partly adsorbed, and partly eliminated. Some antioxidants may exert antioxidant effects locally in the intestine and/or be transformed by the intestinal microflora into more active compounds. As a result, the target tissue levels of dietary antioxidants are substantially lower than those of endogenous antioxidants (96). In addition important chemical properties of antioxidants – i.e., being lipophilic or hydrophilic – influence their cellular and subcellular distribution. Therefore targeting oral antioxidants against selective cellular compartments may help to improve the pharmacological profile of antioxidant drugs and to prevent adverse effects related to the need for higher dosages of native compounds. Examples of cellular-targeted antioxidants include MitoQ and MitoVitE, which are targeted to mitochondria, which represent key sites for ROS generation and oxidative damage in disease progression (97). Once in the mitochondria, they rapidly neutralize free radicals and decrease mitochondrial toxicity. The potential efficacy of cellular-targeted antioxidants compared with their native compounds, however, needs further study before widespread clinical application.

Some antioxidants, such as lipoic or dehydroascorbic acid, can cross the blood-brain barrier. Lipoic acid has been shown to reduce oxidative stress and restore impaired antioxidant levels in vivo (98). N-Acetylcysteine, which is involved both in glutathione synthesis and regeneration as well as in quenching ROS by its thiol groups is a promising therapeutic agent for oxidative damage in Alzheimer's disease (98).

Another approach to reduce oxidative stress is by copper and iron chelators; these agents have been tested with positive results in Alzheimer's disease (99). The pathogenic role of ROS and RNS in mitochondrial damage (100) and cell death induction (101, 102) in amyotrophic lateral sclerosis (ALS) is also becoming increasingly clear. Neurological impairment in ALS may be extraordinary rapid (103), making artificial nutrition support almost invariably necessary, due to severe dysphagia. Evidence exists that the neuronal damage in ALS and other motor neuron diseases may be secondary to disturbances of iron metabolism (100), a view that is strengthened by the high prevalence of the mutation for the hemochromatosis gene *Hfe* in ALS patients. Thus, iron and antioxidant nutritional provision with nutrients could be of major relevance in the clinical management of this increasingly recognized group of neurological disorders.

In addition, although there is no evidence that long-term supply of antioxidants in enteral formulas may be harmful at dosages equivalent to the daily requirement,

TABLE VI - CHEMOTHERAPIC AGENTS THAT CAUSE OR INCREASE CELLULAR OXIDATIVE STRESS

ROS generation
Arsenic trioxide (75)
Anthracyclines (76)
Bleomycin (77)
Bortezomib (78)
Cisplatin (79)
Retinamide (80)
Emodin (81)
GSH depletion
Buthionine sulfoximine (82)
Diethylmaleate (79)
Ascorbic acid (83)
Inhibition of antioxidant enzymes
Mercaptosuccinic acid (GPx) (84)
Aminotriazole (catalase) (84)
Ethacrynic acid, TLX 199 (GST) (85)
2-Methoxyoestradiol (SOD) (86)

GSH = glutathione; GPX= Glutathione peroxidase; GST = Glutathione S-transferase; SOD = superoxide dismutase.

in selected disease states, such as ALS, Parkinson's disease, and Alzheimer's disease, long-term supply of iron-enriched enteral formulas might carry the risk of iron overload and oxidative damage, as is observed in some cases in infant parenteral nutrition (104). This aspect is currently under careful investigation.

TOWARD A SYNTHESIS: ANTIOXIDANT THERAPY AND PATIENT OUTCOME

Clinical and experimental evidence demonstrates that oxidative stress and/or prooxidant/antioxidant imbalance is implicated in the pathogenesis and outcome of critical illness, cancer, and selected neurological diseases. Antioxidant administration and intensive insulin therapy aiming at attaining strict glucose control have been shown to improve outcomes in critically ill patients, especially in ARDS. However, whether this effect is achieved by modulation of oxidative stress or by mechanisms other than the antioxidant is currently unknown. In cancer, there is no evidence of a risk reduction by oral antioxidant administration, and some antioxidants (i.e., beta carotene) may paradoxically increase the risk of cancer, while selenium may be beneficial in a sex-specific fashion. In dementia, supplementation with vitamin E is currently no longer recommended

in the light of the risks versus benefits demonstrated by clinical trials; modulation of oxidative stress by lipoic acid and N-acetylcysteine administration seems more promising. In ALS, enteral nutrition enriched with iron and antioxidants may be beneficial, but the potential effects of long-term iron supplementation need to be specifically addressed in clinical studies.

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