Oxidative Stress: Antioxidants in Degenerative Neurologic and Ophthalmologic Diseases

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Reactive oxygen species, also called prooxidants, are produced physiologically during normal metabolic reactions in the aerobic organism. However, when they are produced in excessive amounts and are not sufficiently detoxified, the steady state balance between prooxidants and antioxidants may be disrupted. An imbalance in favor of the prooxidants and against the antioxidants then occurs. This has been called “oxidative stress” (1–3), and can lead to damage affecting all types of biologic molecules, including DNA, lipids, protein, and carbohydrates. Oxidative stress is thus involved in processes such as mutagenesis, carcinogenesis, cellular dysfunction, and tissue degeneration.

Although it is often suggested that antioxidants may play a critical role in human health, and that oxidative stress could be involved in the pathogenesis of various disorders, attempts to treat diseases with antioxidants have been less than satisfactory (4), and neurodegenerative diseases are no exception. However, there is increasing interest in the potential pathophyslogic role of oxidative stress and mitochondrial dysfunction in Alzheimer disease, Huntington disease, Parkinson disease, and amyotrophic lateral sclerosis. The fact that there is a multitude of oxidants as well as antioxidants with overlapping reactivities makes a biochemically rigorous assessment of the implications of oxidative stress difficult. Moreover, helpful as epidemiologic studies can be, sometimes there is a confounding of associations with cause–effect relations, leading to erroneous conclusions.

The aging process itself is associated with an overall increased oxidant load in the organism (5) and age is a major “risk factor” for neurodegenerative diseases. Accordingly, an involvement of oxidative stress has been suggested in accelerated aging processes such as progeria and Werner syndromes, which are also associated with neurodegenerative processes [for review, see Butterfield et al. (6)]. Studies conducted in patients, animal models, and human necropsy samples have shown that increased blood and tissue levels of biomarkers of oxidative damage to DNA, lipids, and proteins are associated with aging and neurodegeneration. Furthermore, in specific diseases, the gene mutations responsible for the impairment
of antioxidant function are associated with severe neurodegeneration in humans (see below).

Another actively studied phenomenon in the field of oxidative damage and human disease is photooxidative stress experienced by light-exposed tissues. Reactive oxygen species, such as singlet molecular oxygen, are generated in reaction sequences involving the transfer of light energy through photosensitizers to oxygen (7,8). Photooxidative damage to proteins and lipids is thought to play a role in the pathologic events leading to degenerative eye diseases such as age-related macular degeneration (9) and cataract (10). As with neurodegenerative disorders, it has been suggested that antioxidants may prevent or delay the onset of these ophthalmic diseases.

In this chapter, we summarize relevant observations on the potential role of oxidants and antioxidants in both neurologic and ophthalmologic degenerative diseases.

NEURODEGENERATIVE DISEASES

Oxidative Stress and the Brain

There is much interest in the possibility that mitochondrial dysfunction and oxidative damage play a role in the pathogenesis of neurodegenerative diseases (11). Mitochondrial oxidative phosphorylation through the electron transport chain normally couples the reducing equivalents to oxygen with the simultaneous production of adenosine triphosphate (ATP). However, when supplies of oxygen are limited and oxidative phosphorylation slows or fails, the production of reactive oxygen species may increase. The result is a cumulative burden of oxidative damage to key cellular constituents, with widespread ramifications for cellular metabolism.

In addition, as ATP levels fall, a series of events ensues that exacerbates a condition of oxidative stress in the brain (12). These include partial neuronal depolarization, activation of voltage-dependent excitatory amino acid receptors, and opening of the calcium permeable channels linked to these receptors (13). The influx of calcium forms an important link between excitotoxicity and oxidative stress, because intracellular calcium can stimulate phospholipase with subsequent release of arachidonic acid, which may undergo free radical-induced lipid peroxidation and lead to further generation of free radicals. Arachidonic acid and oxygen radicals may, in turn, enhance the release of excitatory neurotransmitters and inhibit their uptake by inactivation of neuronal and glial transport processes, thus promoting a vicious cycle. Furthermore, calcium influx can activate peptidases such as calpain, which catalyzes the enzymatic conversion of xanthine dehydrogenase to xanthine oxidase, ultimately leading to superoxide anion generation.

In this cascade of damaging events, the simultaneous increase of lactic acid favors the liberation of cellular stores of iron, which promotes Fenton reactions to yield hydroxyl radicals. All these reactions lead finally to the so-called excitotoxic cell death (14), which constitutes a critical pathophysiologic mechanism not only in neurodegenerative brain changes (slow excitotoxicity) but also in acute brain injury (acute excitotoxicity).
The brain may be especially prone to oxidative damage not only because it consumes a large amount of the body’s oxygen, but especially because the activity of antioxidant enzymes in brain tissue is relatively low (15).

**Biomarkers of Oxidative Stress and Alzheimer Disease**

Alzheimer disease is the most prominent cause of dementia in the elderly and is clinically characterized by memory dysfunction—loss of lexical access, spatial and temporal disorientation, and impairment of judgment. Histopathologically, Alzheimer disease is characterized by synaptic loss, nerve cell loss (mostly in the cerebral cortex, hippocampus, and amygdala), extracellular deposition of β-amyloid protein (forming senile plaques), and intracellular precipitation of hyperphosphorylated τ protein (forming neurofibrillary tangles). The exact biochemical mechanism of the pathogenesis of this disease is still unknown, but much attention is given to the role of the massive loss of the neurotransmitter acetylcholine (necessary for cognition and memory) and to the possible implication of oxidative stress in the development of Alzheimer disease.

There is impaired energy metabolism in Alzheimer disease (16). Consistently, cerebral blood flow and oxygen utilization have been shown to be decreased in this disorder in a temporoparietal pattern (17). Impaired mitochondrial function may lead to increased free radical–related damage affecting critical cellular key components. There is substantial evidence that mitochondrial function declines with age and is altered in Alzheimer disease (18). Mitochondrial DNA (mtDNA), which encodes subunits of the mitochondrial respiratory enzyme complex, is particularly susceptible to oxidative damage owing to its close proximity to the respiratory chain proteins, limited repair mechanisms, paucity of noncoding sequences, and absence of histones (19).

An increase in DNA, lipid, and protein oxidation products has been shown in blood and necropsy brain samples obtained from patients with Alzheimer disease in comparison with controls [for review, see Markesbery and Carney (20)]. A three-fold increase in 8-oxo-2′deoxyguanosine (8-oxo-dG) has been found in mtDNA isolated from cortical tissue of patients with Alzheimer disease in comparison with nuclear DNA (nDNA) (21). An increased 8-oxo-dG lymphocyte DNA content has also been found in Alzheimer disease donors compared with control subjects (22). This increase has been found recently to be inversely correlated with plasma concentrations of several antioxidant vitamins and micronutrients in patients with Alzheimer disease (23). Immunohistological studies on the antioxidant enzymes superoxide dismutase and catalase have shown that they are colocalized with a subset of neurofibrillary tangles and plaques in Alzheimer disease tissue (24). It has been suggested that the β-amyloid protein itself may cause oxidative damage to neurons [for review, see Butterfield et al. (25)].

**Biomarkers of Oxidative Stress and Parkinson Disease**

As a result of neurodegeneration occurring in the substantia nigra and the striatum and because of dopamine depletion, Parkinson disease is characterized clinically by
bradykinesia, postural instability, gait difficulty, and tremor. Depigmentation, neuronal loss, and gliosis of the substantia nigra are typical brain abnormalities found in Parkinson disease. The mechanisms of cell death in this disorder have not yet been fully elucidated, but increased oxidative stress, abnormal mitochondrial function, and excitotoxicity are perhaps among the most important initiators or mediators of neuronal damage.

Oxidative damage is increased in Parkinson disease. The evidence for an involvement of free radicals comes from the observation that oxidation of dopamine yields potentially toxic semiquinones, and that the accelerated metabolism of dopamine by monoamine oxidase-B may induce an excessive formation of hydrogen peroxide, superoxide anions, and hydroxyl radicals (26).

Further evidence for the role of oxidative stress in Parkinson disease patients comes from studies on the selective toxicity against the substantia nigra of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which induces Parkinson-like symptoms in primates. MPTP acts through its metabolite MPP⁺ to inhibit Complex I of the mitochondrial respiratory chain. Necropsy studies on Parkinson disease brains have shown that a disease-specific and drug-independent defect of mitochondrial Complex I accumulates in the substantia nigra of affected patients (27–29). It has also been suggested that MPP⁺ acts by increasing the vulnerability of cells to oxidative stress (30).

The role of a Complex I dysfunction in Parkinson disease is supported by the finding that Complex I is inhibited in vivo and in vitro by neuroleptic drugs that have extrapyramidal side effects, but not by clozapine, an atypical antipsychotic agent that has minimal, if any, extrapyramidal toxicity (31). Mitochondrial Complex I defects have been also found in muscle (31) and platelets (32) in patients with Parkinson disease. Parkinson disease was found to be associated with increased lipid peroxidation in the brain (33) and with a marked increase in 8-oxo-dG in the caudatum and substantia nigra (11,34).

Biomarkers of Oxidative Stress and Huntington Disease

Huntington disease is a late-onset, progressive neurodegenerative disorder characterized by involuntary movements, cognitive loss, and personality changes. The disease is inherited as an autosomal dominant pattern.

Direct evidence for a defect in oxidative phosphorylation in Huntington disease was obtained applying proton nuclear magnetic resonance spectroscopy in 16 affected patients (35). A three-fold increase in lactate concentrations in the occipital cortex was observed in patients examined; increases were also found in the basal ganglia (35).

Several studies have shown DNA strand breaks in necropsy tissue from patients with Huntington disease, as detected by in situ end labeling of DNA.

Administration of 3-nitropropionic acid, a neurotoxic agent inhibiting succinate dehydrogenase, caused brain lesions accompanied by increased levels of 8-oxo-dG and increased staining for 8-oxo-dG in the basal ganglia (36). Increased 8-oxo-dG
levels have been found in mtDNA from the parietal cortex of patients with Huntington disease (37).

Studies have also shown that the disease is associated with increased levels of malondialdehyde, 3-nitrotyrosine, and heme oxygenase in areas of degeneration (38). It has been proposed that glyceraldehyde-3-phosphate dehydrogenase, which binds specifically to proteins implicated in the pathogenesis of neurodegeneration and apoptosis, including Huntington, may affect neuronal energy production (39).

**Biomarkers of Oxidative Stress in Other Neurodegenerative Diseases**

Amyotrophic lateral sclerosis is a fatal neurodegenerative disease associated with point mutations and lowered activity of Cu–Zn superoxide dismutase in its familial form (40). Other reports have shown an increase in Mn superoxide dismutase protein and activity, in contrast with one study indicating potential gene defects of this enzyme in sporadic amyotrophic lateral sclerosis (41). Several mechanisms have been proposed for its pathophysiology (42), including a trigger exerted by the age-dependent decline in ascorbic acid availability in the presence of the specific superoxide dismutase defect (43).

It has been suggested that oxidative stress is involved in other neurodegenerative diseases, including genetic disorders of the central nervous system such as Friedreich ataxia and Down syndrome. Increased levels of 8-oxo-dG have been found in the urine from patients with Friedreich ataxia, the most common hereditary ataxia related to a gene encoding the protein frataxin (44). Down syndrome, which is caused by trisomy of chromosome 21, is characterized by pathologic brain features similar to those seen in Alzheimer disease. When looking at the consequences of chromosome 21–linked gene overexpression, it has been suggested that superoxide dismutase-1 and amyloid precursor protein genes may contribute to the phenotype of the syndrome. There are studies supporting the role for superoxide dismutase-1 and amyloid precursor protein genes in the pathogenesis of neural abnormalities of Down syndrome (45).

**Is There a Role for Antioxidants in Neurodegenerative Diseases?**

Higher ascorbic acid and β-carotene blood levels have been found to be associated with a better memory performance among subjects older than 65 years (46). On the other hand, lower levels of plasma vitamin C and E (47), as well as lower serum β-carotene and vitamin A (48), have been found in demented patients than in healthy controls. Plasma vitamin C levels are lower in Alzheimer disease patients than in controls, even in the presence of an adequate dietary vitamin C intake (49), and Alzheimer patients have significantly lower cerebrospinal fluid levels of vitamin E than controls (50). It is not known, however, whether Alzheimer disease is associated with a specific plasma antioxidant profile, as has been shown for other nonneurodegenerative diseases (51), because studies conducted so far assessed only a few antioxidants rather than a broad spectrum of water-soluble and lipophilic antioxidant vitamins and micronutrients.
Nonenzymatic antioxidants—particularly dietary antioxidants such as vitamin C, vitamin E, β-carotene, and polyphenols—have been shown to exert positive effects on cognitive performance in several studies (52–54). It has thus been suggested that a balanced diet containing large amounts of antioxidant-rich fruits and vegetables might be an efficient way of decreasing the incidence and the prevalence of dementia in elderly people (55). In the case of flavonoids, a significant inverse relation was found between flavonoid intake and the risk of incident dementia in a cohort of 1,367 elderly subjects followed for 5 years (56). No association between cognitive function and the intake of vitamins C and E was found in the Rotterdam study (57), whereas the cross-sectional observation of a potential protective effect of β carotene against cognitive impairment was made (57). Some of these results, however, may reflect confounding errors, among which calculations of the antioxidant intake with diet or changes in dietary habits may play an important role. Another study suggested that use of high doses of vitamin E and vitamin C supplements may reduce the risk of Alzheimer disease, but the proportion of subjects taking antioxidant supplements was too small to exclude the occurrence of chance (58).

When the effect of the cholinesterase inhibitor donepezil was compared with donepezil plus vitamin E in 19 patients with mild to moderate Alzheimer disease, no clear benefit of vitamin E was observed (59). In a large clinical trial, vitamin E (2,000 IU/d) and/or selegiline (10 mg/d) were given to patients with moderate Alzheimer disease for 2 years (60). Both compounds alone, but not the combination, proved to be effective in lowering the risk of reaching one of the primary endpoints—death, severe dementia, loss of ability to perform activities of daily living, or need for institutional care (60). One trial is currently ongoing on the preventive effect of vitamin E supplementation (2,000 IU/d for 3 years) against the development of dementia in patients with mild cognitive impairment (61). The American Psychiatric Association recommends the use of vitamin E in Alzheimer disease (62). Other clinical trials, including those carried out to assess the effect of extract of Gingko biloba and idebenone in Alzheimer disease, have not led to consistent results [for reviews, see Praticó and Delanty (63) and Rottkamp et al. (64)].

Epidemiologic studies suggest that a high vitamin E intake is associated with a lower risk of developing Parkinson disease [for review, see Ebadi et al. (65)]. Recently, however, it was found that plasma vitamin E levels were similar in Parkinson disease patients and in a control population (66). In a small study in patients with Parkinson disease taking 400 to 3,200 IU of vitamin E daily for an average of 7 years, the disease was significantly milder (better performance in the activities of daily living) in the supplemented group than in nonsupplemented subjects (67). In another study, patients with Parkinson disease receiving high doses of vitamin E and C did not require levodopa to treat their symptoms for 2.5 years longer than untreated patients (68). The DATATOP (deprenyl and tocopherol antioxidant therapy of parkinsonism) study, however, showed that 2,000 IU of vitamin E per day was not beneficial in slowing disease progression in patients with early, untreated Parkinson disease (69).

With respect to Huntington disease, MPTP-induced neurotoxic lesions have been shown to be attenuated by coenzyme Q10 administration in animal models [reviewed
by Beal (70)]. Thus, trials are currently ongoing to assess the potential beneficial effects of coenzyme Q_{10} treatment in patients with Huntington disease. A recent multicenter, randomized clinical trial evaluating the effect of coenzyme Q_{10} or remacemide hydrochloride in patients with early Huntington disease has shown a beneficial trend of coenzyme Q_{10} treatment in slowing the decline in overall functional capacity, but neither treatment has produced significant slowing of functional decline (71).

Superoxide dismutase-1 transgenic mice supplemented with vitamin E have a delay in the onset of clinical symptoms resembling amyotrophic lateral sclerosis (72), and transgenic mouse models of familial amyotrophic lateral sclerosis supplemented orally with coenzyme Q_{10} show a significant increase in life span compared with untreated animals (73). N-acetylcysteine given to patients with amyotrophic lateral sclerosis showed only a trend toward benefit (74). In patients with Friedreich ataxia, raised urinary 8-oxo-dG levels have been found to decrease after treatment with idebenone (44).

Antioxidant deficiencies may be associated with severe neurologic deficits, as in the case of coenzyme Q_{10} deficiency (75). Copper is a cofactor of Cu–Zn superoxide dismutase, and copper deficiency is associated with severe neurologic disorders such as Menke disease and Wilson disease (76). Children with genetic abnormalities of the \( \alpha \) tocopherol transfer protein suffer from severe neurologic dysfunction within 18 to 24 months of age, developing cerebellar ataxia, dysarthria, and abnormal tendon and Babinski reflexes (77). Adults may develop neurologic abnormalities after several years of chronic liver disease or fat malabsorption (78). In both cases, symptoms can be ameliorated by vitamin E supplementation (78).

OPHTHALMOLOGIC DISEASES

**Age-Related Macular Degeneration**

Degenerative processes in the retina and retinal pigment epithelium in the region of the macula lead to age-related macular degeneration, a major cause of visual loss and irreversible blindness among the elderly (79). The macula, the yellow spot of the eye, is the area of maximal visual acuity (9,80). The carotenoid pigments lutein and zeaxanthin are responsible for the yellow color of this tissue. Other dietary carotenoids, including \( \beta \)-carotene, which are present in blood, plasma, and most human tissues (81), are not found in the macula. Epidemiologic studies indicate that an increased dietary intake of the macular carotenoids is related to a lower risk of age-related macular degeneration. An increased risk is correlated with a lowered macular pigment density (82), whereas a lower risk is related with higher serum levels of the xanthophylls lutein and zeaxanthin (83). Further, a relation between a high dietary intake of these carotenoids and a lowered risk of age-related macular degeneration has been reported (84). It has also been shown that lutein and zeaxanthin levels in the macular region are lower in subjects with age-related macular degeneration than in controls (85).

Only limited information is available on the biochemical mechanisms involved in the development of this disease. It has been suggested that photooxidative damage
plays an important role in the pathobiochemistry (9,80). In the presence of appropriate sensitizers, singlet molecular oxygen and further reactive oxygen species may be generated. Drusen, which are characteristic of age-related macular degeneration, are rich in lipids, and they may form when lipofuscin accumulates in the retinal epithelium. Lipofuscin is an indigestible, fluorescent product of lipid oxidation occurring in some of the oxidation products of retinol (86). Excessive blue light may increase its formation. In vitro studies have shown that lipofuscin acts as a photosensitizer (87); upon photoactivation of lipofuscin granules, singlet oxygen and other reactive oxygen species are generated.

Singlet oxygen formation in this reaction is most efficient at wavelengths around 420 nm (blue light). The absorbance maxima of lutein and zeaxanthin are around 450 nm. Both compounds may act as broadband filters, diminishing the intensity of blue light that reaches the macula. Like most carotenoids, they are also very efficient singlet oxygen quenchers and thus capable of scavenging the products of photooxidative reactions.

In homogeneous solutions, other carotenoids such as β-carotene also efficiently absorb blue light. However, in vitro data on membrane models indicate that the filter efficacy of lutein and zeaxanthin is superior to β-carotene and lycopene when the compounds are incorporated into lipid bilayers (88). It was suggested that this increased efficacy is related to the orientation of the incorporated molecules in the liposomal membrane and might be a reason why lutein and zeaxanthin, but not other carotenoids, are used as protective agents in the macula.

Cataract

Cataract is an eye disease that affects the lens and is characterized by opacities that impair visual function (89). Oxidative alterations of lens proteins such as crystallins, with subsequent aggregation and precipitation, appear to be a key step in the formation of cataract lesions. Reactive oxygen species may further inactivate repair systems that are responsible for the elimination of modified proteins and thus accelerate the generation of cataracts. There is increasing evidence that the ubiquitin–proteasome pathway plays a role in the removal of oxidatively damaged lens proteins (90).

In vitro data show that the proteasomal step of the ubiquitin–proteasome system is more susceptible to oxidative inactivation than the ubiquitination step. It could be that the attenuation of the ubiquitin–proteasome pathway activity is at least in part responsible for the cytotoxic accumulation of damaged lens proteins. It has further been proposed that the activities of ubiquitin-activating enzyme and ubiquitin-conjugating enzymes are regulated by cellular redox status (that is, the GSSG:GSH ratio) (91). Thus, changes in the antioxidant status that affect the redox state of a cell might have an impact on the ubiquitin–proteasome pathway by different mechanisms.

Epidemiologic studies provide evidence that an increased intake of a diet rich in antioxidants is associated with a lowered risk of cataract (92). For single antioxidants, the most promising data have been presented for vitamin C. It was shown that
long-term supplementation with ascorbate is associated with a lower incidence (77%) of early lens opacities (93). Decreased levels of vitamin C in the lens are correlated with the severity of cataract.

Recent data provide additional evidence for a specific role of vitamin C (94). After adjustment for other nutrients, only vitamin C intake was significantly associated with the incidence of nuclear opacities. The prevalence of nuclear opacities was less in the highest vitamin C intake group than in the lowest. The study also showed a statistically significant trend of decreasing prevalence of nuclear opacities with increasing duration of vitamin C supplementation.

Vitamin C is the major dietary antioxidant in the lens and is detected in large amounts in this tissue, where concentrations of ascorbate are about ten-fold higher than in the blood. The endogenous antioxidant glutathione is also present in an unusually high concentration in the lens (94), exceeding the plasma level. In contrast, the levels of lipophilic antioxidants in the lens, such as tocopherol and the carotenoids, are much lower and less is known about their possible contribution to lens protection against oxidative damage.

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**DISCUSSION**

Dr. Marquardt: About 10 years ago I heard one of the last talks by Linus Pauling, where he was propagating pharmacologic intakes of vitamin C, vitamin A, and vitamin E for different reasons. What is your opinion on this, and could an increased uptake of antioxidants in the general population be of benefit?
**Dr. Sies:** Linus Pauling's book, *How to Live Longer and Feel Better,* is still a valid exploration of this subject. He overinterpreted himself and was overinterpreted by others, but the basic message that these low-molecular-weight antioxidants are useful is certainly valid. The question is, at what dosage and under what circumstances? In general, Pauling was a pioneer in this field.

**Dr. Scott:** You stated categorically that antioxidants have been shown to be of benefit in Alzheimer disease. Is it your opinion that there have been well-conducted studies where that conclusion can be drawn? Do those studies have sufficient statistical power to answer that question?

**Dr. Sies:** In this area, the basic problem is that we are not looking at these compounds as pharmacologic agents but against a background of antioxidants already present in the tissues and whose activities may well overlap. If you remove one or increase one, everything else will also change, as will the pattern of gene expression of the antioxidant enzymes. So it's very difficult to do studies in this area. Having said that, there are indeed studies that have shown beneficial effects (1-4). If the numbers studied were larger, then the effect would certainly have been clearer, but I think the trend is convincing enough to show there is an effect.

**Dr. Scott:** I believe there may be associations, but they do not prove cause and effect. In your presentation, you made it sound like a proven positive result, but it is not.

**Dr. Sies:** I never said there was a causative effect—in other words that the lack of antioxidants causes Alzheimer disease. That is certainly something entirely different. There is a theory of pathogenesis involving lipid peroxidation in neural cell membranes (5,6), but you are right, this has to be proven in humans and at present that is very difficult to do. On the other hand, without antioxidants life would cease. There is no doubt about that.

**Dr. Scott:** Yes, but one must have properly conducted studies before making statements about benefits. It is possible to do such studies. If scientific journals don't insist on such evidence, then traditions of treatment emerge that are not evidence based and may not have a good scientific basis. Not only may they be untrue, but by clouding the picture, they may even hinder useful treatments from emerging.

**Dr. Sies:** I think there is no doubt that oxidative reactions can be stopped with antioxidants. What you are asking is whether the oxidative reactions are in any way related to the original pathogenesis of a disease. In Down syndrome, for example, there is evidence for excessive activity of superoxide dismutase, so the gene determining SOD activity may be very relevant (7). There are many studies suggesting effects of oxidative reactions in other diseases—Huntington disease is an example—but usually with small numbers, I accept.

**Dr. Micheli:** Is there any way to quantify oxidative stress? There are at least three diseases of the preterm baby where oxidative stress may play a role—retinopathy of prematurity, bronchopulmonary dysplasia, and perhaps necrotizing enterocolitis. It would be very helpful to be able to identify the babies at highest risk.

**Dr. Sies:** A first approximation is the profile of antioxidants in the plasma. If antioxidants are decreased, then this could reflect either a reduced dietary supply or an increased uptake of antioxidants because of reactions with prooxidants. There are some diseases where there are selective patterns of loss of antioxidants, for example cystic fibrosis. The problem is that in many of these diseases the harmful effects are occurring in a specific organ and have little to do with circulating plasma. To obtain information on the rate of generation of prooxidants and the rate of utilization of antioxidants at organ level is very difficult. So what one has to use are the biomarkers for oxidative stress that I showed you for some of the neurologic diseases, which comprise an accumulation of damaged products and are therefore a molecular measure of past oxidative stress. These include the lipofuscins, the carboxymethyl-lysines, malondialdehyde products, and other simple products of the degradation of lipids.
The problem is that, as with the antioxidants themselves, there is no single magic compound that one can measure that will confirm there has been oxidative stress. Oxidative stress occurs in the lipid phase, in the aqueous phase, at the nucleus, or at the mitochondria, so it is very diversified. In order to assess it, one needs different measures of antioxidants and damage products. These assays are relatively difficult because, fortunately for us, prooxidants are scarce. Some assays may be done on exhaled air—ethane and pentane, for example, are markers of lipid peroxidation. These are of course noninvasive, so could be of value in children. There have been clinical studies investigating these volatile products of lipid peroxidation (8).

Dr. Koletzko: Going back to your mention of the potential increase in oxidative stress in Down syndrome, there is already a thriving sales drive in Europe for very expensive megadose preparations of vitamins for people with Down syndrome, promoted by someone who claims to have been a coworker of Linus Pauling. Is there actually any controlled evidence that such an intervention in Down syndrome is justified?

Dr. Sies: There have been studies from Monash University in Melbourne on this topic (9). The problem with this whole field is that it gets taken over by people who do not really understand the subject, just riding the crest of the wave. The basic message of antioxidants is clear to my mind, but the dosage, timing, and applications are not. There is also another aspect, which is the matter of combinations. In fruits and vegetables, for example, there is not a single compound but a mixture of compounds. There are interesting synergistic effects between these compounds, so you need less of one compound if the correct amount of another is present. The best example is the synergism between vitamin E and vitamin C, because the vitamin E radical is repaired by vitamin C, which then becomes vitamin C radical and can be detoxified to nonradical end products. Therefore, the synergism is of great interest.

Dr. Bachmann: Do you know of any neurologic diseases where you think there might be primary involvement of prooxidants rather than secondary changes?

Dr. Sies: The Heidelberg group has formulated an oxidative stress theory of Alzheimer disease. I mentioned already the site for the cleavage of the APP (amyloid precursor protein), but they have also found copper binding sites on the APP, which generate a continual Fenton reaction type of oxidative damage (10). So the idea is that prooxidant generation is the major initial event. I think this is a plausible hypothesis, and the group is trying to find further support for the presence of copper-mediated neurologic disease. For example, no one knows the function of the prion protein, but several groups have now shown that it is a copper-binding protein (11-14); perhaps when it is falsely folded it can become an oxidant.

Dr. Bachmann: Have you looked at Wilson disease in that context?

Dr. Sies: Other people have of course looked at Wilson disease, and there is clear evidence of nitrotyrosine formation in that condition and in hemochromatosis. In all the metal diseases where there is inadequate binding and an increase in free metal, there is an oxidative component in a very prominent pathogenic position.

Dr. Kon: Is there any possibility of measuring metabolites of nitrotyrosine?

Dr. Sies: There are some studies measuring 8-oxo-dG in different parts of the brain. With immunocytchemistry, it is readily demonstrated that nitrotyrosine is formed at sites where you also have DNA damage. Therefore, these are very nice indicators that oxidative damage has taken place. In addition, when preventive treatment is given, there is a decrease in nitrotyrosine formation.

Dr. Scott: I want to return to the question of evidence-based medicine. There is a theory that raised homocysteine is a significant cause of Alzheimer disease, in that it might cause microvascular-type strokes. That is a plausible theory, and there is no doubt that people with
Alzheimer disease have elevated homocysteine. However, the groups that are putting that theory forward are doing a proper placebo-controlled trial, intervening with folic acid to see if it’s true. That is the kind of burden of proof for Down syndrome or Alzheimer disease that we should be looking for from our colleagues. I do not think we should permit people who have studied a few cases of Down syndrome to say that it looks as though some intervention has worked. We need to insist that these studies are properly conducted and that they are not published unless that is the case. The public needs that reassurance from the scientific community. Many things are plausible and might happen, but proof must be provided.

Dr. Sies: It’s a good thing to be scientifically critical, but one can easily turn this around the other way and say, as you said earlier today, that homocysteine might just be a marker of something else rather than a causative factor—but that would still be of interest. The papers I referred to on Alzheimer disease and so on were peer-reviewed and in good journals. I agree that what is translated into journalism and to the general public is the responsibility of the scientist, but I think these initial studies are excellent pilot studies, showing us the way to go in future research. Homocysteine is much more oxidizable than cysteine, and it could be that the key to an elevated homocysteine is an increased radical attack. In the end, we may find important links between our fields of homocysteine and antioxidants.

Dr. Scott: To resolve the issue of whether homocysteine is a marker or a risk factor, there are seven or eight trials in progress at the moment, so an evidence-based approach is being used which will, I think, resolve the issue.

Dr. Kunz: Many of the antioxidants that have been used in clinical studies are synthetic components, and it is known that their effects may be different from natural antioxidants. Could you comment on that?

Dr. Sies: Such antioxidant compounds are used because a patent can be applied. You cannot patent ascorbate or vitamin E in this day and age. The antioxidants selected for use in clinical studies are used as pharmacologic agents. Some of them are quite interesting, particularly N-acetylcysteine. It is clear that redox reactions occur in all cells and the redox balance can be regained with such antioxidants (15,16).

Dr. Daniel: With the experiences of the large β-carotene supplementation trials, nutritionists and health officials are increasingly concerned about the widespread use of supplements, particularly the enrichment of diets with vitamin A, vitamin C, and vitamin E. You mentioned that when metals are present in a free state they produce a prooxidant environment. I believe a study from Düsseldorf has shown that the incidence of iron overload in men is almost the same as the incidence of iron deficiency in women (17). Do we have to worry about men who have a particularly large amount of free iron?

Dr. Sies: Iron overload in men is relevant to the development of cardiovascular problems and should be taken seriously. Regarding β-carotene, there is certainly a plausible explanation for why high β-carotene levels, 4 to 5 μmol/l or more, might be detrimental, whereas in the normal range it has an important protective function (18). For example, it is essential in terms of protection against photooxidative reactions in the skin and the eye, and, I’m sure, in other organs. Your question was on levels, and how high one should go when carrying out a study. Should one use high doses to ensure an effect and risk the possibility of unforeseen potential side effects? These problems are important in designing a study.

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