Antioxidant Therapy: Current Status and Future Prospects

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Abstract: Reactive oxygen species (ROS) are widely believed to cause or aggravate several human pathologies such as neurodegenerative diseases, cancer, stroke and many other ailments. Antioxidants are assumed to counteract the harmful effects of ROS and therefore prevent or treat oxidative stress-related diseases. In this report, recent human studies exploring the efficiency of antioxidants in prevention and treatment of various diseases are reviewed. Few antioxidants including edaravone (for ischemic stroke in Japan), N-acetylcysteine (for acetaminophen toxicity), alfa-lipoic acid (for diabetic neuropathy) and some flavonoids (polyphenolic compounds present in dietary plants), such as micronized purified flavonoid fraction (diosmin and hesperidin) and oxerutins (for chronic venous insufficiency) as well as baicalein and catechins (for osteoarthritis) have found accepted clinical use. However, despite much enthusiasm in the 1980s and 1990s, many well-known agents such as antioxidant vitamins and also more recently developed compounds such as nitrones have not successfully passed the scrutiny of clinical trials for prevention and treatment of various diseases. This has given rise to a pessimistic view of antioxidant therapy, however, the evidence from human epidemiological studies about the beneficial effects of dietary antioxidants and preclinical *in vitro* and animal data are compelling. We have probably wasted too much time on agents like antioxidant vitamins instead of focusing on more disease specific, target-directed, highly bioavailable antioxidants. We here discuss possible reasons for the lack of success in some clinical trials and seek to provide some suggestions to be considered if antioxidant therapy is to succeed as an effective therapeutic strategy.

Keywords: Antioxidant, therapy, clinical trial, vitamin A, vitamin C, vitamin E, edaravone, idebenone, polyphenolic, N-acetylcysteine, Lipoic acid.

INTRODUCTION

Reactive Oxygen Species and Oxidative Stress

Reactive oxygen species (ROS) is a generic term used for a variety of molecules derived from oxygen that react with most of biomolecules and oxidize them. ROS include free radicals such as hydroxyl radical ($OH^{\cdot}$), superoxide anion radical ($O_2^{\cdot-}$) and nitric oxide ($NO^{\cdot}$) as well as non-radicalic molecules such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and peroxynitrite ($ONOO^-$) [1, 2].

Free radicals are atoms or molecules with one or more unpaired electrons that are capable of independent existence (reason of term "free"). The unpaired electrons make free radicals extremely reactive towards other molecules [1]. The reactivity of free radicals varies and their half-lives can range from only one nanosecond for hydroxyl radical to a few seconds. Non-radicalic species in general are more stable, but this can add to their harmful effect, since they can travel longer (as in the case of H_2O_2) and reach more distant targets [3].

Oxidation is a chemical process that involves gain of oxygen or loss of electrons. Oxidation of biomolecules causes them to become damaged and then degraded by physiological processes or malfunction [1].

Free radicals are also involved in important physiological processes; nitric oxide (NO) is protective in vasculature and is an important neurotransmitter in the nervous system [4] and oxygen free radicals are vital to the immune system and important for gene expression, signal transduction and growth regulation [5].

Since we are in constant contact with oxygen, ROS are continuously produced in our body [6], but they are always kept under control and their effect is counteracted by physiological antioxidant defense mechanisms that intercept the ROS, or repair the damage that has already occurred by them. Under normal conditions, the potentially harmful effect of the ROS is successfully restrained by the defense mechanisms. However, the balance between ROS production and antioxidant protective mechanisms may be disturbed in favor of the ROS and a situation called oxidative stress ensues [1, 7, 8] (Fig. (**1**)).

Oxidative stress is now widely believed to be involved in the pathogenesis of major age-related diseases such as neurodegenerative diseases [9-16], cancer [17, 18] and a long list of several other human diseases such as ischemia-reperfusion injury [19], stroke [20, 21], hypertension [3], diabetes [22], rheumatic diseases [23-25] and multiple sclerosis [26].

It is also interesting to observe the oxidative stress problem from an evolutionary point of view [27, 28]. The ROS are very useful for killing of bacteria and viruses [29]. When humans were struggling against epidemics of infectious diseases several centuries ago, it was important to maintain a strong immune system and therefore a heavy use of ROS was a priority of evolution to keep young people from dying. Diseases like cancer and neurodegenerative diseases that are caused by an overproduction of ROS were not important at that point because they happen after the reproductive age and therefore do not affect the survival of human race [27, 28].

Use of Antioxidants

An antioxidant has been defined by Halliwell and Gutteridge as "any substance that delays, prevents or removes oxidative damage to a target molecule" [1]. This definition includes either small molecules such as uric acid or large molecules like albumin. Antioxidants prevent oxidative stress by counter balancing the harmful effects of ROS and therefore it is logical to assume that they are useful in oxidative stress-related disease.

Since the ROS are by definition very reactive towards other molecules, most chemical compounds can react with the ROS and neutralize them. However, a good antioxidant is a molecule that reacts with the ROS at low concentrations and the product of its oxidation is either a stable chemical or can be easily recycled back to an active antioxidant. Other vital characteristics for a compound to act as a good antioxidant *in vivo* is its ability to achieve sufficient concentrations at sites it is supposed to act and also its solubility profile [30].

Antioxidants could be divided to endogenous molecules that are naturally synthesized in the human body or exogenous compounds

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that are mostly produced in plants and are taken up by humans from the diet [28] (Fig. (**1**)).

Epidemiological Studies of Antioxidants

Large prospective cohort epidemiological studies have shown that higher intake of antioxidants in the diet is associated with lower risks of coronary heart disease, certain cancers [31-36] and neurodegenerative diseases [37-39].

Although, the presence of antioxidants has been claimed by many to be responsible for the beneficial effect of vegetables and fruits, it has also been postulated that low content of fat in these foods may be the responsible cause. Most of these studies generally agree on the notion that antioxidants are much more effective in prevention of disease, rather than in the treatment of an already established active pathology.

Aim of the Study

There has been much enthusiasm in the field of free radicals. Antioxidants have been advocated for therapy of a vast range of serious diseases in the 1980s and 1990s, however, in the light of recent negative findings, many doubts have now been raised about the usefulness of administration of single antioxidants [40-48]. Therefore, it is timely to evaluate the recent clinical evidence supporting the use of antioxidants and outline the fields that antioxidants are more likely to be effective.

The aim of this article is to review our current knowledge about the antioxidants that are in clinical use for treatment or prevention of diseases or are close to be approved for use in human. We have also included the last clinical findings about antioxidant vitamins and other antioxidants that have not made their way to routine clinical use, in spite of huge initial enthusiasm. We have looked for explanations for several instances of failure of antioxidant therapy and have provided some future directions, based on our current knowledge.

This review mainly focuses on the last evidence gathered by interventional clinical trials for primary prevention or treatment of diseases. However, covering all published reports in the field of antioxidant therapy is impossible in one review, because of the tremendous number of studies conducted in the recent years. Therefore, we have had to remain mainly focused on larger and more recent clinical studies.

There also exist many antioxidants in the market that should be classified under the category of food supplements and have not been the main scope of this article.

Few concise review articles have been published in the past few years about the general subject of antioxidants and antioxidant therapy [48-50]. We here present a more extensive review of the subject that gives more weight to the antioxidants that are currently in clinical use and also a detailed discussion of the reasons that could explain the lack of success of some clinical interventions with antioxidants.

CLINICAL STUDIES OF ANTIOXIDANTS

Numerous studies have been conducted on various antioxidant agents. We here discuss the last clinical evidence on those already approved for routine clinical use (Table **1**) and also other antioxidants that have been extensively investigated. Food supplements that are widely available on the market also include many antioxidants, but those that are not specifically used for treatment or prevention of specific diseases have not been covered in this review.

Edaravone

Edaravone (3-methyl-l-phenyl-pyrazolin-5-one, MCI-186, Radicut®) (Fig. (**2**)) initially developed by Mitsubishi Tanabe Pharma Corporation (Osaka, Japan) has been approved in Japan since 2001 for reduction of neuronal damage after acute ischemic stroke [51]. Edaravone was developed in the process of searching for "phenol-like" compounds with antioxidant properties. This pyrazolin containing molecule undergoes keto-enol tautomerization and generates phenolic structure [51]. Half of edaravone exists in an anionic form at physiological pH, which is the form that strongly reacts with the ROS in the brain [52]. The products of the reaction

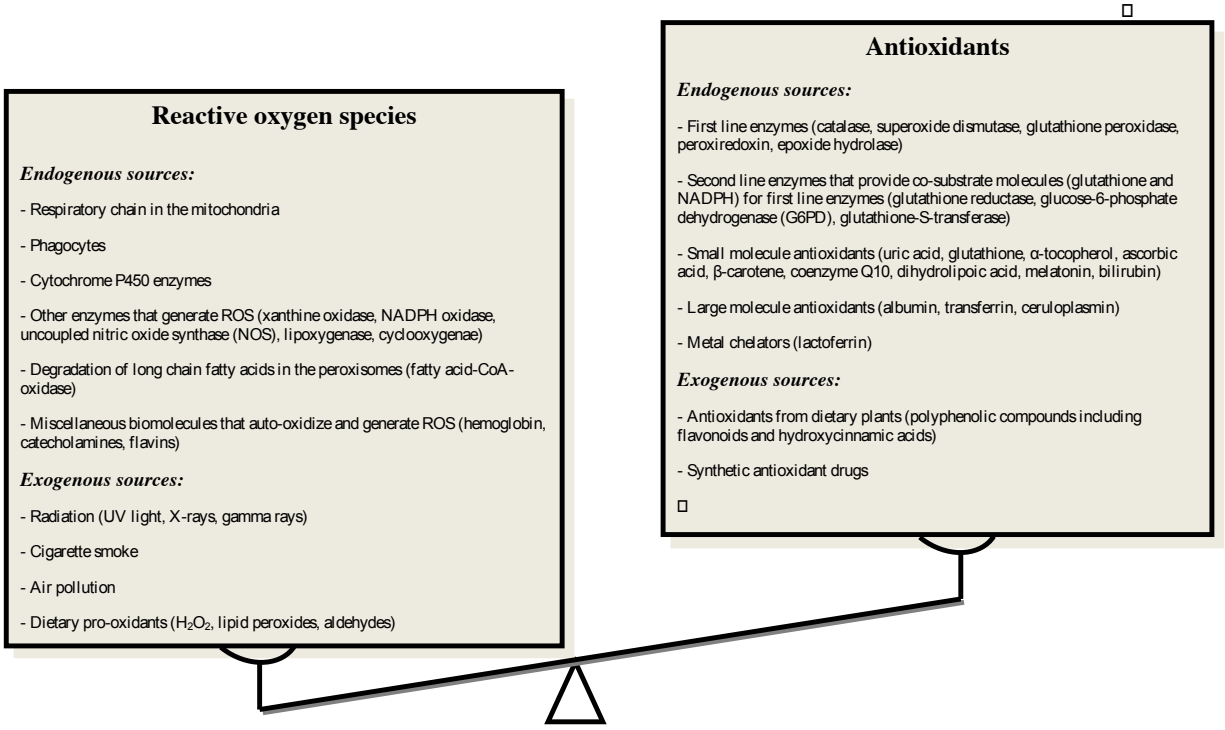

Fig. (1). Antioxidants can counteract the harmful effects of reactive oxygen species.

Table 1. Antioxidant Drugs Approved for Clinical Use in Various Diseases

Antioxidant	Clinical use
Edaravone	Ischemic stroke
Idebenone	Alzheimer disease (?)
N-Aceylcysteine	Acetaminophen overdose, mucolytic, dry eye syndrome
□-Lipoic acid	Diabetic neuropathy
Micronized purified flavonoids fraction (MPFF, Daflon 500®)	Persistent venous ulcers
0-□-hydroxyethyl-rutosides (Venoruton®)	Chronic venous insufficiency
Silibinin (Leaglon®)	Hepatoprotective (?), chemopreventive
Baicalein and catechins (flavocoxid)	Osteoarthritis

of edaravone with free radicals are stable and do not cause oxidation [53].

ROS play an important role in ischemia-reperfusion injury in stroke patients, which is mainly induced by peroxidation of membrane lipids that ultimately leads to neuronal and endothelial cell damage and brain edema [20, 21]. Therefore antioxidants that counteract the effects of ROS are supposed to exert neuroprotective effects. Edaravone has shown *in vitro* antioxidant activity against ROS such as singlet oxygen [54], hydroxyl radical [55, 56] and other ROS [57]. Edaravone has been reported to reduce oxidative damage in rodents' brain after ischemic injury [58] (reviewed by Watanabe and colleagues [51]).

Thrombolytic therapy is the most reputable treatment strategy for ischemic stroke. The aim of thrombolytic therapy is to reestablish the CNS blood flow in the shortest possible time to limit the infarct area and rescue the parts of the brain that are still viable (ischemic penumbra) [59]. However, ischemia-reperfusion injury and free radical damage that follows the recanalization process is very harmful and should be counteracted by neuroprotective agents. Therefore, thrombolytic therapy should be combined with neuroprotection to be more successful in the management of stroke patients [60].

Clinical studies performed on edaravone are limited only to investigations conducted in Japan, since this drug has been available only in this country. However, around 500,000 of stroke patients have been treated with this drug, which provides a very good level of post-marketing experience [51].

In a placebo-controlled double-blind randomized controlled trial (RCT) conducted on 252 ischemic stroke patients, edaravone significantly improved the functional outcome of patients [55]. Other authors have been able to show by magnetic resonance imaging (MRI) that administration of edaravone in 6 patients with extensive ischemic stroke rescues the boundary zone of infarct and reduce brain edema [61]. Very recently, in a retrospective study of 72 patients with acute ischemic stroke, edaravone was shown to dose-dependently enhance the functional recovery [62].

Clinical studies on patients with acute lacunar infarction have also shown efficacy of edaravone in improving the functional outcome; In one recent study on 124 participants, combination of edaravone and conventional therapy was superior to conventional therapy and significantly improved the outcome (especially motor palsy) of patients [63]. A previous study on 70 patients of acute lacunar infarction had similarly shown that administration of this drug improves the functional outcome in these patients [64].

However, there are also studies that do not agree with the above mentioned investigations. The results of a study conducted on 141 patients of cardioembolic stroke that were treated with edaravone and were retrospectively compared with a historical control cohort of 114 patients, early functional improvement was seen only in patients with a mild stroke and not in moderate to severe cases. No improvement was observed in the late stage in this study [65]. In another study conducted on a total of 61 participants of severe carotid-territorial stroke, patients receiving edaravone, when compared to a historical control cohort group, showed delayed formation of infarct and edema and decreased mortality in the acute stage. However, no effect on development of infarct and edema or improvement in the functional outcome was observed in the late stage [66].

Edaravone has also shown efficacy in neonatal hypoxic-ischemic encephalopathy. Interestingly, short term administration of this drug was reported to be more effective than its long term use [67].

Edaravone also reduces brain edema in acute ischemic stroke patients. Mechanisms such as inhibition of vascular endothelial growth factor [68] or inhibition of aquaporin-4 (a membrane water channel) expression [69] have been suggested for this effect.

Oxidative stress is a pathological mechanism shared by many diseases. Therefore, the good thing about the antioxidant drugs is that once they are developed for one disease, they can also be potentially useful for other apparently dissimilar pathologies. Edaravone has been used successfully in animal models of extracerebral diseases such as amyotrophic lateral sclerosis (ALS) [70] ischemic injury to spinal cord, kidney and intestine and a number of other pathologies (reviewed by Watanabe and colleagues [51]. It has also shown beneficial effects in human studies; It was able to prevent cerebral hyperperfusion after carotid endarterectomy [71] and decreased reperfusion injury in acute myocardial infarction patients [72] (reviewed by Higashi and coworkers [73].

Edaravone is a safe drug. In approximately 500000 individuals treated with this agent in 4 years, adverse effects have been observed only in 0.1% of patients [51].

Some studies have reported nephrotoxicity in patients receiving edaravone, but the precise role of edaravone in these cases needs further clarification [74]. It has been reported that 45% of cases of renal toxicity caused by edaravone ultimately recover renal function [74].

Idebenone

Idebenone (2,3-dimethoxy-5-methyl-6-(10-hydroxydecyl)-1,4-benzoquinonenoben, SNT-MC17, CV-2619), is a short chain benzoquinone that is structurally related to coenzyme Q10 (ubiquinone) (Fig. (**2**)) and is a potent antioxidant and electron carrier [75]. It was originally developed by Takeda Pharmaceuticals Company Limited (Osaka, Japan) and was approved in Japan in 1986 for treatment of Alzheimer's disease and other cognitive disorders [76],

Although idebenone have shown efficacy in Alzheimer diseases in some studies [77, 78] and has been better than tacrine [79], due to the lack of sufficient evidence [80] its clinical use for this purpose has been limited. According to the WHO Collaborating Centre

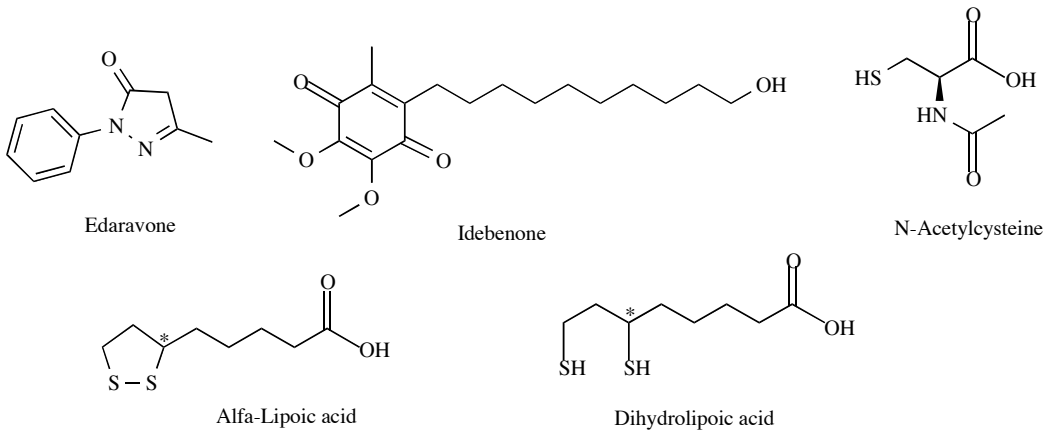

Fig. (2). Structures of some of the antioxidants of common clinical use.

for Drug Statistics Methodology (http://www.whocc.no/), idebenone is classified a psychostimulant and nootropic by the Anatomical Therapeutic Chemical classification system and the Defined Daily Dose (ATC/DDD) methodology.

Idebenone is an analog of coenzyme Q10 that carries a benzoquinone ring. The benzoquinone ring participates in redox reactions. Idebenone is able to function as an antioxidant by inhibition of lipid peroxidation and protection of cell membranes and mitochondria from oxidative stress [81-83]. It is also able to interact with the respiratory chain and electron balance in the mitochondria [76, 84]. It is not very clear which one of the two (antioxidant activity or electron transportation) is the main mechanism responsible for beneficial effects of idebenone [12].

Idebenone has been further developed by Santhera Pharmaceuticals (Liestal, Switzerland) with the trade names of Catena® and Sovrima® for treatment of Friedreich's ataxia (FRDA) and Duchenne muscular dystrophy (DMD).

Several clinical studies have been conducted on the efficacy of idebenone in treatment of FRDA (reviewed in 2 recent articles [76, 84]). In these studies, idebenone consistently improved cardiac hypertrophy, which is an important feature of FRDA. Neurological symptoms have not been improved as much as cardiac problems, however higher doses of idebenone especially in younger patients seem to be much more promising for neurological efficacy [76, 85-87].

In a large double-blind phase III RCT that enrolled 232 patients with FRDA (MICONOS; Mitochondrial Protection with Idebenone In Cardiac Or Neurological Outcome Study) the efficacy and safety of idebenone was tested over a period of 12 months. Idebenone was safe but unfortunately, did not induce any significant change in the functional outcome of treated patients compared to the placebo group [88]. However, in a meta-analysis of 3 Phase II and III studies, trends towards improvement in the neurological function were identified [88].

In a phase II clinical trial on patients with Duchenne muscular dystrophy, idebenone improved the functional outcome including cardiac and respiratory parameters [89].

Other coenzyme Q analogs are currently being developed. One of these analogs, mito-Q has a lipophilic triphenylphosphonium cation conjugated to ubiquinone [90].

Since the launch of idebenone in 1986 till 2009, approximately 8 million people have been exposed to this drug [76]. Some patients have received very high doses (up to 1080 mg) for extended periods of time and overall this drug has been well tolerated. The most common adverse effects are gastrointestinal, while neurotoxicity and cardiotoxicity have not raised concern [76].

N-Acetylcysteine

N-Acetylcysteine (NAC, Acetadote®) (Fig. (**2**)) has been approved by FDA and other regulatory authorities as an antidote for treatment of acetaminophen (paracetamol) overdose. It has also been clinically used as eye drops for dry eye syndrome in the U.K.. NAC has also been used as a mucolytic agent (Mucomyst®) since several decades ago [91] and is now one of the highly prescribed drugs in pediatric patients in Europe. It has also been suggested for therapy of several oxidative stress-related diseases [92].

NAC is the acetylated form of the amino acid cysteine. It is a direct ROS scavenger and also provider of amino acid cysteine, which is the precursor for the rate-limiting step of the synthesis of glutathione (GSH, a tripeptide consisting of cysteine, glycine and glutamate). This function is important for example in acetaminophen poisoning, where hepatic intracellular stores of GSH are depleted and the liver remains vulnerable to oxidative stress [93]. GSH is an important antioxidant that together with enzymes glutathione peroxidase and glutathione-S-transferase participates in cell defense against oxidative stress [92]. NAC has a high first-pass effect in the liver after oral administration and therefore its plasma levels are probably very low. Consequently, it is more likely that NAC is mainly effective through induction of GSH synthesis rather than direct involvement in ROS scavenging [94].

Some systematic reviews and meta-analyses have suggested that NAC prevents exacerbations and improves symptoms in Chronic obstructive pulmonary disease (COPD) patients [94-98] (Table **2**), however, probably with little or no effect on the lung function parameters [96].

The BRONCUS study (Bronchitis Randomized on NAC Cost-Utility Study), a large multicenter trial, showed that oral administration of NAC at a dose of 600 mg daily is ineffective at improvement of lung function and prevention of exacerbations in COPD patients [99]. The low efficiency of NAC shown in the BRONCUS trial might be explained by the relatively low dose (600 mg once daily) used in that study [95]. Other studies such as Idiopathic Pulmonary Fibrosis International Group Exploring N-Acetylcysteine I Annual (IFIGENIA) study have suggested that 600 mg twice daily is safe and more effective [100].

The efficiency of NAC in prevention of radiographic contrast induced nephropathy has been the subject of numerous clinical studies. Several recent systematic reviews and meta-analyses have been published on this subject; some of these reports have found no significant benefit for this compound [101], some have concluded that the results are mixed and inconclusive [94], while others have suggested that NAC protects patients from nephropathy [102, 103].

Table 2. Recent Meta-Analyses of Randomized Controlled Trials (RCTs) of N-Acetylcysteine in Various Diseases

Publication	Number of RCTs	Number of randomized patients	Disease	Results of treatment with NAC	Conclusion
Adabag, et al. 2009* [261]	10	1,163	Post- cardiac surgery	No significant change in ARI incidence, hemodialysis rate or mortality. A trend towards reduced ARI in subjects with baseline chronic kidney disease.	No conclusive evidence on the benefit of treatment
Baker, et al. 2009* [262]	13	1,338	Post-cardiothoracic surgery	Significantly lower incidence of atrial fibrillation. No significant effect on myocardial infarction, stroke, acute renal injury, need for renal replacement therapy, mortality and total hospital length-of-stay.	Treatment partially beneficial
Duijvestijn, et al. 2009 [109]	6	497	Acute upper and lower respiratory tract infections in children without chronic bronchopulmonary diseases	Significant reduction of cough after 6-7 days, but not other symptoms.	Some benefit with little clinical relevance
Nigwekar, et al. 2009* [263]	12	1,324	Post-cardiovascular surgery	No significant change in the incidence of ARF or ARF requiring dialysis, mortality, length of intensive care unit stay, postoperative serum creatinine, creatinine clearance. A trend towards reduction in ARF in studies using intravenous NAC.	No conclusive evidence on the benefit of treatment
McKay, et al. 2008 [110]	6	-	Liver transplantation	Some studies showed improvement in biochemical parameters. No improvement of clinical outcome. Heterogeneous outcome measures and limited sample sizes of studies prevented pooling of the data.	No conclusive evidence on the benefit of treatment
Naughton, et al. 2008* [264]	7	1,000	Post- cardiac surgery	No significant effect on postoperative indices of renal function, mortality, myocardial infarction, atrial fibrillation, stroke. Small significant increase in postoperative blood loss.	No conclusive evidence on the benefit of treatment
Ho et al., 2008 [265]	10	1,193	After major surgery without the use of radiocontrast	No significant decrease in mortality, ARF requiring dialysis or length of intensive care unit stay.	No conclusive evidence on the benefit of treatment
Sutherland et al., 2006 [266]	8	2,214	Chronic obstructive pulmonary disease (COPD)	Significant reduction of the odds of exacerbations.	Treatment beneficial

The meta-analyses of radiographic contrast induced nephropathy are discussed in the text and are not reported here. ARF: Acute renal failure; ARI: Acute renal injury; NAC: N-Acetylcysteine; RCT: Randomized controlled trial.
* Several reports used by these analyses are in common with one another.

In a study by Bagshaw and colleagues, the results of 11 meta-analyses of studies exploring the role of NAC in prevention of contrast-induced nephropathy published before 2006 were gathered [104]. Seven reports found that NAC treatment was beneficial, while 4 of them found the data inconclusive. It has been claimed that there is a significant publication bias on this subject and treatment-effect estimate presented by published manuscripts is more optimistic than that found in unpublished abstracts [105].

In a review of different clinical studies of NAC by Aitio, it was concluded that NAC might be of benefit for immunodeficiency virus infection (see also Table **2**). However, it seems to be ineffective in prevention of cancer recurrence, acute hepatic failure and in intensive care [94]. NAC has also been suggested for treatment of cystic fibrosis [106], idiopathic pulmonary fibrosis (IFIGENIA study) [100] and recently for psychiatric disorders such as depressive symptoms in bipolar disorder [107] and schizophrenia [108]. All these new implications seem very promising, however, further studies are needed before NAC can be routinely used for clinical purposes.

NAC seems to be a safe drug with limited side effects that appear to be mostly anaphylactoid in nature [106, 109, 110].

□-Lipoic Acid

□-Lipoic acid (LA) (Fig. (**2**)) is a naturally occurring dithiol compound that is known as an essential cofactor for mitochondrial bioenergetic enzymes and has different biological properties [111]. LA and its reduced form, dihydrolipoic acid are important endogenous antioxidants (Fig. (**2**)). LA (dexlipotam) has been clinically approved and used for diabetic neuropathy [111, 112]. It has been used in Germany for treatment of symptomatic diabetic neuropathy since several years ago [113, 114]. According to a meta-analysis performed on 4 RCTs (ALADIN I, ALADIN III, SYDNEY, NATHAN II) including 1,258 patients of diabetic polyneuropathy, LA administration for 3 weeks significantly improved symptoms in the feet [115].

Some medications such as zycose that also includes LA has recently been introduced and marketed for management of diabetes [116]. LA has also been shown to improve endothelial function in diabetic patients [117].

Clinical trials of LA in Alzheimer disease are presented in Alzheimer section.

Flavonoids

Flavonoids are a large group of naturally occurring phenolic compounds, which are present at high levels in human diet. They have been extensively studied for their vast antioxidant properties *in vitro* [118-120] and many other biological activities including antitumoral, cardioprotective and antiinflammatory properties [121].

Epidemiological studies have shown that higher dietary intake of flavonoids is protective against cardiovascular diseases [31-35, 122, 123], certain cancers [124] and some other chronic diseases [125].

In a meta-analysis of 133 RCTs exploring the effects of flavonoid-rich foods containing different subclasses of flavonoids on the risk of cardiovascular diseases, it was reported that chocolate improved endothelial functional and lowered blood pressure, while soy protein isolate significantly reduced diastolic blood pressure and LDL cholesterol. Green tea seemed to have some beneficial effects on cholesterol levels, but black tea did not and it even increased blood pressure after acute consumption. However, the data on other flavonoid-rich food sources were not conclusive [126]. Other authors have also reviewed the effects of different flavonoid-rich foods on various human diseases [127].

In addition to these prospective epidemiological studies, preclinical *in vitro* and animal studies have also provided overwhelming evidence about the beneficial effects of flavonoids [121]. However, many flavonoids have failed to pass the scrutiny of clinical trials. In a review of reports by Halliwell and colleagues on the *in vivo* effects of flavonoids fed to human volunteers [128], it was concluded that the sum of evidence does not support the notion that absorbed flavonoids exert systemic antioxidant effect.

Consumption of flavonoid-rich foods increases the total antioxidant capacity of plasma, but it has been argued that this increase may be caused by other reasons such as increased urate in plasma rather than flavonoids [129].

The pharmacokinetics of flavonoids is also an important issue to take into consideration. Flavonoids are extensively metabolized in the liver and GI tract and their hydroxyl group is usually blocked by methylation, glucuronidation and sulphation [130]. The unconjugated form of flavonoids in the systemic circulation rarely reaches the concentration of 1 μM and the conjugated forms are much less potent antioxidants, therefore it is plausible not to expect powerful systemic activity after consumption of flavonoids [27].

However, flavonoids can be very useful for protection of the gastrointestinal tract, since they have much higher concentrations in the stomach and intestine compared to blood levels. They have micromolar concentrations in the rectal water [131]. Since GI tract is constantly exposed to exogenous and endogenous ROS, the presence of flavonoids can be very useful and may explain the lower incidence of gastric and colonic cancer among people who consume more flavonoids in their diets [27].

Here we discuss some classes of flavonoids that have been more successful than others and some of them have also found established clinical use (Table **1**).

Green Tea Catechins

Green tea catechins (GTC) have shown efficiency as chemopreventive agents of prostate cancer in subjects with premalignant lesions of prostate [132]. However, they have shown less efficiency in chemotherapy of prostate cancer (reviewed by Khan and colleagues [133]).

GTC have been reported to have cardioprotective effects, however antioxidant activity may not be the exclusive reason for these effects, because GTC have also several other biological properties including anti-inflammatory, anti-thrombogenic, anti-proliferative and lipid lowering effects [134].

GTC have also shown beneficial effects on abdominal fat loss in several RCTs [135-137].

Soy Isoflavones

Soy isoflavones are an important class of flavonoids that include genistein and daidzein, also classified as phytoestrogens, that have been extensively studied for their beneficial effects on endothelial function [138], osteoporosis [139], endometrial hyperplasia [140], cardiovascular system and homocysteine levels [141].

A meta-analysis of 9 RCTs in postmenopausal women, showed that isoflavone improves endothelial function in women with low baseline flow-mediated dilatation (FMD), a marker of endothelial function, but not in women with high baseline FMD levels [138]. Another meta-analysis of RCTs assessing the effects of soy isoflavone supplementation on bone turnover markers in postmenopausal women, revealed that isoflavone moderately decreased urinary deoxypyridinoline (DPD), a bone resorption marker, but did not affect serum bone alkaline phosphatase and serum osteocalcin, 2 bone formation markers [139]. It should be mentioned that some of the above mentioned effects in postmenopausal women, can be best ascribed to the estrogenic effect of isoflavones and not their antioxidant capacity.

Genistein aglycone has also shown positive effects on endometrial hyperplasia in premenopausal women [140] and on some cardiovascular risk factors and homocysteine levels in postmenopausal women [141].

An RCT conducted on 180 postmenopausal women with prediabetes or early untreated diabetes, treated with soy protein with or without isoflavone supplementation for 6 months, did not find any evidence of favorable effects on glycemic control and insulin sensitivity [142].

Quercetin

Large clinical trials of quercetin are very scarce. In an RCT that was conducted in 1,002 participants, oral quercetin administration for 12 weeks had no significant effect on upper respiratory tract infection rates or symptoms, however, a significant decrease in total sick days and severity of symptoms was found in middle aged and older subjects with high self-reported physical fitness level [143].

In a small RCT recruiting 93 individuals, quercetin showed blood pressure lowering effect in overweight patients [144].

In a review of *in vitro* and animal studies performed by Ossola and colleagues [145], it was concluded that quercetin is unlikely to have any significant efficacy in neurodegenerative disorders and it is better to focus on its application in cerebrovascular diseases.

Micronized Purified Flavonoids Fraction

A micronized purified flavonoid fraction (MPFF) named Daflon 500® (Servier, Neuilly-Sur-Seine, France) consisting of 90% diosmin and 10% flavonoids expressed as hesperidin, has been reported to protect the microcirculation [146, 147] and has been approved for clinical use and widely marketed in different countries.

MPFF undergoes a special pharmaceutical formulation that "micronizes" the drug particles with microwave and makes them more readily absorbable form the gastrointestinal tract and as a result increases the bioavailability [148].

MPFF has been successfully used for treatment of venous leg ulcers caused by microcirculation damage induced by increased ambulatory venous pressure [146, 147, 149, 150] and seems to have the highest clinical benefit among various pharmacological agents in patients with venous diseases [151]. In a meta-analysis of 5

RCTs including 723 patients with venous ulcers who were treated with MPFF, the chance of healing ulcer was 32% better in individuals treated with adjunctive MPFF compared to those treated only with conventional therapy. MPFF treatment was also associated with a shorter time to healing [146]. Other RCTs are reviewed by Kearon and colleagues [152].

Oral MPFF is suggested by American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition) for persistent venous ulcers [152] and has only minor side effects [153]. Another antioxidant, pentoxifylline, is suggested by the American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition) for venous leg ulcers [152].

MPFF, aside from its venotropic effects, has been suggested for other purposes; In a randomized trial recruiting 43 patients with impaired cardiac function who underwent coronary artery bypass grafting, patients who received MPFF showed improvement in some of their clinical and paraclinical parameters [154].

Hydroxyethylrutosides

Hydroxyethylrutosides, also called oxerutins and 0-β-hydroxyethyl-rutosides are semisynthetic hydroxyethyl esters of the famous natural flavonoid rutin (or rutoside). Among these compounds, trihydroxyethylrutoside (troxerutin) has been studied more for its phlebotropic effects [155]. Hydroxyethylrutosides have been successfully used for management of venous diseases [151, 156] and they are presumed to act on the microvascular endothelium and decrease hyperpermeability and edema [157]. Commercially available approved drugs in Europe including Venoruton®, Paroven® and Relvène® are mixtures of hydroxyethylrutosides and are prescribed for chronic venous insufficiency.

Silibinin

Silibinin or silybin is a natural flavonoid that constitutes the major flavonoid of silymarin. Silymarin is a special extract from the fruits of milk thistle (*Silybum marianum*) plant consisting of 3 flavonolignans; silibinin, silydianin, and silychristine. Silibinin is the component with greatest biological activity [158].

Silibinin was once thought of as a single compound, but now is considered as a 1:1 mixture of 2 diastereoisomers, silybin A and silybin B [159].

Silybum marianum is the most extensively studied plant for the management of liver disease [158] and silymarin was first introduced as a hepatoprotective agent (Leaglon®). However, the data on its hepatoprotective activity are mixed and inconclusive.

In a meta-analysis of 14 RCTs that included 1,209 patients with viral, alcoholic and mixed liver diseases, silymarin appeared to be safe and well tolerated, but it did not decrease mortality or improve histology and biochemical liver markers [160]. In another more recent systematic review of clinical trials on silymarin, no evidence was found on the efficiency of silymarin on the evolution of viral hepatitis. However, it was able to significantly reduce some liver enzymes in alcoholic liver disease and in liver cirrhosis patient. Liver-related mortality was significantly decreased in treated patients [161].

Silibinin has shown promising chemopreventive effects in *in vitro* and animal studies [162-164].

It appears that routine use of silibinin or silymarin as hepatoprotective cannot be suggested based on actual clinical evidence, but there is some evidence on the cancer preventing role of silibinin or silymarin in *in vitro* and animal studies.

Flavocoxid

Flavocoxid (Limbrel®) is a proprietary mixed plant extract that contains a 90% pure standardized blend of flavonoids baicalein and catechin and has been used for management of osteoarthritis. It has been classified as a medical food product by FDA [165]. Flavocoxid has dual cyclooxygenase (COX)/5-lipoxygenase (5-LOX) inhibitory activity and also acts on several inflammatory pathways [166]. It has also been reported as effective as naproxen in treatment of knee osteoarthritis [165].

A large open-label post-marketing study named Gauging Osteoarthritis with Limbrel (GOAL), that recruited 1,067 individuals with osteoarthritis demonstrated efficacy in the management of OA and reduction of adverse GI effects for this medication [167].

Other Polyphenolic Compounds

Resveratrol

Resveratrol is a polyphenolic phytoalexin found at high levels in grapes and red wine. It has been reported to have many biological activities and protect against Alzheimer's disease [168, 169] and other diseases including chemoprevention of cancer [170].

Curcumin

Curcumin is a yellow pigment present in the rhizomes of turmeric (*Curcuma longa*) and it has long been used as a food additive and spice in India and elsewhere in the world [168]. Preclinical studies have shown that curcumin has antioxidant and anti-inflammatory properties [171] and it has been proposed as a therapeutic for Alzheimer's diseases [168, 169].

Clinical studies have also shown that curcumin is safe and well tolerated and have suggest a potential therapeutic role in diseases such as colon and pancreatic cancer, inflammatory bowel disease and many other inflammatory diseases [171].

Vitamins

A vitamin is an organic chemical that does not belong to the major groups of food substances (carbohydrates, protein and fat) and is required as a nutrient for human body because our organism is not able to synthesize it in sufficient quantities itself [172].

The most extensively studied antioxidants are vitamins. Antioxidants vitamins including vitamins A, C and E under physiological conditions are very useful for different functions in the human body, and are generally considered safe. Therefore, they can be taken in larger doses and for extended periods of time. Furthermore, they have the advantage of being able to be recycled back to an antioxidant molecule after the reaction with ROS [48].

Several large observational studies involving more than 100,000 volunteers were conducted in the 1990s that studied the effect of intake of different vitamins and the risk of coronary artery diseases (CAD). Most of these studies, but not all, suggested that higher intake of antioxidants significantly lowered the risk of CAD [173-176].

However, interventional studies, as we shall see, have produced conflicting and many times disappointing results. An important limitation of vitamins can be the presence of physiological mechanisms that tightly regulate their tissue levels [48]. Therefore, in pathological conditions that higher tissue levels of antioxidants may be needed, these endogenous control mechanisms could limit the therapeutic role of vitamins.

Vitamins and Mortality

Systematic reviews and meta-analyses performed by Cochrane group investigators have given a significant contribution to our knowledge about the efficiency of vitamin supplementation for primary and secondary prevention of diseases [40, 41, 43, 45, 177].

In the review of Bjelakovic and colleagues, 68 randomized trials conducted on 232,606 adults who were randomized to receive commonly used antioxidants including β-carotene, selenium, vitamins A, C and E were analyzed for the effect of antioxidant on all-cause mortality [178]. This review followed the Cochrane Collabo-

ration method and included primary (healthy subjects) and secondary (diseased individuals) prevention studies. When all trials were considered, antioxidants did not seem to significantly affect mortality. However, when 47 "low-bias" trials were separately analyzed, β-carotene, vitamin A and vitamin E administered alone or in combination, significantly enhanced all-cause mortality. Vitamin C and selenium did not have any significant effect on mortality. The relative risk of these increases in mortality was never higher than 1.16 (in case of vitamin A, 95%CI, 1.10-1.24) [178]. The same investigators analyzed the results of 20 randomized trials of the same antioxidants conducted on 211,818 participants in another study and found that antioxidants significantly enhanced mortality in a fixed-effect model meta-analysis (RR 1.04, 95% CI 1.02–1.07), but not in a random-effect model meta-analysis (RR 1.02, 95% CI 0.97–1.07) [179].

Another meta-analysis performed on 7 large trials of vitamin E involving 81,788 individuals showed that there was no significant difference in cardiovascular mortality when individuals receiving vitamin E were compared to control [180]. The same study included also 8 trials of β-carotene involving 138,113 patients and showed that β-carotene intake was associated with a small but significant increase in all-cause mortality and cardiovascular death [180].

In another large meta-analysis including 19 trials and 135,967 subjects, it was shown that high dose intake of vitamin E (≥ 400IU/day) may increase all-cause mortality [181]. However, other authors have claimed that the increase in mortality caused by vitamin E is questionable [182].

There seems to be consensus that taking vitamin A supplements is especially dangerous in smokers, since it significantly increases the risk of lung cancer [183, 184].

Large secondary prevention trials of vitamin E including Secondary Prevention with Antioxidants of Cardiovascular Disease in Endstage Renal Disease (SPACE) [185], the Cambridge Heart Antioxidant Study (CHAOS) [186], the Heart Outcomes Prevention Evaluation (HOPE) [187], Gruppo Italiano per lo Studio della Sopravvianza nell'Infarto Miocardico (GISSI) [188] have evaluated the effect of vitamin E on mortality rates. In a meta-analysis of these RCTs and other primary and secondary prevention trials, it was concluded that vitamin E supplementation did not significantly affect mortality or risk of cardiovascular diseases [189].

Few investigators have suggested that conflicting data coming from clinical trials may be explained in part by concomitant consumption of other drugs with antioxidant effects such as statins [190] or even the form of vitamin E (RRR-α-tocopherol versus *all-rac*-α-tocopherol) prescribed to patients [191].

Recent large meta-analyses of RCTs investigating the preventive effects of vitamins in various diseases are presented in Table **3**. Vitamins were selected for antioxidant therapy in several studies in the past decades, because they were cheap and available, but they are not the best antioxidant molecules in terms of efficacy. It is clear that many studies agree on the lack of evidence on the beneficial effects of antioxidant vitamins and in some cases even point to harmful effects. Putting these findings with the data on the possibility of increased mortality rate by vitamin consumption has led us to the conclusion that vitamins cannot be used as effective antioxidant therapeutics for human diseases.

Coenzyme Q10

Coenzyme Q10 (CoQ10), or ubiquinone (oxidized form) or ubiquinol (reduced form), is an endogenous lipid, that takes part in the transport of electrons in the mitochondria during the process of respiratory chain reactions [192]. CoQ10 has been suggested for treatment of a variety of diseases including heart failure [193] migraine [194], hypertension [195] and neurodegenerative diseases [196]. Although CoQ10 is considered a safe drug [197], additional studies are still required to prove its clinical usefulness [196].

Nitrones

Nitrones (X-CH=NO-Y) are very good antioxidant molecules that react with oxygen free radicals and form nitroxyl free radical, which is generally much more stable than the oxygen free radical [198, 199].

α-Phenyl-tert-butyl nitrone (PBN) is one of the nitrones that have been extensively studied in animal models [200]. PBN and its derivatives have been successfully tried on several rodent models of cancer and seem to be promising [201]. Nitrones seem also to be effective on animal models of hearing loss [202-204].

NXY-059 (2,4-disulfophenyl-N-*tert*-butylnitrone, disufenton) is a PBN-related nitrone that has been developed by AstraZeneca for treatment of ischemic stroke. A large randomized, double-blind, placebo-controlled clinical trial referred to as the Stroke-Acute Ischemic NXY Treatment I (SAINT I) was conducted on 1,722 patients suffering from acute ischemic stroke from 2003 to 2004. NXY-059 or placebo was intravenously administered to patients within 6 hours after the onset of stroke. NXY-059 significantly reduced disability after 90 days. However, it was not able to significantly change other parameters including mortality and neurological function [205]. Although some concerns existed about the power of this trial, it was hailed as a success in treatment of ischemic stroke. A larger trial recruiting 3,306 patients referred to as SAINT II was conducted in the years 2003-2006 to further investigate the effects of NXY-059, but unfortunately, this trial did not find any evidence of efficacy for any of the end points [47].

The large trial of SAINT II was a significant blow to the use of nitrones for treatment of ischemic stroke, but some investigators have expressed concern over the design of the trial including inappropriate treatment window and inclusion of disparate patients [206]. Clinical use of nitrones still awaits further studies that confirm their effectiveness.

DISEASES THAT MAY BENEFIT FROM ANTIOXIDANT THERAPY

Many diseases have been reported to benefit from antioxidant therapy and covering all of them in one article is not possible. We here tried to discuss some pathologies that may benefit the most from antioxidant therapy.

Neurodegenerative Diseases

The prevalence of neurodegenerative diseases increases with advanced age and as the aging population of the world grows, neurodegenerative diseases become one of the most serious health issues [207]. Currently, no disease-modifying therapy exists for most of neurodegenerative diseases.

The central nervous systems (CNS), including brain, spinal cord and peripheral nerves, is one of the organs particularly susceptible to oxidative stress for several reasons. Neurons have high metabolic rates and therefore produce large amounts of ROS. On the other hand, CNS has high content of polyunsaturated fatty acids, which are very prone to oxidative damage and also contains large amounts of iron, which is involved in the formation of dangerous ROS like hydroxyl radical [12]. A large body of evidence exists on the involvement of oxidative stress in the pathogenesis of Alzheimer disease [9, 10, 12, 13], Parkinson disease [11, 16] and amyotrophic lateral sclerosis (ALS) [14].

Oxidative stress happens early in the pathogenesis of neurodegenerative diseases and is probably one of the key initiating factors of the pathology [12, 15]. The intake of different antioxidants has

been shown to be important in reducing the risk of neurodegenerative diseases [38, 208, 209].

For above-mentioned reasons antioxidants appear to be good candidates for management of neurodegenerative diseases. However, the presence of blood brain barrier (BBB) is an extra obstacle for the use of antioxidants in neurodegenerative diseases. Most of the known antioxidants have difficulty crossing the BBB and an effective antioxidant should also be able to cross readily this barrier.

Alzheimer Disease

The role of antioxidant therapy in Alzheimer disease has been recently reviewed [12].

Idebenone, an antioxidant drug as discussed above, has been reported to be effective in management of Alzheimer disease [77-79], but the evidence on its effectiveness does not seem to be sufficient [80].

Selegiline, a monoamine oxidase inhibitor (MAO-Inhibitor) that has antioxidant properties, and vitamin E, each one alone or combined together were not able to improve Alzheimer's disease Assessment Scale Cognitive Score (ADAS-cog) in Alzheimer disease patients, but could significantly delay the disease progression [210]. Other agents such as clioquinol (a lipid soluble metal chelator that can cross the BBB) [15, 211] and LA has shown promise in clinical trials of Alzheimer disease [212, 213], however these trials have been small and need further confirmation.

Other studies of the efficacy of antioxidant vitamins for prevention of Alzheimer disease and cognitive decline have been less encouraging. In a relatively large study that involved 2,969 individuals aged 65 years or older with no cognitive impairment at the baseline, who self-reported the use of vitamin C, vitamin E or a combination of the two vitamins, none of the vitamins or their combination significantly changed the hazard ratio for development of dementia or Alzheimer disease in an average follow up period of 5.5 years [214].

A systematic review of 22 RCTs (3,442 subjects) that used vitamin B for prevention of cognitive decline, showed no significant effect for the vitamin [215]. In a Cochrane group analysis of 2 clinical trials of individuals with dementia and low serum vitamin B12 levels, treatment with vitamin B12 had no significant effect on cognitive function [216].

A novel approach recently proposed by some research groups consist of the use of bi-functional molecules that contain both amyloid □ binding and antioxidant moieties that are able to cross blood-brain barrier [15]. This approach may compensate for the pitfall of most antioxidant compounds that suffer from poor target specificity [15].

Parkinson Disease

Coenzyme Q10 (CoQ10) has been studied for management of Parkinson disease, but conflicting results have been produced. In a systematic review of 4 large clinical trials that studied the role of CoQ10 in Parkinson disease, 2 of the reports found a small but statistically significant improvement in Parkinson symptoms [217].

In an RCT that recruited 131 individuals with mid-stage Parkinson disease, patients were treated with nanoparticular CoQ10 for 3 months. Although, the treatment was safe, no significant symptomatic improvement was observed in patients treated with CoQ10 compared with the control group [218].

Amyotrophic Lateral Sclerosis

In a meta-analysis performed by the Cochrane group investigators, 9 studies exploring the effect of antioxidant treatment on ALS were analyzed. No significant effect was reported for vitamin E, N-acetylcysteine, combination of L-methionine plus vitamin E or selenium in the individual studies. Similarly, a meta-analysis on all antioxidants combined did not reveal any significant effect on primary or secondary outcome measures [42]. Another antioxidant, pentoxifylline, might be effective in treatment of ALS [219].

Cancer

ROS can damage various biomolecules. The oxidative damage of DNA is especially important in predisposition of humans to malignancy [17]. In fact, the ablation of various antioxidant enzymes in experimental animals increases oxidative stress and can increase the chance of age-related tumor development [17].

Epidemiological studies have shown that antioxidants may have a role in prevention of cancer [220]. However, large interventional studies have produced conflicting results.

In the Linxian study, conducted on 29,584 adults (40-69 years old) 4 regimens of antioxidants were given to 8 groups for 5 years and then the subjects were followed up for an additional 10 years (total of 15.25 years). Each group received a combination of regimens. Subjects who received a certain regimen where then compared to subjects who did not receive that regimen. After 15 years of follow-up, regimen D, which contained selenium, vitamin E and □-carotene, had a small but significant reduction in overall mortality (32.19% compared to 33.62%) and lower gastric cancer mortality (3.84% compared to 4.28%). Treatments seemed to be much more effective in subjects younger than 55 years old. Vitamin A and zinc supplementations were associated with a higher total and cerebrovascular mortality. These findings which were similar to the findings of 5 year follow-up, are also important in showing the durability of the beneficial effects of some antioxidants after a long time [221].

In the Finish study Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study conducted on male smokers, □-tocopherol lowered the incidence of prostate cancer, but □-carotene enhanced the risk of lung cancer and total mortality [184]. However, in a follow up study, the effects of antioxidant vitamins (beneficial or harmful) disappeared during post-intervention follow-up [222].

Another large randomized placebo-controlled trial, Selenium and Vitamin E Cancer Prevention Trial (SELECT) recruited 35,533 healthy men and divided them into 4 groups of selenium, vitamin E, selenium plus vitamin E, and placebo and followed them for a median period of 5.5 years for development of prostate cancer. Vitamin E or selenium, alone or in combination did not decrease the incidence of prostate cancer. There was even a statistically nonsignificant increased risk of prostate cancer in the vitamin E group [223].

Several systematic reviews and meta-analysis have also been performed on clinical studies that explored the role of antioxidants in prevention of cancer. In a systematic review of 20 trials (211,818 participants), □-carotene, selenium and vitamins A, C and E did not result in any significant reduction in cancer risk [179]. In another systematic review that covered studies about the effect of □-carotene supplementation on prevention of lung cancer, □-Carotene was not associated with a reduction of the risk of lung cancer. However, findings of prospective cohort studies have suggested a decrease of the risk of lung cancer but these reductions were mostly small and not statistically significant [224].

Although there is much discrepancy in the data, overall it seems that there is not enough evidence to support the protective effect of vitamins against cancer. The discrepancy between interventional and cohort studies could also reside in the fact that carotenoid measurement in prospective cohort studies is only an index of a healthier lifestyle and diet [224].

Stroke

Stroke is a common disease of the elderly, which affects millions of people in the world and comprises one of the leading causes of death [225]. The majority of the cases are categorized as ischemic type, while the rest are designated as hemorrhagic type stroke [226]. Similar to neurodegenerative diseases that increase in prevalence as the population grows older, stroke is also much more prevalent in the elderly and therefore comprises one of the important health priorities that need immediate attention.

The important role of ROS in ischemic stroke has been studied by many investigators [20, 21] and reviewed in [227] and [228].

There are 2 main approaches for treatment of acute ischemic stroke; thrombolysis and neuroprotection. Neuroprotection strategy is based on the prevention of complex processes that cause ischemic cell death in the infarct area of the brain and its vicinity [225]. Antioxidants have had an important place in the neuroprotection strategy, but there are also several clinical trials that have produced disappointing results [225, 229].

Ebselen, Tirilazad and edaravone have been clinically tested in stroke, but only edaravone has succeeded in the management of stroke and is in clinical use in Japan [225].

Ebselen is a selenium containing compound that has glutathione peroxidase-like activity. Glutathione peroxidase neutralizes hydrogen peroxide, which is one of the important ROS involved in oxidative damage. Small-scale clinical trials with 99 [230] and 150 participants [231] showed only limited neuroprotection in ischemic stroke patients treated with ebselen. The development of ebselen has been terminated because of its limited efficacy [226].

Tirilazad is another antioxidant tested in animal models of stroke and also in humans. There is disagreement between animal studies that have shown beneficial effect for tirilazad on the one hand and human studies that could not provide any evidence about the neuroprotective effect of this compound [232-234].

Oxidative stress is only one of several processes involved in ischemic cell death. Other processes including inflammation, excitotoxicity, failure of ionic pumps and activation of apoptotic pathways also take part in the pathogenesis. Inhibition of one of these cascades may not be enough to control neuronal death [225]. On this basis, multi-functional compounds that block several ischemia-induced processes should prove to increase our chance of success in the management of stroke [225].

WHY MANY ANTIOXIDANTS HAVE FAILED TO SHOW EFFICACY IN INTERVENTIONAL HUMAN STUDIES?

Clinical trials of many antioxidant therapeutics in human volunteers have produced negative or inconclusive results or have shown very little benefit [40-48, 180]. On the other hand, it is difficult to find a disease for which oxidative stress has not been proposed as an important part of etiopathogenesis [48]. The inability of clinical trials to prove the usefulness of antioxidant therapies shows are failure in translating our knowledge of molecular and cellular mechanisms into efficient clinical remedies [30].

The reason of clinical failure of many antioxidants despite the existence of overwhelming evidence on the involvement of oxidative damage in various pathologies still remains elusive. However, we here enlist some possible hypotheses that may explain this phenomenon.

A- Oxidative Stress is Not the Primary Cause of the Disease

Oxidative stress has been implicated in many diseases, but it is vitally important to know whether it is present in the early or in the late stage of tissue injury. In other words, it is crucial to make sure that oxidative damage is the direct initiation factor and not just a byproduct or end product of the disease process [12]. If oxidative stress is a late consequence of the disease, it may not give an adverse contribution to the pathology and its prevention can actually turn out to be harmful [235, 236].

B- Oxidative Stress is Not the Only Cause of the Disease

Oxidative stress may be only one of several processes involved in the pathogenesis of a disease. It has been suggested that the lack of success with antioxidants in lung diseases is in part, because oxidative damage is not the only pathogenic process [237]. Likewise, in ischemic cell death in stroke, other deleterious processes including inflammation, excitotoxicity, failure of ionic pumps and activation of apoptotic pathways also take part in the pathogenesis [225]. On this basis, multi-functional compounds that act on several pathways increase the odds of success [225].

C- Patients do Not Equally Benefit from Antioxidant Therapy

Status of oxidative stress might be very different from one patient to another. Lack of patient selection based on elevated indices of oxidative damage, could mix individuals that benefit from antioxidant therapy with those who do not, and therefore render the final outcome less enthusiastic [22]. The inclusion of patients without biochemical evidence of increased oxidative stress in clinical trials has been suggested as a motive of failure in vitamin E therapy [238].

Pharmacogenomics considerations could also improve the outcomes of clinical trials. For example, selection of patients based on haptoglobin genotype in diabetic patients treated with vitamin E [239] or glutathione-S-transferase genotype in acute respiratory distress syndrome (ARDS)/acute lung injury patients treated with N-acetylcysteine [240], or apo□genotype in over-weight individuals treated with quercetin as a blood pressure-lowering agent [144] could alter the response to antioxidant therapy. The real challenge in this regard, is to establish patient selection methods that can predict who is more likely to benefit from antioxidant therapy [241].

D- Administered Antioxidant is Not Able to Lower Oxidative Stress

Many studies have assumed that the use of antioxidants in humans decreases oxidative stress, but this is not necessarily true. For example, it was shown in a study that feeding Brussels sprouts to human subjects decreases the urinary excretion of DNA oxidation marker [242], but □-carotene , vitamin C and □-tocopherol supplementation do not reduce the oxidation biomarker [243]. Similarly, flavonoids-rich diet [244] or quercetin [245] have failed to reduce markers of oxidative damage in human subjects. It can be even worse; It has been shown that a mixture of antioxidants could increase the oxidative damage to DNA [246]. These observations emphasize the importance of using biomarkers in clinical studies.

We here discuss some factors that may influence the effectiveness of antioxidant therapy in reducing oxidative damage.

Antioxidant Molecule has Low Bioavailability

Low bioavailability is a problem with many antioxidants. Some polyphenolics, for example GTC, may have very low bioavailability [247].

Time and Duration of Therapy are Not Optimal

Populations that are selected for interventional trials of antioxidants often consist of middle aged individuals who have suffered from the consequences of oxidative stress for several decades. It is not logical to assume that with a brief period of antioxidant therapy, those adverse effects can be overturned [248]. It has also been suggested for dietary polyphenols [127] and vitamin E [238] that the duration of interventions ought to be increased in order to more closely reflect the long-term dietary intake of these compounds.

Table 3. Recent Large Meta-Analyses of Randomized Controlled Clinical Trials (RCTs) Exploring the Efficacy of Vitamins A, C and E in Prevention of Various Diseases

Publication	Antioxidants studied	Number of RCTs	Number of randomized participants	Illness	Results	Conclusion
Arain et al., 2010 [267]	Vitamin E	4	94,069	Prevention of colorectal cancer	No significant effect on prevention of cancer.	No conclusive evidence on the benefit of treatment
Myung et al., 2010 [268]	Vitamin E, vitamin C, vitamin A, □-carotene, selenium (alone and in combination)	22	161,045	Prevention of cancer	No significant effect on prevention of cancer. No significant effect according to the type of antioxidant or type of cancer. Significant increase in the risk of bladder cancer in a subgroup meta-analysis of 4 trials.	No conclusive evidence on the benefit of treatment
Evans et al., 2009 [45]	□-carotene and □-tocopherol	3	23,099	Prevention of age-related macular degeneration (AMD)	No significant effect on prevention or delaying the onset of AMD (all trials included). No significant effect when the analyses were restricted to either □-carotene or □-tocopherol.	No conclusive evidence on the benefit of treatment
Bardia et al., 2008 [269]	□-carotene, vitamin E, selenium	12	104,196	Prevention of cancer and mortality	Significant increase in cancer incidence and cancer mortality among smokers by □-carotene. Vitamin E supplementation had no effect. Selenium supplementation might have anticarcinogenic effects in men and thus requires further research.	Selenium may be beneficial
Alkhenizan et al., 2007 [270]	Vitamin E	12	167,025	Prevention of cancer	No significant difference in all-cause mortality, cancer incidence and cancer mortality. Significant reduction in the incidence of prostate cancer.	Beneficial for prostate cancer prevention. Not beneficial for other causes
Polyzos et al., 2007 [271]	Combination of vitamin C and vitamin E for prevention of preeclampsia	4	4,680	Prevention of preeclampsia	No significant effect on the risk of preeclampsia, fetal or neonatal loss, or small for gestational age infant.	No conclusive evidence on the benefit of treatment

Only large studies that included at least 4000 subjects were included. Studies that explored the effect of vitamins on mortality are discussed in the text and are not mentioned here.

However, the optimal duration of therapy may also depend on the type of the disease. While, for example for cancer prevention, long term therapy is probably needed, it has been claimed that long-term antioxidant therapy may not be useful in hypertension. Antioxidants can be beneficial for a short time, but further weakening of ROS formation may result in decreased endothelial NO synthase expression and activity and therefore be deleterious [236].

Oxidative Stress is Hard to Overcome in Certain Diseases

In certain situations such as lung diseases, the balance of pro-oxidant/antioxidant is enormously disturbed and the amount of antioxidant necessary to restore that balance is so high that it is not achievable with regular non-toxic doses of antioxidants [237].

Antioxidant has Poor Target Specificity

The antioxidant agent may be effective for universal targets, but is not able to reach its main target. Many antioxidants suffer from poor target specificity [15].

In diseases like neurodegenerative diseases or cancer, oxidative damage is mainly limited to certain organs and tissues and is not a systemic phenomenon. However, many antioxidants work systemically when administered to these patients [249].

Reaction Products of the Antioxidant are Toxic

Some antioxidants, after reacting with ROS, may turn into free radicals that initiate new reactions [250]. Successful experience with edaravone, for example, is also due to the fact that the oxidation products of the reaction between free radical and the antioxidant molecule are not toxic themselves [51, 53].

A single Antioxidant is Not Enough to Overcome Oxidative Stress

One of the pillars of oxidative stress theory has come from the prospective epidemiological studies, which have shown that antioxidant-rich diets prevent diseases. It is very clear that there are numerous antioxidants of different types in dietary plants that we consume. However, we have used a single antioxidant in many clinical trials. Many investigators have suggested that antioxidants work synergistically when used together [251]. For example, vitamin E supplementation should be concurrent with use of vitamin C to produce maximum antioxidant effect [238].

Physiological Mechanisms Prevent the Achievement of a High Tissue Level of Antioxidant

This phenomenon is especially important in the case of endogenous antioxidants like vitamins, since there are physiological mechanisms that tightly regulate their tissue levels [48]. These endogenous control mechanisms limit the therapeutic action of the antioxidant molecule.

E- Antioxidant Molecule has Harmful Effects that Mask its Useful Antioxidant Actions

Antioxidant molecules like any other compound may have other actions unrelated to their main effect [252]. For example, vitamins, aside from their antioxidant activity, have some undesirable effects that may have been responsible for the increased risk of mortality observed in some studies. For example, □-carotene can have unwanted effects on lipid profile and vitamin E may prevent the increase in high-density lipoprotein-2 (HDL-2) [248].

F- Certain Antioxidants are Not Effective in Well-Nourished Populations

Some of the antioxidants are more effective in undernourished populations. In a study that 51.5% of participants reported zero consumption of citrus fruits, subjects who consumed good amounts of fruit had a lower risk of development of symptomatic asthma [253]. It has been similarly reported that antioxidant vitamins may prevent cancer in subjects with poor or suboptimal nutritional status [220]. On the other hand, in another study conducted on adequately nourished subjects, supplementation with lutein (a carotenoid) and green tea extract, did not alter plasma parameters of oxidative stress [254].

One important conclusion that can be deducted from these studies is that antioxidants are probably more effective in developing countries with higher prevalence of under-nourishment. Indeed, such studies are scarce and could be of very high value in advancement of our knowledge about the relation of nutritional status and effectiveness of antioxidants.

However, we should be careful not to generalize this notion to all antioxidants. In a Cochrane group analysis of 2 clinical trials, it was reported that treatment with vitamin B12 had no significant effect on cognitive function in dementia patients with low serum vitamin B12 levels [216].

G- The Target is Not Well Selected

It is important to remember that human body has already developed extremely efficient antioxidant defense mechanisms. For example, the activity of superoxide dismutase (SOD) enzyme is very high (K ~10^9 M^{-1} S^{-1}) and its intracellular concentration may reach 10 μM [6]. Therefore it is important to choose the right target for therapy. An antioxidant designed to function as a SOD mimetic, is unlikely to be successful, because endogenous SOD enzyme acts very efficiently [48].

FUTURE DIRECTIONS

Failure of clinical trials to prove beneficial effects for antioxidants should challenge us to optimize our clinical studies. Here we discuss actions that could be undertaken in order to improve the success rate of antioxidant clinical trials.

In selection of diseases that we candidate for antioxidant therapy, we should differentiate between those that have oxidative damage as a primary mechanism at the core of their pathogenesis on the one hand and diseases in which oxidative damage represents a late consequence of primary mechanisms. We should therefore be able identify diseases that antioxidant therapy has a higher probability of success. Cancer and neurodegenerative diseases could probably be included in the list of such diseases [12, 28].

Suitable oxidative stress status biomarkers should be employed to measure whether or not oxidative stress is diminished in response to antioxidant therapy. Unless the biomarkers have shown that oxidative stress is decreased, the mere consumption of antioxidants cannot be enough for such assumption. In other words, it is not sufficient to measure only the clinical outcome; It is also mandatory to determine whether or not the antioxidant blocked the oxidative damage [48]. Good biomarkers are needed for this purpose.

In general, earlier intervention seems to have a higher chance of success. Antioxidants do not appear to be very effective when the disease is already well-established and compelling evidence shows that they are more effective in prevention rather than treatment. For example, they are effective in precancerous lesion of prostate [132] but not in metastatic prostate cancer [133].

Oxidative stress usually has different aspects and one single antioxidant may not be able to effectively neutralize the damage induced in various macromolecules and tissues. This leads to the assumption that the combination of different antioxidants might have a synergistic effect, a point that has been made previously in the case of stroke [225] and trauma-induced hearing loss [255] and supports the idea of using plant derived products, which contain a multitude of different molecules, as sources of antioxidants [256, 257].

Targeted delivery of antioxidants to mitochondria is a new exciting field of research that seeks to concentrate antioxidants on the inner membrane of mitochondria in order to protect against mitochondrial oxidative stress, and is finding therapeutic potential for atherosclerosis [258].

An alternative approach, which lies in the category of molecular targeted therapy, is the identification and modulation of disease-specific redox-sensitive signaling pathways, which has been proposed in lung diseases [237]. Antioxidant gene therapy has also recently been proposed as a treatment strategy [259] that can over-

Table 4. Possible Causes of Lack of Success in some Antioxidant Clinical Studies and Plausible Suggestions to Improve the Outcome

Cause of failure of antioxidant therapy	Suggestion
Oxidative stress is not the primary cause of the disease	Selection of diseases in which the involvement of oxidative stress as the core pathology is proved
Oxidative stress is not the only cause of the disease	Application of multifunctional agents Combination of antioxidants with other drugs
Patients do not equally benefit from antioxidant therapy	Stratification of patients by use of biomarkers of oxidative stress to identify and include patients with high levels of oxidative stress
Antioxidant molecule has low bioavailability	Optimization of antioxidant molecule
Time and duration of therapy are not optimal	Optimization of time and duration of therapy Use of biomarkers to monitor the response to antioxidant therapy
Oxidative stress is hard to overcome in certain diseases	Use of combination of antioxidants
Antioxidant has poor target specificity	Use of antioxidants that act on disease specific pathways rather than universal pathways
Reaction products of the antioxidant are toxic	Optimization of antioxidant molecule
A single antioxidant is not enough to overcome oxidative stress	Use of combination of antioxidants
Physiological mechanisms prevent the achievement of a high tissue level of antioxidant	Selection of antioxidants that do not suffer from this shortage (not vitamins)
Antioxidant molecule has harmful effects that mask its useful antioxidant action	Use of better antioxidants
Certain antioxidants are not effective in well-nourished populations	Use of better antioxidants
The target is not well selected	Choose of an appropriate target

come the problem of poor availability of the antioxidant at its target.

Inhibition of enzymes that are involved in oxidative damage, especially NADPH oxidase, whose sole function is ROS production [249] is also another seemingly promising strategy.

It is not cautious to assume that antioxidants are 'Elixirs of life" that prevent every kind of disease and should be taken as much as possible to keep us healthier and younger [235]. We know that pro-oxidants can up-regulate the normal defense systems such as anti-oxidant enzymes like heme-oxygenase-1, peroxiredoxin, catalase, etc., therefore, some level of exposure to pro-oxidants may be helpful as it induces a "super protection" in tissues. ROS may also have some important physiological roles in cell signaling and killing microorganisms. Thus the use of antioxidants can actually turn up to be deleterious to our health in certain conditions [235, 260].

A good thing about antioxidant drugs is that, one antioxidant drug developed for an oxidative stress-related disease is very likely to be effective against other pathologies that have oxidative damage as their etiology: edaravone is a good example of this [51].

CONCLUSION

Several decades have passed since the idea of antioxidant therapy was introduced for the first time. The field of antioxidants turned out to be much more challenging than what was presumed in the beginning. Much effort has been directed to the study of the efficacy of different antioxidants in human diseases, but unfortunately the products of this long process have not been satisfactory. However, the lack of clear cut success in clinical trials does not disprove the crucial role of oxidative stress in diseases. We have learned many things along this way. Once we apply our experience to select the right disease and the right population, design optimized and highly bioavailable antioxidants directed at specific and appropriate targets and choose optimal treatment times and durations, useful therapeutics could emerge for various diseases.

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ABBREVIATIONS

ALS	=	Amyotrophic lateral sclerosis
CNS	=	Central nervous system
CoQ10	=	Coenzyme Q10
COPD	=	Chronic obstructive pulmonary disease
FRDA	=	*Friedreich's* ataxia
GSH	=	Glutathione
GTC	=	Green tea catechins
LA	=	□-Lipoic acid
NAC	=	N-acetylcysteine
MPFF	=	micronized purified flavonoid fraction
RCT	=	Randomized controlled trial
ROS	=	Reactive oxygen species

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