Translational medicine

Is oxidative stress a therapeutic target in cardiovascular disease?

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An abnormal production of reactive oxygen species (ROS) and the subsequent decrease in vascular bioavailability of nitric oxide (NO) have long been proposed to be the common pathogenetic mechanism of the endothelial dysfunction, resulting from diverse cardiovascular risk factors such as hypercholesterolaemia, diabetes mellitus, chronic smoking, metabolic syndrome, and hypertension. Superoxide produced by the nicotinamide dinucleotide phosphate (NADPH) oxidase, mitochondrial sources, or the xanthine oxidase may react with NO, thereby resulting in excessive formation of peroxynitrite, a reactive nitrogen species that has been demonstrated to accelerate the atherosclerotic process by causing direct structural damage and by causing further ROS production. Despite this sound biological rationale and a number of pre-clinical and clinical lines of evidence, studies testing the effects of classical antioxidants such as vitamin C, vitamin E, or folic acid in combination with vitamin E have been disappointing. Rather, substances such as statins, angiotensin-converting enzyme inhibitors, or AT1-receptor blockers, which possess indirect antioxidant properties mediated by the stimulation of NO production and simultaneous inhibition of superoxide production (e.g. from the NADPH oxidase), have been shown to improve vascular function in pre-clinical and clinical studies and to reduce the incidence of cardiovascular events in patients with cardiovascular disease. Today, oxidative stress remains an attractive target for cardiovascular prevention and therapy. However, a deeper understanding of its source, and of its role in vascular pathology, is necessary before new trials are attempted.

Keywords
Oxidative stress • Myocardial ischaemia • Endothelial function

Introduction

Traditionally, the vascular endothelium was thought to primarily function as a selective barrier to the diffusion of macromolecules from the blood lumen to the interstitial space. During the past 20 years, numerous additional roles have been defined for this biologically active tissue, including the regulation of vascular tone, modulation of inflammation, and promotion or inhibition of vascular growth and of platelet aggregation and coagulation. Disturbance in endothelial homeostasis, a condition termed ‘endothelial dysfunction’, is a characteristic feature of patients with coronary atherosclerosis, and more recent studies indicate that it may predict long-term progression of atherosclerotic disease as well as cardiovascular event rate.1 Although the mechanisms underlying endothelial dysfunction are complex and multifactorial, a growing body of evidence suggests that increased production of reactive oxygen species (ROS) may have a pivotal role in this phenomenon, and, in the general idea that correction of the abnormally elevated ROS bioavailability might retard, or prevent, cardiovascular disease, a number of interventions have been proposed to restore the redox status in the vascular milieu. Three approaches may correct the balance between increased oxidative stress and simultaneously decreased vascular nitric oxide (NO) synthesis:

(i) reducing vascular ROS bioavailability by administration of antioxidant compounds;
(ii) increasing vascular NO concentrations via administration of NO donors such as nitroglycerin or mono/dinitrates;
(iii) reducing vascular ROS production and stimulating vascular NO production, e.g. by treatment with statins, angiotensin-converting enzyme (ACE) inhibitors, AT1-receptor blockers, or β-receptor blockers with vasodilating (NO-dependent) properties such as nebivolol.

The present review will summarize the current concepts concerning increased superoxide production in cardiovascular disease and will discuss old and new pharmaceutical concepts for fighting increased oxidative stress in the vascular tissue.
Part I: biochemical and pre-clinical background

Oxidative stress and mechanistic insight into endothelial dysfunction

The endothelium-derived relaxing factor, identified in the mid-1980s with NO or a closely related compound, has potent anti-atherosclerotic properties. NO released from endothelial cells works in concert with prostacyclin to inhibit platelet aggregation, the attachment of neutrophils to endothelial cells, and the expression of adhesion molecules. Further, it inhibits the proliferation of smooth muscle cells. Therefore, the process of atherosclerosis is initiated or accelerated under all conditions in which an absolute or relative NO deficit is encountered, i.e. when NO is insufficiently produced or too rapidly scavenged. The half-life of NO and therefore its biological activity are decisively determined by ROS such as superoxide: this free radical rapidly reacts with NO to form the highly reactive intermediate peroxynitrite (ONOO\(^-\)) in a reaction that is about 10 times faster than the dismutation of superoxide by the superoxide dismutase (rate constant: \(5-10 \times 10^9/M/s\)) (Figure 1). The reaction with superoxide and ONOO\(^-\) formation may thus substantially limit NO bioavailability and its protective effects. ONOO\(^-\) may act as a vasoconstrictor, and most importantly, a cytotoxic molecule may cause oxidative damage to proteins, lipids, and DNA. Further, recent studies also indicate that ONOO\(^-\) may impair the activity of the prostacyclin synthase and the endothelial NO synthase (NOS, discussed subsequently), thus limiting the bioavailability of endothelial mediators with protective functions. Other ROSs such as the dismutation product of superoxide hydrogen peroxide and hypochlorous acid cannot be considered as free radicals, but have a powerful oxidizing capacity, which will further contribute to oxidative stress within vascular tissues.

Cardiovascular risk factors cause endothelial dysfunction via oxidative stress

Endothelial dysfunction is increasingly accepted as a common trait of essentially all cardiovascular risk factors. Impaired endothelial homeostasis has been shown, among others, in the elderly, after chronic or acute smoking, in patients with increased low-density lipoprotein (LDL) or triglyceride levels, in patients with diabetes Type I and II, in hypertensives, and in patients with metabolic syndrome [for review see (1)]. There are several potential abnormalities that could account for such reductions in endothelium-dependent vascular relaxation including—limiting

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**Figure 1** An unbalanced production of nitric oxide and superoxide (\(O_2^{-}\)) secondary to cardiovascular risk factor exposure leads to inappropriate formation of superoxide by the nicotinamide dinucleotide phosphate oxidase, xanthine oxidase, and the mitochondria. Superoxide immediately reacts with nitric oxide to form the highly reactive intermediate peroxynitrite, which causes vascular dysfunction through different mechanisms including nitric oxide synthase uncoupling secondary to BH4 oxidation (insert), tyrosin nitration of the prostacyclin synthase (PGI\(_2\)S), and subsequent inhibition of PGI\(_2\) formation and by inhibiting the activity of the soluble guanylate cyclase (sGC) and therefore nitric oxide signalling. Superoxide per se is also know to potently stimulate the expression of endothelin-1 (ET-1) in endothelial and smooth muscle cells, shifting the balance from vasodilation more towards vasoconstriction.
the discussion to NO-dependent phenomena—changes in the activity and/or expression of the NOS, decreased sensitivity of vascular smooth muscle cells to NO, or increased degradation of NO via its interaction with ROS such as superoxide. Direct (i.e. elevated ROS levels and/or evidence of ROS-derived damage) and indirect (i.e. improvements in endothelial vasomotor responses following the acute intravascular administration of high-dose vitamin C) lines of evidence suggest that among these possible mechanisms, the ROS-determined NO-degradation concept is a very important one.

Of importance, the degree of improvement of endothelial dysfunction in response to vitamin C has been shown to correlate with prognostic endpoints. For instance, patients with established coronary artery disease who show an improvement in endothelial function in response to vitamin C (i.e. patients in whom oxidative stress can be demonstrated) have a worse prognosis when compared with those with low or no vitamin C effects (Figure 2).

This finding strengthens not only the concept that oxidative stress is indeed the key player in determining the degree of endothelial dysfunction, but also that the prognosis of patients with established coronary artery disease is tightly determined by these processes. To date, a number of studies have confirmed the existence of a clear-cut association between endothelial dysfunction and prognosis in a number of settings associated with cardiovascular disease (for review see (1)).

Mechanisms underlying increased oxidative stress

Vascular enzymatic superoxide sources

The most important superoxide sources comprise the so-called nicotinamide dinucleotide phosphate (NADPH) oxidase, the xanthine oxidase, mitochondria, and, under certain conditions, the endothelial NOS.

The NADPH oxidase

The NADPH oxidase is a superoxide-producing enzyme, first characterized in neutrophils, but also expressed in endothelial and smooth muscle cells as well as in the adventitia. This enzyme consists of at least five isoforms (NOX1-5) (Figure 3).

Under certain conditions (e.g. upon angiotensin II stimulation), the activity of the enzyme is increased in endothelial and smooth muscle cells, suggesting that in the presence of an activated renin–angiotensin system (either local or circulating), vascular dysfunction due to increased vascular superoxide production is likely to be expected. Experimental hypercholesterolemia has been shown to be associated with an activation of the NADPH oxidase, and in human saphenous veins of patients with coronary artery disease, a close association has been shown between endothelial dysfunction, clinical risk factors, and the activity of this enzyme. In atherosclerotic arteries, an increased expression of the NADPH oxidase subunit gp91phox and NOX-4, all of which may contribute to increased oxidative stress within the vascular tissue, has been demonstrated.

Interestingly, ACE activity and therefore local angiotensin II concentrations are increased in the shoulder region of atherosclerotic plaques, and inflammatory cells are capable of producing large amounts of angiotensin II along with superoxide. In vessels from hypercholesterolaemic animals and platelets from hypercholesterolaemic patients, an increased expression of the angiotensin II receptor subtype AT1 can also be observed. Thus, both experimental and clinical studies have provided evidence for the stimulation of the renin–angiotensin system in atherosclerosis and for the activation of the NADPH oxidase in the arterial wall. Evidence for an activation of this enzyme in the vasculature has also been derived from multiple experimental animal models of hypertension as well as in different forms of diabetes mellitus.

Xanthine oxidase

Xanthine oxidoreductase catalyses the sequential hydroxylation of hypoxanthine to yield xanthine and uric acid. The enzyme can exist in two forms that differ primarily in their oxidizing substrate specificity. The dehydrogenase form preferentially uses NAD+ as an electron acceptor, but is also able to donate electrons to molecular oxygen. By proteolytic breakdown and thiol oxidation, xanthine dehydrogenase from mammalian sources can be converted to the oxidase form that readily donates electrons to molecular oxygen, thereby producing superoxide and hydrogen peroxide without reducing NAD+. Oxypurinol, an inhibitor of xanthine oxidoreductase, has been shown to reduce superoxide production and to improve endothelium-dependent vascular relaxations in hyperlipidaemia, pointing to an increase in expression and/or activity of the xanthine oxidase. The mechanisms underlying this enzymatic activation remain unclear; however, it has been demonstrated that cytokines can stimulate the expression of xanthine oxidase in the endothelium. Alternatively, increased cholesterol levels might
Mitochondrial superoxide sources
Mitochondrial superoxide generation represents a major intracellular source of ROSs under physiological conditions. Mitochondrial superoxide levels are highly governed by the respiratory rate and by the activity of the manganese superoxide dismutase located in the matrix of the organelle. Stimulation of endothelial cells with angiotensin II, oxLDL, lipid peroxidation products, high glucose levels, free fatty acids, protein kinase C, and cyclic strain is able to stimulate mitochondrial superoxide production, and an inappropriately high mitochondrial ROS production has been shown in a number of settings.

Dysfunctional, uncoupled NOS contributes to endothelial dysfunction: the kindling radical concept
In most situations in which endothelial dysfunction due to increased oxidative stress is encountered, the expression of the NOS has been shown to be paradoxically increased. The demonstration of endothelial dysfunction in the presence of increased expression of NOS indicates that the capacity of the enzyme to produce NO may be limited, a concept that fits well with the observation that the NOS itself can be a superoxide source. A critical role in this NOS-dependent ROS production appears to be played by the levels of tetrahydrobiopterin (BH4) in the endothelial microenvironment: in the absence of this coenzyme, NOS reduces molecular oxygen rather than L-arginine, resulting in the production of superoxide rather than NO, a phenomenon known as ‘NOS uncoupling’. Several hypotheses have been proposed for intracellular BH4 depletion, including an ONOO−-mediated oxidation of BH4, which, in turn, will oxidize BH4 to the BH3 radical, and lead to further ROS and reactive nitrogen species formation (‘kindling radicals’). In addition to ONOO−-mediated oxidation of BH4, a decreased intracellular bioavailability of the NOS coenzyme may result from the inhibition of GTP-CH-I (de novo synthetic pathway) or by inhibition of the so-called salvage pathway involving enzymes such as the sepiapterin synthase, the sepiapterin reductase, or the dihydrofolate reductase (DHFR). Interestingly, downregulation of GTP-CH-I and DHFR, resulting in decreased vascular BH4 levels
and NOS uncoupling, can be observed in the setting of angiotensin II-induced hypertension and diabetes mellitus.\textsuperscript{16} As well, the GTPCH-I is regulated by phosphorylation, and this process is impaired in regions of altered flow,\textsuperscript{17} which might concur to explain the increased bioavailability of ROS and the preferential development of atherosclerosis in regions of turbulent flow. Preclinical studies treating diabetic or hypercholesterolaemic animals with statins and AT1-receptor blockers demonstrated that these compounds are able to inhibit the activity and expression of the NADPH oxidase and recouple NOS by preventing BH\textsubscript{4} oxidation and the downregulation of BH\textsubscript{4} synthesizing enzymes. Similarly, vitamin C is able to recycle BH\textsubscript{4} from its oxidized form (Figure 1), which suggests that the antioxidant effect of this molecule might also be mediated by mechanisms other than direct superoxide scavenging.\textsuperscript{15}

**NOS uncoupling and increased production of asymmetric dimethyl arginine (ADMA)**

Increased concentrations of the arginine-analogue ADMA in cultured endothelial cells or in patients with endothelial dysfunction are associated with increased ROS production.\textsuperscript{18} Since arginine is the substrate of NOS, this association between ADMA and endothelial dysfunction raises the question whether increased ADMA levels are an epiphenomenon of increased ROS production or whether ADMA actually contributes to the oxidative stress burden of the vasculature via uncoupling of NOS. Interestingly, the activity of enzymes responsible for the synthesis of ADMA (such as the S-adNOSylmethionine-dependent protein arginine methyltransferase) and that of ADMA-hydrolysing enzymes (such as dimethylarginine dimethylaminohydrolase) is redox-sensitive.\textsuperscript{18} Thus, oxidative stress in the vasculature may result in the ADMA production and/or inhibition of ADMA degradation, leading to ADMA concentrations that significantly inhibit NOS activity or even uncouple the enzyme, thus triggering another positive feedback mechanism.\textsuperscript{18}

Taken together, the results of the in vitro and pre-clinical data strongly suggest that increased vascular production of ROS contributes substantially to the initiation and continuation of the atherosclerotic process, suggesting that manoeuvres that reduce oxidative stress in vascular tissue should in general beneficially influence not only vascular function, but also the prognosis of patients with cardiovascular disease.

**Part II: translation into clinical practice**

**Epidemiological evidence: prospective cohort studies**

A wide range of prospective cohort studies confirm the above considerations. For instance, the lower cardiovascular mortality observed in Mediterranean populations when compared with Northern European countries has been attributed to differences in the intake of antioxidant-rich foods and beverages.\textsuperscript{19} In line with this, a meta-analysis of cohort studies including almost 400 000 patients\textsuperscript{20} reported that high vitamin E and vitamin C intake was associated with a lower rate of coronary heart disease (Figure 4). The Zutphen Elderly study, conducted in 805 elderly men without prior history of cardiovascular disease, provided the first evidence of a reduced mortality from coronary heart disease in patients with high flavonoid intake, which was confirmed after adjustment for traditional cardiovascular risk factors and antioxidant vitamin intake.\textsuperscript{21} These findings were reproduced by a later cohort prospective study enrolling almost 35 000 postmenopausal women without overt cardiovascular disease.\textsuperscript{22} Although results from the few studies that assessed antioxidant intake based on specific blood assays (rather than on self-reported questionnaires) were more ambiguous, higher quintiles of serum vitamin E (within the physiological range) were associated with lower mortality for cancer and cardiovascular disease after a follow-up of 19 years in 29 092 male smokers enrolled in the ATBC study.\textsuperscript{23} In the roughly 8000 subjects of the NHANES-II study, the lowest quartile of serum vitamin C had an increased mortality for cancer and cardiovascular mortality in men but not in women, who had higher ascorbate levels at baseline.\textsuperscript{24} Notably, the major limitation of these studies was that higher consumption of antioxidant-rich vegetables and fruits is also associated with generally ‘healthier’ lifestyle, including physical exercise, abstinence from smoking, etc.

![Figure 4](image-url) Relative risk and 95% confidence interval (top tertile vs. bottom tertile of antioxidant vitamins intake) of coronary heart disease in a meta-analysis of 15 prospective cohort studies involving 374 488 participants. Adapted from Ye and Song.\textsuperscript{20}
Despite these limitations, the above results prompted great enthusiasm, reinforcing the role of ROS as pathophysiological mechanism and as a possible therapeutic target. Perhaps, the most insidious pitfall in cohort studies is the difficulty in correctly evaluating the importance of confounding factors. For example, in one of the cornerstones of prospective studies that demonstrated the beneficial effect of vitamin C on total mortality, the EPIC study, no adjustment was made for two informative and easily obtainable parameters of socio-economic status and lifestyle such as social class and physical activity. The confounding power of socio-economic status is nevertheless very difficult to quantify, and elimination of this source of error, in order to exclude the hypothesis that high antioxidant intake is simply the marker of a healthier lifestyle, requires robust and accurate study design and statistical analysis.

**Interventional trials on antioxidants vitamins**

Since great expectations were awakened by the above epidemiological studies, a number of interventional trials were conducted between 1996 and 2002, mainly administering vitamin E, in the synthetic or natural form, β-carotene, and vitamin C, alone or in combination, and at different dosages. Clinical trials gave heterogeneous outcomes: some studies showed a benefit of vitamin E supplementation in the secondary prevention of cardiovascular disease and of vitamin E plus C supplementation in slowing carotid intima-media thickening in hypercholesterolaemic patients. Taken together, however, clinical trials to date have failed to demonstrate a beneficial effect of antioxidant supplements on cardiovascular morbidity and mortality. The data were pooled together in a meta-analysis, which demonstrated a substantial lack of efficacy for different doses of β-carotene and vitamin E in diverse population groups (Figure 5). Further attempts have been made with the supplementation of folic acid, which, as discussed earlier, might compensate for the oxidation of the NOS coenzyme BH₄. However, also these studies did not show a prognostic impact of this type of supplementation. Thus, the 2004 AHA Committee for Nutrition, Physical Activity, and Metabolism discouraged the use of antioxidant supplementation for the prevention of cardiovascular disease. Recently, the neutral effect of vitamin C plus E supplementation was confirmed by the results of the Physicians Health Study, which enrolled 14,641 middle-aged male physicians with low prevalent cardiovascular disease, followed up for about 10 years. As a further confounding factor, questions have been raised about the safety of prescribing antioxidant vitamins, with an increased overall mortality associated with β-carotene, vitamin A, and vitamin E supplementation, possibly due to increases in cancer mortality, reported in some of these trials.

In summary, interventional trials did not confirm whether the oxidative stress modification hypothesis is relevant in the clinical setting and, more importantly, did not allow concluding that pharmaceutical correction of the redox status could be used as a safe and effective therapeutic strategy. Moreover, the discrepancy between mechanistic and cohort studies and randomized clinical trials has created, if possible, even greater confusion. It might well be that the antioxidants used were intrinsically flawed. Or else, it could be argued that interventional trials conducted so far were not adequately designed, both for conceptual and for methodological reasons. One crucial issue is: what population is likely to benefit from antioxidant supplementation? The majority of the studies enrolled patients at high risk or who previously experienced a myocardial infarction. These conditions are characterized by established atherosclerotic damage, which is unlikely to regress with antioxidant treatment. In contrast, even in healthy subjects, equalling the positive effects of a lifelong high vegetable and fruit diet simply by a few years-long antioxidant supplementations appears to be a very ambitious endpoint. Moreover, it is still not clear whether the benefit of antioxidant supplementation depends on baseline antioxidant status, because reliable, simple, and easily available biomarkers of oxidative stress, suitable for large population studies, are still unavailable. Finally, the administration of antioxidants is based on the concept that these molecules only have deleterious effects. Instead, low concentrations of some ROSs, particularly hydrogen peroxide, are likely to play a role as physiological intracellular mediators in fundamental processes.

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**Figure 5** Odds ratios (95% confidence interval) of all-cause mortality, cardiovascular death, and all-cause stroke for individuals treated with antioxidant vitamins or control therapy in a meta-analysis of eight interventional trials with β-carotene and seven with vitamin E. Adapted from Vivekananthan et al. [29]
processes such as cell growth and angiogenesis, and an important role of ROS has also been shown in protective mechanisms such as preconditioning.\textsuperscript{34} Thus, in certain settings, ROS quenching might have deleterious implications that offset the impact on endothelial function.

Methodological issues also need to be carefully acknowledged before dismissing the antioxidant hypothesis: for instance, the role of trial design issues, choice of outcome measures, duration of treatment, populations under study, and concomitant therapy should not be underestimated. Examination of these aspects would require a more detailed discussion, given elsewhere;\textsuperscript{35,36} just to limit to some examples, the rate constant between vitamin C and superoxide is 10^7 lower than that between superoxide and NO, and supraphysiological levels of ascorbic acid (in the range of 10 mM, i.e. 10–100 times higher than those physiologically measured at the level of cellular membranes) are required to protect NO against ‘inactivation’ by superoxide.\textsuperscript{37} Thus, vitamin C, at least when orally administered, is too weak an antioxidant to reverse the processes triggered by superoxide anions. In addition, oral administration of vitamin C might not allow achieving biologically active levels, and finally, treatment with vitamin C and vitamin E stimulates the formation of so-called vitamin E and C radicals with subsequent prooxidant effects. Finally, co-administration of statins, a confounding factor that is usually overlooked, can normalize circulating levels of vitamin E, making its administration unnecessary.\textsuperscript{38}

Collectively, these considerations provide a sufficient explanation to the failure of ‘traditional’ antioxidants. Thus, rather than discarding the oxidative stress hypothesis, evidence suggests that we need more hypothesis-driven and rigorous clinical trial designs, guided by a deeper understanding of the complex physiology of ROS. Future research will have to develop newer antioxidant compounds, more specific, with a more favourable pharmacodynamic profile or ancillary effects, or impacting systemic and tissue oxidative stress through different mechanisms.

Are we in the polyphenols era?
The essentially neutral results of these interventional studies discouraged the use of antioxidant vitamin supplementation for the prevention of cardiovascular diseases, whereas the recommendation of a healthy diet, rich in fruits and vegetables and whole brain foods, is still standing.\textsuperscript{39} In line with this concept, attention has been focused on another family of antioxidant compounds, i.e. polyphenols, a group comprising about 8000 different molecules, among which flavonoids are the most studied family. Polyphenols are potent antioxidants abundant in vegetables and particularly in derived products such as chocolate, tea, and wine. This more ‘natural’ approach to antioxidant supplementation seems to be promising, since the antioxidant capacity of these compounds is not simply related to direct ROS scavenging, but also to inhibition of enzymatic sources of oxidative stress and stimulation of endogenous antioxidant enzymes. Benefits from polyphenol-rich foods and beverages are likely to arise from multiple pathways, and the antioxidant power appears to be only one of these.\textsuperscript{40} A meta-analysis of 113 interventional studies for a total of roughly 6000 subjects who underwent different kinds of food/beverages or extracts supplementation at different doses demonstrated that these compounds ameliorate endothelial function, an intermediate endpoint strongly associated with cardiovascular prognosis, both in healthy subjects and in patients with cardiovascular risk factors, while significantly reducing blood pressure.\textsuperscript{41} Despite these promising data, further questions remain to be solved. First of all, it remains unclear how potent are the antioxidant properties of polyphenols and which molecules in this class are the most potent ones. Further, it needs to be clarified whether these compounds possess other properties beyond their chemical antioxidant ones. As well, concentrations of active substances present in food and beverages show remarkable variability due to genetic and agronomic factors, post-harvest handling, and subsequent processing steps. Such problems could theoretically be overcome by using standardized formulations for supplementation, but this field still awaits exploration. As well, in recommending an increase in flavonoid-rich substances such as chocolate and wine consumption, physicians must be careful in balancing caloric and alcohol intake, and this therapeutic approach requires patients’ compliance with chronic lifestyle changes. At the moment, strong evidence obtained with long-term randomized controlled trials is still lacking, and no conclusion on the efficacy and safety of flavonoid supplementation can be reached. Although more promising than other direct antioxidants, whose clinical efficacy is limited by the disadvantageous biochemical properties described previously, the available evidence with flavonoids consists mainly of prospective cohort studies and of mechanistic studies, in vitro or in animals; short-term interventional randomized trials only addressed blood pressure reduction or endothelial function as surrogate endpoints of cardiovascular health. In the meta-analysis mentioned earlier, the positive results on endothelial function and blood pressure were obtained only after several weeks’ administration of certain flavonoid-rich foods, particularly tea and chocolate.\textsuperscript{41} Furthermore, it needs to be mentioned again that an increased antioxidants intake, rather than being the cause of improved outcome, could be simply a marker of a healthier lifestyle. Thus, future research should more clearly address differences between different kinds of polyphenols, in order to identify which type of intervention would constitute the most feasible and effective approach for cardiovascular patients. Additionally, research should aim to clarify whether these encouraging results can be translated into reduction of events in our patients.

Old and new strategies to fight oxidative stress

As mentioned earlier, another strategy for modulating oxidative stress in humans is via exploitation of the pleiotropic properties of drugs directed primarily at other targets. These drugs have indirect antioxidant effects while, at the same time, potentiating endogenous NO production. In line with the role of the renin–angiotensin system in mediating the activation of the NADPH oxidases, ACE inhibitors and angiotensin-receptor blockers have been found to exert non-haemodynamic beneficial effects on endothelial function by inhibiting NADPH oxidase activity, reducing subunits expression and vascular oxidative stress.\textsuperscript{42} Similarly, beyond their lipid-lowering potential, statins are capable of reducing NADPH
oxidase-related ROS production and inducing and activating the NOS. An improvement in the endothelial function of the forearm vasculature was demonstrated only after few days of treatment with statins, i.e. prior to any lipid-reducing effect. Furthermore, both statins and blockers of the renin–angiotensin system are able to reduce mitochondrial oxidative stress. Collectively, there is a general consensus that the beneficial impact of therapy with ACE inhibitors and/or statins is at least in part mediated by properties that are independent of the haemodynamic or cholesterol-lowering effects of these drugs. In an effort to further develop this strategy, given the importance of NADPH oxidase in ROS generation in cardiovascular disease, new drugs, specifically targeted at inhibiting this system, have been developed. Peptide inhibitors of the assembly of the NADPH oxidase complex have shown effectiveness in reducing vascular oxidative stress in experimental models of hypertension. Another possible approach inhibiting the assembly of the intact oxidase by the prevention of phosphorylation of the p47phox subunit is via the administration of protein kinase C inhibitors. This approach appears to be promising in animal and in vitro human studies and is now subject to clinical trials.

Xanthine oxidase, a noteworthy endothelial and cardiac source of superoxide in cardiovascular disease, can be inhibited by an old and inexpensive drug, allopurinol, which has been shown to improve endothelial function and to reduce oxidative stress in a number of cardiovascular diseases. Like for oxypurinol, the antioxidant effect of allopurinol is mediated by both xanthine oxidase inhibition and direct radical scavenging properties. Six-month therapy with oxypurinol was found to reduce mortality in patients with chronic heart failure and high serum urate. In this case, serum urate was a surrogate marker of xanthine oxidase activity (and indirectly of oxidative stress), confirming the utility of stratifying patients to obtain beneficial effects from antioxidant treatments. Novel xanthine oxidase inhibitors have been developed, but their effects in cardiovascular disease have not been tested, complicating the development of these drugs. NAD(P)H oxidases have important physiological functions that should not be impacted by therapies aimed at reducing ROS production. In this sense, as discussed earlier, the fact that ROSs (and their sources) also have regulatory (and in certain cases also protective) functions emphasizes the need for a better understanding of the biology of these molecules before untargeted therapies are attempted.

Targeting NOS uncoupling in order to reduce oxidative stress is possible also in humans, through supplementation of BH4 or of its precursor sepiapterin. BH4 has been shown to improve endothelial dysfunction in smokers, diabetic subjects, hypertensive patients, patients with hypercholesterolaemia, and those with coronary artery disease [for review see (48)]. Folate, which has mild antioxidant properties, could reverse NOS uncoupling, possibly via BH4-dependent mechanisms. Despite these pre-clinical data, the effect of BH4 and related molecules on cardiovascular morbidity and mortality is still unknown, although the possibility of amelioration of cardiovascular prognosis by folate supplementation was definitely ruled out by several randomized controlled trials.

Alternatively, instead of preventing NO degradation through reduction of oxidative stress, a possibility would be to exogenously provide NO via administration of NO-donating substances such as organic nitrates. However, research has shown that these mediators cause endothelial dysfunction and increase oxidative stress through at least three different mechanisms: uncoupling of the mitochondrial respiratory chain, activation of ROS-producing enzymes (including NOS uncoupling), and direct reaction of the nitrate-derived NO with vascular superoxide to form ONOO−. In turn, these mechanisms would accelerate, rather than inhibit, oxidative stress.

Thus, the ideal approach to antioxidant therapy requires stimulation of NO production and simultaneous inhibition of vascular superoxide production. Drugs such as statins, ACE inhibitors, and AT1-receptor blockers, and the NO-releasing β-blocker nebivolol, have been shown to possess such combined antioxidant properties.

Conclusions

The basic principle of pharmacological prevention of cardiovascular events consists of the control of classical risk factors with specific interventions. This clinical approach has led to an improvement in prognosis, although still far below expectations. In other words, the mere control of hypertension, hypercholesterolaemia, or diabetes, although definitely beneficial, leaves a residual risk, which is still greater than that reduced by treatment. Thus, it is necessary to identify different treatment targets, which could more successfully impact on cardiovascular disease.

Oxidative stress is an attractive candidate target, as it represents a common mechanism leading from multiple risk factors to disease. At the present time, however, it is not clear which pathogenetic mechanism should be targeted and/or which kind of compounds should be used. Therefore, retrospectively looking, the attempts made in several clinical trials, which mainly obtained negative results, have to be considered premature. Today, we have to recognize that it may be still too early to test this hypothesis in the clinical setting. Further mechanistic studies, both in animals and in humans, are needed in order to achieve a better understanding of the role of ROS in the pathophysiology of atherosclerosis. Further methods that allow a better characterization of the vascular milieu in humans are necessary. Only a more in-depth knowledge of these important pathophysiological mechanisms can allow identifying and testing a therapeutic strategy aimed at oxidative stress as a possible target of cardiovascular therapy.

Conflict of interest: none declared.

References


