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REVIEW ARTICLE

The influence of sulforaphane on vascular health and its relevance to nutritional approaches to prevent cardiovascular disease

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Abstract Oxidation of low-density lipoproteins (LDL) promotes atherosclerosis by enhancing vascular inflammation and foam cell formation. The corollary is that diets that stimulate endogenous anti-oxidants may protect against atherosclerosis. This review focuses on sulforaphane, an isothiocyanate derived from green vegetables, which induces multiple anti-oxidant enzymes via activation of a transcription factor called Nrf2. Although studies of cultured cells and experimental animals revealed that sulforaphane can suppress inflammatory activation of vascular cells, the potential beneficial effects of sulforaphane in atherosclerosis have not been studied directly. A deeper understanding of vascular responses to sulforaphane may inform nutritional approaches to prevent vascular inflammation and atherosclerosis.

Keywords Preventative medicine · Cardiovascular · Nutrition · Sulforaphane

The influence of oxidants and antioxidants on the development of atherosclerosis

Atherosclerosis is a lipid-driven chronic inflammatory disease [1–4] in which accumulation of cells, lipids and extracellular matrix in the wall of an artery can result in occlusion of the vessel lumen and lead to angina, heart attack or stroke. A critical step in lesion formation is the oxidation of LDL to generate a family of covalent modifications. Oxidised LDL (oxLDL) particles influence atherosclerosis by altering the physiology of endothelial cells (EC), macrophages and other cell types. They promote the recruitment of leukocytes from the bloodstream to the arterial wall by triggering EC expression of adhesion proteins, chemokines and other pro-inflammatory molecules [5–7]. This process involves multiple inflammatory signalling events including induction of intracellular reactive oxygen species (ROS) [6, 8], activation of p38 and ERK MAP kinases [8–10], and activation of the transcription factors NF-κB [6, 11] and STAT3 [7]. Moreover, evidence from our group and others suggests that ROS promote inflammatory activation in part by inactivating enzymes that negatively regulate inflammatory pathways [12–14]. Induction of ROS by oxLDL can also induce EC apoptosis [15–17], and influence vascular tone through uncoupling of eNOS from nitric oxide production [18, 19]. OxLDL can also influence the physiology of macrophages which recognise and internalise oxLDL via scavenger receptors (e.g. CD36, Scavenger Receptor class A (SRA)) [20–23]. Binding of oxLDL to CD36 or SRA initiates a signalling cascade that includes MAP kinase and NF-κB activation [20, 24–28], which drives expression of inflammatory genes. Although clearance of oxLDL by macrophages maintains vascular homeostasis by preventing oxLDL build up, persistent phagocytosis leads to accumulation of intracellular lipids. This process creates activated foam cells that drive atherogenesis by generating oxidant moieties, secreting pro-inflammatory proteins, expressing thrombogenic factors and releasing toxic lipids [2–4].

Biological antioxidants are broadly divided into direct and indirect antioxidants. Direct antioxidants either absorb free radicals (e.g. vitamin E) or transfer electrons in redox-exchange (e.g. vitamin C). Indirect antioxidants, primarily phytochemicals, alter cellular physiology by inducing anti-

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oxidant genes (e.g. heme oxygenase-1 (HO-1), ferritin, thioredoxin, thioredoxin reductase 1, glutathione, superoxide dismutase). Given the central importance of oxidation in atherogenesis, it has been proposed that antioxidants may protect arteries from lesion formation [29–31]. There are several lines of evidence to support this idea: (1) epidemiological studies correlate the consumption of fruit and vegetables rich in anti-oxidants with protection from cardiovascular disease [29–31], (2) in vitro studies revealed that antioxidants exert "anti-atherogenic" effects including suppression of pro-inflammatory activation of EC [32–36] and macrophages [37–39] and inhibition of vascular smooth muscle cell (VSMC) proliferation [40, 41], (3) genetic deletion of antioxidant genes including hemoxygenase-1 (HO-1) [42] and glutathione peroxidase-1 [43] can enhance experimental atherosclerosis, while overexpression of HO-1 [44], thioredoxin [45] or catalase [46] reduces lesion formation, (4) a polymorphism in the promoter of the (antioxidant) HO-1 gene is associated with elevated risk of coronary heart disease in man [47, 48]. The potential utility of antioxidants as therapeutics for the prevention or treatment of atherosclerosis has received considerable attention. However, clinical trials of dietary supplementation with vitamin E did not reveal beneficial effects on cardiovascular health in the general population [30, 31], but gave promising results in some groups of patients with enhanced oxidative load associated with existing coronary artery disease [49] or end-stage renal disease [50]. In view of the disappointing results of direct antioxidants in clinical trials, we suggest that therapeutic strategies aimed at inducing anti-oxidant enzymes indirectly may be more effective. This concept is consistent with studies of probucol, a drug with both lipid-lowering and indirect anti-oxidant effects, which suppressed atherosclerosis in pre-clinical [51, 52] and clinical trials [53, 54]. Importantly, comparison of probucol with control derivatives that retain its anti-oxidant effects but not lipid-lowering effects, indicate that probucol's atheroprotective activity is primarily due to induction of anti-oxidant enzymes in vascular cells [55].

Sulforaphane is an indirect dietary antioxidant

Sulforaphane is a biologically active dietary isothiocyanate that is derived from vegetables in the *Brassica* genus (e.g. broccoli, brussel sprouts, cauliflower, Pak Choi). Recent studies revealed that sulforaphane alters cellular physiology, at least in part, by functioning as an indirect anti-oxidant. This property of sulforaphane is mediated via activation of the transcription factor Nrf2 which is a central regulator of cellular redox [56, 57]. In unstimulated cells, Nrf2 is suppressed by kelch-like ECH-associated protein 1 (Keap1) which targets it for ubiquitination and proteasomal processing. Nrf2 can be activated by sulforaphane and other physiological stimuli which disrupt Keap1-Nrf2 interactions leading to stabilisation and nuclear translocation of Nrf2 [56, 57] (Fig. 1). Following its activation, Nrf2 binds to electrophilic response elements (otherwise known as antioxidant response elements) and induces numerous antioxidants including HO-1, ferritin, thioredoxin, thioredoxin reductase 1 and MnSOD [56–59].

The protective effects of sulforaphane on vascular physiology

The concept that consumption of green vegetables can prevent cardiovascular disease is consistent with epidemiological studies that revealed that dietary intake of broccoli and related vegetables is associated with reduced risk of

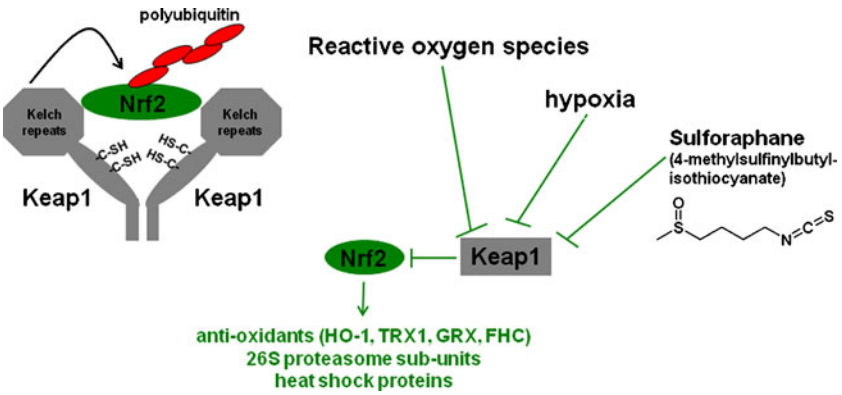

Fig. 1 Regulation and function of Nrf2. Nrf2 is a transcription factor that induces numerous antioxidants (e.g. hemoxygenase-1 (HO-1), thioredoxin (TRX1), ferritin heavy chain (FHC)), 26S proteasome subunits, heat shock proteins and other protective molecules. In unstimulated cells, Nrf2 is inactivated by Keap1 which sequesters Nrf2 in the cytoplasm and targets Nrf2 for ubiquitination and degradation. Nrf2 can be activated by several stimuli (e.g. reactive oxygen species, hypoxia, sulforaphane) that interfere with Nrf2-Keap 1 interaction

coronary heart disease mortality [60–63]. It also consistent with the observation that ingestion of broccoli can reduce oxidation and inflammation in arteries of spontaneously hypertensive stroke-prone rats [64]. Pharmacokinetic studies in humans that revealed that consumption of a single portion of broccoli cress (a particularly rich source of sulforaphane) or mature broccoli can generate plasma sulforaphane concentrations of approximately 1 μM [65, 66] and 60 nM [67], respectively. Plasma sulforaphane concentrations subsequently declined with first-order kinetics (1.77 h half life) to reach low nM concentrations within several hours [65–67]. Although sulforaphane is rapidly cleared from plasma, it is plausible that it may have prolonged effects on vascular physiology as consumption of broccoli can induce antioxidant enzymes for at least 24 h in healthy volunteers [68, 69]. In vitro studies revealed that concentrations of sulforaphane that can be achieved in plasma through consumption of Brassica vegetables can influence the physiology of vascular and inflammatory cells [70–75], suggesting that green vegetable intake may prevent cardiovascular disease in part via generation of sulforaphane.

We recently studied the effects of shear stress and sulforaphane on endothelial activation in aortae of mice challenged with lipopolysaccharide (LPS) [70]. Vascular inflammation and atherosclerosis develop predominantly at branches and bends of arteries which are exposed to non-uniform blood flow which exerts relatively low shear stress on vascular endothelium, whereas regions of arteries that are exposed to high shear stress are protected [76, 77]. Pro-inflammatory activation of EC is reduced at high shear sites compared to low shear regions, thus providing a potential explanation for the distinct spatial localisation of lesions [70, 78, 79]. Our findings revealed that high shear stress at atheroprotected sites reduced key measures of EC inflammatory activation, p38 MAP kinase activation and VCAM-1 expression, by activating Nrf2 [70] (Fig. 2). By contrast, EC at a low shear, atherosusceptible site contained inactive (cytoplasmic) Nrf2 and were prone to pro-inflammatory activation. We therefore examined whether pharmacological activation of Nrf2 using sulforaphane would suppress inflammation at susceptible sites. In cultured EC, sulforaphane suppressed p38 activation, VCAM-1 expression and ROS production via Nrf2 [70, 71]. Similarly, sulforaphane suppressed p38 activation and VCAM-1 expression at atherosusceptible sites in wild-type but not in Nrf2-/- mice, indicating that the anti-inflammatory effects of sulforaphane were Nrf2-dependent [70] (Fig. 2). Thus our observations reveal that inflammation at atherosusceptible regions can be prevented by sulforaphane-mediated activation of Nrf2.

Given observations from our group and others that Nrf2 and its target genes can protect against vascular inflammation [42, 43, 70] it would be expected that ablation of Nrf2 would exaggerate atherosclerosis. Consistent with this, Levonen et al. demonstrated that Nrf2 overexpression in the rabbit aorta reduces inflammation in response to injury [80]. However, paradoxically, a recent report points in the opposite direction, with genetic deletion of Nrf2 suppressing CD36-dependent uptake of modified LDL by macrophages and reducing lesion development in an atherosusceptible strain of mice [81]. This latter study does not however preclude a protective role for Nrf2 during the initiation of atherosclerosis because: (1) early stages of atherogenesis were not studied, (2) the model studied is severe and any protective effects of Nrf2 may have been overwhelmed by the high atherogenic drive. Similarly, although sulforaphane can protect arteries from inflammation, this compound may have deleterious effects on vascular health at high concentrations, e.g. although low concentrations (<1 μM) of sulforaphane can reduce inflammatory activation of EC without altering viability [70, 71], very high concentrations (>10 μM) can trigger EC apoptosis [82–84]. High concentrations of sulforaphane can also induce CD36 in macrophages [85] and therefore may potentially influence the formation of foam cells which play

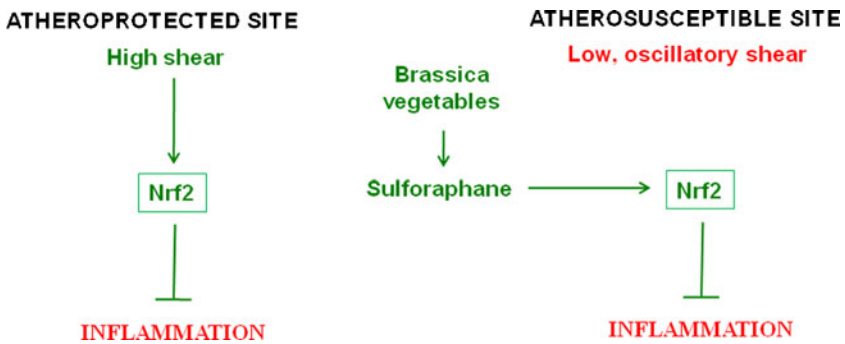

Fig. 2 Model—Consumption of Brassica vegetables may prevent vascular inflammation at atherosusceptible sites. Regions of arteries with relatively uniform geometry are protected from inflammation and atherosclerosis by high shear stress which activates Nrf2. By contrast, branches and bends that are exposed to low, oscillatory shear stress are susceptible to lesion formation. However, dietary consumption of Brassica vegetables may prevent branches and bends from inflammation by activating Nrf2 in endothelial cells

a central role in atherogenesis. Thus it will be important in future studies to assess directly whether relatively low concentrations of sulforaphane (achievable through consumption of green vegetables) can prevent atherosclerosis, without triggering potential deleterious effects on EC viability.

Outlook

There is now strong evidence to suggest that regular consumption of Brassica vegetables may provide a preventative dietary approach to reduce cardiovascular disease risk. This has been obtained partly through epidemiological studies that correlated green vegetable intake with protection. The idea is also consistent with the observation that plasma concentrations of sulforaphane that can be achieved by consumption of green vegetables are sufficient to suppress inflammatory activation of arteries. Clinical trials should now be carried out to test directly whether consumption of green vegetables or bioactive derivatives (e.g. sulforaphane) can prevent the initiation and progression of atherosclerosis. This idea is currently being pursued through a study that will investigate the effects of a diet rich in broccoli on plasma cholesterol levels, blood pressure and augmentation index and pulse wave velocity (measures of arterial stiffness) in subjects with elevated cardiovascular risk (ClinicalTrials.gov identifier NCT01114399). In addition to their scientific value, trials of this nature may also provide value in terms of public perception by reinforcing guidelines that eating fruit and vegetables can prevent cardiovascular disease.

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