Oxidative stress and abdominal aortic aneurysm: Potential treatment targets.

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Abstract

Abdominal aortic aneurysm (AAA) is a significant cause of mortality in older adults. A key mechanism implicated in AAA pathogenesis is inflammation and the associated production of reactive oxygen species (ROS) and oxidative stress. These have been suggested to promote degradation of the extracellular matrix and vascular smooth muscle apoptosis. Experimental and human association studies suggest that ROS can be favourably modified to limit AAA formation and progression. In this article, we discuss mechanisms potentially linking ROS to AAA pathogenesis and highlight potential treatment strategies targeting ROS. Currently, none of these strategies have been shown to be effective in clinical practice.

Key words- abdominal aortic aneurysm, pharmacotherapy, oxidative stress, clinical trial, and animal models

Introduction

Abdominal aortic aneurysm (AAA) is a degenerative disease of the aorta common in people aged > 65 years.(1, 2) AAA is usually asymptomatic until rupture which is frequently fatal.(1-4) However, rupture of small AAAs is rare.(5) The main focus of AAA management is to prevent rupture, therefore current guidelines recommend that patients with large AAAs (>5-5.5cm) undergo endovascular stent graft or open surgical repair.(4, 6) Patients with small AAAs undergo regular imaging to monitor AAA diameter, although up to 70% of these individuals eventually require AAA repair.(2)

AAA is believed to result from an aberrant interaction between genetic factors and the environment which aggravates the normal ageing processes.(1, 2) One of the key features of AAA is vascular wall inflammation associated with significant production of reactive oxygen species (ROS).(3, 6) ROS, which include hydrogen peroxide (H_2O_2), superoxide (O_2^-), and hydroxyl radical ($^{\bullet}OH$) are oxygen-derived chemical molecules with high reactivity.(7, 8) Reports from recent clinical and experimental investigations suggest that oxidative stress, i.e. production of ROS in excess of antioxidant protection, is involved in the vascular degeneration found in AAA.(9-11) Imbalance in the activity of endogenous pro-oxidative enzymes such as nicotinamide adenine dinucleotide phosphate oxidase (NOX) and xanthine oxidase (XO), the mitochondrial respiratory chain and the antioxidant enzymes such as glutathione peroxidase, heme oxygenase (HO), superoxide dismutase (SOD), thioredoxin (TRX), and catalase results in excessive ROS.(12, 13) This is implicated in vascular cell dysfunction, lipid and protein peroxidation, and deoxyribonucleic acid (DNA) damage which can result in permanent cellular damage and death.(7, 12, 13) ROS regulate the degradation and remodelling of the extracellular matrix (ECM) by upregulating proteolytic enzymes such as matrix metalloproteinases (MMPs), (14) activating signalling kinases and transcription factors such as nuclear factor kappa beta (NF-κB) and activator protein 1 (AP-1),(15-17) and promoting vascular smooth muscle cell (VSMC) apoptosis.(18, 19) ROS also regulate fibroblast proliferation and macrophage and mononuclear lymphocyte infiltration.(7, 20, 21)

In this article we discuss mechanisms potentially linking ROS to AAA pathogenesis and review potential treatment strategies targeting ROS in the management of AAA based on experimental and human studies.

Literature Search

A literature search was conducted to identify studies assessing antioxidants as therapeutic targets for AAA using the Embase (1980), MEDLINE (1966), SCOPUS (1996), Web of Science (1965), and Cochrane Library databases (1992) from inception to the 25th of May, 2015. The following search terms were applied either as single or combined searches: “abdominal aortic aneurysm” OR “AAA”, [Title/Abstract] AND “antioxidant treatment” OR “antioxidant targets” OR “antioxidant therapy”, AND/OR “clinical studies” OR “human studies”, AND/OR “animal studies” OR “experimental studies”. Abstracts were analysed for relevance and studies describing the therapeutic potential of antioxidants in AAA pathogenesis were retrieved. Human and animal studies investigating the therapeutic potential of antioxidants in AAA were included. Included article references were hand searched for relevant publications meeting the inclusion criteria. Studies excluded include those in languages other than English and studies unrelated to oxidative stress.

Evidence linking ROS with AAA

ROS such as NO and O_2^- are produced by all vascular cell types principally via membrane-associated-specific enzymes such as NOX, XO, and nitric oxide synthase (NOS). (7, 13) Both animal and human studies have associated elevated concentrations of ROS, or surrogate markers of ROS with AAA. (11, 22-24)

Animal studies

Evidence from different animal models implicates ROS in AAA pathogenesis. Nakahashi and colleagues reported that HO-1 expression was significantly upregulated within the elastase-induced rat model of AAA. (24) They further demonstrated the localisation of HO-1 to infiltrative macrophages within the aneurysmal aortic wall. Similarly, iNOS produced NO has been reported to exacerbate experimental AAA development.(25) Lizarbe *et al.* reported a reduction in aortic diameter and MMP-13 expression in mice administered the iNOS inhibitior, 1400 W.(14) In the angiotensin II (AngII)-induced mouse model of AAA, 8-isoprostane,(23) and 8-hydroxy-2' -deoxyguanosine (8-OHdG), (9) concentrations (markers of oxidative stress) have been reported to be high within the aneurysmal aorta. Similarly, high 8-OHdG content and glutathione peroxidase expression were reported within the calcium chloride ($CaCl_2$)-induced mouse model of AAA. (26) Infiltrating inflammatory cells and their products have been reported to be involved in aortic wall degradation and ensuing AAA formation.(3, 27) (28)

Human associated studies

Dubick and colleagues reported low levels of vitamin C, and lower copper-zinc SOD (CuZnSOD) activity in aortic tissue samples from 29 patients with AAA and 14 patients with atherosclerotic occlusive disease (AOD) compared to non-diseased control aortas.(29) SOD is a family of metalloenzymes including CuZnSOD, manganese SOD (MnSOD), and extracellular SOD (EcSOD) that catalyses the dismutation of O_2^- into oxygen and H_2O_2 thereby maintaining vascular NO concentrations.(30, 31) High MnSOD and low CuZnSOD activities were observed in a study evaluating the effect of hypertension on aortic antioxidant status in human AAA and AOD.(42) In contrast, a separate study suggested that both CuZnSOD and MnSOD activities were lower in infra-renal aortic biopsies collected from patients with intact and ruptured AAAs compared to non-aneurysmal organ donors.(32) This study also suggested that glutathione reductase and glutathione peroxidase were downregulated whilst lipid peroxidation products were high in AAA patients, particularly those with ruptured aneurysms, compared to controls.(32) Aberrant lipid peroxidation has been associated with aortic cell apoptosis and necrosis, (discussed in (33, 34)) which potentially promotes aortic weakening and AAA. Evidence also suggests that dysregulated antioxidant protective mechanisms,(35-42) and/or low levels of exogenous antioxidants (vitamin C, vitamin E) can exacerbate ROS activity.(7, 13, 43) In addition, Miller *et al.* reported high expression of O_2^- and NOX activity in biopsies of human aneurysmal aortas compared with biopsies from adjacent non-aneurysmal aortas.(11) Zhang *et al.* reported high iNOS expression in the media and adventitia of human AAA tissues compared to controls.(44) They suggested that iNOS promotes AAA progression by selectively stimulating the formation and activity of the NO-derived oxidant peroxynitrite ($ONOO^-$) with consequent aortic oxidative injury and AAA.(44)

Collectively, these studies suggest that oxidative stress is associated with the presence of AAA. It remains to be shown whether targeting ROS or their modulators would be an effective strategy in managing AAA.

Potential mechanisms linking oxidative stress with AAA and associated therapeutic targets

Sies defined oxidative stress as an imbalance between ROS and antioxidants in favour of the oxidants leading to cellular injury and damage.(45) There is a close association between oxidative stress and inflammation as evidence suggests that oxidative stress induces inflammation, which in turn potentiates oxidative stress with resultant injury to tissues.(11, 46, 47) The human body is equipped to maintain the oxidant-antioxidant balance to avoid injury in physiological conditions, but potentially not in pathophysiological conditions like AAA (discussed in (45, 48)).

A number of endogenous enzyme systems regulating oxidative stress in AAA have been researched in both pre-clinical and clinical studies in the past decade (Figure 1). The following sections summarise the potential mechanisms linking these pathways to AAA and strategies which potentially, may be used therapeutically to limit AAA (Figure 2).

Xanthine and NADPH oxidases

NOX activity has been reported to be markedly elevated by stimuli thought to be key in the pathogenesis of AAA such as Ang II, TNF-α, mechanical stretch, and endothelin-1 acting both through transcriptional pathways and posttranslational modification of oxidase regulatory subunits.(7, 49) Ang II for example, has been reported to induce mitochondrial dysfunction via a protein kinase C–dependent pathway by activating NOX and forming NO, $ONOO^-$, and O_2^- (Figure 2).(50) Oxidative stress has been reported to enhance expression of angiotensin converting enzyme (ACE) which is responsible for the conversion of angiotensin I (Ang I) to Ang II.(51) In addition, mice overexpressing the NOX catalytic subunit Nox1 within VSMC are reported to have an enhanced response to Ang II, exhibit increased O_2^- and H_2O_2 production and have aortic thickening.(52, 53) Thomas *et al.* employing the Ang II-infused mouse model of AAA reported that deletion of p47phox, a cytosolic subunit of NOX, abrogated NOX activity, reduced production of ROS within the aorta and within infiltrating leukocytes, and significantly reduced the incidence and progression of AAA.(54) In contrast, inhibition of Nox1 has been associated with resistance to aortic dissection and AAA development in mouse models.(55) However, a deficiency in another NOX catalytic subunit, Nox2 was reported to exacerbate Ang II-induced AAA in mice and promote interleukin-1 beta (IL-1β) and MMP-9 expression.(56) Inhibition of IL-1β or IL-1β receptor expression has been reported to confer AAA resistance in mouse models.(57)

XO is also a H_2O_2 and O_2^- generating enzyme formed from the proteolytic breakdown of xanthine dehydrogenase.(13) Experimental evidence suggests XO is expressed by aortic endothelial cells in a NOX dependent manner,(58) and is actively involved in lipid peroxidation and damage to the ECM (discussed in (59)). However there are limited studies investigating the specific role of XO in AAA pathogenesis.

Taken together, these studies suggest a potential mechanism by which NOX may modulate AAA pathogenesis via effects on the renin-angiotensin system (RAS), inflammatory cytokines and MMPs.

Zhang *et al.* reported that cilostazol, a phosphodiesterase III inhibitor that selectively targets cyclic adenosine monophosphate (cAMP), inhibited AAA development in an elastase-induced rat model.(60) Cilostazol is a 2-oxo-quinoline derivative with antithrombotic, vasodilator, antimitogenic and cardiotonic properties and is indicated for intermittent claudication in patients with peripheral arterial disease.(61) Cilostazol was shown to significantly reduce NOX activity and ROS concentration with consequent inhibition of MMP-2 and MMP-9 expression, and NF-κB activation ($P < 0.05$).(60) In a $CaCl_2$ mouse model of AAA, mice orally administered apocynin (a NOX inhibitor) were shown to have reduced MMP-2, MMP-9 and NO metabolite (NO_2 and NO_3) expression within aortic tissues, and decreased aneurysm formation compared to controls.(18) In contrast, Kigawa *et al.* reported that mice deficient in NOX demonstrated increased incidence of AAA, albeit with decreased ROS expression, within an Ang II-induced low-density lipoprotein receptor knock-out ($Ldlr^{-/-}$) mouse model of AAA.(56) Collectively, these studies suggest that medication targeting both NOX and XO pathways may limit AAA formation and progression, although more studies are needed to validate these findings.

Lipoxygenases

Lipoxygenases are non-heme iron–containing enzymes that catalyse the stereospecific deoxygenation of polyunsaturated fatty acids with a 1, 4-cis, cis-pentadiene structure (62, 63) and are also proficient in the generation of ROS, or augmenting leukocyte ROS production.(8, 64) Lipoxygenases are required for the biosynthesis of leukotrienes, which are key inflammatory and chemotactic mediators.(65) For example, inhibition of lipoxygenases including 5- lipoxygenase (5-LO) has been reported to reduce the expression of MMP-2 and -9, and phagocytic macrophage infiltration.(66-68) The proteolytic breakdown of the ECM by MMPs has been reported to be one of the key mechanism involved in AAA pathogenesis (for a detailed review of MMPs and AAA, please see (69)). It has been suggested that by promoting inflammation and the proteolytic degradation of the ECM via effects on MMPs expression and recruitment of inflammatory cells, lipoxygenases may play a role in inducing AAA formation.

A growing body of evidence has implicated lipoxygenases in the pathogenesis of AAA as illustrated in Table 1.(46, 66, 67) A recent study by Bhamidipati *et al.* using an elastase perfused- and an Ang II-infused $Ldlr^{-/-}$ mouse models of AAA demonstrated that 5- lipoxygenase (5-LO) inhibition attenuated AAA formation and progression.(67) The authors reported that genetic deletion of 5-LO within the elastase infused mouse model resulted in a 71% reduction in aortic dilatation compared with wild-type controls. Mice administered 30 mg/kg/day of AZD4407 (a selective 5-LO inhibitor) were also shown to be resistant to AAA formation and progression associated with decreased MMP-9 enzymatic activity and immune cell infiltration within aortic tissue. These findings were further strengthened through studies within the Ang II-infused $Ldlr^{-/-}$mouse model of AAA, were they reported that administration of AZD4407 within the chow for 28 days inhibited 5-LO activity within the circulation resulting in a 54% reduction in aneurysm formation compared to control.(67)

Edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one), a powerful antioxidant indicated as a therapeutic agent for acute cerebral infarction,(70) was reported to significantly reduce the expression of MMP-2, MMP-9 and ROS, and abrogate AAA formation and expansion in the combined elastase-induced and $CaCl_2$-induced rat model of AAA.(71) In addition, earlier studies by Zhao *et al.* found that $ApoE^{-/-}$ mice lacking 5-LO genes and fed a cholic acid-rich diet demonstrated a reduced incidence of AAA.(66) Consistent with this, Ang II-infused $ApoE^{-/-}$ mice deficient in leukotriene B4 receptor 1

(BLT1) were shown to exhibit decreased incidence and size of AAA.(72) In a similar model, a BLT antagonist (CP-105,696) limited AAA formation when administered at the same time as Ang II infusion, but did not limit progression of established aneurysms. (68). Cao *et al.* employing the Ang II-infused $ApoE^{-/-}$ mouse model of AAA, reported that mice deficient in 5-LO or administered a 5-LO activating protein (FLAP) inhibitor (MK-0591) had a similar incidence of AAA development to controls.(73)

A human association study reported higher expressions of leukotriene C4 synthase (LTC4S), 5-LO and FLAP within aneurysmal aortas compared to non-aneurysmal donor aortas.(74) Others have also reported high production of leukotriene B4 (LTB4) by polymorphonuclear leukocytes localised within intra-luminal thrombus samples collected from patients undergoing aneurysm repair.(75) A study involving 613 men aged ≥65 with AAA and 707 randomly selected age-matched controls failed to find an association between 7 single nucleotide polymorphisms in ALOX5AP, the gene encoding FLAP, with human AAA.(76) Together, these reports fail to show a consistent role of lipoxygenase in human and experimental AAA.

Cyclooxygenases

Cyclooxygenases (COX) are the key enzymes involved in the conversion of arachidonic acid to prostaglandins.(77) It has been reported that COX-2 and its metabolite, prostaglandin E2 (PGE2), are highly expressed within aneurysmal tissue and exacerbate VSMC apoptosis.(78-80) COX-2 expression was also reported to be associated with increased macrophage infiltration,(81) and increased MMP-2 expression and aortic remodelling. Collectively, this information suggests that COX-2 may play a significant role in AAA pathogenesis via effects on VSMC integrity and inflammation, stimulating interest in treatments targeting the COX pathway as a means of limiting AAA. Celecoxib, a nonsteroidal anti-inflammatory drug (NSAID), and a selective COX-2 inhibitor, was reported to reduce the incidence and severity of AAA within the Ang II-induced mouse model in both hyperlipidaemic and nonhyperlipidaemic mice.(82) In the same mouse model of AAA, Gitlin *et al.* reported that mice with genetic deletion of COX-2 failed to develop AAA. They reported a 73% and 90% reduction in AAA incidence following 7 and 21 days of Ang II infusion respectively and a marked decrease in the aortic expression of the macrophage marker CD68, MCP-1 and macrophage inflammatory protein-1alpha (MIP-1α).(81) This is in contrast to the study by Cao *et al.* reporting that genetic deletion of COX-2 had no significant effect on AAA development within the Ang II-induced AAA mouse model.(83) However, two recent studies provide evidence that mice administered celecoxib have decreased AAA progression and rupture within the Ang II infused mouse model of AAA.(84, 85) Another selective COX-2 inhibitor, MF-tricyclic, was reported to inhibit MMP-9 expression and consequently AAA growth within an elastase-induced rat model of AAA.(86) In support, two other studies employing the same elastase-induced rat model of AAA reported that indomethacin (a non- selective COX-2 inhibitor) significantly inhibited PGE_2 and MMP-9 expression thereby preserving elastin integrity and reducing AAA expansion with no effect on the inflammatory infiltrate.(87, 88) Conversely, Armstrong and colleagues reported that indomethacin and rofecoxib (a selective COX-2 inhibitor) failed to impede aneurysm expansion within the elastase-induced AAA rat model.(89) Furthermore, in a small case-control study involving 15 subjects on NSAIDs and 63 patients without NSAID, it was reported that AAA growth was significantly reduced in patients receiving NSAIDs compared to controls without NSAIDs.(80) COX inhibitors have been associated with increased incidence of cardiovascular events (myocardial infarction stroke and cardiovascular death), and it is therefore unclear whether they would be safe to give patients with AAA.(90, 91) The

conflicting evidence from experimental studies regarding their therapeutic potential does not currently support their trialling in AAA patients. A summary of studies investigating the role of COX in AAA pathogenesis is given in Table 2.

Nitric oxide synthases

The family of NOS including inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (nNOS) are capable of generating NO and L-citrulline by catalysing the oxidation of L-arginine. Both eNOS and nNOS are selectively expressed whereas iNOS is widely distributed within multiple cell types.(12, 92) NO is an important regulator of cardiovascular homeostasis, however evidence suggest that under specific conditions, exaggerated production of NO by iNOS may lead to tissue damage through several mechanisms including the formation of reactive nitrogen species.(93) In addition, uncoupling of eNOS from its cofactors is associated with increased generation of O_2^-,(93, 94) and consequent tissue damage which has been suggested to be relevant in AAA pathogenesis.(94, 95)

A number of medications which were not developed to modulate NOS have been suggested to have pleiotropic effects on NOS which in part explain their ability to limit AAA within experimental models (Table 3). For example a number of studies suggest that statin therapy may limit AAA development due to its antioxidant and anti-inflammatory effects.(96-102) In an elastase induced rat model of AAA, Kalyanasundaram *et al.* reported that simvastatin administration resulted in the downregulation of several oxidative stress-related genes including SOD2, HO-1, NOS3 and thioredoxin reductase 1 (TRXR1) with consequent AAA inhibition.(96) Simvastatin was also shown to reduce NF-κB, MMP-9 and MMP3 expression.(96) This is supported by the study by Steinmetz *et al.* which demonstrated that simvastatin inhibited AAA formation independent of serum cholesterol levels in the C57BL/6 wild type and the $ApoE^{-/-}$ mice using the elastase-infused mice model of AAA.(97) They reported a simvastatin-mediated decrease in MMP-9 and increase in TIMP-1 expression, but did not measure any ROS.(97) In contrast, others have reported that simvastatin had no significant effect on aortic diameter within the Ang II-induced mouse model of AAA.(103) In two previous studies, fenofibrate, a peroxisome proliferator-activated receptor alpha (PPARα) activator used clinically to reduce triglycerides, was shown to abrogate Ang II-induced AAA within $Ldlr^{-/-}$ and $ApoE^{-/-}$ mice.(104, 105) Fenofibrate upregulated eNOS within the tunica intima and iNOS within the tunica media and adventitia associated with reduced Ang II induced AAA formation.(105) In addition, within a $CaCl_2$ mouse model of AAA, iNOS deficient mice were shown to be partly resistant to AAA formation associated with decreased MMP-2, MMP-9 and NO expression within aortic tissues.(18) In contrast, Gao *et al.* reported that mice deficient in the eNOS cofactor tetrahydrobiopterin (H4B) were prone to Ang II-induced AAA formation.(94) They reported that oral folic acid treatment upregulates the expression and activity of the H4B salvage enzyme dihydrofolate reductase within the endothelium, restores eNOS function and inhibited AAA formation.(94) In a further study employing the Ang II infused $ApoE^{-/-}$ mice model of AAA, Siu and colleagues also demonstrated that oral administration of folic acid limited aortic elastin degradation, macrophage infiltration and significantly inhibited AAA expansion by upregulating eNOS activity.(95) Similarly, deletion of the eNOS gene within $ApoE^{-/-}$ mice has been associated with increased AAA formation.(106) These studies suggest that due to the complexity of these enzymes, the underlying difference in the animal models involved, and the inconsistent findings it is currently unclear how NOS regulation is involved in AAA pathogenesis.

Heme oxygenases and thioredoxin

HO-1 is an enzyme that reduces oxidative stress by catalysing heme to carbon monoxide, biliverdin, bilirubin, and free ferrous iron.(12, 13, 107, 108) The endogenously produced carbon monoxide is reported to serve as a second messenger influencing cellular proliferation, inflammation, and apoptosis.(109)

Nakahashi *et al.* were the first to suggest that HO-1 may be beneficial in limiting AAA development.(24) The authors conducted a microarray analysis to investigate aortic gene expression following a femoral arteriovenous fistula to increase laminar shear and wall strain within an elastase-induced rat model of AAA. They found that flow loading decreased AAA diameter and upregulated HO-1 expression.(24)

Two contradictory case-control studies were found examining the relationship between the HO-1 gene promoter region and AAA prevalence in humans.(110, 111) Short (<25 GT) dinucleotide repeats within the HO-1 gene promoter region are associated with greater HO-1 expression whilst longer (≥25 GT) dinucleotide repeats are associated with lower HO-1 expression in response to recognised stimuli. (112) A study by Schillinger and colleagues suggested that patients with AAA were less frequently carriers of short (< 25 GT) repeats within the HO-1 gene promoter compared to atherosclerotic or healthy controls. The findings suggested that short (<25 GT) dinucleotide repeats within the HO-1 gene promoter region associated with increased HO-1 expression protected against AAA.(110) In contrast, a more recent study by Gregorek *et al.* found more short (< 25 GT) repeats within the HO-1 gene promoter alleles in AAA patients compared to healthy non-aneurysmal controls.(111) Both studies have limitations that may have influenced the results, such as, the small sample sizes (70 AAA patients in the study of Schillinger *et al.* and 117 in the investigation of Gregorek and colleagues), the relatively wide 95 % confidence interval in both studies, and lack of validation of findings. Therefore, it remains to be seen what role HO-1 plays in AAA formation and progression.

Thioredoxin (TRX) is a thiol oxidoreductase and regulator of signal transduction found in endothelial cells and VSMC.(41) Functionally, TRX has been suggested to modulate NOX-mediated generation of O_2^- via interaction with p40Phox,(113) and to decrease IL-1β expression by monocyte-derived macrophages during inflammation.(114) These effects are potentially partly mediated by downregulating macrophage inhibitory factor (MIF).(115)

Only one study was found investigating TRX in AAA patients.(22) In a multicentre cohort study, the authors reported that TRX was present at higher concentrations in the serum of patients with AAA and concentrations were correlated with AAA diameter and growth rate.(22) More investigations into the importance of TRX in AAA using experimental and human association studies are needed to further elucidate the role of TRX.

Superoxide dismutases and catalases

The efficacy of the SOD system has been reported to rely on the ability of catalase or glutathione peroxidase to subsequently catalyse the decomposition of H_2O_2 to water and oxygen, and inhibit the conversion of H_2O_2 to the $^{\bullet}OH$.(13) H_2O_2 has been reported to promote MMP-3, MMP-9 and MMP-12 expression,(116) which have been implicated in ECM degradation important in AAA. The ability of the SOD system to balance H_2O_2 levels could be important in protecting against AAA.

Catalase is an enzyme responsible for the degradation of H_2O_2 to water and oxygen and has cardiovascular protective effects,(117) including inhibition of Ang II-induced aortic thickening.(37) Patients with AAA have been reported to have low catalase expression within circulating neutrophils and plasma.(118) Catalase has been reported to abrogate the H_2O_2-mediated increases in MMP expression and inflammatory cytokine secretion. (117, 119)

Several studies have investigated the relationship between SOD, catalases and AAA as shown in Table 4.(119-124) In a study, employing an elastase-induced rat model of AAA, Sinha *et al*. reported a significant increase in MnSOD levels and MMP-9 activity in aneurysmal aortas during the early stages of aneurysm formation. CuZnSOD and EcSOD levels were similar in AAAs and control aortas.(120) The authors reported that a SOD mimetic, TEMPOL, increased MMP-2 and MMP-9 activity by more than 2-fold in aortic explant cultures, and suggested that strategies aimed at inhibiting oxidative stress during AAA formation should focus on MnSOD.(120) In a recent study employing the $CaCl_2$ induced mouse model of AAA, a standardised extract from *Ginkgo biloba*, EGb 761, reported to possess antioxidant and free radical scavenging properties,(125) was shown to significantly reduce NF-κβ protein expression, MMP-2 and MMP-9 activities within the aorta, inhibit SOD and catalase activity, and decrease aneurysm size.(126) Other studies however report that increased SOD is beneficial against AAA formation.(121) For example, another natural product, diferuloylmethane (curcumin), a phenol found in the dietary spice turmeric,(127) has been reported to exert anti-inflammatory effects via inhibition of ROS production and enhanced SOD expression.(121) Curcumin was also reported to inhibit aneurysm formation.(128) An initial study using the elastase-induced mouse model of AAA showed that curcumin inhibited AAA formation by decreasing aortic inflammation and MMP-9 expression.(129) Similarly, in the Ang II-induced mouse model of AAA, Hao *et al*. reported that oral administration of curcumin significantly decreased aortic macrophage infiltration, MCP-1 and TNF-α concentrations and AAA incidence, and increased SOD activity.(121) Cobalt chloride ($CoCl_2$), an inhibitor of the hypoxia-inducible factor alpha (HIF-1α) degrading prolyl hydroxylase domain protein,(130) was reported to restore SOD and catalase expression within a $CaCl_2$ model of AAA, and inhibit NF-κβ phosphorylation, cytokine expression and consequent AAA formation.(123) Taken together, there is no consistent evidence on the role of SOD in AAA pathogenesis.

A study examining the effect of tamoxifen, a selective oestrogen receptor modulator with potent antioxidant and vasodilator properties,(131) demonstrated that rats receiving 10mg/kg/day of tamoxifen subcutaneously exhibited a marked increase in aortic catalase expression associated with significant inhibition of neutrophil infiltration and AAA expansion compared to controls within the elastase-induced rat model of AAA.(124) The direct irreversible catalase inhibitor 3-amino-1, 2, 4-triazole (AT) was shown to significantly abrogate the aneurysm inhibitory effect of tamoxifen by roughly 30%.(124) In addition, transgenic $ApoE^{-/-}$ mice overexpressing the human catalase gene within VSMC where shown to be resistant to Ang II induced aortic wall remodelling thought to be involved in early AAA formation.(132) This finding was supported by a similar study within the $CaCl_2$ induced mice model of AAA, where both transgenic mice over expressing catalase and mice administered polyethylene glycol-catalase (PEG-catalase) where shown to exhibit decreased MMP activity, decreased VSMC apoptosis and decreased AAA formation.(119) However, mice transgenic for catalase did not have altered H_2O_2 concentrations.(119) These studies suggest that upregulating aortic catalase activity is a putative mechanism to limit AAA progression.

Studies investigating exogenous and phenolic antioxidants

Exogenous antioxidants including folic acid, vitamin C (ascorbic acid), and vitamin E (α-Tocopherol) are reported to modulate ROS production with implications for AAA pathogenesis.(23, 133-136) For example, vitamin E which has been reported to also inhibit COX expression,(133) has been shown to inhibit AAA formation in two different rodent models of AAA.(23, 24, 137) In the Ang II-induced $ApoE^{-/-}$ mice model of AAA, Gavrila *et al.* reported decreased concentrations of markers of oxidative stress and aortic macrophage infiltration along with reduction in maximum AAA diameter and incidence of rupture in mice receiving vitamin E.(23) Gopal *et al.* reported that dietary supplementation with vitamin E and β-carotene confers substantial protection against Ang II induced AAA formation. (137) In an elastase-induced rat model of AAA, Nakahashi and colleagues reported that rats receiving vitamin E had significantly decreased AAA expansion compared to controls.(24) Unfortunately in a randomised double-blind placebo-controlled trial, Tornwall *et al.* failed to show any significant association between vitamin E supplementation and the incidence of AAA.(138) The latter study did not include aortic imaging and simply relied on the clinical presentation of AAA. This study does not therefore rule out a benefit of vitamin E in limiting AAA growth.

Vitamin C is considered to be one of the most efficient water-soluble antioxidant in human plasma with the ability to scavenge ROS, regenerate α-tocopherol and reduce H4B preventing eNOS uncoupling.(12, 43, 139) Shang *et al.* in a study using the elastase-induced rat model of AAA demonstrated that daily intraperitoneal injection of vitamin C (100mg/ml) significantly decreased 8-hydroxylguanine and 8-isoprostane (markers of oxidative stress), MMP-2, MMP-9 and IL-1β expression, upregulated TIMP-1 and -2 expression and abrogated AAA expansion by 26%.(10) In a similar study employing the combined elastase and $CaCl_2$-induced rat model, vitamin C administration was shown to limit AAA formation through downregulation of MMP-9, MCP-1, IL-1β, TNFα, CD68, and ROS (8-hydroxylguanine).(140)

Resveratrol (3,5,4′-trihydroxy-trans-stilbene), a dietary polyphenol commonly found in red wine and grape skin with antioxidant,(141-144) anti-inflammatory,(145) and anti-angiogenic(146) effects has been reported to inhibit experimental AAA.(26, 147) In a $CaCl_2$ induced mice model of AAA, resveratrol (at 100mg/kg/day) was reported to prevent AAA development by attenuating the expression of glutathione peroxidase, MCP-1, TNF-α and CD68 and reducing the activity of MMP-9 and -2.(26) One limitation of this study was the effect of resveratrol at other doses or time points was not reported especially since 100mg/kg/day is quite a large dose within mice. Palmieri *et al.* studied the effect of resveratrol within the elastase-induced rat model of AAA.(147) They reported that male Sprague-Dawley rats receiving 10 mg/kg/day resveratrol one week before until two weeks following AAA induction exhibited significantly decreased CD62L-monocyte subset expansion, CD143 monocyte expression, TNFα expression and MMP-9 activity with consequent inhibition of AAA development.(147) In addition, the polyphenolic flavonoid quercetin, which has antioxidant and anti-inflammatory properties,(148) was reported to reduce the aortic expression of MMP-2, MMP-9, and cathepsin, and limit macrophage infiltration with consequent decrease in AAA diameter.(149) In a second experiment, the authors reported the inhibitory effects of quercetin on AAA incidence via attenuation of oxidative stress and MMP activation was in part through the modulation of c-Jun N-terminal kinase (JNK)/AP-1 signalling.(150)

The potential of modulating oxidative stress to limit AAA growth in patients

The studies noted above provide good evidence that markers of oxidative stress are upregulated in experimental and human AAA. The wide range of oxidative stress pathways upregulated and the number of interactions between these makes it extremely complex to design interventions which can effectively reduce aortic oxidate stress long-term (see Figure 2). This is particularly difficult where high levels of oxidative stress are already present such as within established AAAs. Many of the interventions which have been successful in experimental models have been applied prior to or at the time of AAA induction. However, treatments are needed for established AAAs in patients.(151) Recently a number of strategies which were successfully applied in animal models, such as doxycycline mediated MMP inhibition and use of a mast cell inhibitor, have been reported to be ineffective at limiting AAA growth in patients.(152, 153) It is currently not clear why previous promising strategies to limit AAA growth have not been translated to patients, although suggested reasons include poor design of previous animal studies, inappropriate animal models and ineffective approaches to inhibiting the pathways that were targeted. Given that patients with AAA usually have multiple co-morbidities any treatment to limit AAA growth would need to be very safe. Based on the animal data presented in this review a number of non-toxic antioxidant approaches have potential to limit AAA, such as vitamin E, vitamin C and polyphenolics (curcumin, quercetin, resveratrol). Despite the promising experimental studies there are no clinical trial data to suggest that such simple antioxidant therapies are effective. For example, a clinical trial failed to show any significant association between vitamin E supplement and the incidence of AAA, although no aortic imaging was included in this trial.(138) Whether such simple approaches to modifying oxidative stress can achieve a sustained reduction in aortic oxidative stress is uncertain. Clinical trials to examine the value of these agents in established AAAs are needed to answer this.

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5-LO	5-lipooxygenase
AAA	Abdominal aortic aneurysm
Ang II	angiotensin II
AP-1	Activator protein-1
$ApoE^{-/-}$	apolipoprotein E deficient
$CaCl_2$	Calcium chloride
cAMP	cyclic adenosine monophosphate
$CoCl_2$	Cobalt chloride
COX	Cyclooxygenase
CuZnSOD	Copper-Zinc SOD
ECM	Extracellular matrix
EcSOD	Extracellular SOD
eNOS	Endothelial NOS
FLAP	5-lipooxygenase activating protein
H4B	Tetrahydrobiopterin
HMG-CoA	3-hydroxyl-3-methylglutaryl coenzyme A
HO	Heme oxygenase
IL-1β	interleukin-1β
iNOS	Inducible NOS
JNK	c-Jun N-terminal kinase
MCP-1	Monocyte chemoattractant protein-1
MIP	Macrophage inflammatory protein
MMP	Matrix metalloproteinase
MnSOD	Manganese SOD

NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	nuclear factor-κB
nNOS	Neuronal NOS
NO	Nitric oxide
NOS	Nitric oxide synthase
NOX	Nicotinamide adenine dinucleotide phosphate oxidase
NSAID	nonsteroidal anti-inflammatory drug
PGE2	Prostaglandin E2
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TIMP-1	tissue inhibitor of metalloproteinase-1
TNFα	Tumor necrosis factor α
TNF-α	tumor necrosis factor-α
TRX	Thioredoxin
VSMC	Vascular smooth muscle cells
XO	Xanthine oxidase

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Conflicts of Interest

The authors declare no conflict of interest.

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Figure legends

Figure 1 A Schematic diagram showing the critical balance between oxidants and antioxidants in relation to AAA [modified from [(1)].

Endogenous oxidant systems generate reactive oxygen species (ROS) whilst the antioxidants either detoxify or scavenge ROS. A shift in the balance towards increased oxidant activity, leads to excess ROS and resultant oxidative stress. Oxidative stress with associated tissue damage and other pro-aneurysmal factors may result in AAA.

Abbreviations: *may generate ROS, AAA= abdominal aortic aneurysm, COX= cyclooxygenase, ECM= extracellular matrix, LO= lipoxygenase, HO= heme oxygenase, NOS= nitric oxide synthase, NOX= nicotinamide adenine dinucleotide phosphate oxidase, ROS= reactive oxygen species, SOD= superoxide dismutase, TRX= thioredoxin, VSMC= vascular smooth muscle cells, XO= xanthine oxidase.

Figure 2 Overview of the mechanism leading to oxidative stress and potential treatments to limit oxidative stress associated with AAA.

AAA risk factors include smoking, male gender, advanced age and family history. Pro-inflammatory agents include Ang II. ROS generating enzymes include eNOS, NOX, XO, LO, COX. ROS degrading systems include SOD and catalase. O_2 is converted to O_2^- by NOX. SOD inactivates O_2^- forming H_2O_2, and catalase converts H_2O_2 to H_2O and O_2. Under pro-aneurysmal conditions, H_2O_2 could be converted to $^{\bullet}OH$ by the Fenton and/or the Haber-Weiss reactions. In addition, O_2^- then combines with NO produced by eNOS to form the highly reactive $ONOO^-$. The increase generation of ROS within the aorta results in oxidative stress which leads to enhance ACE expression which promotes Ang II production and resultant positive feedback activation of more pro-oxidant enzymes. Oxidative stress also promotes mitochondria dysfunction, activates signalling molecules, and potentiates inflammation. It also increases pro-inflammatory cytokine secretion with resultant recruitment of effector immune cells, release of proteases, leading to ECM degradation, VSMC apoptosis and consequent AAA development and progression. Key targets currently under investigation include: 1). Preventing eNOS uncoupling using vitamins, 2). Preventing the production of O_2^- using NOX-inhibitors, TRX or genetic approaches, 3). Blocking the COX-2 and/or LO pathways using vitamins and NSAID, 4). Enhancing the effect of SOD using SOD mimetics, folic acid and polyphenolics, 5). Applying catalase activators to improve the detoxification of H_2O_2, 6). Using polyphenolics to inhibit ROS-induced signalling and 7). Employing vitamins, polyphenolics and NSAID to scavenge ROS and prevent oxidative stress.

Abbreviations: 5-LO= 5-lipooxygenase, AAA= abdominal aortic aneurysm, ACE= angiotensin converting enzyme, Ang I= angiotensin I, Ang II= angiotensin II, AP-1= activator protein-1, COX= cyclooxygenase, ECM= extracellular matrix, eNOS= endothelial nitric oxide synthase, Erk1/2= extracellular signal-regulated kinase 1/2, Fe^{2+}= ferrous iron, Fe^{3+}= ferric iron, H_2O_2= hydrogen peroxide, IL-1β= interleukin-1 beta, JNK= c-Jun N-terminal kinase, LO= lipoxygenase, MCP-1=

977 monocyte chemoattractant protein-1, MMP= matrix metalloproteinase, NF-κβ= nuclear factor kappa
978 beta, NO= nitric oxide, NOS= nitric oxide synthase, NSAIDS= nonsteroidal anti-inflammatory drugs,
979 NOX= nicotinamide adenine dinucleotide phosphate oxidase, O_2-= superoxide, ˙OH= hydroxyl
980 radical, $ONOO^-$ = peroxynitrite, ROS= reactive oxygen species, SOD= superoxide dismutase, TNF-
981 α= tumour necrosis factor-α, VSMC= vascular smooth muscle cells, XO= xanthine oxidase. Symbols
982 indicate: ↑increase; ↓decrease.

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1010 **Tables**

1011 **Table 1** Overview of studies assessing the lipoxygenase pathway in AAA

Therapies	Key findings	AAA model	Reference
BLT1 inhibition (genetic).	Inhibition of the 5-LO metabolite, LTB4 mediated-inflammation. Decreased MMP-2 and -9 expression. Decreased inflammatory cell infiltrate and decreased AAA formation.	Ang II-infused $ApoE^{-/-}$ mouse model of AAA.	(72)
5-LO inhibition (genetic and pharmacological).	Decreased MMP-9 activity and effector immune cell recruitment. 71% (genetic) and 54% (pharmacological) reduction in aortic dilatation.	Elastase perfused- and Ang II-infused $Ldlr^{-/-}$ mouse models of AAA.	(67)
None.	Upregulated 5-LO, FLAP, and LTC4S transcripts in aneurysmal aorta compared with non-aneurysmal control aortas.	Human.	(74)
5-LO inhibition genetic deletion).	Decreased MMP-2 and MIP-1α expression. Decreased AAA incidence.	Cholate fed $ApoE^{-/-}$ mice.	(66)
BLT1 inhibition (pharmacological).	Diminished macrophage accumulation, decreased incidence of AAA when administered at the same time as Ang II, but no significant effect on AAA size when administered 14 days after AAA induction.	Ang II-infused $ApoE^{-/-}$ mouse model of AAA.	(68)
Generalised ROS inhibition (endaravone).	Decreased MMP-2, MMP-9, and ROS expression. Reduced AAA formation (when administered from the onset of AAA induction), and expansion (when administered to already established AAAs).	Combined elastase-induced and $CaCl_2$-induced rat models of AAA.	(71)
5-LO inhibition (genetic and pharmacological).	No significant effect on aortic media inflammation or AAA development in 5-LO deficient mice or mice administered FLAP inhibitor (MK-0591).	Ang II-infused $ApoE^{-/-}$ mouse model of AAA.	(73)
None.	No association between 7 single nucleotide polymorphisms in ALOX5AP, the gene encoding FLAP with human AAA.	Human.	(76)

Abbreviations: 5-LO= 5-lipooxygenase, Ang II= angiotensin-II, $ApoE^{-/-}$ = apolipoprotein E-deficient, BLT1= leukotriene B4 receptor-1, FLAP= 5-LO activating protein, $CaCl_2$= calcium chloride, $Ldlr^{-/-}$ = low density lipoprotein receptor knockout, LTB4= leukotriene B4, LTC4S= leukotriene C4 synthase, MIP= macrophage inflammatory protein, MMP== matrix metalloproteinase, ROS= reactive oxygen species.

Table 2 Studies assessing the association of the cyclooxygenase pathway with AAA

Therapies	Key findings	AAA model	Reference
COX-2 inhibition (pharmacological).	Decreased incidence and severity of AAA.	Ang II-infused mouse model of AAA.	(82) (84) (85)
COX-2 inhibition (genetic deletion).	Decreased MCP-1, MIP-1α expression. Decreased macrophage infiltration. Decreased AAA formation.	Ang II-infused mouse model of AAA.	(81)
COX-2 inhibition (genetic deletion).	No significant effect on AAA incidence.	Ang II-infused $ApoE^{-/-}$ mouse model of AAA.	(83)
COX-2 inhibition (pharmacological).	Decreased MMP-9 expression and decreased AAA development. Decreased PGE2 and MMP-9 expression. Decreased inflammatory cell infiltration. Decreased AAA expansion.	Elastase-induced rat model of AAA.	(86) (87) (88)
COX-2 inhibition (pharmacological).	Decreased MMP-9 expression but no significant effect on AAA expansion.	Elastase-induced rat model of AAA.	(89)
None	Significant reduction in AAA growth in patients on NSAIDS compared to patients without NSAIDS.	Human case-control study.	(80)

Abbreviations: Ang II= angiotensin-II, $ApoE^{-/-}$ = apolipoprotein E-deficient, COX-2= cyclooxygenase-2, MCP= monocyte chemoattractant protein, MIP= macrophage inflammatory protein, MMP= matrix metalloproteinase, NSAIDS= nonsteroidal anti-inflammatory drugs, PGE2= prostaglandin E-2, VSMC= vascular smooth muscle cell.

Table 3 Overview of studies assessing the effects of medications known to affect NOS and/or direct NOS inhibition in AAA

Therapies	Key findings	AAA model	Reference
Simvastatin	Decreased MMP-9, MMP-3, and NF-κβ expression. Increased TIMP-1 expression. Decreased AAA formation.	Elastase-induced mouse model of AAA.	(147) (Steinmetz 2005)
Simvastatin	Decreased macrophage infiltration. No significant effect on AAA diameter.	Ang II-infused $ApoE^{-/-}$ and $Ldlr^{-/-}$ mouse models of AAA.	(153) (Golledge 2010)
Fenofibrate	Decreased inflammatory cell and cytokine expression. Decreased VSMC apoptosis. Decreased AAA development.	Ang II-infused $ApoE^{-/-}$ and $Ldlr^{-/-}$ mouse models of AAA.	(105) (120)
iNOS inhibition (genetic).	Decreased MMP-9, MMP-2, and NO expression. Decreased AAA formation.	$CaCl_2$-induced mouse model of AAA.	(18)
H4B and eNOS inhibition (genetic).	Increased MMP-9, MMP-2 expression. Increased macrophage infiltration. Increased AAA formation. Folic acid administration blocked the AAA promoting effect of H4B deficiency.	Ang II-infused mouse model of AAA.	(94)
eNOS upregulation (pharmacological).	Decreased ECM degradation. Decreased macrophage infiltration. Decreased O_2^- production. Decreased AAA expansion.	Ang II-infused mouse model of AAA.	(95)

Abbreviations: Ang II= angiotensin-II, $ApoE^{-/-}$ = apolipoprotein E-deficient, $CaCl_2$= calcium chloride, ECM= extracellular matrix, eNOS= endothelial nitric oxide synthase, H4B= tetrahydrobiopterin, iNOS= inducible nitric oxide synthase, $Ldlr^{-/-}$ = low density lipoprotein receptor knockout, MMP= matrix metalloproteinase, NF-κβ= nuclear factor kappa beta, NO= nitric oxide,

O_2^-= superoxide , TIMP-1= tissue inhibitor of matrix metellaoproteinase-1, VSMC= vascular smooth muscle cells.

Table 4 Overview of studies assessing superoxide dismutase and/or catalase in AAA

Therapies	Key findings	AAA model	Reference
SOD inhibition (pharmacological).	Decreased MMP-2, MMP-9, and NF-κβ expression. Decreased SOD and catalase activity. Decreased AAA size.	$CaCl_2$-induced mouse model of AAA.	(126)
SOD upregulation (pharmacological).	Decreased aortic MCP-1, and TNF-α expression. Decreased macrophage infiltration. Increased SOD activity. Decreased AAA incidence.	Ang II-infused $ApoE^{-/-}$ mouse model of AAA.	(121)
None.	Higher catalase activity. Reduced NF-κβ expression. No effect on SOD activity and H_2O_2 in AAA wall in vitro.	Human.	(122)
Catalase upregulation (pharmacological).	Decreased aortic neutrophil infiltration. Decreased AAA expansion in rats receiving tamoxifen to increase catalase expression. A catalase inhibitor (AT) blocked the AAA protective effects of tamoxifen.	Elastase-induced rat model of AAA.	(124)
Catalase upregulation (genetic).	Transgenic mice overexpressing catalase exhibited increased H_2O_2 degradation and decreased aortic wall remodelling.	Ang II-infused mouse model of AAA.	(163) (Maiellaro_rafferty, 2011)
Catalase upregulation (genetic and pharmacological).	Decreased MMP activity. Decreased VSMC apoptosis. Decreased AAA formation.	$CaCl_2$-induced mouse model of AAA.	(119)

Abbreviations: Ang II= angiotensin-II, $ApoE^{-/-}$ = apolipoprotein E-deficient, AT=3-amino-1, 2, 4-triazole, $CaCl_2$= calcium chloride, H_2O_2= hydrogen peroxide, MMP== matrix metalloproteinase, MnSOD= manganese superoxide dismutase,NF-κβ= nuclear factor kappa beta, SOD= superoxide dismutase, TNF-α= tumor necrosis factor-α, VSMC= vascular smooth muscle cells.

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