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The Role of Oxidative Stress in Cerebral Aneurysm Formation and Rupture

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Abstract

Oxidative stress is known to contribute to the progression of cerebrovascular disease. Additionally, oxidative stress may be increased by, but also augment inflammation, a key contributor to cerebral aneurysm development and rupture. Oxidative stress can induce important processes leading to cerebral aneurysm formation including direct endothelial injury as well as smooth muscle cell phenotypic switching to an inflammatory phenotype and ultimately apoptosis. Oxidative stress leads to recruitment and invasion of inflammatory cells through upregulation of chemotactic cytokines and adhesion molecules. Matrix metalloproteinases can be activated by free radicals leading to vessel wall remodeling and breakdown. Free radicals mediate lipid peroxidation leading to atherosclerosis and contribute to hemodynamic stress and hypertensive pathology, all integral elements of cerebral aneurysm development. Preliminary studies suggest that therapies targeted at oxidative stress may provide a future beneficial treatment for cerebral aneurysms, but further studies are indicated to define the role of free radicals in cerebral aneurysm formation and rupture. The goal of this review is to assess the role of oxidative stress in cerebral aneurysm pathogenesis.

Keywords

Aneurysm; inflammation; oxidative stress; NADPH oxidase; reactive oxygen species; subarachnoid hemorrhage

INTRODUCTION

Cerebral aneurysms occur in 2–3% of humans [1, 2]. Aneurysm rupture leading to subarachnoid hemorrhage (SAH) occurs in 6 to 20 per 100,000 people annually [3–6]. Both

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

aneurysm formation and rupture may be significantly elevated in high risk populations [7, 8]. Mortality following SAH may be above 50%, and of those who survive, only 30–45% return to their previous jobs, and up to 30% remain disabled [9–11]. The exact mechanisms behind aneurysm formation and rupture are unclear, but inflammation and tissue degeneration are key elements. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been linked to a number of vascular diseases. Formation following normal metabolism, increased expression secondary to inflammation, or reduced antioxidant defense capacity can result in oxidative stress with progressive tissue and cellular injury. The goal of this review is to assess the role of oxidative stress in the pathophysiology of cerebral aneurysm formation and rupture.

INFLAMMATION AND OXIDATIVE STRESS

Many studies provide support for the role of inflammation in the formation, progression, and rupture of cerebral aneurysms [12–16]. There is likely a multifactorial effect of the coordinated inflammatory actions of leukocytes, cytokines, adhesion molecules, immunoglobulins, and complement. Endothelial dysfunction is an early step in aneurysm formation followed by vascular smooth muscle cell (VSMC) phenotypic modulation, extracellular matrix remodeling, and cell death with resultant vessel degeneration, dilation, and rupture. Oxidative stress is an important element of the pathophysiology of inflammation, as endothelial dysfunction, incorporation of immune cells into vessel walls, and VSMC proliferation and migration all involve free radical production. Oxidative stress is injury due to increased production and/or decreased removal of free radicals. Thus, imbalance between production and destruction of free radicals is a key element of oxidative stress. Imbalance in free radicals leading to oxidative stress can result in deoxyribonucleic acid (DNA) damage, cellular toxicity, and apoptosis [17]. A number reviews have described the regulation of oxidative stress in great detail, and the reader is referred to a number of recent articles for a comprehensive review [17–20]. In this manuscript, we review existing studies demonstrating increased oxidative stress in cerebral aneurysms, possible sources of free radicals, potential pathways of oxidative injury, and the therapeutic implications for aneurysm treatment.

POSSIBLE SOURCES OF ROS GENERATION IN CEREBRAL ANEURYSMS

There are a wide number of sources for possible oxidative stress in cerebral aneurysm formation and rupture (Table 1; Fig. 1). Evidence suggests that vascular nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidases and cyclooxygenases/lipoxygenases are the primary sources of the free radicals superoxide ($O_2^{\bullet-}$) and hydrogen peroxide H_2O_2 in cerebral arteries [21, 22]. Vascular NADPH oxidases are present and functionally active in endothelial cells, VSMC, and adventitial cells of cerebral arteries. Several factors present in cerebral aneurysms could potentially induce vascular production of superoxide and hydrogen peroxide by NADPH oxidase, including cytokines, mechanical stress, and growth factors [23]. Moreover, free radicals themselves may induce NADPH oxidase in a “feed-forward” mechanism of amplification of free radical production. Studies have shown that cyclooxygenases (COX) and lipoxygenases, enzymes involved in the synthesis of prostanoids and hydroperoxides respectively from arachidonic acid, are also able to generate free radicals in cerebral arteries [24–27]. This could provide an important source of free radicals in cerebral aneurysms, as COX-2 was found to be abundantly expressed in the wall of human cerebral aneurysms [28].

Another potentially significant source of free in the cerebral circulation is nitric oxide synthase (NOS) [21, 29]. When $O_2^{\bullet-}$ is produced with $NO^{\bullet}$, they form the peroxynitrite

(ONOO^{•-}), a highly reactive product that results in protein nitration and inactivation of several vasculoprotective enzymes [30].

Endothelial NOS (eNOS) is constitutively expressed in endothelial cells and its activity is associated with the cytoprotective effects of NO [31]. On the other hand, inducible NOS (iNOS) is expressed in macrophages and smooth muscle cells in inflammatory states and its activity is associated with the cytotoxic effects of NO [30, 32]. In fact, iNOS generates high levels of NO that promote oxidative stress [29, 30, 32]. In addition, endothelial NOS (eNOS) generates O₂^{•-} rather than NO when it becomes uncoupled from its cofactors, L-arginine or tetrahydrobiopterin (BH4) [29, 33]. Moreover, ONOO^{•-} has been shown to oxidize BH4 and lead to uncoupling of eNOS, suggesting a feed-forward mechanism that amplifies free radical generation within vascular cells [29, 34]. Thus, NOS may be an important source of RNS and ROS in cerebral aneurysms that could contribute to aneurysm formation and progression through several mechanisms [35,36].

The local environment within the wall of cerebral aneurysms may be particularly favorable to free radical formation. Infiltrating leukocytes, particularly macrophages and neutrophils, can generate large amounts of O₂^{•-}, H₂O₂, and HOCL via NADPH oxidase and myeloperoxidase. In addition, VSMC and endothelial cells may produce O₂^{•-} via vascular NADPH oxidase and several other pathways. Hemodynamic forces have also been shown to induce free radical production in aneurysm walls, resulting in matrix metalloproteinase activation and vascular wall remodeling [12, 37, 38]. Several cytokines in the walls of cerebral aneurysms, especially Tumor Necrosis Factor (TNF)-alpha, may also activate production of O₂^{•-} and H₂O₂ by upregulation of NADPH oxidase activity [39, 40]. Collectively, these data suggest that oxidative stress in cerebral aneurysm walls is intimately linked to inflammation and hemodynamic stress.

POSSIBLE PATHWAYS OF OXIDATIVE STRESS INDUCED INJURY IN CEREBRAL ANEURYSMS

While low levels of free radicals contribute to normal vascular function, high levels create a proinflammatory local environment and mediate cell injury. ROS appear to affect several steps in the cascade of events leading to aneurysm formation/rupture from endothelial dysfunction to aneurysm wall degradation.

Several lines of evidence indicate that cerebral aneurysm formation begins with endothelial dysfunction in response to hemodynamic stress [37, 41, 42]. In the early stage of aneurysm formation, endothelial cells undergo a series of pro-inflammatory changes that include expression of chemotactic cytokines, upregulation of adhesion molecules, and downregulation of tight junction proteins (occludin and ZO-1) [12, 37, 43]. These early changes in endothelial function resulting in macrophage recruitment and migration can be modulated by O₂^{•-} and H₂O₂, primarily through activation of Nuclear Factor Kappa-B (NF-κB) [44–47]. In addition, free radicals can stimulate invasion of monocytes into the vascular wall by increasing production of monocyte chemoattractant peptide-1 (MCP-1) [48], a seemingly important chemoattractant of macrophages in cerebral aneurysms whose inactivation was shown to halt aneurysm formation in animal models [49]. These changes induced by free radicals are particularly relevant with regard to cerebral aneurysm pathogenesis because macrophage infiltration is the hallmark of cerebral aneurysms. Indeed, macrophage depletion was shown to reduce the prevalence of cerebral aneurysms in mice [50]. Collectively, these data suggest that free radicals may induce endothelial dysfunction and promote macrophage recruitment into vessel walls, thus amplifying the inflammatory response.

Macrophages and other inflammatory cells secrete a variety of pro-inflammatory cytokines and release matrix metalloproteinases (MMPs) that contribute to vessel wall remodeling, weakening, and dilation. In animal models, inhibitors of either MMPs [51] or cathepsins [52] successfully blocked the progression of cerebral aneurysms, highlighting the key role of these proteinases in aneurysm pathogenesis. The expression and activity of MMPs are at least in part ROS-dependant, with early remodeling requiring NADPH oxidase, while late remodeling being more dependent on ONOO^{•-} generated by eNOS [19, 53–55]. Importantly, MMP-2 and -9, two major human proteinases that play a crucial role in aneurysm formation, [51] are regulated by free radicals including ONOO^{•-} produced by VSMCs, endothelium and macrophages [19]. Thus, free radicals can induce the expression of MMPs causing vessel wall remodeling and breakdown.

Vascular smooth muscle cells constitute an integral part of the inflammatory reaction associated with cerebral aneurysm formation [42]. VSMC initially undergo phenotypic modulation from a contractile to pro-inflammatory/pro-matrix remodeling phenotype resulting in myointimal hyperplasia, inflammation, and vessel wall degeneration. Subsequent apoptosis and VSMC death eventually lead to a hypocellular thin wall with increased cerebral aneurysm susceptibility to rupture. We have previously demonstrated that NADPH induced O₂^{•-} is able to induce VSMC phenotypic modulation, and this will be discussed in further detail in a subsequent section. In addition, free radicals may induce VSMC apoptosis and reduce collagen synthesis with weakening of arterial walls [19, 45, 56]. Indeed, VSMC apoptosis was found to be attenuated by antioxidants such as N-acetylcysteine or pyrrolidine dithiocarbamate, implicating free radicals in the apoptotic response [57]. In addition to O₂^{•-} and H₂O₂, NO and other ONOO^{•-} may induce apoptosis of VSMC [30]. Thus, ROS can modulate VSMC phenotypic switching and apoptosis, two events that are pivotal to aneurysm formation and rupture.

Atherosclerotic changes are reportedly present in all saccular cerebral aneurysms [58, 59]. In small aneurysms, atherosclerotic lesions are characterized by diffuse intimal thickening composed primarily of SMC whereas larger aneurysms display more advanced atherosclerotic lesions composed predominantly of macrophages, lymphocytes, and mature smooth muscle cells (SMC) [59]. The pathogenesis of cerebral aneurysms may therefore be intimately linked to that of atherosclerosis. NADPH, myeloperoxidase, and lipoxygenase produce O₂^{•-}, H₂O₂, and ONOO^{•-} involved in lipid peroxidation including oxidation of low density lipoproteins (LDL) [17, 20]. LDL has significant pro-atherogenic effects and itself may activate NADPH to further produce O₂^{•-} and H₂O₂ [23]. Thus, by inducing peroxidation of lipoproteins, free radicals promote atherosclerosis and possibly also aneurysmal changes in cerebral vessels.

Cigarette smoke is a major risk factor for cerebral aneurysm formation and rupture, with almost 80% of patients who sustain an aneurysmal SAH reporting a significant history of smoking [60, 61]. Cigarette smoke is a major source of free radicals including activation of NADPH and production of O₂^{•-} and H₂O₂ [62, 63].

This can arise directly from the gas/tar phase, activated leukocytes, or endogenous sources of ROS such as xanthine oxidase, uncoupled eNOS, and the mitochondrial electron transport chain. [63, 64] Free radicals are thought to be the main mechanism through which cigarette smoke induces vascular pathology. [65] Accordingly, antioxidants have been shown to reverse the proinflammatory and proathrogenic attributes associated with cigarette smoke [66–71]. Thus, cigarette smoke-induced oxidative stress may be an integral part of cerebral aneurysm pathogenesis.

Alcohol is another well-established risk factor for SAH that may also act through free radical-mediated mechanisms. Alcohol increases the risk of aneurysm rupture independently of cigarette smoking, age, and history of hypertension [72, 73]. Accrued data suggest that free radicals produced by alcohol exposure in the central nervous system play important roles in neuroinflammation and neurodegeneration [74–76]. Alcohol especially at levels attained in heavy drinkers was shown to affect human brain endothelial cells and the permeability of the blood-brain barrier through ROS formation [74, 77, 78]. Much work, however, remains to be done to determine whether alcohol-induced oxidative stress could directly induce aneurysmal changes in cerebral vessels.

Hypertension is another risk factor for aneurysm formation and SAH [60, 72, 79]. It is estimated that of all SAH, about 25% of cases are attributable to hypertension [72]. In addition, hypertension is a risk factor for new aneurysm formation after treatment of a ruptured aneurysm [73]. Evidence suggests that $O_2^{\bullet-}$, H_2O_2 , and $ONO_2^{\bullet-}$ produced through NADPH oxidase and uncoupled eNOS may play a particularly important role in the pathogenesis of hypertension, although multiple other factors may also be involved [80–83]. ROS may increase cerebrovascular tone by direct effects on the contractility of VSMC and by influencing the regulatory role of endothelium. For instance, $O_2^{\bullet-}$ may react with NO in the cerebral circulation thus eliminating its vasodilatory effects. ROS may also enhance sympathetic efferent activity from the cerebral nervous system through excessive central angiotensin II stimulation or a loss of NOS and antioxidant enzymes [80]. Evidence, however, for a direct link between free radicals, hypertension, and aneurysm formation/rupture has yet to emerge.

OXIDATIVE STRESS IN THE PATHOGENESIS OF CEREBRAL ANEURYSMS

Oxidative stress has been linked to a number of vascular diseases including hypertension, abdominal aortic aneurysms, and atherosclerosis. Additionally, as atherosclerosis and hemodynamic stress are key components of both abdominal aortic and cerebral aneurysms, it is possible that there is also a similar pathological role for oxidative stress in both processes. As there are a number of possible sources and mechanisms whereby oxidative stress can contribute to the pathogenesis of cerebral aneurysms, researchers have sought to determine whether oxidative stress was directly involved in cerebral aneurysm formation and rupture.

To assess whether oxidative stress contributes to the pathogenesis of cerebral aneurysms, investigators turned to animal models of cerebral aneurysm formation. In these experiments, aneurysms are induced through ligation of a single common carotid artery and posterior branches of the bilateral renal arteries [84, 85]. Animals are then fed β -aminopropionitrile, an inhibitor of lysyl oxidase that catalyzes the cross-linking of collagen and elastin. In a study by Aoki *et al.* [86], the intima and media of cerebral arterial walls in rats one month after aneurysm induction was dissected with laser microdissection and changes in gene expression were analyzed using microarrays in both induced and control rats. In this model, three months after induction almost all animals experienced aneurysm formation. At one month, there was early evidence of aneurysm formation including disruption of internal elastic lamina. Differential expression at one month demonstrated alterations in gene expression at an early point of aneurysm formation where pathological changes in arterial walls progress rapidly. Using microarrays, differential expression of ROS genes has been found in the intima including NADPH oxidase, superoxide dismutase, catalase, peroxiredoxin 2, aldehyde dehydrogenase, transaldolase, epoxide hydrolase, cytoplasmic, eptidylglycine α -amidating monooxygenase, flavin containing monooxygenase, and peroxiredoxin [86]. Additionally, there was differential expression of a number of ROS

genes in the media: inducible nitric oxide synthase, arachidonate 15-lipoxygenase, transaldolase, epoxide hydrolase, and peroxiredoxin.

A number of studies have looked more closely at specific alterations in genes regulating ROS in animal models of cerebral aneurysms. In a follow up study by Aoki et al.,[87] loss of cyclooxygenase-2 (COX-2) or prostaglandin E receptor 2 (EP2) reduced the incidence of cerebral aneurysm formation in rats and mice. As both COX and EP2 have significant relationship with a number of key inflammatory cytokines including NF- κ B, it is unclear whether aneurysmal inhibition was due to alteration in oxidative stress and/or key inflammatory mediators.

In a study by Fukuda *et al.* [35] the role of iNOS was investigated in a rat model of aneurysm formation. Inducible NOS was immunohistochemically located at the origin of aneurysms but was not found in normal arteries. Endothelial NOS was also found to be decreased compared to control arteries. NO is normally produced by eNOS and plays a role in vasodilation, prevention of platelet aggregation, and smooth muscle growth and differentiation. As discussed above, iNOS is induced during inflammatory states and leads to oxidative stress as $O_2^{\bullet-}$ reacts with $NO^{\bullet}$ to form peroxynitrite ($ONOO^{\bullet-}$), a key modulator of lipid peroxidation including oxidation of LDL. The authors found that expression of nitrotyrosine, a marker of peroxynitrite, was increased in aneurysms. Additionally, a NOS inhibitor aminoguanidine decreased both early aneurysmal changes and the incidence of aneurysm formation.

As aminoguanidine is a non-selective inhibitor of NOS, the exact role of alterations in eNOS, inducible NOS (iNOS), or neuronal NOS (nNOS) remain unclear. Inducible NOS is not constitutively active, but following vascular injury, it generates 100–1000 fold more NO than its counterparts [88–90]. To better elucidate the role of iNOS in the pathogenesis of cerebral aneurysms, Sadamasa et al.[36] induced aneurysms in iNOS knockout mice and comparable controls. No significant difference was found in the overall incidence of cerebral aneurysms between the two groups, but iNOS knock out mice had significantly smaller aneurysms. Thus, although iNOS is not necessary for cerebral aneurysm formation, it may play a key role in aneurysm progression.

In iNOS knock out mice, the number of apoptotic smooth muscle cells was also significantly decreased [35]. As SMC help generate elastic fibers [91], their death leads to decreased structural element production and may propagate aneurysm progression [92]. Additionally, as the number of apoptotic cells has been shown to correlate with the probability of rupture of cerebral aneurysms [93, 94], iNOS alterations may play a role in aneurysmal hemorrhage and may represent a suitable therapeutic target.

Arterial blood flow is a continuous generator of hemodynamic force. NO is generated by NOS constitutively to mitigate hemodynamic stress [95]. Hemodynamic alterations are noted to be a key element of aneurysm formation and may lead to generation of oxidative stress. In rat aneurysm models, Fukuda *et al.* [35] demonstrated that decreased shear stress reduced iNOS expression and early aneurysmal changes. Wall shear stress is the product of shear rate and Newtonian viscosity. At high shear stress, blood acts as a Newtonian liquid, and the major determinants of blood viscosity is hematocrit. Batroxobin is a defibrogenic agent that decreases viscosity. Treatment with batroxobin lead to decreased tissue damage, iNOS expression, and attenuation of early aneurysmal changes [35]. Thus, although hemodynamic alterations may be a key element behind oxidative stress through altered expression of NOS, further experiments are necessary to better elucidate the underlining mechanisms.

To directly assess the role of oxidative stress in cerebral aneurysm pathogenesis, alterations in ROS have been quantified in a number of animal experiments [96, 97]. Superoxide production as assessed by dihydroethidium staining were found to be dramatically increased in CA walls compared to controls. Additionally, the oxidative product of DNA induced by ROS, 8-hydroxy-2-deoxyguanosine (8-OHdG8-OHdG), was increased in aneurysmal walls. In further experiments by Aoki *et al.* [97], expression of heme oxygenase-1 and the NADPH p47phox subunit was increased in cerebral aneurysms. Additionally, Superoxide dismutase-1 (SOD-1) which was initially present decreased during aneurysm progression and became significantly lower than in controls during aneurysm formation. Further experiments have also found that estrogen may mediate the expression of NADPH oxidase in aneurysms and provide a potential link between oxidative stress and the increased incidence of aneurysms in women [96].

In experiments in rats, cells expressing the P47phox subunit of NADPH in aneurysmal walls were primarily macrophages and smooth muscle cells [97]. Macrophage infiltration has been shown to be a key element of cerebral aneurysm formation [49–51]. Macrophages may contribute to cerebral aneurysm formation both through increasing expression of key inflammatory cytokines and production of ROS including $O_2^{\bullet-}$ and hypochlorous acid (HOCL). In p47phox knock out mice, the size of induced aneurysms was significantly smaller and macrophage infiltration into aneurysmal walls was significantly inhibited [97]. P47phox knock out mice had significantly decreased $O_2^{\bullet-}$ production. This leads to inhibition of expression of MCP-1 and NF- κ B, two key inflammatory mediators that have been found to be necessary for cerebral aneurysm formation [49, 97, 98]. Thus, excess free radical production may play a key role in activation of inflammatory cytokines required for cerebral aneurysm formation.

Treatment with Edaravone, a free radical scavenger, significantly decreased cerebral aneurysm size following induction [97]. Additionally, disruption of the internal elastic lamina was decreased as was degeneration of the media. Edaravone treatment significantly decreased expression of NF- κ B, MCP-1 and VCAM, all of which help macrophages infiltrate aneurysmal walls. Correspondingly, following Edaravone treatment, decreased numbers of macrophages were found in cerebral aneurysms. This resulted in decreased expression of $O_2^{\bullet-}$.

Although the link between production of free radicals and macrophages in cerebral aneurysms has received attention, the role of smooth muscle cells has received less focus. We have found that smooth muscle cell phenotypic modulation is a key element of aneurysm formation whereby smooth muscle cells change from a differentiated phenotype characterized by expression of contractile elements to an inflammatory/matrix remodeling phenotype [99, 100]. In both cultured cerebral vascular SMCs *in vitro* or rat carotid arteries *in vivo*, exposure to cigarette smoke extract or treatment with TNF- α leads to decreased expression of smooth muscle contractile genes (SM-MHC and SM- α Actin) and upregulation of pro-inflammatory genes (iNOS, MCP-1, MMPs, VCAM) and NADPH and $O_2^{\bullet-}$ NADPH oxidase overexpression via adenovirus transfection results in decreased expression of smooth muscle contractile genes (SM-MHC and SM- α Actin). Treatment with superoxide dismutase, anti-sense NADPH oxidase adenovirus, and small inhibitor NADPH RNA reversed cigarette smoke induced pro-inflammatory/matrix remodeling changes. In an *in vivo* rat aneurysm induction model, there is early similar vascular smooth muscle cell phenotypic modulation including decreased expression of SM- α -actin with an increase in expression of KLF4, MMP-2, MMP-3, MMP-9, MCP-1, iNOS and VCAM-1 compared to Circle of Willis vessels from age-matched controls.

OXIDATIVE STRESS IN HUMAN CEREBRAL ANEURYSMS

There have been few studies to directly assess oxidative stress in human cerebral aneurysms. In a resected human aneurysm, iNOS and nitrotyrosine, a marker of ONOO^{•-}, were increased in SMCs at the origin of the aneurysmal orifice as compared with other blood vessels [35]. A primary step in demonstrating that oxidative stress is involved in the pathogenesis of cerebral aneurysms is localization of free radicals to aneurysms rather than just global upregulation in patients who have cerebral aneurysms. A number of microarray studies have compared gene expression in samples from unruptured cerebral aneurysms and compared them to control cerebral blood vessels in patients with and without cerebral aneurysms. Although results have not been consistent across all studies, alterations in expression of a wide variety of genes governing ROS balance has been demonstrated [101–106]. Further studies will be necessary to investigate the specific pathways of alterations amongst free radical producing genes within unruptured cerebral aneurysms.

Although cerebral aneurysms occur in 2–3% of humans, [1, 2] rupture occurs in only 6 to 20 per 100,000 people annually [3–6]. Not all cerebral aneurysms act in a similar fashion; some remain quiescent for many years while some rapidly progress to hemorrhage. An important objective is to discern alterations in free radicals that contribute to aneurysmal rupture. Few studies have assessed alterations in ROS function in ruptured versus unruptured cerebral aneurysms, although ROS are known to increase significantly after SAH and can contribute to cerebral vasospasm. A number of microarray studies have compared gene expression in unruptured and ruptured cerebral aneurysms and found alterations in ROS genes [101–103, 105–107]. Shi *et al.* [106] found a wide variety of alterations in the NADPH complex genes, SOD, and Hypoxia-inducible factor (HIF) as well as a number of other factors associated with production of ROS and oxidative stress. Further studies will be necessary in humans to assess alterations in gene expression and mechanisms behind oxidative stress leading to SAH.

Few studies have further assessed the role of oxidative stress in unruptured human intracranial aneurysms. In addition to alterations during *in vivo* animal experiments, Aoki *et al.* [87] found alterations in COX-2, mPGES-1, and EP2 in endothelial cells in 5 unruptured human cerebral aneurysms and compared their findings with those of cadaver specimens. More recently Hasan *et al.* [108] found upregulation of COX-2 and Microsomal Prostaglandin E2 Synthase-1 (mPGES-1) in walls of ruptured human cerebral aneurysms.

A number of studies have analyzed plasma, cerebrospinal fluid, and brain parenchyma levels of key markers of oxidative stress following aneurysmal SAH, and have found that the balance of oxidative species is significantly altered immediately following aneurysm rupture. In the study by Gaetani *et al.* [109], the total SOD, Glutathione peroxidase (GP), and the SOD to GP ratio were altered in gyrus rectus or temporal operculum of patients following aneurysmal SAH as compared to those with unruptured aneurysms. In the study by Lin *et al.* mean or peak levels of F2-isoprostanes, a specific marker of lipid peroxidation, metabolites of nitric oxide (NO), and ONOO^{•-} in CSF and plasma were significantly higher in SAH patients than in controls [110]. Other studies have found alterations in antioxidant vitamins following aneurysmal subarachnoid hemorrhage versus controls [111].

From these studies it is unclear if altered balance in formation and removal of oxidative species contributes to aneurysmal rupture or is associated with the inflammatory reaction following SAH. Due to the association of antioxidant vitamins and a possible protective role in aneurysm rupture, a number of population based cross-sectional studies have been carried out. A case-control study in Japan found that the greatest risk for aneurysmal SAH was in patients with a history of smoking and less frequent intake of antioxidant soy products [112].

Other case control-studies have found a decreased risk of SAH in patients with increased green tea intake, which may also act as an antioxidant [113]. Further studies have found that higher composite antioxidant intake scores calculated according to individual intake of each food group (rice, tea, soy products, vegetables, and fruits) was associated with decreased incidence of aneurysmal SAH [114]. Although studies provide support for the role of oxidative stress in aneurysmal SAH and the protective role of antioxidants, these case-control studies still provide significant room for confounding effects between patients with and without aneurysmal SAH.

THERAPEUTIC IMPLICATIONS FOR OXIDATIVE STRESS IN CEREBRAL ANEURYSMS

Although there are a number of studies in animal models and humans that provide evidence of the role of oxidative stress in the pathogenesis of cerebral aneurysms, there have been no human randomized clinical trials to directly assess the presence of oxidative stress or the role of antioxidant therapies. A number of antioxidants are available and used in alternative pathology. An exhaustive review is recently available [115]. Despite the promise of the above studies, further analysis is necessary to assess the role of oxidative stress in the risk of rupture of aneurysms rather than just formation. This will help identify patients that could specifically benefit from antioxidant therapy, particularly in patients deemed high risk for surgical intervention or in cases where the risks of intervention outweigh the risks of observation. Additionally, prior to a randomized clinical trial, identification of an efficacious therapeutic option that reduces oxidative stress in patients with cerebral aneurysms would be beneficial. This could be assessed through studies testing various antioxidants in patients prior to undergoing surgical clipping of cerebral aneurysms. After a beneficial agent is determined, a randomized clinical trial could be undertaken to determine the role of oxidative stress in the pathogenesis of cerebral aneurysms.

CONCLUSION

A number of studies have provided evidence of the role of oxidative stress in cerebral aneurysm formation, progression, and rupture. Oxidative stress likely contributes to the pathogenesis through vessel wall degradation, through promotion of an inflammatory environment, alteration in flow hemodynamics, upregulation of smooth muscle cell phenotypic modulation and ultimately cell death, and induction of matrix remodeling. As such, antioxidant therapy may be efficacious in inhibition of cerebral aneurysm formation and rupture.

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Fig. (1).
Potential mediators of oxidative stress in cerebral aneurysm pathogenesis.

Table 1

Summary of Major Pathways of Oxidative Stress in Cerebral Aneurysm Pathogenesis

Major Pathways	Major Inflammatory Mediators
Atherosclerosis	Oxidized LDL NADPH Myeloperoxidase COX LIPOX NOS IL1 β TNF α IL-6 MCP-1 Selectins and adhesion molecules
Endothelial dysfunction, hemodynamic stress and hypertension	NADPH NF- κ B COX NOS VCAM IL-8 mPGEs-1 angiotensin II
VSMC pro-inflammatory, pro-matrix remodeling phenotypic modulation. Apoptotic cell death.	NADPH NOS NF- κ B KLF4 TNF α VCAM MMP MCP
Chronic inflammatory reaction, vessel wall remodeling and damage, apoptotic cell death	NADPH NOS MCP-1 MMP VCAM IL-1 β , IL-8, IL-12 NF- κ B