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Oxidative Stress in Atrial Fibrillation: An Emerging Role of NADPH Oxidase

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Abstract

Atrial fibrillation (AF) is the most common cardiac arrhythmia. Patients with AF have up to seven-fold higher risk of suffering from ischemic stroke. Better understanding of etiologies of AF and its thromboembolic complications are required for improved patient care, as current anti-arrhythmic therapies have limited efficacy and off target effects. Accumulating evidence has implicated a potential role of oxidative stress in the pathogenesis of AF. Excessive production of reactive oxygen species (ROS) is likely involved in the structural and electrical remodeling of the heart, contributing to fibrosis and thrombosis. In particular, NADPH oxidase (NOX) has emerged as a potential enzymatic source for ROS production in AF based on growing evidence from clinical and animal studies. Indeed, NOX can be activated by known upstream triggers of AF such as angiotensin II and atrial stretch. In addition, treatments such as Statins, antioxidants, ACEI or AT1RB have been shown to prevent post-operative AF; among which ACEI/AT1RB and Statins can attenuate NOX activity. On the other hand, detailed molecular mechanisms by which specific NOX isoform(s) are involved in the pathogenesis of AF and the extent to which activation of NOX plays a causal role in AF development remains to be determined. The current review discusses causes and consequences of oxidative stress in AF with a special focus on the emerging role of NOX pathways.

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1. Atrial fibrillation and oxidative stress

Cardiac arrhythmias refer to abnormal rate or rhythm of the heartbeat caused by perturbed electrophysiology of the myocardium. Among many types of clinically significant arrhythmias, atrial fibrillation (AF) is most common; affecting 2.7 to 6.1 million adults in 2010 in the United States [1], among which 14–16% die of ischemic stroke [1]. AF incidence increases with aging. In particular, the percentage of stroke associated with AF rises steeply from 1.5% at age 50–59 years to 23.5 % at age 80–89 years [2]. AF also develops in 25–45% of patients with previous heart attack or cardiac surgery [3]. Several risk factors for AF have been identified including cardiopulmonary diseases (congenital heart disease [4], heart failure [5], valvular heart disease [6], hypertropic cardiomyopathy [1, 7]), hypertension [8], metabolic diseases (diabetes [3] or obesity [9]), hyperthyroidism [10], and heavy alcohol consumption [11]). Detailed molecular mechanisms underlying development of AF however, have remained elusive. Anti-arrhythmic drugs including β-blockers, Amiodarone, Dronedarone, Dofetilide, and Sotalol have been widely used for treatment of AF by blocking β-adrenergic receptors or ion channels [12]. Although therapy with these drugs is beneficial, many have been found to have limited long-term efficacy, off target side effects, or drug induced pro-arrhythmic effects [13].

Therefore, better understanding of molecular mechanisms underlying AF is essential for development of novel therapeutic strategies. Increasing evidence has demonstrated that oxidative stress likely plays a role in the pathogenesis of AF [14]. In myocardial tissues, increased levels of ROS such as superoxide and H_2O_2 have been found to be associated with AF [15–18], corresponding to a decrease in nitric oxide bioavailability [19]. The ratio of oxidized GSSG to reduced glutathione, and the ratio of oxidized cysteine to reduced cysteine, both of which as markers of oxidative stress, have been found increased in the blood samples of patients with AF [20]. Increased ROS levels result in damage to proteins, lipids, and DNA, and potentiate inflammation by augmenting cytokine production from activated inflammatory cells, which in turn further induces tissue damage. ROS are not only implicated in inflammation, but also involved in cardiac structural and electrical remodeling, all of which increase susceptibility to AF. For example, it has been shown that hydroxyl radical (OH^-) and peroxynitrate ($ONOO^-$) mediate oxidative damage of myofibrils in AF [21, 22], which in turn contributes to structural remodeling of atria. Atrial electrical remodeling has been known to be associated with intracellular calcium overload [23–25]. Oxidative stress induction of mitochondrial DNA damage in AF causes calcium overload by modulating calcium handling proteins or channels [26], which can promote atrial remodeling. On the other hand, treatment with antioxidants such as Vitamin C [27] and N-acetylcyctine (NAC) [28] has been shown to prevent post-operative AF [29]. Oral Vitamin C treatment was found beneficial in reducing early recurrence and inflammation after electrical cardioversion of persistent AF [30]. What remains to be defined, however, are the molecular mechanisms responsible for increased ROS production in AF, i.e. the enzymatic sources and their regulation; as well as better definition of the ROS-mediated downstream events, i.e. detailed pathways as to how calcium handling is modulated. Evidences accumulated in the past twenty years have shown that ROS generated in the cardiovascular system are primarily derived from NADPH oxidase (NOX), mitochondria, xanthine oxidase, and uncoupled eNOS [31, 32]. Among these enzymatic systems/complexes, NOX has emerged as a major initiating source for increased ROS production in cardiovascular

diseases. In particular, latest studies have implicated a correlative and likely important causal role of NOX with AF, which will be discussed in depth in the current review.

2. NOX in the heart: NOX2 vs. NOX4

The NAD(P)H oxidases (NOXs) are a family of multi-subunit enzymatic complexes. NOX isoforms [NOX1–5 or dual oxidase 1–2 (Duox)] recruit separate regulatory subunits for enzymatic assembly and activation [33]. NOX1–4 require p22phox ("phox" stands for *ph*agocyte *ox*idase) as the membrane binding partner for their activation. NOX1 is composed of p47phox and/or its homologue NOXO1, as well as NOXA1, p40phox, and Rac1. Similarly, NOX2 (gp91phox in leukocytes) requires p47phox, p67phox, p40phox, and Rac1/2 (Rac2 in leukocytes) for enzymatic assembly and activation. NOX3 is activated when NOX organizers (p47phox or NOXO1), activators (p67phox or NOXA1), and Rac1 are assembled and bound to NOX3. NOX4 activation however only requires p22phox binding but recently found regulated by Poldip2 and Tks5, which are cytosolic regulators [34]. Unlike other isoforms, NOX5 does not require any other subunits including the only membrane component p22phox for its activation [35, 36]. Of note, NOX5 does not exist in rodents but in humans, in which it is activated in a calcium-dependent manner [37–39]. Duox has a NOX2-like catalytic subunit and dual functions of acting as an oxidase, and as a peroxidase in the presence of H_2O_2. The pathophysiological regulation and function of each NOX isoform remains to be fully elucidated; but it is evident that some isoforms of NOX enzymes are pivotal in normal biological responses such as cell growth and gene regulation [34, 35, 40]. Excessive activations of these enzymes however contribute to cardiovascular pathogenesis.

Of note, tissue specific distribution of NOX isoforms has been documented [36, 41]. The heart expresses primarily NOX2 and NOX4 isoforms [42]. Recent studies have shown that NOX1 is also expressed in LAA [18] and left ventricle [43] at mRNA level and protein levels respectively, even though its expression appears to be much lower than NOX2 and NOX4 [44–47]. NOX2 is expressed in endothelial cells, cardiomyocytes, and fibroblasts, whereas NOX4 is expressed in all these cells and vascular smooth muscle cells. NOX2 and NOX4 have distinct cellular localization and consequences of activation. NOX2 is localized in plasma membrane and produces superoxide. Although it is still controversial, NOX4 has been found in intracellular compartments such as endoplasmic reticulum (in endothelium [48]) and mitochondrion (in myocardium [45]), and it has been suggested to produce H_2O_2 rather than superoxide due to its subcellular localization (i.e. only H_2O_2 that diffuses out of these compartments gets detected) [49, 50]. More importantly, each isoform has cell specific roles and often determines the specific reactive oxygen species (ROS) produced in the context. For instance, NOX4 promotes apoptosis in cardiomyocytes by generating H_2O_2 [45], while it also induces H_2O_2-dependent proliferation in fibroblasts [51], both of which contribute to pathogenesis of heart failure [52]. The role of NOX2 in angiotensin II (Ang II)-induced cardiac hypertrophy and fibrosis has been well established based on data from NOX2 knockout mice [47]. Recent data from NOX4 knockout [52] or transgenic mice [45] however demonstrated that NOX4 contributes to cardiac LV dysfunction, hypertrophy, and fibrosis [45, 52]. Consistently, NOX4 overproduction has been reported in heart failure patients with AF [18]. On the other hand, it should be noted that inducible deletion of endothelial NOX4 led to impaired angiogenesis and endothelial dysfunction [53]. Endothelium specific NOX4 transgenic mice demonstrated H_2O_2-mediated hyperpolarization and non-NO mediated vasodilation, resulting in lower blood pressure [54]. In the heart NOX4 overexpression has also been found to contribute to angiogenesis via H_2O_2 dependent mechanisms [55]. These observations seem consistent with previous notions that H_2O_2 can activate eNOS dependent or independent mechanisms to mediate physiological or compensatory vasodilatations [56–59]. Therefore, whereas cardiac

activation of NOX4 has been reported to be pathogenic or protective, endothelial activation of NOX4 maybe protective.

3. Role of NOX in AF

AF is associated with oxidative stress [60]. Table 1 summarizes systemic or myocardial oxidative stress observed in AF patients and experimental models. It should be noted that each study used different atrial tissues and/or at different stages of AF.

Animal studies

In a porcine model of pacing-induced AF, nitric oxide (NO) bioavailability was reduced, implicating a potential oxidative stress-mediated degradation mechanism in atrial tissue [19]. The study by Dudley et al. further demonstrated NOX-dependent ROS production, and highly upregulated Rac1 expression, in a similar model [61]. The expression of the other NOX isoforms/subunit, NOX1, NOX2, NOX4, and p22phox, was however unchanged. Nonetheless, Adam et al. also found increased Rac1 GTPase activity in AF [62]. Cardiac specific Rac1 overexpression in mice (RACET mice) resulted in AF in 44% and 75% of the mice at 10 month and 16 month old respectively, characterized by ECG analysis, and this response was reversed by Statin treatment. The animals also exhibited obvious cardiac hypertrophy and increased atrial collagen content [62]. A recent study by Yagi et al. demonstrated that Pitavastatin reduces not only incidence of Ang II-induced AF and left atrial enlargement, but also fibrosis and cardiac hypertrophy, via downregulation of Rac1 activity in eNOS null mice [63]. In addition, Reilly S et al. suggested that initial NOX activation likely accounts for early development of AF, whereas mitochondrion and uncoupled eNOS are involved in permanent AF [64]. In this study of pacing-induced AF in goats, NOX2 and p22phox expression were increased 2 weeks after AF induction but returned to baseline 6 months later. Taken together, the studies described above clearly demonstrate NOX activation in AF, which is highly dependent on Rac1 activity. However, it should be noted that mice with transgenic overexpression or knockout on different NOX isoform(s), have yet been employed to confirm whether particular NOX isoform(s) contribute to pathogenesis of AF. Therefore, usage of transgenic animal models may provide additional evidences for a potential causal role of specific isoform(s) of NOX in the development of AF.

Clinical studies

In agreement with animal studies, analysis of human LA myocardium in patients with AF showed significant upregulation of Rac1 GTPase and NOX activity compared to those in sinus rhythm (SR) (n=8) [62]. Likewise, Kim et al. also investigated sources of superoxide production in right atrial appendage (RAA) homogenates or isolated myocytes from 15 patients with AF (6 patients with persistent AF, 4 patients with persistent AF developed from paroxysmal AF, 5 patients with paroxymal AF) [16]. Superoxide production was inhibited in the presence of Diphenyleneiodonium (DPI), an inhibitor of flavin-containing oxidases, or Apocynin, an inhibitor of NOX by blocking p47phox translocation (not specific for NOX isoforms), implicating NOX-derived superoxide production in AF [16]. To a smaller degree, L-NAME-sensitive superoxide production was also increased, implicating a potential downstream role of uncoupled eNOS [16]. In a later study by Kim et al., the authors also showed that NADPH-driven superoxide production, reflective of NOX activity, was significantly increased in RAA from patients with postoperative AF [17]. Similarly, NADPH-driven superoxide in RAA was found attenuated by short term treatment of Atorvastatin (40 mg/day), indicating Rac1-dependent NOX activation in postoperative AF [65]. Chang and colleagues recently demonstrated borderline but significant correlation between NOX2-driven superoxide in LAA and left atrial enlargement in AF patients. They

also observed NOX2 upregulation in RAA of AF patients and correlations between NOX2 upregulation, with myolysis and hypertrophy, implicating a possible role of NOX2-derived oxidative stress in atrial remodeling in AF [15].

In other studies, Reilly and colleagues demonstrated that NOX activation was increased along with increased expression of p22phox and NOX2, while eNOS was still coupled, in postoperative AF patients [64]. But in patients with permanent AF, expressions of NOX2, p22phox, NOX4 and NOX5 were not altered; and levels of superoxide production inhibitable by Rotenone and L-NAME were increased compared to SR, indicating increases in mitochondrion and uncoupled eNOS-derived superoxide in permanent AF [64]. In another recent study, it was found that mRNA expression of neither NOX2 nor NOX1 was increased in LAA of 18 AF patients [18]. Instead, NOX4 expression was significantly increased, and correlated well with a marked elevation in H_2O_2 production and higher blood pressure, implicating a potential role of NOX4 and NOX4-derived H_2O_2 in AF, particularly in those with hypertension [18]. NOX4 expression was also increased by Ang II stimulation in HL-1 atrial cells [18]. However, it cannot be excluded that NOX4 increase in this study might be in part associated with the severe pathological remodeling of the heart in these end stage heart transplant patients. Interestingly, recent data have shown that tachypacing of HL-1 atrial cells upregulates NOX2 and NOX4, leading to oxidative stress and myofibril degradation [66] (more see below for cellular studies). Taken together, it is evident that NOX derived ROS are involved in the pathogenesis of AF, although it remains unclear which NOX isoform(s) is(are) responsible in different types of AF. It is also possible that different NOX isoforms and other downstream sources of ROS including mitochondrion and uncoupled eNOS, are involved at different stages of the disease. It is known that regulation of one NOX isoform can affect other isoforms and their regulatory partners [52, 67, 68]. Therefore, larger trials with application of more selective, NOX-specific and NOX isoform-specific inhibitors are required to resolve these issues in future studies. Cell-type specific analyses of the NOX isoforms in heart tissues may also provide additional mechanistic information.

Cellular studies

In vitro studies using HL-1 atrial myocytes have implicated a role of NOX4 as well as NOX2 in increased TGFp1 expression, intracellular oxidative stress, and calpain activation for myofibril degradation [66]. In particular, tachypacing increased NOX2 and NOX4 protein expression. Tachypacing (at 4Hz, 24hrs) induced ROS production was also suppressed in both NOX2 and NOX4 siRNA transfected cell, indicating a role of NOX2 and NOX4 containing NOX as a source of oxidative stress in AF at cellular model. Additionally, neutralization of TGFp1 with specific antibody prevented NOX induced ROS production following tachypacing. These data seem to suggest that TGFp1 mediated NOX2/NOX4 activation induces subsequent atrial myocyte remodeling via myofibril degradation aside from TGFp1-smad3-myolysis pathway. Zhang and colleagues recently demonstrated that Ang II increases H_2O_2 production and NOX4 expression in HL-1 atrial cells, which is similar to findings observed in AF patients [18]. Given the well known effect of Ang II in structural remodeling in AF and the previously established role of H_2O_2 in inflammation and thrombosis, these data indicate that NOX4-derived H_2O_2 is possibly involved in electrophysiological changes promoting AF, as well as development of its thromboembolic complications.

4. Role of ROS in mediating initiation of AF and thromboembolic complication: AF begets AF as NOX begets NOX

Despite the clear link between oxidative stress and AF, it remains unclear whether oxidative stress is a causal factor for AF, or, conversely, a consequence of AF.

Pulmonary veins (PV) have been recognized as the sites of origin of premature atrial extrasystoles that can initiate AF [69, 70]. Indeed, ablation therapy has been known to be effective in patients with paroxysmal AF by disconnecting electrical conduction from PV to LA. Of note, H_2O_2 has been demonstrated to trigger irregular electrical firing of PV cardiomyocytes [71]. Moreover, NOX might be the source for increased H_2O_2 production. It is worth mentioning that H_2O_2 is cell permeable and known to diffuse to adjacent cells and tissues. On the other hand, in post-operative AF, oxidative stress induced by ischemia/reperfusion and mechanical stretch during cardiac surgery has been suggested to be a factor promoting electrical remodeling. For instance, mechanical stretch induces Ang II, which acts on multiple ion channels by stimulation of AT1R. This response is known to specifically destabilize cardiac myocyte Kv4.3 channel mRNA by activating NOX [72, 73]. Moreover, Ang II is a potent stimulator of NOX, which promotes ROS generation and oxidative modifications of protein targets, such as oxidative activation of CaMKII [31, 32, 74]. It has been reported that Ang II infusion leads to oxidation of Met 281/282 residues in the regulatory domain of CaMKII, which transforms CaMKII into a constitutively active form, leading to secondary electrical remodeling by inducing calcium overload via activation of L-type calcium channel and ryanodine receptor 2 (RyR2) hyperphosporylation [74, 75]. In the same study, expression of ox-CaMKII was found increased in atria of AF patients, indicating a potential role of ROS induced CaMKII activation in AF. Higher levels of ox-CaMKII were associated with sinoatrial node dysfunction in patients with heart failure [74]. Primary electrical disturbance induced by increased calcium loading can trigger electrical remodeling accompanied by structural remodeling of myocardium, which can subsequently lead to irreversible fibrosis. Thus oxidative stress is not only an early event in primary electrical remodeling, but also involved in secondary structural remodeling and consequent changes in electrophysiology. A similar relationship exists between inflammation and AF [76]. Calcium overload or Ang II receptor 1 activates NFkB, which is an inflammatory transcription factor and a known contributor of AF initiation and maintenance. Recently, NFκB has been shown to downregulate transcription of the cardiac sodium channel in response to oxidative stress [76], consistent with an Ang II-NOX-NFkB axis. Indeed, H_2O_2 can activate NF-kB. In addition, NF-kB is a transcription factor for TNF-α, iNOS, IL-1β, and MMPs, all of which are involved in structural remodeling and inflammation. Taken together, these data seem to indicate ROS could well be upstream of AF initiation, by contributing to electrical and structural remodeling, and activation of inflammatory pathways. As discussed earlier, activation of NOX in AF could lead to ROS production and possible downstream activations of uncoupled eNOS and mitochondrion [16, 45, 77–79]. It is proposed therefore, NOX might have an important role in “AF begets AF” [80].

5. Interventions potentially targeting NOX in AF

The spectrum of antioxidant treatments in AF has been reviewed extensively [29]. As summarized in Table 2, treatment with Vitamin C, Vitamin C in combination with Vitamin E or N-acetylcysteine (NAC), each of which has antioxidant actions, reduced post-operative AF. However, evidences as to whether general antioxidant treatments are effective to prevent AF are conflicting. Therefore, approaches targeting more specific ROS-generating pathways have received increasing attention.

Inhibition of RAS to inhibit initial activation of NOX

The RAS system is known to activate NOX to result in marked oxidative stress, and therefore a possible trigger of AF. It has been established that Ang II induces NOX activation [32] and consequent eNOS uncoupling in endothelial cells [81], hypertensive mice [82], and diabetic animals [83]. Similar to the findings that Ang II upregulates NOX4/AT1 expression and H_2O_2 production in HL-1 atrial cells [18], it was reported that Ang II induction of superoxide was attenuated by AT1RB Losartan in HL-1 cells [84]. AT1 receptor expression was found markedly increased in left atrium of patients with AF compared to those in SR [85]. Activation of the AT1 receptor leads to activation of plasminogen activator inhibitor (PAI-1) and tissue factor (TF), which in turn mediates thrombosis and fibrinolysis [86, 87]. In addition, stimulation of AT1 receptor activates monocyte chemoattractant protein (MCP-1), vascular adhesion molecule (VCAM-1), intracellular adhesion molecule (ICAM-1) and tumor necrosis factor α (TNF-α) [88]. Of note, elevated ICAM-1 expression occurs in patients with post-operative AF [89]. Endothelial VCAM-1 expression was also found increased in RAA of AF patients [90]. Pre-treatment with Olmesartan attenuated RAP (rapid atrial pacing) induced VCAM-1 expression in human atrial tissue slices [90]. Olmesartan significantly prevented RAP-induced downregulation of endocardial tissue factor platelet inhibitor (TFPI), thrombomodulin, and eNOS at protein levels without affecting mRNA expression of these proteins [91]. Upregulation of PAI-1 by RAP was also inhibited by AT1 receptor blockade. A recent study by Bodiga S. et al. reported accelerated adverse remodeling of the extracellular matrix and worsening of systolic function, along with increased NOX activity and increased superoxide production in response to pressure overload in ACE2 null mice, which was attenuated by p47phox depletion [92]. This finding suggests that Ang II-mediated NOX2 activation may serve as an early regulator of cardiac remodeling and that targeting ACE2 might be an effective therapeutic strategy. Furthermore, a recent meta-analysis based on 23 randomized trials showed that RAS inhibition with either ACEI or ARB is effective in preventing AF, despite considerable variation among different trials [93]. Taken together, targeting Ang II-mediated oxidative stress may be effective in attenuating several processes including atrial fibrosis, inflammation, electrical and structural remodeling of AF, all of which are involved in the pathogenesis of AF. What underlies the effectiveness on AF prevention of Ang II signaling attenuation could be inhibition of the NOX pathway.

Statins as NOX inhibitor

Statin therapy has been shown to decrease NOX activation by downregulation of Rac1 subunit. Statins and Probucol, which act as antioxidants in addition to their lipid lowering effects, reduced AF incidence [94]. Prubucol reduces cholesterol and inhibits oxidation of LDL cholesterol. It has been shown to cross membranes easily and act as a scavenger of oxygen radicals. In a dog AF model, levels of malondialdehyde and calpain 1, which contribute to structural remodeling, were both abrogated by Prubucol treatment [95]. Simvastatin attenuated Ang II-stimulated superoxide production in HL-1 atrial myocytes [84]. Likewise, it was shown that Probucol inhibits NOX activation [94]. A recent clinical study suggested that levels of myocardial superoxide and peroxynitrate are associated with length of hospitalization and duration of ionic support, whereas short term treatment with Atorvastatin (40 mg/day) reduces myocardial superoxide and peroxynitrate ($ONOO^-$) due to decreased NOX activation in RAA from SR patients, suggesting that Statin usage for a longer period perhaps would have some benefits in preventing post-operative AF through inhibition of NOX [96]. Reilly S et al. also showed that Atovastatin treatment is effective to inhibit Rac1-dependent NOX activation in postoperative AF but not in permanent AF [64]. Taken together, Statins seem effective in preventing post-operative AF at least to some extent, potentially via modulation of NOX activity. Nonetheless, the efficacy of Statins in the prevention of AF is not yet conclusive [97].

6. Conclusions

AF has become more and more prevalent and now a major public health problem [98]. Although AF is clearly associated with aging, and cardiovascular conditions such as hypertension, mitral valve disease and heart failure, the underlying molecular mechanisms have remained elusive [98]. Recent developments suggest that AF is promoted by atrial structural and electrical remodeling, and that AF itself further augments these responses to perpetuate AF [99, 100]. The known risk factors for AF have been linked to oxidative stress, although it is still unclear whether oxidative stress is the initiating factor for AF. Antioxidant treatment and interventions targeting Ang II signaling appear to have some preventive effects on post-operative AF [101, 102], and sometimes on recurrence and new onset of AF [103, 104]. Latest advances from experimental and clinical approaches seem to suggest that NOX isoforms such as NOX2 and NOX4 are major sources of ROS production in AF. NOX-derived ROS, superoxide and H_2O_2, activate several processes including atrial inflammation, fibrosis, and structural and electrical remodeling as shown in Figure 1. These processes confer upstream activators of NOX such as Ang II and atrial stretch, forming a vicious cycle of NOX activation promoting AF, and AF promoting NOX activation. Therefore, inhibition of specific isoforms of NOX may serve as a novel therapeutic strategy for breaking the NOX-AF vicious cycle. So far, NOX2 and NOX4 have been identified as the specific NOX isoforms involved in AF. Nontheless, further investigations using specific inhibitors or siRNAs targeting different NOX isoforms, and knockout and transgenic animals targeting different NOX isoforms, are necessary to further elucidate detailed, NOX isoform(s)-dependent mechanisms involved in AF pathogenesis, and subsequently novel therapeutic strategies for the prevention and treatment of AF.

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Highlights

- Oxidative stress has been implicated in the pathogenesis of AF;
- Activation of NADPH oxidase (NOX), particularly isoforms 2 and 4, occurs in humans with AF and experimental models of AF;
- NOX can be activated by upstream substrates of AF such as Ang II and atrial stretch;
- Inhibition of NOX by ACEI/AT1RB or Statins may have beneficial effects in preventing post-operative AF

Figure

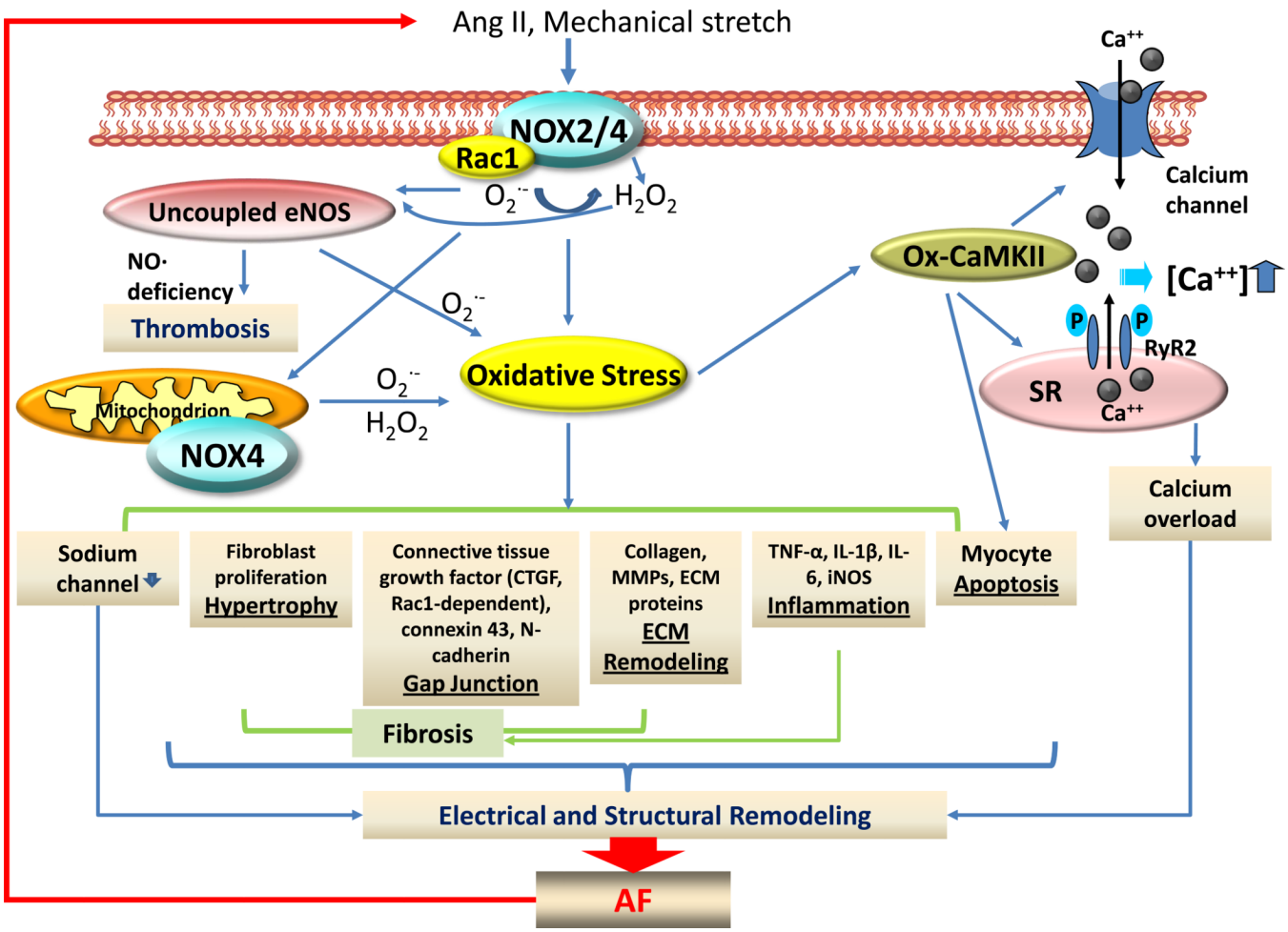

Figure 1.

Role of NADPH oxidase (NOX)-derived oxidative stress in atrial fibrillation (AF). Production of superoxide and H_2O_2 from activated NADPH oxidasae (NOX) isoforms 2 and 4 (NOX2, NOX4 respectively) leads to activation of downstream reactive oxygen species (ROS)-generating systems including mitochondrion and uncoupled eNOS, resulting in sustained oxidative stress which in turn stimulates myocyte apoptosis, atrial inflammation, fibrosis, and structural and electrical remodeling. Examples of key mediators downstream of increased ROS production include oxidized CaMKII (Ox-CaMKII) and activated nuclear factor kB (NF-kB). ox-CaMKII activates ryanodine receptor 2 (RyR2) hyperphospohrylation, which in turn causes secondary electrical remodeling and calcium overload-induced cardiac injury. NF-kB is a well known redox-sensitive transcriptional factor for inflammation and structural remodeling by activating TNF-alpha, iNOS, IL-1β, and MMPs. All these processes confer to NOX activators such as Ang II and atrial stretch, thus forming vicious cycle of NOX activation promoting AF, and AF promoting NOX activation. Endocardial nitric oxide deficiency due to oxidative stress and eNOS uncoupling may also contribute to thromboembolic complications of AF.

Table 1

Summary of studies on oxidative stress in AF

Experimental Model	Analyzed Tissues	Oxidative Stress Markers	Analyses	Ref
Animal Studies				
Porcine RAP (1 week)	LA, LAA	↓Bioavailable NO	NO specific microeletrode	[19]
	LA	↓eNOS	Western blot	
		↑PAI −1		
Swine RAP (1 week)	LAA	↑superoxide dependent on NOX and XO (NOX>XO)	ESR, cytochrome C reduction	[61]
	LA	↑Rac1 expression	Western blot	
Goat RAP (2 weeks/6 months)	LA	↑NOX dependent superoxide (2 weeks)	Lucigenin-enhanced chemiluminescence	[64]
		↑Mitochondria derived (6 months)		
	RA	↑superoxide in 6 months-AF		
		↑Mitochondria and uncoupled eNOS derived		
Clinical Studies				
AF Patients (permanent AF, n=6; permanent from paroxymal n=4, paroxymal AF, n=5)	RAA	↑NOX activation	Lucigenin-enhanced chemiluminescence	[16]
		↑eNOS uncoupling	DHI staining	
AF Patients with mitral regurgitation (persistent AF, n=8; SR, n=8)	RAA	↑superoxide	Lucigenin-enhanced chemiluminescence	[15]
		↑NOX activation -NOX 2	RT-PCR (no change)	IHC
		↑NOX 2		
AF patients (SR=99, AF =71) CABG surgery	RAA	↑NOX activation	Lucigenin-enhanced chemiluminescence	[17]
Persistent AF patients (n=8)	RAA	↓peroxiredoxin	Mass spectrometry	[105]
		↑NADH dehydrogenase flavoprotein	Microarray	
		↑Ubiquinol cytochrome C reductase core protein 1		
Persistent AF patients (n=15)	RA	↑NOX in RA (↓NOX in serum)	Nitrite assay (Griess reaction)	[106]
		↑iNOS/eNOS	western blotting	
		↑3-nitrotyrosine	immunostaining	
Persistent AF (n=26)	RA	↑Rotenone/L-NAME inhibited superoxide - NOX2, NOX4, NOX5	Lucigenin-enhanced Chemiluminescence Western & real timePCR	[64]

Experimental Model	Analyzed Tissues	Oxidative Stress Markers	Analyses	Ref
Post-operative AF (n=32)	RA	↑ NOX dependent superoxide ↑ p22phox, NOX2 ↓ H4B level	Lucigenin-enhanced chemiluminescence Western blot HPLC	[64]
AF patients (n=18) non-AF (n=17)	LAA	↑H_2O_2 ↑NOX4 - NOX1, NOX2	Amplex Red Real time PCR	[18]

Table 2

Summary of studies on antioxidant treatments in AF

Antioxidant	Effects	Action mechanism	Experimental Model	Ref
Clinical Studies				
Vitamin C	↓incidence of postoperative AF - incidence of postoperative AF	Downregulation of NADPH oxides (transcriptional or post translational level)	coronary artery bypass grafting patients	[107] [108]
Vitamin E with Vitamin C	↓ncidence of AF	Scavenges ROS	Patients undergoing coronary artery bypass	[109]
Statins	↓incidence of postoperative AF, ↓incidence of postoperative AF ↓incidence of postoperative AF	Counteracts the arrhythmogenic effects of angiotensin, reduces cholesterol, antioxidant, anti-inflammatory, anti-apoptosis, reduces myocardial O_2 and ONOO reduces C-reactive protein levels	coronary artery bypass grafting patients	[110, 111] [65] [112, 113]
n-3 Polyunsaturated fatty acids (PUFAs)	↓incidence of postoperative AF, - incidence of postoperative AF	increases electrical stability	cardiac surgery patients	[114, 115] [116–118]
Carvedilol	↓incidence of postoperative AF	increases myocardial levels of the antioxidant enzymes superoxide dismutase and glutathione peroxidase,	heart failure patients	[119]
N-acetylcysteine	↓incidence of postoperative AF	ROS scavenger	Patients undergoing coronary artery bypass and/or valve surgery	[28]