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Defining the role of oxidative stress in atrial fibrillation and diabetes

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Oxidative stress and atrial fibrillation

Atrial fibrillation (AF) is the most common form of arrhythmia, currently estimated to affect over 33 million individuals worldwide.[1] The incidence of AF continues to grow, and unfortunately current treatment strategies (e.g., catheter ablation, ion channel blockers) may have limited efficacy and/or adverse effects. Thus, understanding the underlying source(s) of AF represents an important strategy for the future treatment of AF.

AF may result from abnormalities in atrial electrical activity generated from improper impulse formation or propagation. This inappropriate activity is primarily associated with electrical and/or structural remodeling (e.g., ectopic activity or altered conduction caused by fibrosis). While the molecular mechanisms of AF are not completely understood, key factors that result in AF have been identified. One such factor is oxidative stress. Oxidative stress occurs when reactive oxygen species (ROS) generation exceeds degradation. Primary sources of ROS within the heart and associated with AF include mitochondria, NADPH oxidase, and xanthine oxidase.[2, 3] Furthermore, in AF, there may be additional sources that elevate ROS production (e.g. monoamine oxidase).[4] Finally, in AF, down regulation of enzymes that degrade ROS (e.g., superoxide dismutase and glutathione peroxidase) exacerbate oxidative stress phenotypes.[5] Subsequently, elevated ROS may modify ion channel activity to increase AF susceptibility. For example, elevated ROS results in shortening of the atrial action potential duration (APD) by increasing the transient outward current (I_{to})[6] and producing delayed after depolarizations (DADs) by increasing sarcoplasmic reticulum (SR) Ca^{2+} release via enhanced ryanodine receptor (RyR) activity.[7]

NF-κB in atrial fibrillation

A critical molecule relevant for AF pathogenesis is nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). NF-κB is a transcription factor that modifies a multitude of genes including many associated with inflammation, a key contributor to AF. Interestingly, NF-κB is now known to be redox sensitive.[8] Thus, NF-κB couples the redox state of the cell to modify gene transcription. NF-κB directly regulates myocyte targets

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directly associated with arrhythmia susceptibility. For example, the promoter region of the cardiac sodium channel (*SCN5A*) has an NF-κB binding site that, upon binding, results in decreased *SCN5A* mRNA abundance and protein expression.[9] The decrease in *SCN5A* expression may result in decreased Na^+ current amplitude and slowed conductance, a key substrate for re-entry. Moreover, NF-κB gene regulation may also be involved in fibrosis. For example, knockout of a subunit of the NF-κB family (c-Rel) prevented fibrosis induced by four weeks of angiotensin infusion.[10] Along with c-Rel, activation of NF-κB also results in the up-regulation of the transforming growth factor (TGF-β) pathway and the induction of fibrosis.[11] As with *SCN5A* reduction, fibrosis is a key substrate for AF. While studies of NF-κB in AF are in their infancy, these data are highly suggestive that NF-κB activation is a key contributor to AF.

Atrial fibrillation and diabetes

A risk factor for the occurrence of AF is diabetes. It is proposed that the greater incidence of AF in individuals with diabetes is due to increased oxidative stress and NF-κB signaling.[12, 13] A new study published in this issue of *Journal of Cardiovascular Electrophysiology* addresses this very topic.[14] Fu *et al.* using a rabbit model of diabetes (alloxan-induced) investigated the effects of anti-oxidant (probucol) treatment for 8 weeks on the incidence of AF and associated molecular mechanisms. The authors observed that diabetic hearts (using a Langendorff preparation) showed greater induction of AF following burst pacing. This induction of AF was greatly reduced by the anti-oxidant treatment. Interestingly, probucol did not change the atrial effective refractory period, suggesting no change in the electrical remodeling, but significantly reduced fibrosis. Upon further investigation, the authors discovered that anti-oxidant treatment reduced NF-κB and TGF-β levels. It should be noted that for most measured parameters, the effects of probucol were modest at best. Unfortunately, this is in line with clinical trials showing that anti-oxidant treatment for AF may show limited benefits. [15, 16] There are potentially many reasons why anti-oxidants may provide minimal protection against AF. For example, ROS does not function autonomously but interacts with additional molecules such as nitric oxide (NO).

Nitroso-redox balance and atrial fibrillation

ROS and NO operate in tandem (nitroso-redox balance) to modulate cardiovascular function.[17] Therefore, a major factor for why anti-oxidants may be ineffective in treating AF is that NO bioavailability is decreased in diabetes and AF.[18, 19] Hence, simply lowering ROS (anti-oxidant therapy) is not sufficient to reverse phenotypes without counter-balancing its partner molecule (i.e., NO).[17]

There are three isoforms of the enzymes that produce NO termed NO synthase (NOS) and all three are expressed within the cardiovascular system. The decrease in NO bioavailability observed with AF is due to a decrease in the endothelial NOS (eNOS) isoform.[20] Additionally, in AF, eNOS becomes “uncoupled” that leads to the generation of ROS instead of NO.[19] Notably, we and others have illustrated that loss of eNOS results in DADs[21] as well as nitroso-redox imbalance leading to the generation of arrhythmias.[22] For unknown reasons, Fu *et al*. reported that NOS and NO were increased in their AF diabetic

model.[14] It will be important for the authors to resolve this discrepancy in relation with previous findings in order to better link AF and diabetes pathogenesis.

Atrial fibrillation and CaMKII

A second mitigating factor why anti-oxidants are unsuccessful is that chronic ROS elevation may have already caused irreversible downstream remodeling so that lowering ROS levels by itself will be ineffective. This may also be the case with diabetes and AF. Calcium and calmodulin (CaM)-dependent protein kinase (CaMKII) is a multifunctional serine/threonine kinase in heart. CaMKII is a holoenzyme formed by 6–12 subunits in a wheel-like structure. Each monomer contains a catalytic domain, a regulatory domain that contains an auto-inhibitory domain, and an oligomerization domain. This kinase is activated when the Ca^{2+}/CaM complex binds to the regulatory domain and displaces the auto-inhibitory region. Once activated, CaMKII can autophosphorylate on threonine 287 to maintain activity even when the Ca^{2+}/CaM complex dissociates. Beyond Ca^{2+}/CaM, it is now established that there are secondary activators of CaMKII. Pivotal work from the Anderson group has shown that CaMKII is activated, independent of Ca^{2+}, via oxidation.[23] Relevant for this editorial, the cellular conditions in diabetes are conducive for CaMKII activation. Specifically, the diabetic heart is under oxidative stress[13] and CaMKII is activated in the diabetic heart via oxidation.[24, 25]

Previous work has established that oxidative activation of CaMKII triggers AF.[26] The molecular mechanism of CaMKII causing AF is via the formation of DADs via RyR serine 2814 phosphorylation to enhance diastolic SR Ca^{2+} leak. Indeed, this pathway has been found to be up-regulated in AF patients.[27] In addition to DADs, the CaMKII induced Ca^{2+}-handling abnormalities play a role in other substrates for AF (e.g. reentry). Hence, CaMKII, through this mechanism as well as its role in regulating fibrosis may be the missing link between oxidative stress and AF in the diabetic heart. In summary, CaMKII is an important mediator of AF and we have shown that NO is also able to modulate CaMKII activity.[28] However, additional studies are warranted to further examine the effects of nitroso-redox imbalance on CaMKII activation.

In conclusion, Fu *et al.* provide compelling preliminary results that support a link between oxidative stress and NF-κB inducing AF. However, as the authors note, this anti-oxidant treatment, may have limitations for disease treatment. Clearly, additional future work is necessary to better understand the links between nitroso-redox balance, NF-κB, CaMKII, and AF in diabetes.

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