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FORUM EDITORIAL

Challenges, Progresses, and Promises for Developing Future NADPH Oxidase Therapeutics

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Abstract

NADPH oxidase (NOX) enzymes show great potential as therapeutic pharmacological targets. This Forum revolves around the roles of specific NOX isoforms in oxidative stress-mediated pathologies, available NOX antagonists/agonists as well as the potential side effects of NOX inhibition and the requisite identification of novel oxidative biomarkers as a measure of NOX activity in patients. In addition, an original article reports the discovery of a novel small molecule NOX2 inhibitor. Finally an attractive and innovative therapeutic approach for modulating NOX activity through the inhibition of the proton channel Hv1 is discussed. *Antioxid Redox Signal.* 23, 355–357.

ALMOST THREE DECADES have passed since the first NOX (NADPH oxidase) isoform was formally identified (8). The recognition that an enzyme system dedicated to the generation of reactive oxygen species (ROS) was required for health and that its absence in phagocytes led to chronic granulomatous disease (CGD) was the first confirmation that ROS are not only toxic by-products of mitochondrial activity but are also essential for normal human physiology. Further evidence of the physiological roles of ROS came a decade later when other NOX isoforms were identified and cloned from a wide range of tissues (1, 3). A great deal of research has been performed as to untangle the complex roles of NOX-derived ROS in physiology and disease, and much evidence indicates that NOX enzymes are valid pharmacological targets for a wide range of conditions. Despite this, as of today, there are still no molecules specifically targeting NOX available in the clinic.

This Forum of *Antioxidants & Redox Signaling* focuses on recent advances in the NOX field and highlights the potential of NOX enzymes as pharmacological targets for the development of novel drugs. NOX are transmembrane enzymes with the sole known purpose of catalyzing the formation of ROS. There are seven NOX isoforms with distinct tissue distribution and activation mechanisms. They regulate various redox-dependent physiological mechanisms, such as neutrophil microbial killing (NOX2), inner ear otoconia formation (NOX3), thyroid hormone synthesis (DUOX2), and many others (2). The expression levels of NOX enzymes are often increased in pathological states, including cardiovascular, neurological, and fibrotic diseases, among many others. Whether this increased expression translates into oxidative damage and contributes to pathology is not always clear. However, it has been proposed that NOX enzymes might be among the long-sought sources of pathological oxidative damage (5). We are still at the early stages of the comprehension of the full range of biological roles of NOX-derived ROS. In the absence of specific pharmacological inhibitors, patients and rodents carrying spontaneous loss-of-function mutations or genetically engineered knockout animal models have been valuable for identifying some of the roles of NOX enzymes. The knowledge gained from these studies supports the concept that specific drugs modulating the NOX activity (both agonists and antagonists depending on the indication) are needed for both research purposes and ultimately as potential therapeutics.

This Forum consists of seven review articles and one original research article. The goal is to present data supporting NOX as a pharmacological target and to discuss some of the key challenges that academic groups and pharmaceutical companies are facing in the development of novel NOX therapeutics. This includes updates on the current evidence supporting NOX as a target in a number of indications, overviews of available and new NOX inhibitors, consideration of the potential for NOX agonists in some indications, discussions of

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the potential side effects, and consideration of oxidative biomarkers as a potential measure of NOX activity in patients.

The review of Wieczfinska *et al.* introduces this Forum by pointing out the utility of NOX inhibitors in lung disorders. They summarize the expression pattern and regulatory mechanisms of the NOX isoforms present in the lung and discuss their potential implication in a wide range of diseases, such as inflammatory diseases (asthma, ARDS, COPD), fibrotic diseases (pulmonary fibrosis), genetic disorders (sickle cell disease, cystic fibrosis), or diseases caused by bacterial or viral infections. They discuss the successes and failures of antioxidants used in lung pathologies and the potential for NOX inhibitors to offer improvements.

The review by Carbone *et al.* describes one of the most debated roles of NOX enzymes, the role in stroke. The impact of inhibition of NOX1, NOX2, or NOX4 in animal models of ischemic stroke has been evaluated by different research groups, but the outcomes have varied. The review provides comprehensive information on the animal research performed with knockout animals and available small molecules. It decrypts the differences between the protocols and the readouts used. Interestingly, it extends its scope to available clinical trials performed with small-molecule scavenging ROS, such as the potent antioxidant Edaravone, and other molecules, such as statins, possibly interfering with the NOX pathway.

A major problem, which can arise from NOX inhibition, is interference with the neutrophil's antibacterial defense. Genetic mutations affecting the NOX2 complex lead to a primary immune deficiency called CGD. CGD patients have a defective neutrophil oxidative burst and are prone to recurrent infections and the development of granuloma. In this context, the use of NOX2 inhibitors as therapeutics seems contraindicated. Diebold *et al.* challenge this conception and comprehensively discuss the conditions where NOX2 inhibitors could be beneficial. These span a wide range of disorders involving acute and chronic inflammation ranging from hypertension, pulmonary disorders, ischemia and ischemia/reperfusion injury, a number of neurological conditions, and muscular disorders. Data from NOX2-deficient animals and from patients carrying mutations in NOX2 are assembled to evaluate the risk versus benefit of the feasibility of the clinical use of a NOX2 inhibitor.

A limitation for the development of NOX therapeutics is the absence of reliable methods to measure NOX activity in tissues [as recently confirmed by Rezende *et al.* (7)] and the availability of predictive NOX-dependent biomarkers. Violi and Pignatelli present current knowledge on laboratory methodologies measuring oxidative modifications in physiological fluids in the field of cardiovascular diseases. Clinical data from their own laboratory link NOX activity and the presence in the blood of specific molecules, such as isoprostanes, which are oxidation products of arachidonic acid. They also discuss the evidence that NOX2 can be used as a biomarker itself during progression of cardiovascular disease. It is unfortunate that NOX-dependent biomarkers have so far been insufficiently explored. The identification of surrogate markers as a laboratory measure of the effects of a treatment targeting NOX would represent a key benchmark for the development of NOX therapeutics and would be mostly valuable to provide evidence of effectiveness of new treatments and for patient stratification in future clinical trials.

Although much of the interest is focused on the observation that excessive NOX-derived ROS production is detrimental, Hultqvist *et al.* draw our attention to certain autoimmune conditions, such as rheumatoid arthritis, systemic lupus erythematosus, and autoimmune demyelination, where enhancing ROS production might be beneficial. More than a decade ago, a positional cloning study identified a polymorphism of *Ncf1* (coding for the $p47^{phox}$ subunit of NOX2) that regulates arthritis severity in a rat model (6). At the time, the findings that ROS can be anti-inflammatory sounded provoking, as ROS were considered as proinflammatory by essence. However, this concept was further confirmed in mice and by the fact that carriers of NOX2 mutations are more susceptible to autoimmune diseases. In this review, the authors describe in an educational way the evidence gained from a complex series of adaptive T-cell transfer experiments in a rat model of arthritis, which argue for a NOX2-dependent regulation of T-cell activity. A phenotypic screening approach used to identify NOX2 agonists is detailed in this review and the benefits and limitations of a NOX2 agonist approach are discussed.

Altenhöfer *et al.* provide an informed and opinionative snapshot of the current knowledge on small-molecule candidate NOX inhibitors. The authors critically address the reported mode of action of these small molecules as well as their potential off-target and nonspecific activities. They come to the conclusion that most molecules available are insufficiently described in terms of use *in vivo* and are not specific or not compatible with clinical use. Interestingly enough, in an attempt to identify common chemical structures among different available molecules, they identify typical scaffolds common to a number of molecules, in particular, the ones developed by the pharmaceutical companies. Finally, the pros and cons for inhibition of each isoforms are discussed as well as the diseases which would benefit the most from NOX therapeutics.

Seredenina *et al.* present an original twist to this Forum by describing evidence supporting an interesting alternate drug target in the quest to regulate NOX function: the Hv1 voltage-gated proton channel. Upon activation, NOX2 causes membrane depolarization due to the transfer of electrons through the plasma membrane and accumulation of protons in the cytoplasm through oxidation of NADPH. These effects are counterbalanced by the proton channel Hv1, which extrudes H^+ into the extracellular space. Thus, Hv1 inhibition represents a mechanism of modulation of the activity of the oxidase. Hv1 inhibitors are expected to have fewer potential side effects than NOX2 inhibitors as Hv1-deficient mice present much milder immune deficit than NOX2-deficient mice. Clinically relevant compounds that are specific for proton channels are not yet available, however, drug discovery programs are currently being undertaken. Applications of HV1 inhibitors overlap with the indications described by Diebold *et al.* for NOX2 inhibitors and include not only inflammatory diseases, autoimmune disorders, neurological disorders, pulmonary diseases but also expand to other diseases independent of NOX activity, such as cancer, where Hv1 may sustain extracellular acidification.

Finally, the original article featured in this Forum by Hirano *et al.* describes the identification and characterization of the novel small-molecule NOX inhibitor GSK2795039. The development of well-characterized and specific tools is

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not a trivial step as it greatly enhances the amount of research performed on a given target (4). Unlike the broad-spectrum flavoprotein inhibitor diphenyleneiodonium, which is still today the *in vitro* gold standard for NOX inhibition in the absence of sufficiently validated molecules, GSK2795039 is selective for NOX2 over other sources of ROS, including the other NOX isoforms, xanthine oxidase and nitric oxide synthase. Importantly, GSK2795039 shows NADPH competitive inhibition mode of action indicating that NADPH-binding sites are sufficiently distinct between NOX isoforms for selective inhibition. Furthermore, its pharmacokinetic profile allows for *in vivo* NOX2 inhibition in a model of paw inflammation and shows therapeutic benefit in an animal model of cerulein-induced pancreatitis.

As of today, with the scarcity of specific small-molecule NOX inhibitors and the use of molecules with multiple potential modes of action in the cell, much of the reliable knowledge on the role of NOX enzymes in disease rests on *in vitro* cellular models and genetically modified rodents. We look forward to the emergence of fully validated small-molecule NOX modulators, which can be applied for further validation of the role of NOX in pathological animal models. We hope that this Forum will help and motivate researchers, clinicians, and pharmaceutical companies to deliver on the promise of modulating NOX activity as a novel therapeutic approach.

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Author Disclosure Statement

Vincent Jaquet holds shares from Genkyotex SA, a company developing NOX inhibitors.

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Abbreviations Used

ARDS = acute respiratory distress syndrome
COPD = chronic obstructive pulmonary disease
DUOX2 = dual oxidase 2
NOX = NADPH oxidase
ROS = reactive oxygen species
CGD = chronic granulomatous disease