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REVIEW ARTICLE

Defence mechanism of nitric oxide against Tuberculosis

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ABSTRACT
This review summarizes the importance of Nitric Oxide (NO) in treatment of Mycobacterium tuberculosis (Mtb), the causative mediator of tuberculosis (TB), is globally known as one of the most vital human pathogens. Mtb approximately infects nearly one third of the world's population with many subjects having a latent infection. Thus, from an estimated 2 billion people infected with Mtb, less than 10% may develop indicative TB. This indicates that the host immune system may hinder pathogen replication in most infected individuals. When Mtb enter the lungs of the host, it primarily encounters resident alveolar macrophages which can engulf and consequently eliminate intracellular microbes via a abundance of bactericidal mechanisms including the generation of free radicals like reactive oxygen and nitrogen species. NO, a key anti-mycobacterial molecule is detected in the exhaled breath of patients infected with Mtb. Recent knowledge regarding the regulatory role of NO in airway function and Mtb proliferation paves the way of exploiting the valuable effects of this molecule for the treatment of airway diseases.
Key word: Nitric Oxide, Tuberculosis, Diagnosis, Treatment

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INTRODUCTION

Tuberculosis (TB) is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). *Mycobacterium tuberculosis* (Mtb), spreads when people who are sick with TB expel bacteria into the air; for example, by coughing. It classically affects the lungs (pulmonary TB) but can also affect other sites (extrapulmonary TB). India accounts for 27% of the total global TB burden. Intensified efforts are required to improve reporting of detected TB cases and access to diagnosis and treatment [1]. Presently the only TB vaccine we have is Bacille Calmette Guerin (BCG) and its efficacy in pulmonary TB is variable in adolescents and adults. There is an urgent need of an alternative to BCG as vaccine.

The smallest signalling molecule known is Nitric oxide (NO), produced by three isoforms of NO synthase (NOS; EC 1.14.13.39). These use L-arginine and molecular oxygen as substrates and need the cofactors reduced nicotinamide-adenine-dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and (6R) 5,6,7,8-tetrahydrobiopterin (BH4). NOS bind calmodulin and contain haem. In response to lipopolysaccharide, cytokines, or other agents inducible NOS (NOS II) are expressed in many cell types. Inducible NOS generates huge amount of NO that have cytostatic effects on parasitic target cells. Inducible NOS contributes to the disordered inflammatory diseases and septic shock. Endothelial NOS (eNOS, NOS III) is expressed in endothelial cells. It keeps blood vessels dilated, controls blood pressure, and has numerous other vasoprotective and anti-atherosclerotic effects [2].

ROLE OF NITRIC OXIDE (NO) and PEROXYNITRITE (ONOO) IN ANTI-MTB IMMUNITY

NO plays an important role in bacteriostatic and bactericidal processes as part of the host defense mechanisms against pulmonary infections [3]. For example, inflammatory stimuli can enhance NO release via the up-regulation of the inducible form of NOS (iNOS orNOS2) within inflammatory macrophages [4].

NO is transformed into extremely Reactive Nitrogen Species (RNS) such as NO3– and NO2– within infected macrophages to compel bacterial death. The term, Reactive Oxygen Intermediates (ROI) refers to the reduction products of oxygen and include superoxide (•O2), hydrogen peroxide (H2O2), and the hydroxyl radical (•OH). These reactive products also form reactive conjugates with halides and amines, as well as with NO, giving rise to the production of peroxynitrite (ONOO–) [5] (Figure 1).

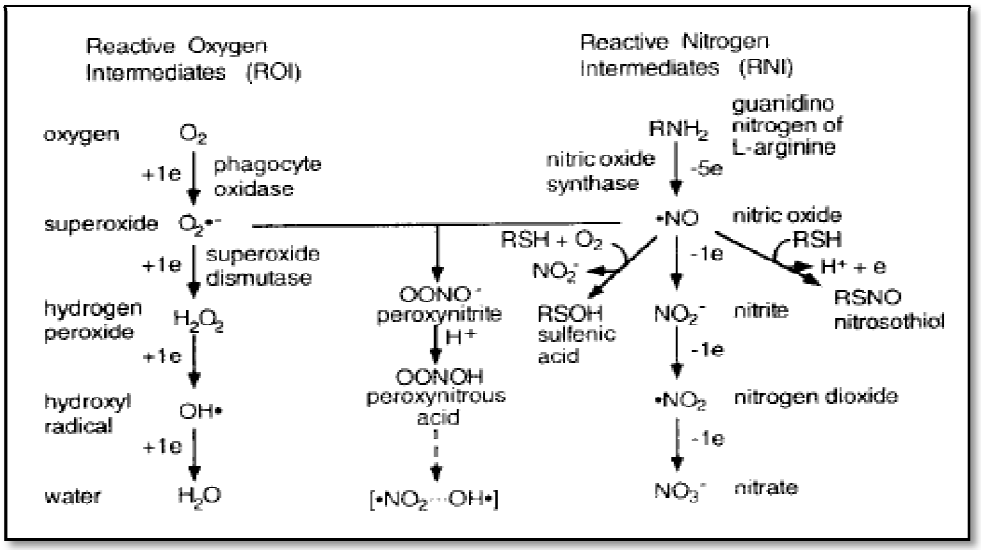

Figure 1: ROI and RNI production in mammalian cells: parallel but connecting paths. Nitroxyl anion (NO2), a one-electron reduction product of nitric oxide (zNO), is unlikely to arise from zNO under physiologic conditions, but is considered by few investigators to be a primary and more toxic product of NOS **[6].** Reaction of RNI with cysteine sulfhydryls can lead either to *S*-nitrosylation or to oxidation to the sulfenic acid, or to disulfide bond formation (not shown), all of which are potentially reversible. Peroxynitrite anion (OONO2) and peroxynitrous acid (OONOH) have distinct patterns of reactivity **[7],** but for ease, the text refers to both as peroxynitrite. OONOH spontaneously decomposes via species resembling the reactive radicals, hydroxyl (OHz) andyor nitrogen dioxide (zNO2). When L-arginine is limiting, NOS can produce superoxide (O2 .) along with zNO, favoring the formation of peroxynitrite **[8].**

To synthesize NO, the NOS enzyme goes through two steps. First step, NOS hydroxylates L-arginine to Nv-hydroxy-L-arginine (which remains largely bound to the enzyme). Second step, NOS oxidizes Nv-hydroxy-L-arginine to L-citrulline and NO [9].

The bactericidal effect of NO in human tissue macrophages may be direct or indirect via RNS [10]. BCG-inoculated alveolar macrophages (AM) from pulmonary fibrosis patients express higher levels of NOS2 protein, mRNA and peroxynitrite [11]. Interferon (IFN)-gamma as well as other inflammatory stimuli increase NO production by stimulating inducible nitric oxide synthase (iNOS). Higher levels of the NO precursor, L-arginine (L-arg) also enhances NO production. NO may act directly or in combination with superoxide (•O2–) to form peroxynitrite (ONOO•), to kill mycobacteria (Mtb) within the phagosome [12]. These data highlight the significant role of NOS2 and of reactive nitrogen intermediates (RNI) in protecting mycobacterium bacilli infection of macrophages [13].

As per the above mentioned ex vivo and in vivo data prominence the significance of NO in TB infection, we review here the mechanisms by which NO regulates TB pathogenesis, the prospective use of NO as a diagnostic of early infection and the future of NO-based therapeutic interventions.

Inducible NOS, when induced in macrophages, produces large amounts of NO, which represent a major cytotoxic principle of those cells [14]. In addition, higher concentrations of NO, as produced by induced macrophages, can directly interfere with the DNA of target cells and cause strand breaks and fragmentation [15]. NO is an endogenous molecule produced at different sites throughout the body [16]. The molecule is chemically active and is efficient against a variety of pathogens including Mtb. Various mechanism are used for killing Mtb in vivo, such as phagosomelysosome membrane fusion along with granzyme, granulysin, and perforin production and acidification of the phagosomes [17]. These, together with ROI-mediated antimicrobial mechanisms, facilitate in killing Mtb. The exact role of ROI in Mtb killing is difficult to accurately distinguish as peroxynitrite is unsuccessful in rodents and different strains of Mtb have differing sensitivity to NO. However, studies in rodent cells may not give accurate insight into human disease as they generally produce greater quantities of NO compared to human cells although this may also relate to the culture conditions used [18]. It is important, therefore, that future studies investigating

the role of NO and ROI in Mtb killing should be performed in primary human AMs in addition to experiments being performed *in vivo* [19].

iNOS broadly regulates the macrophage transcriptome during Mtb infection, activating antimicrobial pathways while also limiting inflammatory cytokine production. The transcription factor hypoxia inducible factor-1a (HIF-1a) was recently shown to be critical for IFN-g–mediated control of Mtb infection. We found that HIF-1a function requires NO production, and that HIF-1a and iNOS are linked by a positive feedback loop that amplifies macrophage. Furthermore, we found that NO inhibits NF-kB activity to prevent hyperinflammatory responses. NO activates vigorous microbicidal programs while also limiting damaging inflammation. IFN-gamma signaling must carefully regulate well-organized immune response to avoid excessive tissue damage, and the study identifies NO as a key player in establishing this balance during Mtb infection [20].

NO prevents Mtb growth and the consequent inflammatory response. NO can also directly modulate inflammation to impact upon Mtb growth and function [21]. The vital role of inflammation in the control of Mtb infection is further established by the ability of thymoquinone (TQ), an essential compound of Nigella sativa (black cumin) [22], to suppress Mtb-induced bacterial replication and inflammation in human and murine macrophage cell lines [23].

In patients with pulmonary tuberculosis, more-severe disease and delayed mycobacterial clearance is observed with impaired pulmonary NO bioavailability. Actions to boost pulmonary NO demand investigation as adjunctive tuberculosis treatments [24].

DIAGNOSTIC ANALYSIS OF NO AND NO METABOLITES

Presence of NO in exhaled breath has been described in literature. Exhaled NO is increased in patients with several lung diseases has been reported in various studies. FeNO values between men and women has significant difference; with a higher level in men (range 2.6–28.8 ppb) compared to women (range 1.6–21.5 ppb) at expiratory flows of 50ml/s. The mechanisms hidden behind this difference may reveal an effect of estrogen on NOS2 expression but more research is required in this field [25]. NO metabolites have been frequently used as an indirect measurement of the production of NO in vivo. The emergence of NOS enzymes or levels of NO in different compartments may also portray a good biomarker for disease. A higher level of NOS2 mRNA expressed in BAL macrophages from patients with Mtb has been linked to higher FeNO levels in the patient [26]. Changes in serum levels of nitrites and nitrates as well as NOS2 activity in blood neutrophils may be another prognostic tool to predict the treatment outcome of TB infection [27].

FACTORS INVOLVED IN NO PRODUCTION

The susceptibility or resistance to in vitro infection with the H37Rvt strain of Mtb depends on the expression of the solute carrier family, gene. The differential capability of resistant and susceptible macrophages to produce NO in response to Mtb is the consequence of this [28].

NOS2 is primarily distributed in newly formed phagosomes following receptor-mediated uptake of latex beads opsonised with either complement products or IgG and not consistently distributed within macrophages [29]. The production of NO by AMs in TB patients may have an auto-regulatory role which, through the activation of the transcription factor nuclear factor (NF)-kB, potentiates the generation of pro-inflammatory cytokines. Significant increase in nitrite levels in advanced TB patients compared with controls observed [30]. The inhibitor of NF-kB, IkBa, confirmed that the IkBa kinase (IKK)–NF-kB signaling pathway enhanced IFN-g- and Mtb lipoarabinomannan-induced NOS2 promoter activity and NO expression [31].

FUTURE OF NO-BASED THERAPY THROUGH GENE THERAPY

The transfer of a specific gene to the host tissue to intrude in a disease process, with resultant mitigation of the symptoms is gene therapy. Dysfunction of Nitric oxide synthase enzyme has been implicated in several types of cardiovascular diseases so the Nitric oxide synthase gene therapy has been the focus of numerous studies. In animal models of vascular tone, ischaemia–reperfusion injury, intimal hyperplasia, and restenosis, gene delivery of NOS isoforms (eNOS, iNOS, or nNOS) has concentrated effects of research. Vascular gene delivery proved to be therapeutically beneficial in many pre-clinical models of cardiovascular disease. Inhibition of intimal hyperplasia and enhanced reendothelialization in injured blood vessels is taken care by Endothelial NOS. The future long-term goal is to decode the benefits of NOS gene therapy seen in animal models into clinical practice. To improve delivery systems and to minimize negative side effects further work is required along this way [32,33].

CONCLUSION

There have been significant increases in our understanding of the mechanisms by which NO can be used in anti-TB therapy. Definitely, NO-donating drugs have therapeutic potential in a number of human diseases including TB [34]. Investigation of the effect of these novel agents and Mtb nitrate reductase inhibitors should be undertaken in primary human cells under physiological conditions.

For rapid readout of drug action it is also important to monitor effectively that sufficient NO is delivered to the target cell within the airway. To monitor drug efficacy and enable variable dosing to minimize any potential side-effect issues measurements of NO are essential**.** Not only treatment but also the potential to cure active and latent tuberculosis polymeric nanoparticles ideally delivered by the inhaled route for pulmonary TB might show promising result and may be improved by structure based design to produce agent(s) for same [35]. Yet, the role of inflammation in Mtb pathophysiology must also be considered when treating patients. Increased understanding of the role of NO in Mtb pathophysiology has provided great insight into many aspects of disease mechanisms and elucidated potential novel NO treatments.

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