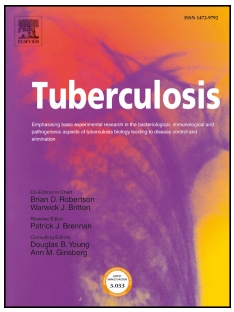
Accepted Manuscript



Reactive Nitrogen and Oxygen Species: Friend or foe in the tuberculosis fight

Jordana Galizia, Marcelo A. Martí

PII: S1472-9792(18)30420-7

DOI: 10.1016/j.tube.2018.10.001

Reference: YTUBE 1762

To appear in: *Tuberculosis*

Received Date: 2 October 2018

Accepted Date: 7 October 2018

Please cite this article as: Galizia J, Martí MA, Reactive Nitrogen and Oxygen Species: Friend or foe in the tuberculosis fight, *Tuberculosis* (2018), doi: https://doi.org/10.1016/j.tube.2018.10.001.

Reactive Nitrogen and Oxygen Species: friend or foe in the tuberculosis fight.

Jordana Galizia & Marcelo A. Martí

Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina
Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina

To whom correspondence should be sent: marti.marcelo@gmail.com

We have read with great joy the letter to the editor, written by Yew *et al*., which analyzes and compares several recent articles [1–4] showing the intimate relationship between *Mycobacterium tuberculosis*' (*Mtb*) redox status - mainly due to the presence of Reactive Nitrogen and Oxygen Species (RNOS) - and tubercular drug efficacy. Given the fact that tuberculosis (TB) is an infectious disease for which there is a limited arsenal of effective drugs, it is crucial to find new drugs or to somehow enhance pre-existing treatments. In this context, it is of great relevance to understand: how the redox status of mycobacteria can be modulated using small molecules, how this modulation impacts in both the bactericidal/static effect of available drugs used in TB treatment and how the presence of RNOS affects mycobacteria (eventually killing them) and relates to induction and escape of *Mtb* dormancy. The picture is however more complicated, since also the host's redox status needs to be considered and has recently been shown to be a key determinant of TB treatment outcome [5,6]. Therefore, and as it will be presented briefly below, the question of whether RNOS presence is positive or negative in the context of TB treatment is still a matter of intense debate and research.

On one hand, nitrosative stress generated by RNOS, activates the expression of the DOS (Dormancy System) regulon. This is a two component system whose sensors are the DosS and DosT heme-containing histidine kinases that modulate DosR cognate receptor activity. However, which is the precise signal detected by the sensors is still an open question [7,8] and new stress responses and defense systems are continuously discovered [9]. The DOS regulon is responsible for the shift of *Mtb*, from aerobic to anaerobic state, survival of the cells during hypoxia-induced dormancy and reactivation of the replicative state upon re exposure to oxygen [10]. On the other hand, there is plenty of evidence that RNOS present antimicrobial activity within many species such as *B. subtilis*, *E. coli* [11], *S. aereus* [12] and even *M. tuberculosis* [13]. More specifically, recently our group showed

how HNO (as well as NO) exerts a mycobactericidal effect [1] even when it is applied in successive sub-inhibitory doses.

Another important but still poorly understood issue related to RNOS and *Mtb*, are the molecular mechanisms underlying the observed effects. From a biochemical point of view, the molecular mechanisms of *Mtb*'s RNOS defense mediated by key proteins such as superoxide dismutase, catalase, alkylhydroperoxide reductases and the truncated hemoglobin N, among others, are well established [8]. However, the molecular targets that are responsible for the mycobactericidal/static effect of RNOS are still unknown. Most importantly, there is no consensus if RNOS kill bacteria due to nonspecific and widespread damage to DNA, lipids and/or proteins or due to the inhibition of specific biological targets.

Finally, two important facts hamper the interpretation of the observed effects in terms of their underlying molecular mechanisms. The first, concerns bacterial population diversity. Apparently opposing observations could be explained by the presence of persisters. Unlike genotypically resistant mutants, these cells are phenotypically drug and possible stress tolerant. Persisters are in a dormant state, as indicated by their gene expression profile and low translation levels [14]. However upon regrowth, persisters reestablish a population that conserves the same antibiotic susceptibility as the original population [15]. As noted by Yew *et al*., RNOS are key candidates to modulate the presence and shift between growing and persistent bacteria, and this relationship is expected to be an intense area of future research.

The second issue, usually overlooked, is related to the fact that RNOS are elusive interconvertible species and determining which is the main RNOS present in a given experimental condition is not that simple. In particular, recent findings show that while it is generally assumed that the presence of nitric oxide (NO) leads to oxidative damage, in a reductive environment (due to, for example, the presence of thiols or aromatic alcohols), NO is readily converted to nitroxyl (HNO) thus potentially leading to different biological effects [16,17]. In this context, in order to define whether the presence of specific RNOS is good or bad for TB treatment, further research into the specific molecular targets and effects of each RNOS in a controlled biological environment is needed.

References

[1] J. Galizia, M.P. Acosta, E. Urdániz, M.A. Martí, Evaluation of nitroxyl donors' effect on mycobacteria, Tuberculosis. 109 (2018) 35–40. doi:10.1016/j.tube.2018.01.006.

[2] D.A. Lamprecht, P.M. Finin, A. Rahman, B.M. Cumming, S.L. Russell, S.R. Jonnala, J.H. Adamson, A.J.C. Steyn, Turning the respiratory flexibility of Mycobacterium tuberculosis against itself, Nat. Commun. 7 (2016) 1–14. doi:10.1038/ncomms12393.

[3] C. Vilchèze, T. Hartman, B. Weinrick, P. Jain, T.R. Weisbrod, L.W. Leung, Enhanced respiration prevents drug tolerance and drug resistance in Mycobacterium tuberculosis, PNAS. 114 (2017) 4495–4500. doi:10.1073/pnas.1704376114.

[4] K. Sikri, P. Duggal, C. Kumar, S. Dhingra, A. Vashist, Redox Biology Multifaceted remodeling by vitamin C boosts sensitivity of Mycobacterium tuberculosis subpopulations to combination treatment by anti-tubercular drugs, Redox Biol. 15 (2018) 452–466. doi:10.1016/j.redox.2017.12.020.

[5] W.W. Yew, D.P. Chan, A. Singhal, Y. Zhang, S.S. Lee, Does oxidative stress contribute to adverse outcomes in HIV-associated TB?, J. Antimicrob. Chemother. 73 (2018) 1117–1120. doi:10.1093/jac/dkx509.

[6] B.B. Mishra, R.R. Lovewell, A.J. Olive, G. Zhang, W. Wang, E. Eugenin, C.M. Smith, J.Y. Phuah, J.E. Long, M.L. Dubuke, S.G. Palace, J.D. Goguen, R.E. Baker, S. Nambi, R. Mishra, M.G. Booty, C.E. Baer, S.A. Shaffer, V. Dartois, B.A. McCormick, X. Chen, C.M. Sassetti, Nitric oxide prevents a pathogen-permissive granulocytic inflammation during tuberculosis, Nat. Microbiol. 2 (2017) 1–11. doi:10.1038/nmicrobiol.2017.72.

[7] A. Kumar, J.S. Deshane, D.K. Crossman, S. Bolisetty, B. Yan, I. Kramnik, A. Agarwal, A.J.C. Steyn, Heme Oxygenase-1-derived Carbon Monoxide Induces the Mycobacterium tuberculosis Dormancy Regulon, J. Biol. Chem. 283 (2008) 18032–18039. doi:10.1074/jbc.M802274200.

[8] B.M. Cumming, D.A. Lamprecht, R.M. Wells, V. Saini, J.H. Mazorodze, A.J.C. Steyn, The Physiology and Genetics of Oxidative Stress in Mycobacteria, Microbiol Spectr. (2014) 1–22. doi:10.1128/microbiolspec.MGM2-0019-2013.Correspondence.

[9] S. Nambi, J.E. Long, B.B. Mishra, R. Baker, K.C. Murphy, A.J. Olive, H.P. Nguyen, S.A. Shaffer, C.M. Sassetti, The Oxidative Stress Network of Mycobacterium tuberculosis Reveals Coordination between Radical Detoxification Systems, Cell Host Microbe. 17 (2015) 829–837. doi:10.1016/j.chom.2015.05.008.

[10] R.L. Leistikow, R.A. Morton, I.L. Bartek, I. Frimpong, K. Wagner, M.I. Voskuil, The Mycobacterium tuberculosis DosR Regulon Assists in Metabolic Homeostasis and Enables Rapid Recovery from Nonrespiring Dormancy, J. Bacteriol. 192 (2010) 1662–1670. doi:10.1128/JB.00926-09.

[11] R. Yadav, S. Goldstein, M.O. Nasef, W. Lee, U. Samuni, Synergistic activity of acetohydroxamic acid on prokaryotes under oxidative stress: The role of reactive nitrogen species, Free Radic. Biol. Med. 77 (2014) 291–297. doi:10.1016/j.freeradbiomed.2014.09.020.

[12] L.S. Nobre, L.M. Saraiva, Effect of combined oxidative and nitrosative stresses on Staphylococcus aureus transcriptome, Appl. Microbiol. Biotechnol. 97 (2013) 2563–2573. doi:10.1007/s00253-013-

4730-3.

[13] T.R. Garbe, N.S. Hibler, V. Deretic, Response of Mycobacterium tuberculosis to reactive oxygen and nitrogen intermediates., Mol. Med. 2 (1996) 134–142.

[14] H.L. Torrey, I. Keren, L.E. Via, J.S. Lee, K. Lewis, High Persister Mutants in Mycobacterium tuberculosis, PLoS One. (2016) 1–28. doi:10.1371/journal.pone.0155127.

[15] N.Q. Balaban, J. Merrin, R. Chait, L. Kowalik, S. Leibler, Bacterial persistence as a phenotypic switch., Science (80-.). 305 (2004) 1622–1625. doi:10.1126/science.1099390.

[16] S.A. Suarez, M. Muñoz, L. Alvarez, M.F. Venâncio, W.R. Rocha, D.E. Bikiel, M.A. Marti, F. Doctorovich, HNO Is Produced by the Reaction of NO with Thiols, J. Am. Chem. Soc. 139 (2017) 14483–14487. doi:10.1021/jacs.7b06968.

[17] M. Hamer, S.A. Suarez, N.I. Neuman, L. Alvarez, M. Muñoz, M.A. Marti, F. Doctorovich, Discussing Endogenous NO$^{\bullet}$/HNO Interconversion Aided by Phenolic Drugs and Vitamins, Inorg. Chem. 54 (2015) 9342–9350. doi:10.1021/acs.inorgchem.5b01347.