Published in final edited form as:

Neuromolecular Med. 2016 September ; 18(3): 483–486. doi:10.1007/s12017-016-8402-1.

Does Concurrent Use of Some Botanicals Interfere with Treatment of Tuberculosis?

William R. Folk^{1, #}, Aaron Smith, Hailong Song^{2,3}, Dennis Chuang^{2,3}, Jianlin Cheng⁴, Zezong Gu^{2,3}, and Grace Sun^{1,2,3}

¹Department of Biochemistry, University of Missouri, Columbia MO 65211

²Department of Pathology and Anatomical Sciences, University of Missouri, Columbia MO 65211

³Center for Translational Neurosciences, University of Missouri, Columbia MO 65211

⁴Department of Computer Science, University of Missouri, Columbia MO 65211

Abstract

Millions of individuals with active TB do not receive recommended treatments, and instead may use botanicals, or use botanicals concurrently with established treatments. Many botanicals protect against oxidative stress, but this can interfere with redox-dependent activation of isoniazid and other prodrugs used for prophylaxis and treatment of TB, as suggested by results of a recent clinical trial of the South African botanical *Sutherlandia frutescens* (L.) R. Br. (Sutherlandia). Here we provide a brief summary of Sutherlandia's effects upon rodent microglia and neurons relevant to tuberculosis of the central nervous system (CNS-TB). We have observed that ethanolic extracts of Sutherlandia suppress production of reactive oxygen species (ROS) in rat primary cortical neurons stimulated by NMDA, and also suppress LPS- and interferon γ (IFN γ)-induced ROS and nitric oxide (NO) production by microglial cells. Sutherlandia consumption mitigates microglial activation in the hippocampus and striatum of ischemic brains of mice. RNAseq analysis indicates that Sutherlandia suppresses gene expression of oxidative stress, inflammatory signaling and toll-like receptor pathways that can reduce the host's immune response to infection and reactivation of latent *Mycobacterium tuberculosis*. As a precautionary measure, we recommend that individuals receiving isoniazid for pulmonary or cerebral TB, be advised not to concurrently use botanicals or dietary supplements having antioxidant activity.

Keywords

Tuberculosis; Botanicals; Sutherlandia; Isoniazid; ROS; Nitric Oxide; Antioxidant

In 2014, approximately 9.6 million people had active TB, which killed 1.5 million people and ranked alongside HIV as a global leading cause of death by infectious agents. Current first line treatment regimens for TB rely upon isonicotinic acid hydrazide (isoniazid), which can be accompanied by hepato- and neuro-toxicities that negatively impact patient adherence and, ultimately, outcomes. Poor adherence to recommended treatments or use of

[#]Corresponding author: folkw@missouri.edu; tel: 1-573-884-2921; fax: 1-573-882-5635.

inappropriate treatments may lead to multiple and extreme drug resistant (MDR/XDR) TB, whose prevalence is increasing (WHO 2015). Approximately a third of those individuals with active TB do not receive recommended treatments and instead, may use botanicals, and many more may use botanicals concurrently with established treatments to help address side effects and to improve health. This is especially true of individuals who are resource-limited and/or have strong attachments for traditional practices. (WHO 2013)

isoniazid

While there are claims that some botanicals may interfere with TB infection/activation or accentuate proven treatments, a recent placebo-controlled randomized clinical trial (RCT) (Wilson, Goggin et al. 2015) of *Sutherlandia*, a botanical widely used in southern Africa for treatment of symptoms of HIV and TB (van Wyk and Albrecht 2008; Davids, Blouws et al. 2014; Sutherlandia.org 2016) has provided unsettling evidence for interference with isoniazid preventive therapy (IPT), a first line regimen used worldwide for prevention and treatment of TB.(WHO 2015) In this RCT, the mean and total burden of infection (BoI; defined as infection-related events in each participant) increased in the *Sutherlandia* (active) arm, where two cases of active pulmonary TB occurred in subjects, despite their receiving IPT(Wilson, Goggin et al. 2015). Additional research is critically needed to determine whether *Sutherlandia* and other botanicals interfere with isoniazid activation in treatment of pulmonary TB and of central nervous system (CNS) TB. Also, other drugs used for treatment of TB (ethionamide, bicyclic nitroimidazoles such as PA-824) require metabolic activation (Baulard, Betts et al. 2000; Singh, Manjunatha et al. 2008) and could be susceptible to interference by botanicals, as might be other infections treated with drugs susceptible to oxidants.

Isoniazid is peroxidatively activated by the *Mycobacterium tuberculosis* KatG catalase-peroxidase so as to form adducts with host-derived NAD⁺ and NADP⁺ that, together with other reactants, inhibit mycolic acid biosynthesis and other pathways essential for bacterial growth.(Timmins and Deretic 2006; Vilcheze and Jacobs 2007) Isoniazid activation also generates nitric oxide (NO)(Timmins, Master et al. 2004; Timmins, Master et al. 2004) which, together with NO generated by cellular metabolism, has been strongly implicated in latency and killing of *M. tuberculosis*. (Singh, Manjunatha et al. 2008; Yang, Yuk et al. 2009; Kumar, Farhana et al. 2011; Voskuil, Bartek et al. 2011; Mishra, Rathinam et al. 2013)

ROS and reactive nitrogen species (RNS) are important for the host immune and inflammatory responses. Chronic inflammatory responses mediated by activated microglial cells in the CNS have important roles in the pathogenesis of numerous neurodegenerative diseases, including Alzheimer's Disease and Parkinson's Disease. (Rogers, Mastroeni et al. 2007; Qian and Flood 2008; Calabrese, Cornelius et al. 2009; Tsang and Chung 2009; Streit 2010) Various inflammatory mediators and cytotoxic molecules, such as IL-1 α , IL-6, TNF- α , ROS and RNS released by activated microglial cells in response to infection, injury, or endotoxins may trigger neuronal damage and cell death. (Block, Zecca et al. 2007; Pacher, Beckman et al. 2007; Yang, Lee et al. 2007; Olin, Armien et al. 2008; Rock, Olin et al. 2008; Brown and Neher 2010; Graeber and Streit 2010; Saenz, Hernandez-Pando et al. 2013; Isabel and Rogelio 2014; Francisco, Hsu et al. 2015)

Sutherlandia, like many botanicals, contains significant antioxidant and free radical scavenging activities that protect cells against oxidative stress by scavenging ROS and RNS and by preserving intracellular reduced glutathione (GSH/GSSG) ratios (Koleva et al, 2002; Fernandes et al, 2004; Katerer and Eloff, 2005, Tobwala et al, 2014). Additionally, botanicals modulate cellular production of ROS and RNS, and as detailed in this issue of NeuroMolecular Medicine and elsewhere, these properties are generally viewed as being neuroprotective. However, the concern that botanicals might interfere with current treatment regimens of TB that utilize isoniazid and other prodrugs requiring activation by ROS and RNS has not previously been considered. Here we provide a brief summary of relevant recent and ongoing studies focusing upon Sutherlandia's effects upon aspects of rodent microglia and neurons relevant to CNS infection by *M. tuberculosis*.

Haematogenous dissemination of *M. tuberculosis* from primary pulmonary infection and the formation of small foci in the CNS are clinically significant, especially in high TB prevalence countries where CNS-TB is one of the top ten causes of death in children. (Rock, Olin et al. 2008; Thwaites and Schoeman 2009; Chin and Mateen 2013) In some individuals, foci rupture and release bacteria into the subarachnoid space causing meningitis; in other individuals, foci enlarge to form tuberculomas without meningitis. The timing and frequency of these events in relation to primary pulmonary infection is dependent upon age, immune status and other factors. (Rock, Olin et al. 2008; Thwaites and Schoeman 2009; Chin and Mateen 2013) With cerebral TB, microglia, neurons and oligodendrocytes are targeted by the invading bacilli (Randall, Hsu et al. 2015) and consequent tissue damage and neurodegeneration is caused by infection and excessive cellular mediated immune response and inflammation. (Rock, Olin et al. 2008; Saenz, Hernandez-Pando et al. 2013; Isabel and Rogelio 2014)

We have observed that ethanolic extracts of Sutherlandia suppress production of ROS in rat primary cortical neurons stimulated by NMDA, and also, these extracts suppress LPS- and interferon γ (IFN γ)-induced ROS and NO production by microglial cells. Its action on microglial cells appears to be mediated through inhibition of p-ERK1/2 and NF-kB signaling pathways. (Jiang, Chuang et al. 2014) Also, Sutherlandia consumption mitigates microglial activation in the hippocampus and striatum of ischemic brains of mice, and is accompanied by reduced expression of p47phox, a subunit of NADPH oxidase, and p-ERK1/2. (Chuang, Cui et al. 2014) It is possible that these anti-oxidative and anti-

inflammatory effects of *Sutherlandia*, while generally protective of uninfected cells, reduce the host's immune response to infection by *M. tuberculosis* and promote reactivation of latent *M. tuberculosis*. Additionally, recent studies by others with a human cell culture model of the blood brain barrier (BBB) suggests that *Sutherlandia* extracts exacerbate HIV-associated neuroinflammation and promote monocyte migration across the BBB (Africa and Smith 2015); this might increase the dissemination of bacilli into the CNS of HIV infected individuals who are especially vulnerable to TB.

Recent analyses of gene expression of non-CNS cells infected by *M. tuberculosis* or stimulated by elicitors provide important insights about genes and pathways important for *M. tuberculosis* replication and latency. Furthermore, experimentally verified intra-species and inter-species protein interactions have been used to predict human-*M. tuberculosis* interactions, that were further filtered using genes known to be differentially expressed during infection; and host proteins and pathways involved in apoptosis and NO production were found to be especially highly represented. (Rapanoel, Mazandu et al. 2013) To begin to relate these seminal findings to effects of *Sutherlandia* upon gene expression of infected cells, we have analyzed patterns of gene expression of rodent microglial cells alone, or stimulated with LPS + IFN γ , together with ethanolic extracts of *Sutherlandia*. (Sun et al, unpublished) Total RNA was isolated, purified and used to generate sequencing libraries, and sequenced and analyzed by RNAseq analyses as described by (Lu, Starkey et al. 2015). Detailed assessment of these experiments will be reported separately, but using Ingenuity® Pathway Analysis software, we compared experimental groups through canonical signaling pathways and through genes grouped to common diseases and functions. One pathway of particular interest was the “*Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses*”, which exhibited significant activation by LPS+IFN γ treatment, but this activation was suppressed by concurrent exposure to *Sutherlandia* extracts. More generally, *Sutherlandia* extract inhibited gene expression of activated leukocytes and lymphocytes, regardless of stimulus. We observed significant reduction of expression of NFkB and iNOS gene subunits and genes regulated by pERK and pERK1/2, and genes for certain inflammatory cytokines and proteins related to toll-like receptors, such as interferon β 1 (IFNB1), chemokine ligand 5 (CCL5), interleukin 6, 15 and 27 (IL6, IL15, IL27), dual specificity phosphatase 1 (DUSP1), inducible NO synthase (NOS2), suppressor of cytokine signaling (SOCS1), CD69 and CD80 antigens (CD69, CD80), and prostaglandin-endoperoxide synthase 2 (PTGS2). Several of these results concur with the observations of protein expression in microglia and neurons described in the previous section, and others remain to be confirmed by analysis of protein expression.

Together, these observations indicate that *Sutherlandia*, in addition to quenching ROS and RNS, modulate gene expression of a variety of immune-function genes that may predispose to *M. tuberculosis* infection, reactivation from latency and the consequent inflammatory responses. Clearly, these preliminary findings require confirmation and extension in *M. tuberculosis* infected cells and animals, so as to fully understand the potential for *Sutherlandia* and other botanicals to interfere with treatment of TB in humans by isoniazid and other prodrugs requiring metabolic activation, and whose therapeutic activities include production of RNS. However, as a precautionary measure, we recommend that individuals receiving IPT and other drugs requiring metabolic activation for pulmonary TB and

isoniazid treatment for cerebral TB, be advised not to concurrently use botanicals or dietary supplements having antioxidant activity. Further research of infections of the nervous system (John, Carabin et al. 2015) should include studies of the use of botanicals and dietary supplements by the public.

Acknowledgments

We thank members of the MU Center for Botanical Interaction Studies for advice and technical assistance. Financial support was provided by grant P50AT006273 from the National Center for Complementary and Integrative Health (NCCIH) and the Office of Dietary Supplements (ODS) and the University of Missouri. The contents are solely the responsibility of the authors and do not necessarily reflect the views of the sponsors.

References

- Africa LD, Smith C. *Sutherlandia frutescens* may exacerbate HIV-associated neuroinflammation. *J Negat Results Biomed.* 2015; 14:14. [PubMed: 26187042]
- Baulard AR, Betts JC, et al. Activation of the pro-drug ethionamide is regulated in mycobacteria. *J Biol Chem.* 2000; 275(36):28326–28331. [PubMed: 10869356]
- Block ML, Zecca L, et al. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci.* 2007; 8(1):57–69. [PubMed: 17180163]
- Brown GC, Neher JJ. Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. *Mol Neurobiol.* 2010; 41(2-3):242–247. [PubMed: 20195798]
- Calabrese V, Cornelius C, et al. Nitric oxide in cell survival: a janus molecule. *Antioxid Redox Signal.* 2009; 11(11):2717–2739. [PubMed: 19558211]
- Chin JH, Mateen FJ. Central Nervous System Tuberculosis: Challenges and Advances in Diagnosis and Treatment. *Curr Infect Dis Rep.* 2013
- Chuang DY, Cui J, et al. Dietary *Sutherlandia* and elderberry mitigate cerebral ischemia-induced neuronal damage and attenuate p47phox and phospho-ERK1/2 expression in microglial cells. *ASN Neuro.* 2014; 6(6)
- Davids D, Blouws T, et al. Traditional health practitioners' perceptions, herbal treatment and management of HIV and related opportunistic infections. *Journal of Ethnobiology and Ethnomedicine.* 2014; 10(1):1–14. [PubMed: 24398225]
- Francisco NM, Hsu NJ, et al. TNF-dependent regulation and activation of innate immune cells are essential for host protection against cerebral tuberculosis. *J Neuroinflammation.* 2015; 12:125. [PubMed: 26112704]
- Graeber MB, Streit WJ. Microglia: biology and pathology. *Acta Neuropathol.* 2010; 119(1):89–105. [PubMed: 20012873]
- Isabel BE, Rogelio HP. Pathogenesis and immune response in tuberculous meningitis. *Malays J Med Sci.* 2014; 21(1):4–10. [PubMed: 24639606]
- Jiang J, Chuang DY, et al. *Sutherlandia frutescens* ethanol extracts inhibit oxidative stress and inflammatory responses in neurons and microglial cells. *PLoS One.* 2014; 9(2):e89748. [PubMed: 24587007]
- John CC, Carabin H, et al. Global research priorities for infections that affect the nervous system. *Nature.* 2015; 527(7578):S178–186. [PubMed: 26580325]
- Kumar A, Farhana A, et al. Redox homeostasis in mycobacteria: the key to tuberculosis control? *Expert Rev Mol Med.* 2011; 13:e39. [PubMed: 22172201]
- Lu Y, Starkey N, et al. Inhibition of Hedgehog-Signaling Driven Genes in Prostate Cancer Cells by *Sutherlandia frutescens* Extract. *PLoS One.* 2015; 10(12):e0145507. [PubMed: 26710108]
- Mishra BB, Rathinam VA, et al. Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1 β . *Nat Immunol.* 2013; 14(1): 52–60. [PubMed: 23160153]
- Olin MR, Armien AG, et al. Role of nitric oxide in defense of the central nervous system against *Mycobacterium tuberculosis*. *J Infect Dis.* 2008; 198(6):886–889. [PubMed: 18627273]

- Pacher P, Beckman JS, et al. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev*. 2007; 87(1):315–424. [PubMed: 17237348]
- Qian L, Flood PM. Microglial cells and Parkinson's disease. *Immunol Res*. 2008; 41(3):155–164. [PubMed: 18512160]
- Randall PJ, Hsu NJ, et al. Mycobacterium tuberculosis infection of the 'non-classical immune cell'. *Immunol Cell Biol*. 2015; 93(9):789–795. [PubMed: 25801479]
- Rapanoel HA, Mazandu GK, et al. Predicting and analyzing interactions between Mycobacterium tuberculosis and its human host. *PLoS One*. 2013; 8(7):e67472. [PubMed: 23844013]
- Rock RB, Olin M, et al. Central nervous system tuberculosis: pathogenesis and clinical aspects. *Clin Microbiol Rev*. 2008; 21(2):243–261. table of contents. [PubMed: 18400795]
- Rogers J, Mastroeni D, et al. Neuroinflammation in Alzheimer's disease and Parkinson's disease: are microglia pathogenic in either disorder? *Int Rev Neurobiol*. 2007; 82:235–246. [PubMed: 17678964]
- Saenz B, Hernandez-Pando R, et al. The dual face of central nervous system tuberculosis: a new Janus Bifrons? *Tuberculosis (Edinb)*. 2013; 93(2):130–135. [PubMed: 23305698]
- Singh R, Manjunatha U, et al. PA-824 kills nonreplicating Mycobacterium tuberculosis by intracellular NO release. *Science*. 2008; 322(5906):1392–1395. [PubMed: 19039139]
- Streit WJ. Microglial activation and neuroinflammation in Alzheimer's disease: a critical examination of recent history. *Front Aging Neurosci*. 2010; 2:22. [PubMed: 20577641]
- Sutherlandia.org. Sutherlandia.org. 2016. from <http://sutherlandia.org/index.html>
- Thwaites GE, Schoeman JF. Update on tuberculosis of the central nervous system: pathogenesis, diagnosis, and treatment. *Clin Chest Med*. 2009; 30(4):745–754, ix. [PubMed: 19925964]
- Timmins GS, Deretic V. Mechanisms of action of isoniazid. *Mol Microbiol*. 2006; 62(5):1220–1227. [PubMed: 17074073]
- Timmins GS, Master S, et al. Nitric oxide generated from isoniazid activation by KatG: source of nitric oxide and activity against Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2004; 48(8):3006–3009. [PubMed: 15273113]
- Timmins GS, Master S, et al. Requirements for nitric oxide generation from isoniazid activation in vitro and inhibition of mycobacterial respiration in vivo. *J Bacteriol*. 2004; 186(16):5427–5431. [PubMed: 15292144]
- Tsang AH, Chung KK. Oxidative and nitrosative stress in Parkinson's disease. *Biochim Biophys Acta*. 2009; 1792(7):643–650. [PubMed: 19162179]
- van Wyk BE, Albrecht C. A review of the taxonomy, ethnobotany, chemistry and pharmacology of *Sutherlandia frutescens* (Fabaceae). *J Ethnopharmacol*. 2008; 119(3):620–629. [PubMed: 18761068]
- Vilcheze C, Jacobs WR Jr. The mechanism of isoniazid killing: clarity through the scope of genetics. *Annu Rev Microbiol*. 2007; 61:35–50. [PubMed: 18035606]
- Voskuil MI, Bartek IL, et al. The response of mycobacterium tuberculosis to reactive oxygen and nitrogen species. *Front Microbiol*. 2011; 2:105. [PubMed: 21734908]
- WHO. Traditional Medicine Strategy 2014–2023. Geneva: World Health Organization; 2013.
- WHO. Recommendation on 36 Months Isoniazid Preventive Therapy to Adults and Adolescents Living with HIV in Resource-Constrained and High TB- and HIV-Prevalence Settings: 2015 Update. Geneva: 2015.
- WHO. Global Tuberculosis Report 2015. Geneva: World Health Organization; 2015.
- Wilson D, Goggin K, et al. Consumption of *Sutherlandia frutescens* by HIV-Seropositive South African Adults: An Adaptive Double-Blind Randomized Placebo Controlled Trial. *PLoS One*. 2015; 10(7):e0128522. [PubMed: 26186450]
- Yang CS, Lee HM, et al. Reactive oxygen species and p47phox activation are essential for the Mycobacterium tuberculosis-induced pro-inflammatory response in murine microglia. *J Neuroinflammation*. 2007; 4:27. [PubMed: 18036262]
- Yang CS, Yuk JM, et al. The role of nitric oxide in mycobacterial infections. *Immune Netw*. 2009; 9(2):46–52. [PubMed: 20107543]