

Nitric Oxide and Staphylococcus aureus Biofilms: Defining their Intricate Relationship in Chronic Rhinosinusitis

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Dedicated to my parents Ted and Teret

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Statements of Authorship

NOTE: Statements of authorship appear in the print copy of the thesis held in the University of Adelaide Library.

Thesis Abstract

This thesis aims to address the relationship of *Staphylococcus aureus* (*S. aureus*) biofilms to the endogenously produced gas nitric oxide (NO) in chronic rhinosinusitis (CRS). While *S. aureus* biofilms are associated with recalcitrance and high severity in CRS, the naturally elevated NO gas is significantly lower in sinuses of CRS patients. However, the relationship of these 3 important factors in CRS aetiopathogenesis is poorly defined. To further clarify this host-microbe-environment (NO) relationship, this thesis first looks into the history of each factor, the roles they play in other disease processes, and the most recent clinical findings and applications in current literature. Building on this foundation, the projects emanating from this thesis hoped to fill in some gaps in knowledge of these 3 components, identifying that all are linked to disease manifestation, and that each can mutually contribute to CRS pathogenesis.

The first project was designed to establish a clearer description of the relationship between NO and *S. aureus* biofilms. Utilizing *S. aureus* strains from CRS patients, these were grown as biofilms and exposed to various NO concentrations mimicking NO levels measured in healthy sinuses vs. CRS patients. We demonstrated the dualistic effects of NO on biofilm growth: increased at lower NO concentrations mimicking diseased sinuses, and anti-biofilm effects at higher concentrations similar to measurements in healthy sinuses. These findings became a stepping stone for the potential design of NO as a therapeutic agent in *S. aureus*-associated CRS.

But first, further characterization of NO's role on the host immune response was needed. The 2nd and 3rd projects aimed to define the host-NO relationship, focusing on the genes involved in NO regulation within the sinonasal mucosa. Because NO is

considered one of the reactive oxygen species (ROS), major players of the innate immune response, genes involved in ROS/innate immunity were investigated. CRS patients, with or without polyps, were sub-classified as either with or without *S. aureus* biofilms, allowing a separate analysis of the role *S. aureus* biofilms play in the alteration of gene expression. The results showed that *S. aureus* biofilm presence associates with a significant difference in the certain gene expressions which have specific roles in NO regulation. This indicates that the microorganism may alter or contribute to an impaired localized innate immune response in the sinuses, or alternatively favor growth in genetically susceptible individuals. Although the cause-effect timeline was not established, these results will serve as baseline for future gene and protein studies that will further increase our understanding of the NO-CRS pathophysiology.

Lastly, building on the therapeutic potential of NO as an anti-biofilm agent, we aimed to design a suitable NO-based topical agent against *S. aureus* biofilms. The 4th project tested a multitude of liposome-encapsulated NO formulations in-vitro with the best formulation tested for safety and efficacy in a sheep model of rhinosinusitis. These projects were designed with an aim for future clinical trials, to test a novel NO-based topical agent, which can be used as a safe and efficacious topical sinus rinse to benefit CRS patients.

Declarations

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution awarded to Camille Jardeleza. To the best of my knowledge and belief, this work contains no material previously published or written by another person, except where due reference has been made in the text.

I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I acknowledge that copyright of published works contained within this thesis resides with the copyright holders of those works.

Dr. Camille Jardeleza

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Publications arising from this Thesis

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International Forum of Allergy and Rhinology 2011, 1(6): 438-44.

Gene expression differences in nitric oxide and reactive oxygen species regulation point to an altered innate immune response in chronic rhinosinusitis

Jardeleza C, Jones D, Baker L, Miljkovic D, Boase S, Tan NC, Vreugde S, Tan LW, Wormald PJ.
International Forum of Allergy and Rhinology 2013, 3(3): 193-8.

Inflammasome gene expression alterations in *Staphylococcus aureus* biofilm-associated Chronic Rhinosinusitis

Jardeleza C, Miljkovic D, Baker L, Boase S, Tan NC, Koblar SA, Zalewski P, Rischmueller M, Lester S, Drilling A, Jones D, Tan LW, Wormald PJ, Vreugde S.
Rhinology 2013, 51(4): 315-22.

Liposome-encapsulated ISMN: A novel nitric oxide-based therapeutic agent against *Staphylococcus aureus* biofilms

Jardeleza C, Rao S, Thierry B, Gajjar P, Vreugde S, Prestidge C, Wormald PJ.
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Liposome-encapsulated nitric oxide donor: a novel topical treatment for *Staphylococcus aureus* biofilm-associated rhinosinusitis

Jardeleza C, Thierry B, Rao S, Rajiv S, Drilling A, Miljkovic D, Paramasivan S, James C, Vreugde S, Prestidge C, Wormald PJ
Prepared for submission

Awards arising from this Thesis

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Research Day, The Queen Elizabeth Hospital / Basil Hetzel Institute, Adelaide SA, Oct 2010

BASIC RESEARCH SCIENCE AWARD WINNER for best scientific paper

American Rhinologic Society, San Francisco Ca, USA, Sept 2011

RONALD GRISTWOOD MEDAL, Best South Australian ENT Registrar Presentation for Research, Adelaide, SA, Nov 2012

NATIONAL HEALTH AND MEDICAL RESEARCH FOUNDATION, Successful

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Presentations arising from this Thesis

The role of nitric oxide in the pathophysiology of *Staphylococcus aureus* biofilm formation in chronic rhinosinusitis

Basil Hetzel Institute Post Graduate Seminar, Adelaide, July 2010

The role of nitric oxide in the pathophysiology of *Staphylococcus aureus* biofilm formation in chronic rhinosinusitis

The Queen Elizabeth Hospital / Basil Hetzel Institute Research day, Adelaide Oct 2010

The Effects of Nitric Oxide on *Staphylococcus aureus* Biofilm Growth and its Implications in Chronic Rhinosinusitis

ASOHNS, Annual Scientific Meeting, Melbourne, April 2011

The role of nitric oxide on *Staphylococcus aureus* biofilm formation in chronic rhinosinusitis

Basil Hetzel Institute Post Graduate Seminar, Adelaide, May 2011

The Effects of Nitric Oxide on *Staphylococcus aureus* Biofilm Growth and its Implications in Chronic Rhinosinusitis

American Rhinologic Society Annual Scientific Meeting, San Francisco Ca, USA
September 2011

Gene expression differences in nitric oxide regulation point to an altered innate immune response in chronic rhinosinusitis

The Australian Society for Medical Research SA Scientific Conference, Adelaide, June 2012

The role of nitric oxide on *Staphylococcus aureus* biofilm growth in chronic rhinosinusitis

Endoscopic Sinus and Skull Base Surgery Course August 2-4 2012, St. Vincent's Hospital, Sydney NSW, August 2012 - Invited speaker

The Efficacy of Liposome-encapsulated Nitric Oxide on *Staphylococcus aureus* biofilms in Chronic Rhinosinusitis

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Gene expression difference in nitric oxide and reactive oxygen species regulation point to an altered innate immune response in chronic rhinosinusitis

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The Effects of Nitric Oxide on *Staphylococcus aureus* Biofilm Growth and its Implications in Chronic Rhinosinusitis

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Management of the recalcitrant sinus infection

With NC Tan, 15th Advanced FESS course, Adelaide, November 2012 – Invited speaker

Gene expression difference in nitric oxide and reactive oxygen species regulation point to an altered innate immune response in chronic rhinosinusitis

ASOHNS, Annual Scientific Meeting, Perth, March 2013

Liposome-encapsulated Nitric Oxide against *Staphylococcus aureus* biofilms in a rhinosinusitis sheep model

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The Effects of Nitric Oxide on *Staphylococcus aureus* Biofilm Growth and its Implications in Chronic Rhinosinusitis

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Abbreviations Used in the Thesis

AdSA: Adenosine synthase A	Nasal Polyps
AERD: Aspirin Exacerbated Respiratory Disease	CRSwNP: Chronic Rhinosinusitis with Nasal Polyps
AFS: Allergic Fungal Sinusitis	Cyclic GMP: Cyclic Guanosine Monophosphate
AIM2: Absent in Melanoma 2	DAMP: Danger-Associated Molecular Patterns
ASC: Apoptosis-associated Speck-like protein with a CARD	DC: Dendritic Cells
bNOS: Bacterial Nitric Oxide Synthase	DNA: Deoxyribonucleic Acid
B- P-: Biofilm negative Polyp negative	DPPG: Dipalmitoylglycerophosphoglycerol
B- P+: Biofilm negative Polyp positive	dsDNA: Double stranded DNA
B+ P-: Biofilm positive Polyp negative	DUOX1: Dual Oxidase 1
B+ P+: Biofilm positive Polyp positive	EDRF: Endothelium Derived Relaxing Factor
CARD: Caspase Recruitment Domain	EGFR: Epidermal growth factor
CASP5: Caspase-5	eNOS: Endothelial Nitric Oxide Synthase
CAT: Catalase	EPS: Extracellular Polymeric Substance
CCL2: Chemokine Ligand 2	ESS: Endoscopic Sinus Surgery
CCL20: Chemokine Ligand 20	FISH: Fluorescence in situ hybridization
CF: Cystic Fibrosis	GTN: Glyceryl Trinitrate
CFTR: Cystic Fibrosis Transmembrane Conductance Regulator	<i>H. influenzae:</i> <i>Haemophilus influenzae</i>
CLSM: Confocal Scanning Laser Microscopy	HPLC: High Performance Liquid Chromatography
CRS: Chronic Rhinosinusitis	
CRSsNP: Chronic Rhinosinusitis without	

HSP90AA1: Heat shock Protein 90 KDa α	NLR: NOD-like receptors
IE: Infective Endocarditis	NLRP3: NOD-like receptor pyrin domain containing protein
IgE: Immunoglobulin E	NME5: Non-metastatic cell 5
IgG: Immunoglobulin G	NO2: Nitrite
IL-1β: Interleukin 1- β	NO3: Nitrate
IL-18: Interleukin 18	NOD: Nucleotide Oligomerization Domain
IFN-γ: Interferon- γ	nNOS: neuronal Nitric Oxide Synthase
iNOS: Inducible Nitric Oxide Synthase	NO: Nitric Oxide
ISMN: Isosorbide Mononitrate	NOS: Nitric Oxide Synthase
LPS: Lipopolysaccharide	OME: Otitis Media with Effusion
LTA: Lipoteichoic acid	ONOO-: Peroxynitrite
LFNO: Liposomal-formulated nitric oxide donor	OXR1: Oxidoreductase 1
MBEC: Minimum Biofilm Eradication Concentration	<i>P. aeruginosa:</i> <i>Pseudomonas aeruginosa</i>
<i>M. catarrhalis:</i> <i>Moraxella catarrhalis</i>	PALS: Phase analysis light scattering
MLV: Multilamellar vesicles	PAMP: Pathogen-associated molecular patterns
MRSA: Methicillin Resistant <i>Staphylococcus aureus</i>	PANX1: Pannexin 1
N₂O: Nitrous Oxide	PDI: Polydispersity Index
NF$\kappa$$\beta$: Nuclear factor kappa beta	PCD: Primary Ciliary Dyskinesia
NADPH: Nicotinamide adenine dinucleotide phosphate	PCR: Polymerase Chain Reaction
NCF2: Neutrophil cytosolic factor 2	PRDX2: Peroxiredoxin 2
	PRDX5: Peroxiredoxin 5
	PRDX6: Peroxiredoxin 6

<p>PRNP: Prion protein</p> <p>PRR: Pattern-recognition receptors</p> <p>PSTPIP1: proline-serine- threonine phosphatase interacting protein 1</p> <p>PYCARD: PYD and CARD Domain containing gene</p> <p>qRT-PCR: Quantitative Real Time PCR</p> <p>RANKL: Receptor activator of nuclear factor kappa-β ligand</p> <p>RAST: Radioallergosorbent test</p> <p>RLH: RNA-sensing RIG-like helicases</p> <p>RNS: Reactive Nitrogen Species</p> <p>ROS: Reactive Oxygen Species</p> <p><i>S. aureus:</i> <i>Staphylococcus aureus</i></p> <p>SCV: Small Colony Variants</p> <p><i>S. pneumoniae:</i> <i>Streptococcus pneumoniae</i></p> <p>TH1: T-Helper 1</p> <p>TH2: T-Helper 2</p> <p>TLR: Toll-Like Receptor</p> <p>TNFSF11: Tumor necrosis factor member 11</p>	<p>ULV: Unilamellar vesicles</p> <p>XIAP: X-linked inhibitor of apoptosis protein</p> <p>ZBP1: Z-DNA-binding protein</p>
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Figure 6.1: Average heart rate (HR) and Mean arterial pressure (MAP) of control vs. treatment sheep at different sinus flushing times.

Figure 6.2: Histologic analysis of the control vs. treatment sheep sinus (LFNO) comparing degree of inflammation, epithelial thickness and acute inflammation.

Figure 6.3: Average biofilm biomass of control vs. treatment sinus within each sheep group showing only the LFNO group with statistical significance.