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Investigation of the Pro-Oxidative and Pro-Inflammatory Interactions of Cobalt, Palladium, Platinum and Vanadium with Human Neutrophils *In Vitro*

by

Heidi Fickl
BSc (UNISA), MSc (Pret)

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For all my children

Thomas and Annette, Stephan and Mikyoung, Nikita, Fettuccini, Katzi, and Ozzie.

Declaration

To my knowledge the work contained in this thesis is original and was undertaken by myself with occasional assistance as indicated in the acknowledgements. The interpretation and analysis of data were also my primary responsibilities.

It is being submitted for the degree of Doctor of Philosophy at the University of Pretoria. It has not been submitted before for any degree or examination at any other university.

Signed: _____

Date : _____

Summary

Heavy metals have been implicated in the increased incidence of cardiopulmonary diseases in industrialised countries. Inhalation of metals and metal-containing compounds is associated with pulmonary inflammation and tissue injury. Mining is South Africa's largest industry sector and occupational asthma, rhinitis, conjunctivitis and eczema are common amongst refinery workers. The research presented in this thesis has been designed to investigate the possible pro-oxidative and pro-inflammatory interactions of cobalt-, palladium-, platinum- and vanadium salts with human neutrophils *in vitro.*

The primary objectives of the study were to investigate: i) the effects of vanadium in the +2, +3, +4, and +5 valence states on hydroxyl radical formation by activated neutrophils (Co^{2+}, Pt^{4+}, and Pd^{2+} had been investigated in previous studies and were found to have no effect) using electron spin resonance spectroscopy); ii) the effects of all of the test metals on spontaneous activation of NF-κB, as well as on activation of cytosolic signalling cascades which converge on NF-κB, and on activation of other transcription factors which cooperate with NF-κB to optimize gene transcription, using an electrophoretic mobility shift assay (NF-κB) and a Bio-Plex® suspension bead phosphoprotein analysis procedures respectively; iii) effects of the metals on synthesis of interleukin-8 (IL-8) using the Bio-Plex® suspension bead system; and iv) effects of the metals on the Ca^{2+}-mobilizing and chemotactic activities of C5a, IL-8, and the pneumococcal toxin, pneumolysin, using spectrofluorimetric and modified Boyden chamber procedures respectively. The most significant findings were as follows:

Exposure of neutrophils to vanadium resulted in formation of hydroxyl radical, one of the most powerful and damaging free radicals in biological systems, whose formation by phagocytes is normally stringently controlled. This may put individuals with pre-existing airway inflammation such as cigarette smokers, asthmatics, and those with chronic obstructive pulmonary disorders at high risk for vanadium toxicity.

None of the metals caused nuclear translocation of NF-κB, activation of related cytosolic signalling cascades, phosphorylation of transcription factors AFT and STAT-3, or synthesis of IL-8.

Palladium was found to attenuate the calcium-mobilizing and chemotactic properties of C5a and Interleukin-8, both essential in the host's innate immunity, as they attract neutrophils to the site of infection to clear the invading micro-organisms by phagocytosis, which may favour colonization with microbial pathogens.

Palladium also inactivates pneumolysin, a key virulence factor of *Streptococcus pneumoniae.* Palladium attenuated the pore-forming interactions of pneumolysin with human neutrophils, attenuating both influx of Ca^{2+} and activation of the NF-κB signalling pathway.

These previously unidentified interactions of vanadium and palladium with key cellular and humoral components of the innate host defence system may contribute to the adverse health effects of exposure to these heavy metals.

Samevatting

Edelmetale word geïmpliseer in die verhoging van kardiopulmenêre siektes in industriële lande. Die inaseming van metale en metaalbevattende stowwe word geassosieer met pulmonêre inflamasie en weefselskade. Die mynbedryf is Suid-Arika se grootse industriële sektor, met beroepsasma, renitis, konjuktivitus en ekseem, wat algemeen onder raffinaderywerkers voorkom. Die navorsing wat in hierdie tesis aangebied word, is ontwerp om die moontlike pro-oksidatiewe en pro-inflammatoriese interaksie van kobalt-, palladium-, en vanadiumsoute met menslike neutrofiele *in vitro*, te ondersoek.

Die primêre fokus van die studie was om die volgende te ondersoek : i) die gevolge van vanadium in die +2, +3, +4 en +5 valensie-vlakke op hidroksiel-radikale formasie deur geaktiveerde neutrofiele (Co^{2+}, Pt^{4+} en Pd^{2+} was ondersoek in vorige studies en het geen effek gehad nie) deur elektron-tol-resonansie spektroskopie te gebruik; ii) die effek van al die toetsmetale op die spontane aktivering van NF-κB, sowel as die aktivering van die sistoliese seinkaskades, wat inwerk op NF-κB en op die aktivering van ander transkripsiefaktore wat saamwerk met NF-κB om geen transkripsie te optimiseer deur gebruik te maak van ‘n elektroforese-mobiliteitsverskuiwings-essay (NF-κB) en ‘n Bio-Plex® korrelsuspensie fosfoproteïen analise onderskeidelik; iii) Effekte van die metale op die sintese van interleukin 8 (IL-8) met behulp van die Bio-Plex® korrelsuspensie-sisteem; en iv) effekte van die metale op die Ca^{2+} mobilisering en chemotaksiese aktiwiteit van C5a, IL-8 en die pneumomokokale toksien, pneumolisien deur spektrofluorometriese en gemodifiseerde Boyden kamer prosedures onderskeidelik. Die mees beduidende bevindings is as volg :

Vanadium bevorder die vorming van hidroksie-radikale, een van die kragtigste en skadelikste vry-radikale in biologiese sisteme, wat se formasie gewoonlik streng beheer word deur fagosiete. Dit plaas individue met bestaande lugweg-inflammasie, soos sigaretrokers, asmatiese persone asook dié met kroniese obstruktiewe pulmonêre siektes teen ‘n hoë risiko vir vanadium toksisiteit.

Geen van die metale het nukleêre translokasie van NF-κB, aktivering van verwante sistoliese sein-kaskades, fosforilisasie van transkripsiefaktore AFT en STAT-3, of sintese van IL-8 veroorsaak nie.

Palladium verswak die Kalsium mobilisering en chemotaksiese eienskappe van K5a en IL-8, wat beide noodsaaklik is in die gasheer se intrinsieke immuniteit, aangesien hul die neutrofiele na die area van infeksie lok en sodoende die invallende mikro-organismes deur fagositose verwyder, wat voordelig kan wees vir kolonisasie deur mikrobiologiese patogene.

Palladium inaktiveer ook pneumolosien, wat 'n belangrike virilente faktor van *Streptococcus pneumoniae* is. Palladium verswak die toksien se vermoë om porieë in die menslike neutrofiel te vorm, dit verswak beide die invloei van Ca^{2+} en die NF-κB seinweg.

Hierdie interaksies van vanadium en palladium met belangrike sellulêre en humorale komponente van die gasheer se intrinsieke beskermingssisteem, wat nog nie voorheen geidentifiseer is nie, mag 'n bydrae lewer tot nadelige gesondheidstoetande met blootstelling aan die swaar metale.

Publications

Parts of this thesis have been published in the following papers:

Fickl H, Cockeran R, Steel HC, Feldman C, Cowan G, Mitchell TJ, Anderson R Pneumolysin-mediated activation of NFκB in human neutrophils is antagonized by docosahexaenoic acid. *Clinical and Experimental Immunology*. 2005; 140: 274-281.

Fickl H, Theron AJ, Grimmer H, Oommen J, Ramafi GJ, Steel HC, Visser SS, Anderson R. Vanadium promotes hydroxyl radical formation by activated human neutrophils. *Free Radical Biology and Medicine.* 2006; 40: 146-155.

Fickl H, Theron AJ, Mitchell TJ, Feldman C, Anderson R. Palladium Attenuates the Pro-Inflammatory Interactions of C5a, Interleukin-8 and Pneumolysin with Human Neutrophils. *Journal of Immunotoxicology*. (manuscript no: J-4-2-8-07 - in press).

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Abbreviations

Akt	alternative name for PKB
AP-1	activator protein-1
APC	antigen presenting cell
ATF	activating transcription factor
ATP	adenosine 5-triphosphate
BAL	bronchoalveolar lavage
BPI	bactericidal/permeability increasing protein
C	complement
CD	cluster of differentiation
CIAP	cellular inhibitors of apoptosis
COX	cyclooxygenase
CRC	colorectal cancer
DAG	diacylglycerol
DHA	docosahexaenoic acid
DISC	death-inducing signalling complex
DMPO	5,5-dimethyl-1-pyrroline *n-oxide*
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPI	diphenylene iodonium chloride
EBV	Epstein-Barr virus
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EGTA	ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid
EMSA	electrophoretic mobility shift assay
ER	endoplasmic reticulum
ERK	extracellular regulated kinase
ESR	electron spin resonance spectroscopy
FADD	fas-associated death domain
FMLP	N-formyl-L-methionyl-L-leucyl-L-phenylalanine
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
gp120	viral envelope glycoprotein

gp350	viral envelope glycoprotein
GSK-3β	glycogen synthase kinase-3β
HA	influenza hemagglutinin
HAGG	heat-aggregated IgG
HeLa	cell line of human cancer cells
HLA	human leukocyte antigen
H_2O_2	hydrogen peroxide
HBSS	Hanks' balanced salt solution
HBV	hepatitis B virus
HCl	hydrochloric acid
HCV	hepatitis C virus
HEK	human embryonic kidney cell line
HIV	human immunodeficiency virus
HOCl	hypochlorous acid
HTLV	human T-cell leukaemia virus
ICAM	intercellular adhesion molecule
IFN	interferon
IKAP	IKK-associated protein
IkB	inhibitory kappa B
IKK	IκB kinase
IL	interleukin
IP_3	inositol-1,4,5-triphosphate
IRAK	IL-1 receptor-associated kinase
IRS	insulin receptor substrate
JNK	C-jun-amino-terminal kinase
LECL	Lucigenin-enhanced chemiluminescence
LPS	lipopolysaccharides
MAPK	mitogen-activated protein kinase
MBL	mannan-binding lectin
MEKK1	mitogen-activated protein kinase/extracellular signal-regulated kinase
MIP-2	macrophage inflammatory protein-2
MPO	myeloperoxidase
MyD88	myeloid differentiation primary response gene
mRNA	messenger ribonucleic acid

NADPH	nicotinamide adenine dinucleotide phosphate, reduced
NEMO	NF-κB essential modulator
NF-κB	nuclear factor kappa B
NIK	NF-κB-inducing kinase
NLS	nuclear localization sequence
O_2	oxygen
OH^-	hydroxyl radical
PAF	platelet-activating factor
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PI3-K	phosphatidylinositol 3-kinase
PKA	protein kinase A
PKB	protein kinase B
PKC	protein kinase C
PKR	double-stranded RNA-dependent protein kinase
PLA_2	phospholipase A_2
Ply	pneumolysin
PMA	phorbol 12- myristate 13-acetate
PMNL	polymorphonuclear leukocytes
PTK	protein-tyrosine kinase
PTP	protein-tyrosine phosphatase
PR3	proteinase3
RAST	radio allergro sorbent test
RHR	Rel homology region
RIP	receptor-interacting protein
RNA	ribonucleic acid
ROS	reactive oxygen species
RRV	rotavirus
SEM	standard error of the mean
SOD	superoxide dismutase
STAT	signal transducer and activator of transcription
TAK1	transforming growth factor-β-activated kinase
Tat	transactivating protein

TCA	trichloracetic acid
TGF	transforming growth factor
TIR	Toll-IL-1receptor homology domain
TLR	Toll-like receptor
TNF	tumour necrosis factor
TNFR	TNF-receptor
TOLLIP	Toll-interacting protein
TRADD	TNFR-associated death domain
TRAF	TNF-receptor-associated factors
TRAIL	TNF-related apoptosis-inducing ligand
VP4	viral capsid protein