Investigation of the Effects of Moxifloxacin on Human Neutrophils and Mononuclear Leucocytes *in vitro*

by

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DECLARATION

I declare that the work contained in this dissertation is my original work and has not been presented for a degree in any other institution. It is being submitted in fulfilment for the MSc degree at the University of Pretoria.

Signed: _____ Date: _____

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SUMMARY

Moxifloxacin is considered to be a broad-spectrum fluoroquinolone due to its activity against both gram positive and gram negative bacteria. Importantly this agent is currently being evaluated in ongoing clinical trials in South Africa and South America as a treatment for pulmonary tuberculosis, with the specific objective of decreasing the duration of chemotherapy. However, relatively little is known about the effects of moxifloxacin on host defenses, particularly innate protective mechanisms, involving neutrophils.

The primary theme of the laboratory research presented in this dissertation was to investigate the role of moxifloxacin in modulating the host immune system, specifically neutrophil protective functions, as well as lymphocyte proliferation and cytokine production (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL13, IL-17, IFN-γ, GM-CSF, G-CSF, TNF-α, and MCP-1).

The generation of reactive oxidants and elastase release by neutrophils activated with the chemoattractant, fMLP, or the phorbol ester, PMA, were assayed using luminol- and lucigenin-enhanced chemiluminescence (LECL) and colorimetric procedures, while alterations in cytosolic Ca^{2+} concentrations were monitored by radiometric ($^{45}Ca^{2+}$) procedures. Moxifloxacin (1-20 μg/ml) was found to have no significant priming or inhibitory effects on oxidant generation by human neutrophils activated with fMLP or PMA, while elastase release was increased at the highest concentrations of the antibiotic. The magnitude of efflux or store-operated Ca^{2+} influx was unaffected following activation of neutrophils with fMLP.

Moxifloxacin at all concentrations tested, did not affect either lymphocyte proliferation or CD25 expression by PHA-activated mononuclear leukocytes (MNLs). Similarly, none of the cytokines measured were significantly affected by moxifloxacin, either in the absence or presence of PHA, compatible with a lack of effect of this agent on Th1 and Th2 lymphocytes.

In conclusion, this study suggests that moxifloxacin, at therapeutic doses, does not affect the protective functions of human neutrophils and lymphocytes.

SAMEVATTING

Moksifloksasin word beskou as ‘n breë spektrum fluoroquinoloon met aktiwiteit teen beide gram positiewe en gram negatiewe bakterieë. Dit is noemenswaardig dat hierdie agent tans in kliniese proewe in Suid Afrika en Suid Amerika getoets word as behandeling vir pulmonêre tuberkulose, met die spesifieke doel om die duur van chemoterapie te verminder. Daar is egter relatief min bekend oor die uitwerking van moksifloksasin op gasheerverdediging, veral intrinsieke beskermende meganismes soos neutrofiele.

Die hooftema van die laboratorium navorsing wat in hierdie verhandeling aangebied word, is om die rol van moksifloksasin in die modulering van die gasheer immuunsisteem te ondersoek veral met betrekking tot neutrofiel beskermende funksies, sowel as limfosiet proliferasie en sitokien produksie (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-17, IFN-γ, GM-CSF, G-CSF,TNF-α and MCP-1).

Die produksie van reaktiewe oksidante en vrystelling van elastase deur neutrofiele, geaktiveer deur die leukolokmiddel, fMLP, of die forbol ester, PMA, is getoets deur gebruik te maak van luminol- en lusigenin-verhoogde chemiluminessensie en kolorimetriese prosedures. Veranderinge in sitosoliese Ca^{2+} konsentrasies is gemeet met behulp van radiometriese ($^{45}Ca^{2+}$) prosedures. Moksifloksasin (1-20μg/ml) het nie ‘n betekenisvolle sensitiserende of inhiberende uitwerking op oksidant generasie van mens neutrofiele geaktiveer met fMLP of PMA gehad nie terwyl elastase vrystelling verhoog is by die hoogste konsentrasies van die antibiotika. Moksifloksasin het ook nie die effluks of stoor-operatiewe Ca^{2+} influks in neutrofiele geaktiveer met fMLP, geaffekteer nie.

Moksifloksasin het by alle konsentrasies getoets, nie limfosiet proliferasie of CD25 uitdrukking deur PHA-geaktiveerde mononukleêre leukosiete,geaffekteer nie. Eweneens is geen van die sitokiene gemeet, betekenisvol geaffekteer deur moksifloksasin in die afwesigheid of teenwoordigheid van PHA nie. Hierdie resultaat toon dat die antibiotika nie ‘n effek op Th1 en Th2 limfosiete het nie.

Ten slotte, dui die studie aan dat mokifloksasin by terapeutiese dosisse geen uitwerking op die produktiewe funksies van mens neutrofiele en T-limfosiete het nie.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Ab	Antibody
Ag	Antigen
ANOVA	Analysis of variance
APCs	Antigen presenting cells
ATP	Adenosine 3', 5-triphosphate
Ca^{2+}	Calcium ion
$[Ca^{2+}]i$	Concentration of intracellular calcium
$^{45}Ca^{2+}$	Calcium-45 chloride
Ca^{2+}-ATPase	Calcium-adenosine 3', 5'-triphosphatase
$CaCl_2$	Calcium chloride
CB	Cytochalasin B
CD	Cluster of differentiation
CG	Cathepsin
CGD	Chronic granulomatous disease
Cl^-	Chloride ion
CSF	Colony stimulating factor
CTL	Cytotoxic T lymphocyte
DMSO	Dimethyl sulphoxide
EGTA	Ethylene glycol-bis (beta-amino-ethyl-ether)-N, N, N', N'-tetraacetic acid
ER	Endoplasmic reticulum
FCS	Fetal calf serum
Fe^{2+}	Ferrous ion
Fe^{3+}	Ferric ion
FITC	Fluorescein isothiocyanate
FMLP	N-formyl-L-methionyl-L-leucyl-L-phenylalanine
GM-CSF	Granulocyte/macrophage colony stimulating factor
GTP	Guanosine triphosphate
H^+	Proton
^3H	Thymidine (tritiated)
HBSS	Hanks' balanced salt solution
HLA	Human histocompatibility leukocyte antigen

H_2O_2	Hydrogen peroxide
HOCL	Hypochlorous acid
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
iNOS	Induced nitric oxide synthase
IP_3	Inositol1, 4,5-triphosphate
IP_3-ICR	IP_3-induced Ca^{2+} release
$iPLA_2$	Ca^{2+}-intended phospholipase A_2
IP_3ROC	IP_3 receptor-operated channel
KDa	kiloDalton
Licigenin	bis-N-methylacridinium nitrate
Luminol	5-amino-2,5-dihydro-1,4-phthalazinedione
LECL	Lucigenin-enhanced chemiluminescence
LPA	Lymphocyte proliferation assay
LPS	Lipopolysaccharide
mAb	Monoclonal antibody
MCP-1	Monocyte chemotactic protein-1
MHC	Major histocompatibility complex
MPO	Myeloperoxidase
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidized form)
NaOH	Sodium hydroxide
NE	Neutrophil elastase
NF-$_k$B	Nuclear transcription factor-kappa B
NH_4Cl	Ammonium chloride
NO	Nitric oxide
NRS	Nucleotide releasing substrate
O_2	Oxygen
O_2^-	Superoxide anion
$^1O_2$	Singlet oxygen
.OH/HO^-	Hydroxyl radical
p22 ^{phox}	Protein/polypeptide phagocyte oxidase, 22kDa molecular weight

PBS	Phosphate-buffer saline
PHA	Phytohaemagglutinin
PMA	Phorbol-12-myristate 13-acetate
PMNL	Polymorphonuclear leukocyte
Rho-GDI	Guanosine nucleotide dissociation inhibitor
RIA	Radioimmunoassay
ROCC	Receptor-operated Ca^{2+} channel
ROI	Reactive oxygen intermediate
ROS	Reactive oxygen species
SEM	Standard error of the mean
SER	Sarco-endoplasmic reticulum
SERCA	Sarco-endoplasmic reticulum Ca^{2+}-ATPase
SNF	Supernatant fluid
SOC	Store operated channel
SOCC	Store-operated calcium channels
SOCE	Store-operated Ca^{2+} entry
SOD	Superoxide dismutase
TCR	T-cell receptor
Th	T helper cell
TNF-α	Tumor necrosis factor alpha
TRPC	Transient receptor potential channel
VGCC	Voltage-gated Ca^{2+} channels CIF - Ca^{2+} influx factor