

**THE HIV-1 Tat PROTEIN AND ADVERSE DRUG REACTIONS:  
A MODEL SYSTEM UTILIZING JURKAT T CELLS AND  
SULPHAMETHOXAZOLE-HYDROXYLAMINE**

(Spine title: HIV-1 Tat AND ADVERSE DRUG REACTIONS)

(Thesis format: Integrated-Article)

by

**Kaothar O. Adeyanju**

Graduate Program in Microbiology and Immunology

A thesis submitted in partial fulfilment  
of the requirements for the degree of  
Doctor of Philosophy

The School of Graduate and Postdoctoral Studies  
The University of Western Ontario  
London, Ontario, Canada

© Kaothar O. Adeyanju 2011

## **Abstract**

In 2009 approximately 2.6 million people became infected with the Human Immunodeficiency Virus (HIV). In addition to the estimated 33.3 million people currently living with the virus, this makes HIV/ AIDS an epidemic of unprecedented scale in modern times. Treatment of HIV infection requires antiretroviral agents as well as a number of other drugs such as antimicrobials. Hypersensitivity adverse drug reactions (ADRs) to a variety of drugs are common in HIV-infected individuals, but the antimicrobial Sulphamethoxazole remains a major culprit. Hypersensitivity ADRs cause significant morbidity, with the skin and liver most commonly affected and are among the top causes of death in the developed world. While the pathophysiology of drug hypersensitivity in general remains incompletely understood, ADRs to Sulphamethoxazole have been linked to one of its reactive metabolites SMX-HA. Previous work from our lab has also shown that the HIV-1 Tat protein plays a role in SMX-induced hypersensitivity ADRs. We sought to determine if altering the amount of Tat would have an effect on cellular toxicity. We also wanted to find out how Tat affects toxicity and what region of the protein mediated those effects.

We created fusion proteins of Tat and its deletion mutants with green fluorescent protein and placed them in an inducible vector which was subsequently used to create stably transfected Jurkat T cell lines. These cell lines were differentially induced for Tat expression and then used in assays for cellular toxicity and oxidative stress in the absence and presence of SMX-HA. We found that cellular toxicity was dependent on the variant of Tat used. In the preliminary report, the first exon of the Tat protein was able to augment T cell death caused by the addition of SMX-HA, and that the cell death occurred

via apoptosis. This cell death took place without alteration to the cellular redox state. In later experiments using a different Tat variant, only the full-length protein affected cell death after SMX-HA treatment. Also, expression of the full-length protein was able to cause an increase in ROS generated after incubation with SMX-HA. None of the deletion mutants had this effect.

To try to further elucidate the effects of HIV-1 Tat on the cellular redox state, a set of experiments were carried out to detect the consequences on thiol proteins of Tat expression in the presence and absence of SMX-HA. Following Tat expression and incubation of the Jurkat cells with either vehicle or SMX-HA, the cells were disrupted in the presence of iodoacetamide and the lysates applied to two-dimensional gel electrophoresis. In the absence of SMX-HA, the Tat-expressing cell lines were already under a fair amount of oxidative stress compared to the parent cell line and the HIV-infected cell line. Also in untreated cells, a small number of protein thiols were already oxidized. Exposure of the Tat-expressing cells to 200 $\mu$ M SMX-HA led to a dramatic increase in thiol protein oxidation.

**Keywords:** HIV-1 Tat, sulphamethoxazole, sulphamethoxazole-hydroxylamine, hypersensitivity adverse drug reactions, Jurkat T cell, redox, thiol, two-dimensional gel electrophoresis.

## **Co-Authorship**

### **Chapter 2: HIV Tat potentiates cell toxicity in a T cell model for Sulphamethoxazole-induced adverse drug reactions.**

Drs. Dekaban and Rieder supervised the project and aided in the preparation of the manuscript. Adriana Krizova is a co-first author having contributed equally to this manuscript. (Figures 2.1A, 2.1B, 2.2A, 2.2B, 2.3, 2.4A and 2.4B).

### **Chapter 3: Cytoplasmic distribution of HIV-1 Tat sensitizes Jurkat T cells to Sulphamethoxazole-hydroxylamine induced toxicity**

Drs. Dekaban and Rieder supervised the project and aided in the preparation of the manuscript. Dr. Bend also aided in the preparation of the manuscript. I was responsible for carrying out all the experiments in this Chapter with technical assistance from Wilfrid Chan and Lindsey Chow.

### **Chapter 4: Detection of oxidant sensitive thiol proteins in HIV-1 Tat-expressing cells by redox two-dimensional electrophoresis**

Drs. Dekaban and Rieder supervised the project and aided in the preparation of the manuscript. Dr. Bend also aided in the preparation of the manuscript. I was responsible for carrying out all the experiments in this Chapter with technical assistance from Kathy Mao.

All manuscripts in this thesis were written by K. Adeyanju and edited based on comments/ contributions from the co-authors.

## **Dedication**

This work is dedicated to my parents who always encouraged me to work hard in pursuit of my goals.

## **Acknowledgements**

I would like to extend my deepest gratitude to Dr. Gregory Dekaban for giving me the opportunity to pursue my graduate studies in his laboratory. Your patience, guidance and encouragement have helped improve my aptitude for hypothesis-driven research and develop my analytical and critical thinking skills. The training I have received here will serve me well in my future endeavours and the friendships forged have made it a truly enriching experience.

I would also like to thank my co-supervisor Dr. Michael Rieder for your guidance and generosity throughout my time in your lab. A gracious thanks to members of my advisory committee Dr. Joaquin Madrenas and Dr. Jack Bend for challenging me during my committee meetings, but also providing insight and valuable discussion.

A special thank you to members of the Dekaban laboratory both past (Carmen Simeirea, Phillipe Gilbert, Andrew Peters, Jin Su, Terlika Pandit, John Barrett, Katherine Bielas, Monty McKillop, Sakina Thawer, Chris Tarola, Lauren DiMenna, Mia Merrill-Segal and Bryan Au) and present (Christy Willert, Sonali de Chickera and Ryan Buensuceso) for their support, friendship and discussions, both scientific and otherwise.

And also to former and current members of the Rieder laboratory especially Dr. Jane Tucker, Nancy Chen, Steven Thompson, Parvaz Madadi, Nada Tabara, Meiyen Lee, Wilfrid Chan, Kathy Mao, Lindsey Chow and Anda Marcu for their assistance and camaraderie throughout my doctoral studies.

## **TABLE of CONTENTS**

CERTIFICATE OF EXAMINATION.....	ii
ABSTRACT.....	iii
CO-AUTHORSHIP.....	v
DEDICATION.....	vi
ACKNOWLEDGEMENTS.....	vii
TABLE OF CONTENTS.....	viii
LIST OF TABLES.....	xii
LIST OF FIGURES.....	xiii
LIST OF ABBREVIATIONS, SYMBOLS, NOMENCLATURE.....	xv

## **CHAPTER 1: Introduction**

1.1	Introduction.....	2
1.2	HIV Genome and Replication.....	3
1.3	Transactivator of Transcription.....	5
1.4	HIV Infection and Pathogenesis.....	14
1.5	HIV and Adverse Drug Reactions.....	15
1.6	Proposed Mechanism of Adverse Drug Reactions.....	19
1.7	Oxidative Stress and Redox Proteomics.....	35
1.8	Research Hypothesis and Objectives.....	44
1.9	References.....	46

**CHAPTER 2: HIV Tat potentiates cell toxicity in a T cell model for Sulphamethoxazole-induced adverse drug reactions**

2.1 Introduction.....	59
2.2 Materials and Methods.....	63
2.2.1 Cell lines.....	63
2.2.2 Confocal microscopy.....	64
2.2.3 Real-time PCR analyses of Tat/ GAPDH mRNA levels.....	64
2.2.4 Immunoblot analysis.....	65
2.2.5 MTT cell viability assay.....	66
2.2.6 Flow Cytometry analysis of GFP expression and apoptosis.....	67
2.2.7 Statistics.....	68
2.3 Results.....	69
2.3.1 Characterization of Doxycycline-inducible cell lines.....	69
2.3.2 Cell viability is further decreased in the presence of Tat following treatment with SMX-HA.....	75
2.3.3 Apoptosis is a mechanism of SMX-HA toxicity.....	78
2.4 Discussion.....	86
2.5 References.....	91

**CHAPTER 3: Cytoplasmic distribution of HIV-1 Tat sensitizes Jurkat T cells to Sulphamethoxazole-hydroxylamine induced toxicity.**

3.1 Introduction.....	96
3.2 Materials and Methods.....	99
3.2.1 Cell lines of HIV-1 Tat and deletion mutants.....	99



3.2.2	Confocal microscopy.....	100
3.2.3	Dose response and time course of doxycycline induction.....	101
3.2.4	Real-time PCR analyses of Tat/GAPDH mRNA levels.....	101
3.2.5	Immunoblot analysis.....	102
3.2.6	MTT cell viability assay.....	102
3.2.6	Detection of Reactive Oxygen Species (ROS).....	103
3.2.7	Statistics.....	104
3.3	Results.....	105
3.3.1	Construction of TatGFP cell lines.....	105
3.3.2	Intracellular localization of TatGFP constructs.....	105
3.3.3	Characterization of the TatGFP fusion constructs.....	109
3.3.4	Quantitative RT-PCR of HIV Tat and TatGFP specific mRNA.....	113
3.3.5	Cell viability of Tat-expressing cell lines after treatment with SMX-HA.....	119
3.3.6	Generation of ROS by Tat-expressing cell lines.....	127
3.4	Discussion.....	132
3.5	References.....	145

**CHAPTER 4: Detection of oxidant sensitive thiol proteins in HIV-1 Tat-expressing cells by redox two-dimensional electrophoresis.**

4.1	Introduction.....	150
4.2	Materials and Methods.....	153
4.2.1	Cell lines.....	153

4.2.2 Purification of polyclonal antibodies from serum of SMX-HA-KLH immunized rabbits.....	154
4.2.3 Slot blots.....	154
4.2.4 Sample preparation for redox 2D gel electrophoresis.....	156
4.2.5 Protein assay.....	156
4.2.6 Non-reducing/reducing two-dimensional SDS/gel electrophoresis.....	157
4.2.7 Silver staining and image analysis of 2D gels.....	157
4.2.8 Statistics.....	158
4.3 Results.....	159
4.3.1 Quantification of SMX-HA-induced haptentation in Tat-expressing Jurkat T cells.....	159
4.3.2 Detection of ROS-sensitive thiol proteins.....	166
4.4 Discussion.....	192
4.5 References.....	209
 <b>CHAPTER 5: Discussion and Conclusion</b>	
5.1 Discussion and Conclusion.....	216
5.2 Future Studies.....	225
5.3 Conclusions.....	227
5.4 References.....	228
 <b>APPENDIX</b> .....	 232
<b>CURRICULUM VITAE</b> .....	236

## LIST OF TABLES

Table 2.1	Expression of TatGFP shifts the dose-response curve to the left.....	80
Table 3.1	LC <sub>50</sub> values of the various cell lines used in the MTT cell toxicity assays.....	126
Table 4.1	Summary of protein spots resolved in the redox 2D gels.....	169

## LIST OF FIGURES

Figure 1.1	The structure of the HIV-1 genome and LTR.....	8
Figure 1.2	Functional domains of the HIV-1 Tat protein.....	12
Figure 1.3	Folate synthesis and the sites of TMP-SMX action.....	21
Figure 1.4	Proposed mechanism of the hapten hypothesis in SMX-induced adverse drug reactions .....	24
Figure 1.5	The metabolism of Sulphamethoxazole .....	26
Figure 1.6	A comparison of the mechanism behind the hapten hypothesis and the p-i concept in stimulating T-cells.....	32
Figure 1.7	Redox two-dimensional gel electrophoresis.....	42
Figure 2.1	Expression of GFP and the TatGFP fusion protein.....	71
Figure 2.2	Increases in the concentration of doxycycline results in increased TatGFP expression.....	74
Figure 2.3	TatGFP expression decreases cell viability upon exposure to SMX-HA..	77
Figure 2.4	TatGFP expression in combination with SMX-HA results in the induction of apoptosis.....	84
Figure 3.1	Confocal images showing localization of the various Tat constructs.....	108
Figure 3.2	Time course of TatGFP expression following differential doxycycline induction.....	111
Figure 3.3	Expression of the protein from the Tat constructs after differential induction.....	115
Figure 3.4	Quantification of Tat mRNA in the various cell lines.....	118
Figure 3.5	The effect of Tat constructs on cell toxicity in the presence of SMX-HA.....	123
Figure 3.6	Reactive oxygen species generated in the presence of SMX-HA and the Tat constructs.....	129
Figure 4.1	Representative film and slot blots showing SMX-HA-induced haptentation.....	161

Figure 4.2	SMX-HA induced haptentation in Jurkat T cell lines.....	165
Figure 4.3	Redox two-dimensional SDS-PAGE of thiol proteins from various control cell lines.....	171
Figure 4.4	Redox two-dimensional SDS-PAGE of thiol proteins from various cell lines induced with 0ng/ml doxycycline.....	174
Figure 4.5	Redox two-dimensional SDS-PAGE of thiol proteins from various cell lines induced for 40hrs with 200ng/ml or 400ng/ml doxycycline prior to drug treatment.....	176
Figure 4.6	Redox two-dimensional SDS-PAGE of thiol proteins from various cell lines induced for 40hrs with 1000ng/ml doxycycline prior to drug treatment.....	178
Figure 4.7	Redox two-dimensional SDS-PAGE of thiol proteins from various control cell lines after treatment with SMX-HA.....	182
Figure 4.8	Redox two-dimensional SDS-PAGE of thiol proteins from various cell lines induced with 0ng/ml doxycycline for 40hrs then treated with 200 $\mu$ M SMX-HA.....	187
Figure 4.9	Redox two-dimensional SDS-PAGE of thiol proteins from various cell lines induced with 200ng/ml or 400ng/ml doxycycline for 40hrs then treated with 200 $\mu$ M SMX-HA.....	189
Figure 4.10	Redox two-dimensional SDS-PAGE of thiol proteins from various cell lines induced with 1000ng/ml doxycycline for 40hrs then treated with 200 $\mu$ M SMX-HA.....	191
Figure 4.11	The catalytic cycle of 2-cys peroxiredoxins.....	201
Figure 5.1	The rationale for increased incidence of ADRs in HIV patients.....	223

## LIST OF ABBREVIATIONS, SYMBOLS, NOMENCLATURE

2D	Two or second dimension
ADR	Adverse Drug Reaction
ANT	adenine nucleotide translocase
AIDS	Acquired Immunodeficiency Syndrome
APC	antigen presenting cell
ART	antiretroviral therapy
ARV	anti-retrovirals
BSA	Bovine serum albumin
CBP	CREB-binding protein
CDK9	cyclin-dependent kinase 9
C <sub>P</sub>	peroxidatic cysteine
C <sub>R</sub>	resolving cysteine
CT	computed tomography
CYP	cytochrome P450
DMSO	dimethyl sulfoxide
DTT	Diththiothretol
EGFP	enhanced green fluorescent protein
ER	endoplasmic reticulum
FI	fusion inhibitors
GSH	glutathione
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HAD	HIV-associated dementia
HDAC	histone deacetylases
HNE	4-hydroxynonenal
HIV	Human Immunodeficiency Virus
iNOS	inducible nitric oxide synthase

IN	integrase
LC <sub>50</sub>	lethal concentration, 50%
LTR	Long terminal repeat
MA	Matrix
MMP	matrix metalloproteinases
mtDNA	mitochondrial DNA
MTT	(3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
MW	molecular weight
Ni	Nickel
NF-κB	Nuclear factor kappa B
NLS	nuclear localization sequence
NPC	nuclear pore complexes
NO	nitric oxide
NRTI	nucleoside reverse transcriptase inhibitors
NNRTI	non-nucleoside reverse transcriptase inhibitors
O <sub>2</sub> <sup>-</sup>	superoxide anion
O <sub>2</sub> <sup>*-</sup>	superoxide radical
PBMC	peripheral blood mononuclear cells
PCP	Pneumocystis pneumonia
PI	protease inhibitors
p-i	pharmacological interaction
PR	Protease
PSH	protein thiol
PSSP	protein disulphide
PSOH	sulphenic acid
PSO <sub>2</sub> H	sulphinic acid
PSO <sub>3</sub> H	sulphonic acid

Prx	Peroxiredoxins
PTD	protein transduction domain
P-TEFb	positive transcriptional elongation factor b
PTP	permeability transition pore
RCM	radio contrast media
RGD	arginine, glycine, asparagine
ROS	reactive oxygen species
RNS	reactive nitrogen species
RRE	Rev response element
RT	reverse transcriptase
SH	sulphydryl groups
SMX	Sulphamethoxazole
SMX-HA	Sulphamethoxazole-hydroxylamine
SMX-NO	sulphamethoxazole-nitroso
SOD	superoxide dismutase
SJS	Steven-Johnson Syndrome
SRX	sulphiredoxin
TAR	Tat activation region
TBP	Tata-binding protein
TCC	T cell clones
TCR	T cell receptor
TEN	Toxic epidermal necrolysis
TFIIH	transcription factor IIH
TMP	Trimethoprim
TNF	Tumour Necrosis Factor
Trx	thioredoxin
UNAIDS	United Nations Program on HIV/ AIDS



UTI            urinary tract infections  
VDAC        voltage-dependent anion channel