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

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by

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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 200μM 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.

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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_P	peroxidatic cysteine
C_R	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_2O_2	Hydrogen peroxide
HAD	HIV-associated dementia
HDAC	histone deacetylases
HNE	4-hydroxynonenal
HIV	Human Immunodeficiency Virus
iNOS	inducible nitric oxide synthase

IN	integrase
LC_{50}	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_2^-	superoxide anion
$O_2^{\bullet -}$	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_2H	sulphinic acid
PSO_3H	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