Hypersensitivity of HIV-1-Infected Cells to Reactive Sulfonamide Metabolites Correlated to Expression of the HIV-1 Viral Protein Tat

Jacqueline Arp, Michael J. Rieder, Brad Urquhart, David Freeman, M. Jane Tucker, Adriana Krizova, David Lehmann, and Gregory A. Dekaban

The BioTherapeutics Group, Robarts Research Institute London, Ontario, Canada (J.A., M.J.R., G.A.D.); Section of Paediatric Clinical Pharmacology, Departments of Paediatrics (M.J.T.), Physiology and Pharmacology (A.K., B.U.), and Medicine (D.F.), Children’s Hospital of Western Ontario, University of Western Ontario, London, Ontario, Canada; and Departments of Medicine and Pharmacology, State University of New York, Health Sciences Center Syracuse, Syracuse, New York (D.L.)

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ABSTRACT

Impairment of human immunodeficiency virus (HIV)-infected cells to deal with reactive drug metabolites may be a mechanism for the increased rate of adverse drug reactions seen in AIDS. HIV Tat protein expression may be associated with increased oxidative stress within HIV-infected cells. To determine the relationship between expression of HIV Tat and sensitivity to reactive drug metabolites, we studied toxicity of sulfamethoxazole (SMX) and its reactive hydroxylamine intermediate (SMX-HA) in lymphocytes transfected with the HIV tat gene.

Over a concentration range from 0 to 400 μM SMX-HA, there was a significant concentration-dependent increase in cell death in transfected cell lines expressing Tat compared with controls. Jurkat T cells transfected with a dose-dependent inducible tat gene showed increased toxicity in response to SMX-HA as more Tat expression was induced. Enhanced sensitivity to SMX-HA was accompanied by significantly lower concentrations of total intracellular glutathione compared with controls (P < 0.05). Sensitivity to reactive drug metabolites in HIV-infected cells seems to be mediated by the viral protein Tat.

Adverse drug reactions (ADRs) are important problems for people living with human immunodeficiency virus (HIV) (Rieder and Dekaban, 2000; Tantisiriwat and Powderly, 2000). The rate of ADRs to sulfonamide drugs among people with AIDS is as high as 50% compared with 5% in the general population (Carr et al., 1993a,b; Rieder et al., 1997). Sulfonamide-induced ADRs among HIV-infected individuals are primarily hypersensitivity reactions, characterized by a severe rash and often associated with involvement of organs such as liver, kidney, or bone marrow (Rieder et al., 1988, 1995; Hess and Rieder, 1997). People with AIDS are at an increased risk for hypersensitivity ADRs to many drugs, including other anti-infectives, anticonvulsants, and antiretroviral drugs (Carr et al., 1993a,b; Koopmans et al., 1995).

The increased risk for ADRs seems to parallel progression of HIV infection to AIDS. During the asymptomatic stage, people positive for HIV are not at a higher risk for hypersensitivity ADRs than the general population. However, as the patient’s CD4+ cell count falls and viral load increases, risk for ADRs increases, according to most investigators (Carr et al., 1993a; Rieder et al., 1995; Reilly et al., 1999; Rieder and Dekaban, 2000; Rabaud et al., 2001; Elisazewicz et al., 2002; Sterling et al., 2003). Thus, factors associated with a decline in CD4+ count, such as increasing viral load, may be associated with increase in ADR risk.

Both plasma and peripheral blood mononuclear cells of AIDS patients exhibit significantly decreased levels of glutathione and concomitant marked increases in oxidized products (Buhl et al., 1989; Choi et al., 2000). Impairment of HIV-infected cells to deal with oxidative stress may explain the compromised ability of the cells to deal with reactive drug metabolites, generated during oxidative metabolism of the parent drug (Rieder et al., 1988, 1989, 1995; Repetto et al., 1996). The HIV-associated decrease in glutathione (GSH) content can result from diminished GSH synthesis or an impaired GSH reduction system.
increased rate of loss. GSH consumption may increase in HIV infection as a result of increased oxidative stress (Favier et al., 1994; Pace and Leaf, 1995; Walmsley et al., 1997). The systemic decrease in total GSH content and decreased metabolic labeling of GSH in HIV-infected individuals suggest reduced GSH biosynthesis (Helbling et al., 1996; Jahoor et al., 1999). Thus, decreased GSH content may allow for development of oxidative stress rather than the converse.

We have previously demonstrated that HIV infection of T cells is associated with increased cellular sensitivity to reactive drug metabolites (Rieder et al., 1995). Recent work has suggested expression of the HIV protein Tat is associated with increased oxidative stress (Flores et al., 1993; Westendorp et al., 1995a; Opalenik et al., 1998). Tat is a 14-kDa protein encoded by HIV type 1 (HIV-1) that serves as a transcriptional/post-transcription transactivator essential for efficient viral replication (Dingwall et al., 1989; Khashan- chi et al., 1994). In addition to regulating HIV gene expression and replication, Tat has been reported to influence cellular phenotype by affecting expression of cellular genes (Li-Weber et al., 2000). HIV-1 Tat has been found to modulate the redox status of cells, lowering the total and reduced GSH concentration in vitro in various cell lines and decreasing the total sulfhydryl content in vivo in a Tat transgenic mouse model (Choi et al., 2000). Further investigation of this transgenic mouse model has revealed that Tat expression was associated with a linear decrease in hepatic and erythrocyte glutathione content accompanied by a decrease in the glutathione synthetase regulatory subunit mRNA and protein content, resulting in an increased sensitivity of the synthetase to glutathione feedback inhibition (Choi et al., 2000).

This, together with the documented down-regulation of manganese superoxide dismutase by Tat, may explain the observed reduction in GSH content in HIV-infected cells (Flores et al., 1993). Tat can cause both enhanced activation of nuclear factor-κB and increased susceptibility to apoptosis by increasing sensitivity to apoptotic signals through up-regulation of FLICE/Caspase-8 and CD95L expression in T cells (Campioni et al., 1995; Westendorp et al., 1995a,b; Demarchi et al., 1996; Bartz and Emerman, 1999; Kelly et al., 1999).

In this study, we sought to determine the relationship between expression of HIV viral protein Tat and cellular sensitivity to reactive drug metabolites using sulfamethoxazole as a model compound. This is the first report to investigate the relationship between viral protein expression and cellular sensitivity to reactive drug metabolites.

Materials and Methods

Cell Lines. The following cell lines used in this study were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health. The HIV-infected lymphoblastic CD4+ cell line Jurkat J1.1 was originally from Dr. Thomas Folks, whereas the uninfected parent cell line Jurkat E6.1 was originally from Dr. Arthur Weiss. The Jurkat J1.1 and the parent Jurkat E6.1 cell lines were maintained in Complete RPMI 1640 medium (supplemented with 1% L-glutamine [Invitrogen, Carlsbad, CA], 20% fetal calf serum [Invitrogen], and 100 units/ml penicillin G and streptomycin [Invitrogen]). The HIV-1 Tat-expressing stable cell line Jurkat-tat was originally from Drs. Antonella Caputo, William Haseltine, and Joseph Sodroski and was grown in Complete RPMI 1640 medium plus 800 μg/ml Geneticin (G418; Invitrogen) to ensure the maintenance of Tat expression.

Drug Incubations. All tissue culture cells were harvested during logarithmic growth (minimum 97% viability), washed repeatedly in Hank’s balanced salt solution, resuspended in Hank’s balanced salt solution and pipetted into either 96-well plates at densities of 8 × 104 cells/well for the cell viability assessments or into six-well plates at corresponding densities of 5 × 105 cell/well for the glutathione quantitation. Cells were incubated in the presence and/or absence of various concentrations of freshly prepared solutions of sulfamethoxazole-hydroxylamine (SMX-HA) or the parent drug sulfamethoxazole (SMX) for 2 h at 5% CO2 and 37°C. A series of final SMX-HA concentrations [in 1% DMSO (v/v)] was 0, 25, 50, 100, 200, 300, and 400 μM; that of SMX was 400 μM; whereas the vehicle control final concentration was maintained throughout all the assays at 1% DMSO except where specified differently. All treatments were performed and measured in quadruplicate. After drug exposure, the supernatants were removed and the cells were incubated in the Complete RPMI 1640 medium for 18 h at 5% CO2 and 37°C.

Drug Cytotoxicity Assessment. Cell viability was determined by flow cytometry of Jurkat E6.1 cells stained with pBiG plasmid containing a tat gene fused to GFP or GFP alone that were induced in a concentration-dependent manner by doxycycline, 0 to 1000 ng/ml. The stable tat-transfected cells were incubated with vehicle alone or increasing concentrations of doxycycline for 38 h, harvested, and analyzed for Tat expression using Western blots and 10 μg of total protein for each differentially induced population. The blots were probed with both anti-tat antibodies and anti-β-actin antibody. These cells were also differentially induced with doxycycline for 38 h and treated with vehicle alone, 1000 μM SMX, and 50 or 200 μM SMX-HA for 2 h. They were subsequently incubated overnight in Complete RPMI 1640 medium, harvested, and stained with Annexin V-PE, a marker of apoptosis, and 7AAD, a viable cell marker (apoptosis detection kit I; BD Biosciences Pharmingen, San Diego, CA).

Quantitation of Total Intracellular Glutathione. High-performance liquid chromatography with fluorescence detection (HPLC-FD) was used to determine the total amount of intracellular glutathione in the transfected and parent Jurkat cell lines. After a 2-h exposure of 1.5 × 105 cells to either vehicle control or 200 μM SMX (final concentration 1% DMSO [v/v]), the cultures were washed and allowed to recover in complete media for 18 h at 5% CO2 and 37°C. Triplicate aliquots of the treated cells were individually harvested and washed three times in HEPES-buffered saline solution (Invitrogen). Cell suspensions were then prepared for total glutathione quantitation after a slightly modified method described by Jacobsen et al. (1994) involving the simultaneous sodium borohydride reduction of disulfide bonds and derivatization of sulfhydryl groups with monobromobimane followed by HPLC-FD. Using a Hewlett Packard 1090 liquid chromatograph, the reduced and derivatized samples were injected as 10-μl aliquots onto a C8 column (10 cm × 3.2 mm i.d., 5 μm) at a flow rate of 0.5 ml/min and column temperature of 40°C. The mobile phase consisted of a 4% acetonitrile/25 mM ammonium formate buffer, pH 9.8 with formic acid. After chromatographic separation, the thiolbimane adducts were detected fluorometrically with a Shimadzu fluorescence detector with the excitation wavelength set at 390 nm and the emission wavelength set at 480 nm.
Western Blot Detection of HIV-1 Tat. Jurkat-tat and Jurkat E6.1 cells at a density of 8 × 10^5 cells/well were incubated in the presence of a series of SMX-HA concentrations ranging from 0 to 400 μg/mL or to the parent drug SMX at a final concentration of 400 μM (all in a final v/v of 1% DMSO) for 2 h, followed by the removal of the drug-containing supernatants and further incubation in Complete RPMI 1640 medium for 18 h at 5% CO_2 and 37°C. Cells were harvested and lysed in cold extraction buffer (pH 7.6; 100 mM NaCl, 10 mM sodium phosphate, 1% (v/v) Triton X-100, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) sodium dodecyl sulfate, and 1 mM EDTA) in the presence of a serine and cysteine protease inhibitor cocktail (Complete; Roche Diagnostics, Indianapolis, IN). After the addition of an equal volume of 8 M urea dithiothreitol-complete Laemmli loading buffer, the samples (pool of 10 wells) were sonicated briefly. HPLC-purified HIV-1 Tat protein (0.45 μg; mol. wt. 14,000 Da) was concomitantly run on each Tricine-6 M urea 15% SDS-polyacrylamide gel electrophoresis to serve as a positive control. The HIV-1 Tat protein was originally from Dr. John Brady supplied through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health. Supernatant of cell lysates and purified Tat protein were electrophoresed for 7 h at 50 V in a Tricine buffer system and transferred from the gel to an Immobilon P membrane (Millipore Corporation, Billerica, MA). Blots were blocked in a solution of 5% skim milk powder at room temperature for 5 h. Blots were incubated in an anti-Tat antibody cocktail diluted in blocking buffer, overnight at 4°C with rocking. The anti-Tat antibody cocktail consisted of the mouse monoclonal NT3 2D1 1 antibody (IgG1a; final concentration of 10 μg/mL) specific for the N-terminal 15-mer peptide of HIV-1 Tat (amino acid [aa.] 73–86), and the HIV-1_HIVIII_HIVtat monomeric antibody 15.1 (IgG_1a; final concentration of 42.5 pg/mL) specific for the epitope spanning aa. 1 to 16 of the HIV-1 Tat protein. Both Tat monoclonal antibodies were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health. Blots were then washed in blocking buffer and exposed to the secondary antibody solution consisting of goat anti-mouse IgG conjugated to hors eradish peroxidase (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) at a final dilution of 1:8200 for 1 h at room temperature. The blots were then washed and exposed to the enhanced chemiluminescence substrate (Amersham Biosciences Inc., Piscataway, NJ) according to the manufacturer’s instructions.

Statistics. GraphPad InStat programs (GraphPad Software Inc., San Diego, CA) were used to calculate mean and standard error of the means for the toxicity data, and mean and standard deviations for the cytometry data. This program was also used to perform one-way analysis of variance and Dunnett’s post hoc multiple comparison tests.

Results

The first objective of the current study was to determine whether the differential toxicity to sulfamethoxazole metabolites previously observed between HIV-infected and uninfected cells was a tissue culture cell type-specific phenomenon or truly a consequence of HIV infection. HIV-infected Molt3_HIVIM lymphoblastoid (CD4^+) cells had previously been shown to exhibit increased sensitivity to sulfonamide-reactive metabolites compared with the uninfected parental cell line (Rieder et al., 1995). In the current study, we investigated the toxicity of sulfamethoxazole hydroxylamine on HIV-infected T4-lymphoblastoid Jurkat J1.1 (CD4^+) cells. To determine the viability of the cell lines after exposure to the sulfamethoxazole and its hydroxylamine metabolite, uninfected Jurkat E6.1 and HIV-infected Jurkat J1.1 cells were incubated with SMX-HA at varying concentrations for 2 h, allowed to recover during an overnight incubation, and then exposed to MTT. As expected, when cytotoxicity was assessed by the measurement of MTT dye conversion, Jurkat E6.1, Jurkat J1.1, and stably transfected Jurkat tat cells exposed to 400 μM parent compound sulfamethoxazole exhibited no significant differences in viability (92% versus 90 and 70%, respectively, P > 0.05 in both cases). In contrast, Jurkat E6.1, the HIV-infected Jurkat J1.1 cell lines both exhibited concentration-dependent toxicity after exposure to the reactive metabolite SMX-HA (Fig. 1). The Jurkat J1.1 cell line exhibited significantly greater levels of sensitivity and cell death to SMX-HA at all concentrations above 200 μM, with a calculated (Graph Pad InStat) LC50 of 62.0 ± 39.5 μM SMX-HA, compared with the uninfected parent cell line E6.1 that demonstrated a LC50 of 280.8 ± 59.4 μM. This increased toxicity to SMX-HA exhibited by the HIV-infected Jurkat J1.1 cells is consistent with our previous findings with HIV-infected Molt3_HIVIM cells and further suggested that the exhibited hypersensitivity to SMX-HA was a direct consequence of HIV infection. The Tat-expressing Jurkat-tat cell lines (LC50 of 55.4 ± 16.7 μM) were studied concomitantly with Jurkat E6.1 and HIV_HIVinfected Jurkat J1.1 cells and exhibited a greater degree of dose-dependent toxicity to the drug metabolite SMX-HA (0–400 μM) (Fig. 1).

To examine the level of Tat expressed during drug treatment, Western blotting was performed and correlated to increasing drug concentrations. The samples analyzed on the Western blot shown in Fig. 2 were collected from cell cultures initially plated at the same density before drug exposure for 2 h followed by an 18-h recuperation period. At the time of cell harvest for Western blot analysis, parallel samples were assessed for cell viability by the MTT dye conversion assay. To reflect the viability effects of the drugs, the collected cell lysate equivalents destined for Western blot analysis were not corrected for total protein content. Purified HIV-1 Tat protein was simultaneously immunoblotted to serve as a positive control. Upon incubation of the blot with an anti-Tat antibody cocktail, untreated Jurkat-tat cell cultures (0 μM hydroxylamine) and those exposed to the parent drug sulfamethoxazole (400 μM SMX), both exhibiting greater than 92% viability, expressed the 14,000-Da Tat protein in comparable amounts (Fig. 2). The level of the Tat expression detected decreased as the drug concentration increased and cell viability decreased.

To further demonstrate the increased cell death in stably transfected Jurkat E6.1 cells, we transfected Jurkat E6.1 cells with an inducible plasmid containing tat fused to GFP or with GFP alone. Increased induction with doxycycline showed a concentration-dependent increase in Tat-GFP and GFP expression. The cells were induced with vehicle alone or increasing concentrations of doxycycline for 38 h, harvested, and analyzed by Western blot for the expression of Tat protein, and the expression of β-actin as a protein-loading control. The Western blot (Fig. 3) clearly demonstrates that Tat-GFP expression from the pBIG vector platform is inducible in a dose-dependent manner and not expressed in the absence of doxycycline. Flow cytometric analysis also demonstrated a dose response of induction of both Jurkat Tat-GFP and control GFP to be in the range of 31 to 500 ng/mL doxycycline, with a plateau beginning at 250 ng/mL. Furthermore, the more doxycycline-induced Tat-GFP there was in the Jurkat-transfected cells, the more susceptible the cells were
to SMX-HA-mediated toxicity, but not to SMX or DMSO. Increasing levels of control GFP expression induced in stably transfected cells by increasing doses of doxycycline did not affect the viability of these cells (>95% viable). However, over the same range of doxycycline, the tat-GFP-transfected Jurkat cells showed a consistent and repeatable increase in cell death on exposure to increasing doses of SMX-HA, but not to SMX. As shown in Table 1, vehicle-treated cells transfected but not induced were viable (90–96.7%), but increasing both the expression of Tat-GFP and the concentration of drug in the incubation caused increased cell death, as evidenced by increased staining with both by Annexin V and 7AAD staining (Table 1). Flow cytometry results indicate the amount of tat-GFP fusion protein present in the cells makes the cells more susceptible to toxicity of sulfa metabolites. No synergistic toxic effects of doxycycline and SMX-HA were found in Jurkat E6.1 or Jurkat-tat cells using the MTT assay. These effects on cell viability were not seen in control cells that expressed only GFP (data not shown).

HPLC-FD was used to determine the total intracellular glutathione concentration of both the transfected and parent Jurkat cell lines. After a 2-h exposure of cultures to either vehicle or 200 μM SMX (final concentration 1% DMSO (v/v)), and 18-h recovery in complete media, triplicate aliquots of the treated cells were prepared for total glutathione quantitation. Total cellular glutathione was determined by borohydride reduction of disulfide bonds and derivatization of sulfhydryl groups (thiols) with monobromobimane followed by HPLC-FD. Figure 4 demonstrates that the intracellular concentrations of total GSH in the Tat-expressing Jurkat-tat cells were significantly lower than those found in their corresponding parental cell lines (P < 0.03), both in the presence and absence of the control parent drug SMX. Upon exposure to both sets of control conditions, the Tat-expressing Jurkat cells possessed the lowest concentrations of total GSH, exhibiting only 23 ± 1% of the total GSH concentration compared with its parent cell line Jurkat E6.1.

**Discussion**

ADRs are common and important problems for people living with HIV (Hess and Rieder, 1997). There is an increase in sulfonamide ADR risk associated with HIV infection, including a significantly increased risk for sulfonamide hypersen-
We have previously suggested that reactive drug metabolites seem to be the key elements in the pathogenesis of ADRs to drugs such as the sulfonamides. This seems to involve increased cellular sensitivity to reactive drug metabolites, and this increased cellular sensitivity is more marked among HIV-infected cells. It seemed likely that clinical hypersensitivity to sulfonamides was related to HIV-specific alterations in cellular homeostasis, which would ultimately place the HIV-infected cells at greater risk for cellular injury resulting from various reactive chemical species, including sulfonamide reactive intermediates (Koopmans et al., 1995; Rieder et al., 1995; Hess et al., 1999). It seems that this increased risk for cellular injury was related to factors unique to HIV infection, which could include the expression of proteins specific to HIV-infected cells. Thus, to test whether the altered ability of HIV-infected cells to detoxify reactive sulfa drug metabolite intermediates was a direct consequence of a fundamental HIV viral function, we decided to focus on Tat protein due to its important role in viral gene expression and replication, the ability of Tat to alter host gene expression, and its known proapoptotic activity.

We have demonstrated that expression of Tat produced an increased cellular sensitivity to reactive drug metabolites equivalent to that seen among HIV-infected cells. This sensitivity occurred over drug concentrations that would be predicted to be found in the tissues of patients being treated clinically with the sulfonamides (i.e., 25–100 μM). The concentration of metabolite in tissues has not been directly measured, but it is certainly highest at the site of metabolite generation. Cells expressing increasing susceptibility to drug metabolites with increasing levels of Tat suggest that Tat may be the most likely candidate for these effects. The dose-response curve for toxicity associated with incubation with reactive drug metabolites in the presence of Tat is not as smooth as would be anticipated if there was a simple linear relationship between toxicity and Tat expression. This suggests that toxicity of reactive metabolites in the context of HIV infection and Tat expression is likely to be a complex process. Thus, although Tat seems to be an important contributor to the toxicity of reactive metabolites, there may be additional molecular determinant(s) of this toxicity. More experiments are needed to determine whether the amount of increased cell death in the transfected cells versus the HIV-infected cells is due to changes in glutathione levels, an increased intracellular concentration of Tat or a combination of both factors in the transfected cells.

The marked toxicity experienced by the Tat-expressing

Fig. 2. Western blot analysis revealed that the HIV-1 Tat protein continues to be expressed in Jurkat-tat cells in detectable amounts, after a 2-h exposure to either 400 μM SMX or low concentrations (0–100 μM) of the reactive metabolite SMX-HA. All cells were initially plated at the same density of 8 × 10^4/well before drug exposure for 2 h and followed by an 18-h recuperation period, at which time the cell viability was assessed. To reflect the toxicity effects of the drugs, the collected cell lysate equivalents were not corrected for total protein content. The blot was incubated in a primary anti-Tat antibody cocktail consisting of the mouse monoclonal NT3 2D1 1 antibody (final concentration of 10 μg/ml) specific for the N-terminal 15-mer peptide of HIV-1 Tat (a.a. 73–86), and the HIV-1BH10 Tat mouse monoclonal antibody 15.1 (final concentration of 42.5 pg/ml) specific for the epitope spanning a.a. 1 to 16 of the HIV-1 Tat protein. Purified HIV-1 Tat protein was concomitantly immunoblotted to serve as a positive control.

Fig. 3. A, cell lysates of JurkatpBIG tatGFP cells treated with different doses of doxycycline were probed with anti-tat or anti-β-actin antibodies. Differential tatGFP expression depended on the dose of doxycycline. Cell populations were induced with 0 (lane 1), 31 (lane 2), 62 (lane 3), 125 (lane 4), 250 (lane 5), 500 (lane 6), or 1000 (lane 7) ng/ml doxycycline per milliliter of media. The experiment was repeated three times. B, densitometric reading of Western blots shows percentage of maximal (JurkatpBIG tatGFP induced with 1000 ng/ml doxycycline) tatGFP expression. The data are presented as mean ± standard deviation.
TABLE 1

Cells expressing increasing concentrations of Tat protein show an increasing susceptibility to sulfamethoxazole metabolite

<table>
<thead>
<tr>
<th>SMX-HA Concentration</th>
<th>0 μM</th>
<th>50 μM</th>
<th>200 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninduced Live</td>
<td>88.3 ± 10.7</td>
<td>81 ± 7</td>
<td>73.5 ± 4</td>
</tr>
<tr>
<td>Annexin V +</td>
<td>5.0 ± 1.5</td>
<td>5.7 ± 1.9</td>
<td>9.6 ± 1.7</td>
</tr>
<tr>
<td>7AAD +, Annexin V +</td>
<td>5.9 ± 8.2</td>
<td>10.4 ± 4.0</td>
<td>15.4 ± 6.0</td>
</tr>
<tr>
<td>31 μM doxycycline Live</td>
<td>92.4 ± 1.1</td>
<td>51.3 ± 14.9</td>
<td>38.7 ± 16.6</td>
</tr>
<tr>
<td>Annexin V +</td>
<td>6.6 ± 0.7</td>
<td>15.3 ± 5.1</td>
<td>14.3 ± 5.6</td>
</tr>
<tr>
<td>7AAD +, Annexin V +</td>
<td>1.0 ± 0.4</td>
<td>29.8 ± 13.5</td>
<td>43.0 ± 13.1</td>
</tr>
<tr>
<td>500 μM doxycycline Live</td>
<td>85.4 ± 5.8</td>
<td>55.6 ± 21.4</td>
<td>40.1 ± 27.7</td>
</tr>
<tr>
<td>Annexin V +</td>
<td>9.0 ± 0.3</td>
<td>17.6 ± 7.1</td>
<td>18.2 ± 4.7</td>
</tr>
<tr>
<td>7AAD +, Annexin V +</td>
<td>5.0 ± 5.3</td>
<td>25.5 ± 13.3</td>
<td>40.9 ± 23.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SMX Concentration</th>
<th>1000 μM</th>
</tr>
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<tbody>
<tr>
<td>Uninduced Live</td>
<td>97.6</td>
</tr>
<tr>
<td>Annexin V +</td>
<td>1.55</td>
</tr>
<tr>
<td>7AAD +, Annexin V +</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Fig. 4. HPLC-FD was used to determine the total intracellular glutathione amounts of both the transfected and parent Jurkat cell lines. After a 2-h exposure of cultures (1.5 × 10^6 cells/well) to either vehicle alone or 200 μM SMX (final concentration 1% DMSO [v/v]), and 18-h recovery in complete media, triplicate aliquots of the treated cells were then prepared for total glutathione quantitation (micromolar per liter). Quantitation by HPLC-FD revealed that intracellular concentrations of total GSH in Jurkat-tat cells were significantly lower than those found in the parent cells (23 ± 2% upon comparison; P < 0.03), both in the presence and absence of the control parent drug SMX (200 μM SMX or vehicle alone, respectively).

transfected Jurkat-tat cells to SMX-HA was also observed upon Western blot analysis of the differentially drug-treated cells. This demonstrated that the Tat expression in Jurkat-tat cells was unaltered, both in the presence of the parent drug SMX and the solvent vehicle 1% DMSO that was common to all of the tested drug-exposure culture conditions. Incubation of Jurkat-tat cells with varying concentrations of the reactive SMX-HA metabolite was associated with an increasing degree of cell death and was reflected in a concomitant decrease in the amount of Tat protein present. Preliminary results with another Tat-expressing cell line [CEM-tart cells received from Drs. Herbert Chen, Terence Boyle, Michael Malim, Bryan Cullen, and H. Kim Lyerly; and parental CEM-SS obtained directly from Dr. Peter Nara (Frederick Cancer Research and Development Center, Frederick, MD)] yielded similar results (data not shown). Thus, this seems to be a consistent result in this third human T cell line.

Increased cell death on exposure to sulfa metabolites was also seen when transfected Jurkat cells were induced to express Tat in a dose-dependent manner. The increased expression of Tat was documented by Western blot and flow cytometry and provided a correlation between expression of Tat protein and increased susceptibility to SMX-HA. The Annexin V data suggest the cells died by apoptosis; however, further experiments are needed to confirm this mechanism of cell death.

With respect to potential targets for Tat, our previous work and the work of others strongly suggests a possible link with the intracellular thiol glutathione. We have previously demonstrated that thiols modulate the effect of reactive sulfonamide metabolites (Rieder et al., 1988). Recent reports have suggested that Tat expression is associated with increased oxidative stress by lowering the total and reduced GSH concentrations in vivo in a Tat transgenic mouse model (Choi et al., 2000). Thus, we were interested in determining whether the total intracellular GSH concentrations of the transfected Jurkat-tat cell line used in this study also exhibited altered GSH levels, since these had not yet been documented. We have demonstrated that expression of Tat significantly decreased total intracellular GSH concentrations. Under both control culture conditions tested, the cell viability of both cell lines was greater than 97%, thus the
observed differences in total GSH were not merely reflections of differences in cell number or cytotoxicity. It has been thought for some time that factors expressed during viral infection may play a role in the induction of adverse drug reactions, but there has been little evidence supporting this theory. It is unlikely that the HIV infection itself is contributing an increased immunostimulation to co-administered drugs because the increased risk for ADRs is so late in the infection process when immune suppression is clearly evident (Coopman et al., 1993), but this is a factor to consider. We have demonstrated that expression of the HIV Tat significantly increases cellular sensitivity to reactive drug metabolites and significantly decreases intracellular GSH concentration. This is the first demonstration of a potential mechanism for the previously described increased cellular sensitivity in the setting of HIV infection. The levels of other compounds affecting oxidative stress may also have a role in the incidence and severity of ADRs in HIV-infected patients. In particular, one study demonstrates that a deficiency in plasma ascorbate is associated with decreased detoxification of sulfam metabolites in HIV-infected individuals (Trepanier et al., 2004). This suggests that altered intracellular homeostasis may be key in the pathogenesis of the increased rate of ADRs seen in the setting of HIV infection. Future work will need to better define the effect(s) and target(s) of Tat in the induction of enhanced cellular sensitivity. In future experiments, to further confirm the Tat-mediated increase in sensitivity to reactive drug metabolites, we hope to use Tat inhibitor/antagonists such as Ro24-7429 (7-chloro-5–1H-pyrrol-2-yl-3H-benzo[e][1,4]diazepin-2-ylmethylamine) to determine whether this antagonist is capable of inhibiting the enhanced drug sensitivity demonstrated in the Tat expressing cells. Our findings detailing the association of Tat protein with increased susceptibility to adverse drug metabolic reactions does not preclude the possibility that other HIV proteins may also contribute to this phenomenon, or be involved in the modulation of the cellular oxidative status. Thus, future research will need to include the development of transfected cell lines expressing other HIV genes for similar sensitivity testing and glutathione quantitation. As well, the effects we have studied have been in relation to total cellular glutathione, which is well known to act by cycling between reduced and oxidized forms. Thus, it would be of great interest to dissect the effect(s) of Tat expression on the kinetics of GSH synthesis and cycling.

The recognition that Tat may be instrumental in viral modulation of host oxidative status underscores the potential importance of this HIV protein. This effect may be direct as has been demonstrated here in HIV-infected cells. However, Tat can also be secreted from infected cells and taken up by uninfected neighboring cells. Tat can then effect transcellular transactivation of gene expression (Marcuzzi et al., 1992; Frankel and Pabo, 1988). Hence, host tissues not actually infected may nevertheless have their oxidant status modified and thereby escalate the progression of the disease. Remembering that HIV infection is a dynamic process, with active traffic in viral particles, it can be appreciated that there would be marked changes in the expression of Tat, with viral protein expression increasing dramatically as the HIV infection progresses to AIDS. If this viral regulatory protein modulates, at least in part, the ability of HIV-infected cells to deal with oxidative stress, such as that produced by reactive drug metabolites, this provides a mechanistic framework for the increased risk of hypersensitivity and adverse drug reactions as HIV infection progresses. This would also explain why the effective control of HIV viral load through the use of antiretroviral therapy seems to lower the risk of developing the most severe adverse reactions associated with drug therapy (Ryan et al., 1998). Understanding the molecular mechanism(s) responsible for these effects will be important in designing safer and more effective therapy for patients with HIV infection and other chronic viral infections.

References
Opaleten SR, Ding Q, Mallery SH, and Thompson JA (1998) Glutathione depletion


Address correspondence to: Dr. M. Rieder, Department of Paediatrics, Children's Hospital of Western Ontario, 800 Commissioner's Rd. East, London, ON, Canada N6C 2V5. E-mail: mrieder@uwo.ca