Detection of Drug Bioactivation in Vivo: Mechanism of Nevirapine—Albumin Conjugate Formation in Patients

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ABSTRACT: The non-nucleoside reverse transcriptase inhibitor nevirapine (NVP) is widely used for the treatment of human immunodeficiency virus type 1 (HIV-1), particularly in developing countries. Despite its therapeutic benefits, NVP has been associated with skin and liver injury in exposed patients. Although the mechanism of the tissue injury is not yet clear, it has been suggested that reactive metabolites of NVP may be involved. The detection of NVP mercapturate in the urine of patients undergoing standard antiretroviral chemotherapy indicates that NVP undergoes bioactivation in vivo. However, covalent binding of drug to protein in patients remains to be determined. In this study, we investigate the chemical basis of NVP protein adduct formation by using human serum albumin (HSA) and glutathione S-transferase pi (GSTP) as model proteins in vitro. In addition, HSA was isolated from serum samples of HIV-1 patients undergoing NVP therapy to measure NVP haptenation. Mass spectrometric analysis of 12-sulfoxyl-NVP-treated HSA revealed that the drug bound selectively to histidine (His146, His242, and His338) and a cysteine residue (Cys34). The reaction proceeds most likely by a concerted elimination—addition mechanism. This pathway was further confirmed by the observation of NVP-modified Cys47 in GSTP. Importantly, the same adduct (His146) was detected in HSA isolated from the blood of patients receiving NVP, providing direct evidence that NVP modifies protein in vivo, via the formation of a reactive metabolite.

INTRODUCTION

Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor that is widely prescribed in patients with HIV infection. Despite its therapeutic benefits, severe skin and liver injury has been reported in approximately 15−20 and 1−5%, respectively, of NVP-exposed patients.1,2 These rare clinically important adverse drug reactions (ADRs) lead to discontinuation of treatment in 6−8% of patients.3,4 Although the mechanism of NVP-mediated tissue injury is not yet fully defined, an immune mechanism involving the drug-specific activation of patient T-cells has been proposed.5 NVP also causes a rash in female Brown Norway rats;6−9 in this model, tissue injury has been shown to be dependent on drug-specific activation of CD4+ T-cells.8

NVP undergoes extensive metabolism in humans, forming a number of hydroxyl metabolites [2,3,8, and 12-hydroxy-NVP (Scheme 1)].10,11 Several possible bioactivation pathways have been proposed: arene oxygenation might yield reactive epoxides; positions 3 and 8 are para to a nitrogen, and oxidation of the hydroxyl metabolites could lead to the formation of a quinone imine intermediate (pathway I); enzymatic oxidation of NVP forming a radical at position 12 could form a quinone methide (pathway II);10 sulfation of 12-hydroxy-NVP (12-OH-NVP) followed by the dissociation of sulfate could also form a quinone methide (pathway III).7 These reactive intermediates are electrophilic and have the potential to form covalent adducts with nucleophiles in proteins.

Several studies have investigated NVP protein conjugation in vitro and in animal models. NVP has been shown to bind to liver tissue and plasma proteins in rats using14C-labeled drug.12 NVP has also been shown to form a glutathione adduct in human liver microsomes and CYP3A4 cultures.10 The bioactivation of NVP in patients was further confirmed through the detection of NVP mercapturate in human urine.13 These experiments provided evidence that reactive metabolites of NVP are formed in experimental systems and drug-exposed patients; however, how these intermediates bind to protein has not been clearly defined.

Recently, Antunes et al. demonstrated that 12-mesyloxy-NVP, a chemical surrogate of 12-OH-NVP, formed covalent adducts with human serum albumin (HSA) and hemoglobin (Hb) in vitro.14 Multiple adducts involving cysteine, lysine, histidine, serine, tryptophan, and the N-terminal valine of Hb were identified as possible binding sites. In addition, NVP-modified N-terminal valine in Hb was also detected in erythrocytes in humans taking a therapeutic dose of NVP.15 However, these findings do not accurately mirror the true hapten formed in vivo as 12-mesyloxy-NVP is more reactive
than 12-sulfoxyl-NVP. Furthermore, because Hb has been shown to catalyze typical monooxygenase reactions, including aromatic and aliphatic hydroxylations, a local high level of reactive metabolite may be formed, leading to covalent modification of Hb.16,17 The aims of this study were (a) to determine the chemical basis of NVP haptenation using HSA and GSTP as model proteins and (b) to investigate whether the same hapten is formed in patients receiving NVP.

■ MATERIALS AND METHODS

HSA (97–99% pure) and all standard reagents were purchased from Sigma-Aldrich (Poole, Dorset, U.K.); trypsin was purchased from Promega (Southampton, Hampshire, U.K.), and LC–MS grade solvents were obtained from Fisher Scientific (Loughborough, Leicestershire, U.K.). 12-Sulfoxyl-NVP was kindly provided by J. P. Uetrecht (University of Toronto, Toronto, ON).

Patients. Plasma samples were obtained from Liverpool Therapeutic Drug Monitoring Registry, which has received ethics approval from The Northwest Multicenter Ethics Committee. The nine patients recruited (Table 1) had achieved steady state on a nevirapine-based regimen at a does of either 200 mg twice daily or 400 mg once daily and were known to be fully compliant. Whole blood was collected in lithium heparin-containing tubes. Five milliliters of blood was centrifuged at 700 g and 4 °C for 6 min to yield plasma. Plasma was then heat-inactivated (58 °C for 40 min) and stored at −20 °C.18

Quantification of 12-OH-NVP in Patient Plasma. Plasma (50 μL) was mixed with 10 μL of an internal standard (acetaminophen). The protein was precipitated by using ice-cold methanol and centrifuged at 14000 rpm for 15 min. The supernatants were collected and concentrated in a Speedvac (Eppendorf, Hamburg, Germany). Five milliliters of blood was centrifuged at 700 g and 4 °C for 6 min to yield plasma. Plasma was then heat-inactivated (58 °C for 40 min) and stored at −20 °C.18

Table 1. NVP-Modified HSA and GSTP Peptides Detected in Vitro

<table>
<thead>
<tr>
<th>protein</th>
<th>observed mass (amu)</th>
<th>theoretical mass of unmodified peptide (amu)</th>
<th>mass increase (amu)</th>
<th>peptide sequence</th>
<th>amino acid residue</th>
<th>adducts detected in vitro</th>
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</thead>
<tbody>
<tr>
<td>HSA</td>
<td>2349.9</td>
<td>2085.8</td>
<td>264.1</td>
<td>VH*TECHGDLECADDR</td>
<td>His242</td>
<td>–</td>
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<tr>
<td></td>
<td>1731</td>
<td>1466.8</td>
<td>264.2</td>
<td>RH*PYYSVLLLR</td>
<td>His338</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2162.2</td>
<td>1898</td>
<td>264.1</td>
<td>RH*PYYPAPLFFAK</td>
<td>His146</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2696.4</td>
<td>2432.3</td>
<td>264.1</td>
<td>ALVIQAQLQQC*SFEHVK</td>
<td>Cys34</td>
<td>+</td>
</tr>
<tr>
<td>GSTP</td>
<td>1342.6</td>
<td>1078.6</td>
<td>264</td>
<td>ASC*LYGLQLPK</td>
<td>Cys47</td>
<td>+</td>
</tr>
</tbody>
</table>

Scheme 1. Possible Metabolic Pathways for Nevirapine

![Scheme 1](image-url)
and 2 mM ammonium acetate in 15 min was applied at a flow rate of 0.2 mL/min. The multiple-reaction monitoring (MRM) transitions were as follows: 267/226.1 (NVP), 283.1/265 (12-OH-NVP). A series of 12-OH-NVP standards ranging from 1 nM to 1 μM were also prepared in a 50% methanol/0.1% TFA (v/v) mixture and provided standard curves with R^2 values of >0.99.

**Concentration-Dependent Modification of Serum Albumin by 12-Sulfoxyl-NVP in Vitro.** 12-Sulfoxyl-NVP freshly dissolved in 50% methanol/phosphate buffer (v/v) was incubated with HSA (1 mg/mL) in phosphate buffer at 37 °C for 16 h; the drug-protein molar ratios were 1:1, 10:1, 50:1, and 100:1. Modified protein was precipitated twice with methanol to remove noncovalently bound drug, resuspended in 50 μL of phosphate buffer, and then reduced with 10 mM dithiothreitol and alkylated with 55 mM iodoacetamide. The protein was precipitated with methanol once more and then dissolved in 100 μL of 50 mM ammonium bicarbonate, and 165 μg (1.25 nmol) of protein was digested with 16 μg of trypsin overnight at 37 °C. The samples were processed for mass spectrometry (MS) analysis as described previously.

**Expression and Affinity Purification of Histidine-Tagged Human GSTP.** The cDNA for histidine-tagged human GSTP (His-GSTP) was cloned into vector pET-15b (Novagen) and expressed as described previously. Transformed BL21 cells were grown on lysogeny broth (LB) medium containing ampicillin (50 μg/mL). A single colony was inoculated into 50 mL of LB medium and grown at 37 °C until the absorbance at 600 nm reached approximately 0.5. An aliquot of this preculture was added to 1 L of medium, which was then eluted with 12 mM HCl and precipitated with 55 mM iodoacetamide. The protein was centrifuged at 13000 rpm for 10 min, and the supernatant was collected and stored at ~80 °C.

**In Vitro Modification of His-GSTP with 12-Sulfoxyl-NVP.** Purified His-GSTP captured on nickel beads was incubated with a range of molar ratios of 12-sulfoxyl-NVP to protein (1:1 to 100:1) in phosphate buffer (pH 7.4) for 16 h. The beads were then washed with 5 × 100 μL of phosphate buffer. The modified protein was subjected to on-bead tryptic digestion followed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis.

**Isolation of Serum Albumin from Plasma by Affinity Chromatography.** HSA was isolated by affinity chromatography, as described previously. A POROS anti-HSA affinity cartridge (Applied Biosystems, Foster City, CA) attached to a Vision Workstation (Applied Biosystems) was used to affinity capture HSA, which was then eluted with 12 mM HCl and precipitated with methanol. The samples were processed for LC–MS/MS analysis as described above.

**Mass Spectrometric Characterization of NVP-Modified Protein.** In vitro samples were reconstituted in 10 μL of 2% ACN and 0.1% formic acid (v/v), and 2 μL of the sample was delivered into a QSTAR Pulsar i hybrid mass spectrometer (AB Sciex) by automated in-line liquid chromatography [integrated LCPackings System, 5 mm C18 nanoprecolumn and 75 μm × 15 cm C18 PepMap column (Dionex)] via a nanoelectrospray source head and a 10 μm inner diameter PicoTip (New Objective). A gradient from 5% ACN and 0.05% TFA (v/v) to 48% ACN and 0.05% TFA (v/v) in 60 min was applied at a flow rate of 300 nL/min, and MS and MS/MS spectra were acquired automatically in positive ion mode using information-dependent acquisition (IDA; Analyst, AB Sciex). Survey scans of 1 s were acquired for m/z 400–2000, and the three most intense ions were selected for MS/MS, with accumulation times of 1 s and a dynamic exclusion of 40 s.

Patient samples were reconstituted in 2% ACN and 0.1% formic acid (v/v), and 2.4–5 pmol aliquots were delivered into a QTRAP $500 hybrid quadrupole-linear ion trap mass spectrometer (AB Sciex) by automated in-line liquid chromatography [U3000 HPLC System, 5 mm C18 nanoprecolumn and 75 μm × 15 cm C18 PepMap column (Dionex)] via a 10 μm inner diameter PicoTip (New Objective). A gradient from 2% ACN and 0.1% formic acid (v/v) to 50% ACN and 0.1% formic acid (v/v) in 70 min was applied at a flow rate of 300 nL/min. The ion spray potential was set to 2200–5500 V, the nebulizer gas to 18, and the interface heater to 150 °C. MRM transitions specific for drug-modified peptides were selected as follows: the mass/charge ratio (m/z) values were calculated for all possible peptides containing a histidine or cysteine residue together with a fragment mass of 265 amu corresponding to the cleaved NVP molecule, or with a characteristic b or y fragment ion. MRM transitions were acquired at unit resolution in both the Q1 and Q3 quadrupoles to maximize specificity; they were optimized for collision energy and collision cell exit potential, and the dwell time was 50 ms. MRM survey scans were used to trigger enhanced product ion MS/MS scans of drug-modified peptides, with Q1 set to unit resolution, dynamic fill selected, and dynamic exclusion for 20 s. Total ion counts were determined from a second aliquot of each sample analyzed by conventional LC–MS and used to normalize sample loading on column. Relative quantification was performed by comparing the relative normalized intensity of MRM peaks for each of the modified residues across samples.

## RESULTS

**Quantification of NVP Metabolites in Patient Plasma.** To explore the relationship between the formation of NVP reactive metabolites and protein adducts in patients, 12-OH-NVP and 12-sulfoxyl-NVP were quantified in plasma by LC–MS. A synthetic 12-OH-NVP standard (Figure S1a of the Supporting Information) was used to construct a standard curve (Figure S1b of the Supporting Information). 12-OH-NVP and NVP were detected in blood from all patients recruited in this study (Figure S1c,d of the Supporting Information). The concentration varied from patient to patient, ranging from 158.5 to 621.1 ng/mL (Table 2). In contrast, the sulfate of 12-OH-NVP was not detectable in any of the samples, which was most likely because of hydrolysis during the heat inactivation process.

**In Vitro Modification of HSA by 12-Sulfoxyl-NVP.** To determine the chemical basis of NVP protein binding, HSA was incubated with 12-sulfoxyl-NVP at drug-protein molar ratios of

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**Table 2. Details of Patients Who Participated in This Study**

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<tr>
<th>patient</th>
<th>age</th>
<th>dose (mg)</th>
<th>frequency (h)</th>
<th>time postdose (h)</th>
<th>NVP concentration (ng/mL)</th>
<th>12-OH-NVP concentration (ng/mL)</th>
<th>NVP 12-sulfate</th>
<th>protein adducts detected</th>
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<td>400</td>
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Figure 1. continued
The cysteine residue is rendered as a red sphere. Showing the NVP binding sites detected in vitro. The protein is rendered as ribbons; modified histidine residues are rendered as blue spheres, and the cysteine residue is rendered as a red sphere.

Figure 1. MS/MS analysis of NVP-modified HSA peptides identified in vitro and in patients. (a) MS/MS spectra of the trypic RH*PYFYAPELFFAK peptide modified by NVP at His146 marked with an asterisk. Characteristic fragment ions from the NVP group are circled. (b) MS/MS spectra of the ALVLIAQYLQQC*PEDHVK trypic peptide modified by NVP at Cys34 marked with an asterisk. (c) MS/MS spectra of the NVP-modified His146 HSA peptide detected in a patient. (d) Semiquantitative analysis of NVP-modified His146 showing a linear relationship between the drug concentration and the normalized ion count for the modified peptide. (e) Three-dimensional structure of HSA showing the NVP binding sites detected in vitro. The protein is rendered as ribbons; modified histidine residues are rendered as blue spheres, and the cysteine residue is rendered as a red sphere.

1:1 to 100:1. LC−MS/MS analysis of the trypic digests revealed four NVP-modified peptides after incubation at the highest concentration of 12-sulfoxyl-NVP, but only one modified peptide (145RH*PYFYAPELFFAK159) with a drug:protein ratio of 1:1 (Table 1). Three NVP-modified peptides contained a histidine residue, namely, His146, His242, and His338. Figure 1a shows a representative MS/MS spectrum for a triply charged ion at m/z 721.7, corresponding to the trypic peptide 145RHPYFYAPELFFAK159 with an additional mass of 264 amu. The peptide sequence was confirmed by a product ion spectrum that generated partial singly charged y and b series ions. The modification was confirmed by b2* (peak at m/z 558.2) and b4* (peak at m/z 818.5), with addition of 264 amu, indicating that NVP binds to His146. The immunion ion of histidine with mass adduction of 264 amu (peak at m/z 374) further confirmed the modification of His146. The mass spectra of other NVP-modified peptides are shown in the Supporting Information. In addition to histidine modification, 12-sulfoxyl-NVP was also found to form an adduct with Cys34 of HSA. Modification of Cys34 resulted in a triply charged ion at m/z 899.8, corresponding to the 25ALVLIAQYLQQCPEDFVK peptide with an additional mass of 264 amu. The sequence of the peptide was confirmed by the MS/MS spectrum shown in Figure 1b, and the cysteine modification was confirmed by the y8 ion corresponding to Cys34 plus 264 amu (m/z 1238.5), followed sequentially by the y9 (m/z 1349.6) and y10 (m/z 1460.5) ions. Cys34 has an extremely low pKₐ and is the only free cysteine residue in HSA; therefore, it is not surprising to find that it was the only one modified by 12-sulfoxyl-NVP.

The modification of HSA by 12-sulfoxyl-NVP was found to be concentration-dependent. A semiquantitative analysis of modification at each site was performed by determining the area under the curve for the extracted ion counts for the modified peptides, followed by normalization of the ion intensity using the total ion count for the sample. There was an approximately linear relationship between the drug:protein ratio and the normalized ion count for each modified peptide (Figure 1d).

NVP-Modified Histidine Residues Were Detected in Patient Serum Albumin. LC−MS/MS analysis was used to analyze the binding of NVP to HSA isolated from the blood of HIV patients receiving NVP at a dose of either 200 mg twice daily or 400 mg once daily for at least 2 weeks. The 145RH*PYFYAPELFFAK159 trypic peptide with a modification at His146 was detected in four of nine patients. The peptide sequence was confirmed by LC−MS/MS as shown in Figure 1c. The modification was confirmed by the presence of the b2* (peak at m/z 558.2) and b4* (peak at m/z 818.5) ions, with addition of 264 amu, indicating that NVP binds to His146 (Figure 1c).

In Vitro Modification of GSTP by 12-Sulfoxyl-NVP. To further characterize the 12-sulfoxyl-NVP protein binding adducts, GSTP contains several reactive cysteine residues was chosen as a model protein. His-tagged GSTP was expressed in transformed BL21 cells and purified by isolation on nickel beads. The protein was then characterized by SDS−PAGE and MALDI-TOF mass spectrometry. A band with a molecular mass of approximately 23−25 kDa was detected following Coomassie blue staining (Figure S2a of the Supporting Information) and was identified as GSTP following trypic digestion and peptide mass fingerprinting (Figure S2b,c of the Supporting Information).

His-GSTP captured on nickel beads was exposed to a range of molar ratios of 12-sulfoxyl-NVP to protein (1:1 to 100:1). LC−MS/MS analysis of tryptic digests revealed the unmodified Cys47-containing 45ASCILYGQLPK34 peptide (Figure 2a, +2 ion at m/z 540.3). Modification of Cys47 by 12-sulfoxyl-NVP resulted in a doubly charged peptide ion at m/z 672.3 that eluted at 23 min, equating to a mass increase of 264 amu compared to the mass of the unmodified peptide that eluted at 18 min. The sequence of the NVP-modified 45ASCILYGQLPK34 peptide was confirmed by the MS/MS spectrum shown in
The modification was confirmed by the presence of the b3* ion (m/z 526.1) with the addition of 264 amu followed sequentially by the b4* (m/z 639.2), b5* (m/z 802.2), b6* (m/z 859.6), and b7* (m/z 987.5) ions, indicating binding of NVP to Cys47. The immonium ion of Cys with a mass addition of 264 amu (m/z 338) provided further evidence of the modification of Cys47.

The modification of Cys47 was detectable at the lowest drug:protein molar ratio (1:1), while the extent of the modification increased when the drug:protein molar ratio was increased to ≥10:1 (Figure 2c). A linear relationship between the concentration of 12-sulfoxyl-NVP and the relative levels of the modified peptide was observed (Figure 2c). Human GSTP contains four cysteine residues (Cys14, -47, -101, and -169), each with a different reactivity (Figure 2d), but Cys47 is the most reactive one (pKₐ of 3.5−4.2). Thus, the modification of Cys47 by 12-sulfoxyl-NVP was as expected.

**DISCUSSION**

Treatment of HIV-1 infections with NVP is associated with skin and/or liver injury. It has been hypothesized that bioactivation of NVP and subsequent binding of reactive
metabolites to protein plays an important role in the pathogenesis. Although NVP−mercapturate metabolites and NVP−Hb adducts have been detected in patients, evidence of the exact binding interactions in humans is lacking. In this study, we have used model proteins to define the chemical basis of NVP binding by mass spectrometry and have confirmed that 12-sulfoxyl-NVP selectively binds to histidine and cysteine residues. More importantly, we have shown that NVP generates albumin adducts in patients via formation of reactive metabolites.

NVP formed covalent adducts in HSA isolated from the blood of HIV patients through the selective modification of histidine residues. As liver is the primary site for metabolism, liver proteins will be the main targets for the reactive NVP metabolites. Thus, it is possible that NVP−HSA conjugates are formed in the liver and then circulated to plasma. Alternatively, NVP metabolites formed in liver may translocate from liver to blood and subsequently bind to plasma protein. Either way, because of the difficulties in detecting NVP reactive metabolites in patients and determining which metabolites bind to proteins, NVP−HSA adducts may serve as an important biomarker for monitoring the exposure to NVP reactive metabolites.

In the Brown Norway rat model, the incidence of skin rash was found to be correlated with the level of 12-OH-NVP but not with 2-OH-NVP, 3-OH-NVP, or 4-COOH-NVP.7 It is worth noting that 12-OH-NVP is not protein reactive per se, and bioactivation is a prerequisite for the formation of covalent adducts in skin. One leading bioactivation pathway being hypothesized is that the circulating 12-OH-NVP is converted into a reactive 12-sulfoxyl-NVP intermediate by sulfotransferase present in skin.7 The adducts formed by 12-sulfoxyl-NVP may serve as an antigen and eventually trigger immune responses that lead to tissue injury. However, no experimental data have shown that 12-sulfoxyl-NVP forms protein adducts.

To probe the chemical basis of binding of 12-sulfoxyl-NVP to proteins, in vitro studies were performed using HSA and GSTP as model proteins because both proteins are reactive targets for numerous electrophilic compounds.25 In this study, 12-sulfoxyl-NVP was found to form protein adducts selectively with histidine and cysteine residues in HSA, and a cysteine residue in GSTP. No lysine adducts were detected even when a 100-fold excess of 12-sulfoxyl-NVP was used. HSA has long been known to exhibit great affinity for many ligands26 and act as an endogenous target and/or quencher for numerous electrophilic compounds such as penicillins, α,β-unsaturated aldehydes, and acyl glucuronides.27−31 Adducts could be formed in HSA with various nucleophilic amino acid residues, including lysine, cysteine, and histidine. In particular, Cys34 and His146 have been identified as major nucleophilic sites in HSA for a variety of electrophilic compounds.28,32,33 In this study, three NVP-modified histidine residues in HSA were

![Scheme 2. Possible Pathways of Binding of 12-Sulfoxyl-NVP to Proteins](image-url)
identified when HSA was incubated with high concentration of 12-sulfoxyl-NVP; however, His146 was the only modified residue when a drug:protein ratio of 1:1 was used, indicating His146 is the primary binding site for 12-sulfoxyl-NVP. The preference of the drug for different amino acid residues in HSA is known to be driven at least in part by the noncovalent interaction of the protein, with the initial noncovalent interaction positioning the drug in favorable orientations to facilitate covalent binding with potential amino acid residues. Thus, the three-dimensional shape of the drug, as well as its inherent chemical reactivity, will determine the selectivity of covalent binding as demonstrated in the literature.39,28,34−36 The reaction of 12-sulfoxyl-NVP with proteins may occur through direct nucleophilic substitution (pathway A in Scheme 2). However, previous studies have shown that 12-sulfoxyl-NVP had low reactivity toward nucleophiles.24 Thus, it is possible that its reaction with HSA may proceed by a different mechanism. Considering possible neighboring groups in the microenvironment of the protein, we therefore proposed a concerted elimination–addition mechanism (pathway B). In this respect, it is plausible that histidine residues or some other basic residues in protein act as a general base, abstracting the proton from amide and promoting the elimination of the sulfoxyl group to form a quinone methide intermediate.

Previous studies have demonstrated that patients who have higher levels of 12-OH-NVP in blood were at high risk of skin injury. However, in other studies, a relationship between the plasma concentrations of NVP (or its oxidative metabolites) and NVP-induced tissue injury was not observed.57 Interestingly, this study also reveals no correlation between the plasma concentration of NVP or 12-OH-NVP and the formation of protein adducts. As the plasma concentrations of NVP (metabolites) do not reflect the quantity of antigen formed in patients, it is therefore necessary to establish the quantitative relationship between antigen formation and the observed adverse reaction(s).

In summary, in our work we show that 12-sulfoxyl-NVP binds selectively to histidine and cysteine residues in proteins in vitro. Furthermore, the same adducts were detected in HSA isolated from the blood of NVP-treated patients. Despite this, the relationship between adduct formation and disease pathogenesis is yet to be defined. Apart from covalent binding, other disease status or host factors may play an important role. Recent studies demonstrated that polymorphisms in CYP2B6 and MHC class I alleles were associated with a high risk for NVP-induced skin rash, whereas MHC class II alleles (e.g., HLA-DRB1*01 and HLA-DQB1*05) were found to be associated with NVP-induced hepatic reactions.38,39 Therefore, an improved understanding of NVP-mediated tissue injury requires a multidisciplinary approach that relates drug metabolism, drug−protein binding, and immune function to clinical outcome.

ASSOCIATED CONTENT

Supporting Information
Figures S1−S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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ABBREVIATIONS

NVP, nevirapine; HIV-1, human immunodeficiency virus type 1; HSA, human serum albumin; GSTP, glutathione S-transferase pi; ADRs, adverse drug reactions; 12-OH-NVP, 12-hydroxyl-NVP; Hb, hemoglobin; MS, mass spectrometry; LB, lysisogeny broth; LC−MS/MS, liquid chromatography−tandem mass spectrometry

REFERENCES


