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Evidence for nevirapine bioactivation in man: Searching for the first step in the mechanism of nevirapine toxicity

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ABSTRACT

Despite its efficacy, including in the prevention of vertical transmission, the antiretroviral nevirapine is associated with severe idiosyncratic hepatotoxicity and skin rash. The mechanisms underlying nevirapine toxicity are not fully understood, but drug bioactivation to reactive metabolites capable of forming stable protein adducts is thought to be involved. This hypothesis is based on the paradigm that drug reactive metabolites have the potential to bind to self-proteins, which results in drug-modified proteins being perceived as foreign by the immune system. The aim of the present work was to identify hemoglobin adducts in HIV patients as biomarkers of nevirapine haptenation upon bioactivation. The ultimate goal is to develop diagnostic methods for predicting the onset of nevirapine-induced toxic reactions.

All included subjects were adults on nevirapine-containing antiretroviral therapy for at least 1 month. The protocol received prior approval from the Hospital Ethics Committees and patients gave their written informed consent. Nevirapine-derived adducts with the *N*-terminal valine of hemoglobin were analyzed by an established liquid chromatography–electrospray ionization-tandem mass spectrometry method and characterized on the basis of retention time and mass spectrometric fragmentation pattern by comparison with adduct standards prepared synthetically. The nevirapine adducts were detected in 12/13 patient samples, and quantified in 11/12 samples (2.58 ± 0.8 fmol/g of hemoglobin).

This work represents the first evidence of nevirapine-protein adduct formation in man and confirms the ability of nevirapine to modify self-proteins, thus providing clues to the molecular mechanisms underlying nevirapine toxicity. Moreover, the possibility of assessing nevirapine-protein adduct levels has the potential to become useful for predicting the onset of nevirapine-induced adverse reactions.

1. Introduction

Since 1996, the inception of combined antiretroviral therapy (cART) has changed the prognosis of human immunodeficiency virus (HIV) infection from a lethal disease to a chronic condition in properly medicated patients. However, HIV-positive individuals still face obstacles associated with chronic treatment, including adherence to a daily administration schedule, loss of therapeutic efficacy and drug-induced toxicity. Moreover, increased concerns are emerging, regarding the long-term adverse effects of cART (Powles et al., 2009), that have been linked to the premature onset of aging observed in HIV-infected patients (Deeks, 2009). Powles et al. (2009) showed an epidemiologic association between the chronic treatment with non-nucleoside reverse transcriptase inhibitors (NNRTI) and a higher incidence of non-AIDS-defining cancers.

Abbreviations: cART, combined antiretroviral therapy; CID, collision-induced dissociation; GSH, glutathione; Hb, hemoglobin; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; 12-OH-NVP, 12-hydroxy-nevirapine; LC–ESI-MS/MS, liquid chromatography–electrospray ionization-tandem mass spectrometry; MHC, major histocompatibility complex; NNRTI, non-nucleoside reverse transcriptase inhibitor; NVP, nevirapine.

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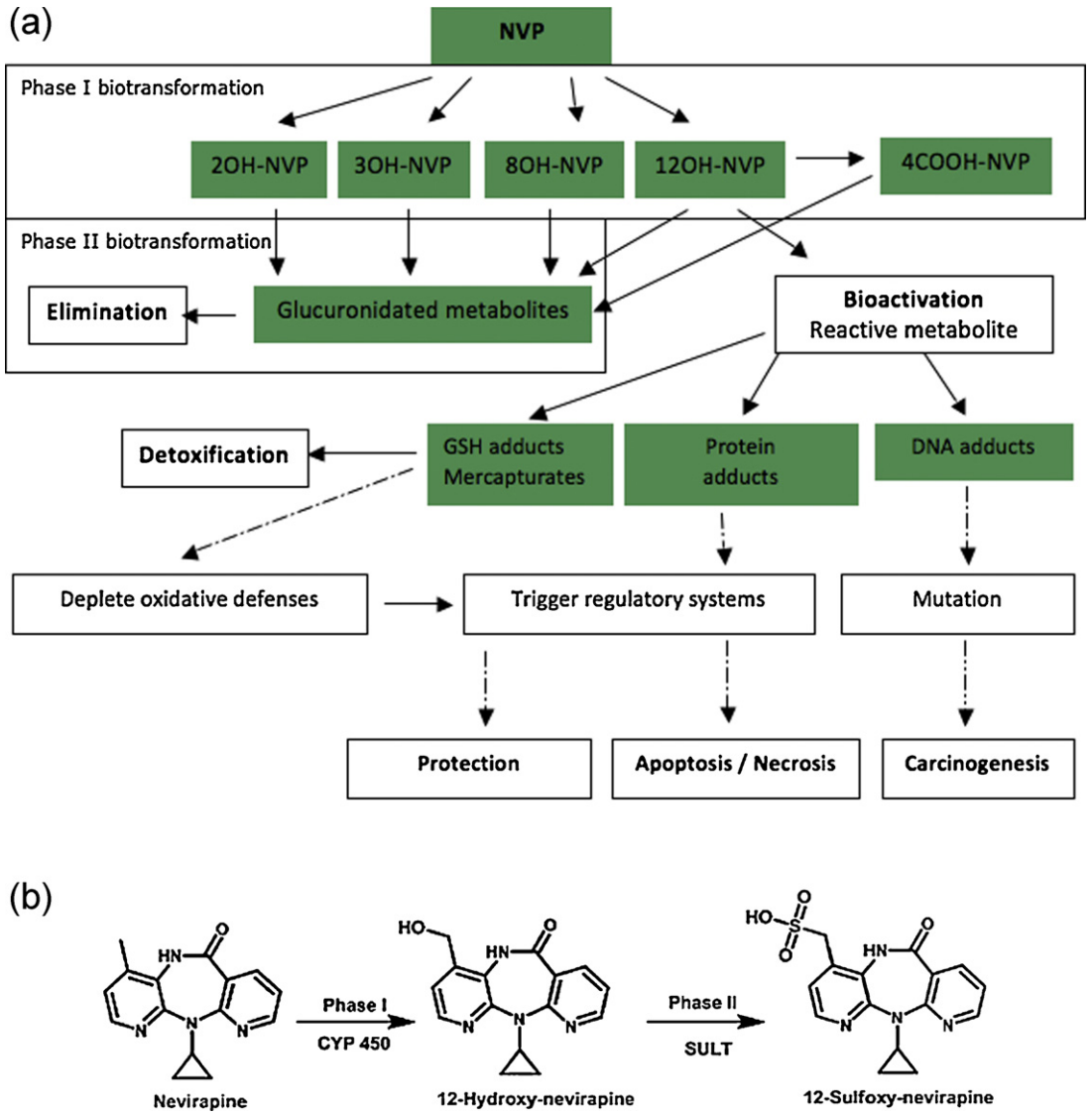

Fig. 1. Metabolic pathways of NVP. (a) Metabolic pathways of NVP, involving Phase I oxidation and subsequent Phase II glucuronidation. The proposed pathway for *in vivo* bioactivation of 12-OH-NVP to electrophilic species involved in the formation of covalent adducts thought to be at the onset of NVP-induced toxic events is also shown. (b) Conversion of NVP to 12-OH-NVP *via* CYP 450-mediated Phase I metabolism and subsequent Phase II activation to 12-sulfoxy-NVP. CYP 450, cytochrome P450; SULT, sulfotransferase.

Nevirapine (NVP, Fig. 1) was the first NNRTI approved by the US Food and Drug Administration for the treatment of HIV type-1 infection, as part of cART (Food and Drug Administration, 1996). Currently, NVP is still the most prescribed NNRTI in the world, and remains the most prescribed antiretroviral in countries with limited economic resources, partly due to its low cost (Ades et al., 2000; Lockman et al., 2007). The favorable metabolic profile is one of the therapeutic advantages of NVP (Ruiz et al., 2001; Clotet et al., 2003), rendering it suitable for use in patients with diabetes, dyslipidemia or metabolic syndrome comorbidities. Moreover, the low incidence of adverse drug reactions in the central nervous system (Medrano et al., 2008), allows NVP use in the context of psychiatric disorders or addiction to narcotic drugs. Furthermore, one of the most relevant benefits of NVP is its efficacy in the prevention of mother-to-child transmission of the HIV-1 infection, with the drug being commonly prescribed to pregnant women and their children (Ades et al., 2000; Medrano et al., 2008; Perinatal HIV Guidelines Working Group, 2009). However, NVP use has been associated with restrictive idiosyncratic hepatotoxicity and cutaneous hypersensitivity (Taiwo, 2006; Medrano et al., 2008; De Lazzari et al., 2008). Severe, life-threatening, and even fatal cases of hepatotoxicity (Cattelan et al., 1999) have been described.

Concern about NVP adverse reactions arose following case reports of liver failure in individuals on post-exposure prophylaxis (Johnson and Baraboutis, 2000; Centers for Disease Control and Prevention, 2001) and in asymptomatic HIV-infected patients with well preserved immunity, administered NVP-containing first line cART (Cattelan et al., 1999; Stern et al., 2003). These adverse reactions are more frequent during the first 6 weeks of treatment and women (including those who are pregnant and of Asian ethnicity) seem to be at increased risk of developing NVP-related toxicities (Ho et al., 1998; Antinori et al., 2001; Bersoff-Matcha et al., 2001). Given that immunocompetence is regarded as an additional risk factor for the development of these reactions (De Lazzari et al., 2008), it is recommended that the drug should be initiated in cART-naïve-women with a CD4 cell count below 250 cells/mm^3 and below 400 cells/mm^3 in men (Thompson et al., 2010). For the same reason, NVP is not recommended as part of post-exposure