Toxicology Letters 224 (2014) 416–423

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Mini review

Bioactivation to an aldehyde metabolite—Possible role in the onset of toxicity induced by the anti-HIV drug abacavir

Nádia M. Grilo[a], Catarina Charneira[b], Sofia A. Pereira[a,**], Emília C. Monteiro[a], M. Matilde Marques[b], Alexandra M.M. Antunes[b,*]

[a] Centro de Estudos de Doenças Crónicas (CEDOC), NOVA Medical School, Universidade NOVA, 1169-056 Lisboa, Portugal
[b] Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, 1049-001 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 11 September 2013
Received in revised form 25 October 2013
Accepted 28 October 2013
Available online 5 November 2013

Keywords:
Abacavir
Aldehyde metabolite
Drug bioactivation
Protein adducts
Cardiotoxicity
Hypersensitivity reactions

ABSTRACT

Aldehydes are highly reactive molecules, which can be generated during numerous physiological processes, including the biotransformation of drugs. Several non-P450 enzymes participate in their metabolism albeit alcohol dehydrogenase and aldehyde dehydrogenase are the ones most frequently involved in this process. Endogenous and exogenous aldehydes have been strongly implicated in multiple human pathologies. Their ability to react with biomacromolecules (*e.g.* proteins) yielding covalent adducts is suggested to be the common primary mechanism underlying the toxicity of these reactive species.

Abacavir is one of the options for combined anti-HIV therapy. Although individual susceptibilities to adverse effects differ among patients, abacavir is associated with idiosyncratic hypersensitivity drug reactions and an increased risk of cardiac dysfunction. This review highlights the current knowledge on abacavir metabolism and discusses the potential role of bioactivation to an aldehyde metabolite, capable of forming protein adducts, in the onset of abacavir-induced toxic outcomes.

Contents

1. Introduction

Human immunodeficiency virus (HIV) infection is currently considered a chronic disease in developed countries. The drastic change in HIV prognosis that occurred in the last two decades is credited to the indubitable benefits of combined antiretroviral therapy (cART). However, in light of the current therapeutic options, once the HIV-infected patient starts cART the treatment will persist throughout life. Consequently, the choice of appropriate long-term treatment options for an aging population has become a new challenge.

Contrasting with acute cART-induced toxic events, the long-term toxic outcomes remain mostly unknown. However, in a scenario of lifelong cART usage, the potential increase in the incidence of such adverse effects cannot be neglected. In fact, the expected negative impact on clinical outcomes, which will ultimately affect the life quality and expectancy of HIV patients, is an emerging concern. Decreased adherence to cART and a need for

* Corresponding author at: Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisboa, Portugal. Tel.: +351 21 8417627; fax: +351 21 8464455.
** Corresponding author at: Centro de Estudos de Doenças Crónicas (CEDOC), NOVA Medical School, Universidade NOVA, Campo dos Mártires da Pátria, 130, 1169-056 Lisboa, Portugal. Tel.: +351 21 8803000; fax: +351 21 8851920.
E-mail addresses: sofia.pereira@fcm.unl.pt (S.A. Pereira), alexandra.antunes@tecnico.ulisboa.pt (A.M.M. Antunes).

0378-4274/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2013.10.036

additional clinical interventions are expected to be further consequences of these long-term toxic effects.

Currently, first-line cART is always composed of at least three drugs, two of which are nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs). Abacavir (ABC, Scheme 1) is one of the anti-HIV drugs of this class and ABC-based regimens have a significant role in HIV-treatment guidelines, due to the antiretroviral efficacy of the drug and its availability in one-pill fixed-dose combinations (Thompson et al., 2010; EACS, 2012). Consequently, the widespread prescription of this drug prompts concerns about ABC-induced toxic outcomes.

2. Abacavir pharmacology

ABC is a 2′-deoxyguanosine nucleoside analogue with anti-HIV type-1 and type-2 activities (Daluge et al., 1997; Hervey and Perry, 2000; Saag et al., 2008). The drug is recommended to both adults and children and is commercially available as ABC sulphate alone (*Ziagen*®) or as part of two-drug (ABC/lamivudine (3TC; *Kivexa*®) and three-drug (ABC/3TC/zidovudine; *Trizivir*®) pill combinations. The recommended oral dose of ABC for adults is 600 mg daily, administered either as 300 mg twice daily or 600 mg once daily.

As a pro-drug, the pharmacological effect of ABC is only achieved upon intracellular conversion to its active metabolite, carbovir thriphosphate (CBV-TP; Scheme 1) (Faletto et al., 1997). This biotransformation occurs *via* a stepwise anabolism, involving enzymes that are not implicated in the metabolism of the other NRTIs (Faletto et al., 1997). ABC is initially phosphorylated to abacavir 5′-monophosphate (ABC-MP) by adenosine phosphotransferase, followed by deamination *via* cytosolic enzymes to form carbovir 5′-monophosphate (CBV-MP). Subsequent phosphorylations originate carbovir diphosphate (CBV-DP) and triphosphate (CBV-TP), *via* guanylate kinase and nucleoside diphosphate kinase activities, respectively. The pharmacologically active metabolite, CBV-TP, competes with the endogenous 2′-deoxyguanosine triphosphate for incorporation into the viral nucleic acid and following incorporation it terminates the DNA chain extension (Hervey and Perry, 2000; Yuen et al., 2008).

ABC is readily absorbed upon oral intake (Daluge et al., 1997) and has an absolute oral bioavailability in adults of about 83% (Chittick et al., 1999). The maximal plasma concentration (4.7 μg/mL) is reached in less than 1 h after intake (Weller et al., 2000). The extent of ABC binding to plasma proteins is about 50% and the drug readily distributes into erythrocytes and penetrates the cerebrospinal fluid (McDowell et al., 1999). While ABC has a short plasma half-life (1.5 h), the half-life of intracellular CBV-TP is longer than 12 h (Daluge et al., 1997; Hawkins et al., 2005).

2.1. Abacavir excretion versus bioactivation

ABC is extensively metabolized in the liver to more hydrophilic, excretable products. Primary ABC biotransformation pathways comprise phase II glucuronidation, yielding the inactive glucuronide metabolite (ABC-glucuronide, Scheme 2), along with phase I oxidation, mediated by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), yielding a carboxylate (ABC-carboxylate). The glucuronide and the carboxylate metabolites are primarily eliminated in the urine, accounting for 66% of the net ABC dose. An additional 15% of the dose is converted into minor metabolites (McDowell et al., 1999).

ABC undergoes bioactivation to reactive aldehyde metabolites. ABC metabolism to ABC-carboxylate involves a two-step oxidation *via* an aldehyde intermediate (unconjugated ABC-aldehyde, Scheme 2), which rapidly tautomerizes to a thermodynamically more stable conjugated ABC-aldehyde. This double bond migration (isomerization), along with the epimerization of the unconjugated ABC-aldehyde, explains the formation of isomers 1 and 2 of the ABC-carboxylate upon incubation of ABC with different human alcohol dehydrogenases (ADH) (Walsh et al., 2002) and in subcellular fractions of EBV-transformed human B-cells incubated with ABC (Bell et al., 2013a).

The first indirect mass spectrometric-based evidence for the formation of the reactive ABC metabolites was provided a decade ago by Walsh et al. (2002), upon *in situ* trapping of an aldehyde derivative with methoxylamine in incubations of [^{14}C]ABC with different human ADHs. These authors also proposed that ADH-mediated ABC metabolism could result in covalent binding to human serum albumin. However, synthetic standards of the two isomeric ABC-derived aldehydes, prepared in our laboratory by oxidation of ABC under *Swern* conditions in tetrahydrofuran, only became available in 2011 (Charneira et al., 2011). This enabled a comprehensive study of the reactivity of the two isomeric aldehydes toward bionucleophiles. This synthetic approach allowed definitive evidence for the isomerization of the unconjugated ABC-aldehyde to the conjugated ABC-aldehyde, both in buffer solutions and in incubations of ABC with rat liver cytosol. In addition, it indicated that the conjugated ABC-aldehyde is the electrophilic metabolite capable of reacting with proteins, either through Schiff base formation with nitrogen-based bionucleophiles (*e.g.* lysine or the *N*-terminal valine of hemoglobin), or upon *Michael*-type 1,4-addition of cysteine residues (Fig. 1). Very recently, in a follow-up of this work, we demonstrated for the first time that this bioactivation-haptenation pathway occurs in man (Grilo et al., 2013), by identifying the ABC-valine *Edman* adduct (Fig. 1) in the hemoglobin of HIV-infected patients treated with abacavir. Our previous efforts, concerning not only the synthesis of ABC-protein adduct standards (Charneira et al., 2011) but also the development of adequate mass spectrometry-based methodologies to assess these adducts at levels expected *in vivo* (Charneira et al., 2012), were pivotal to this achievement. The recent study by Bell et al. (2013a), who demonstrated that ABC undergoes oxidative bioactivation in subcellular fractions of human antigen presenting cells (APCs), provided further support to the hypothesis that protein-reactive aldehyde metabolites may be generated in immune cells *in vivo*.

Taking into consideration the frequent involvement of ADH in the oxidation of aldehydes to carboxylic acids (Olson et al., 1996), Walsh et al. (2002) screened *in vitro* the metabolic capability of several human isoforms of ADH (then designated αα, β1β1, β2β2, γ2γ2, π, σ and χ) for the transformation of ABC into its aldehyde and carboxylic acid metabolites. While an ABC-derived aldehyde was trapped in incubations with the αα and γ2γ2 isoforms, only the αα isoform effected the two sequential oxidation steps to the ABC-carboxylate and its two isomers, mirroring the *in vitro* cytosolic profiles. More recently, the expression of a number of ADHs of classes I (ADH1A, ADH1B, and ADH1C), II (ADH4), III (ADH5), and V (ADH6) was tested in several subsets of immune cells from human peripheral blood (Adam et al., 2012) but with the exception of ADH5, that was found to be ubiquitously expressed, no relevant ADH activity was detected. This is in line with an earlier report that the mRNA of ADH5 is moderately expressed in human peripheral leukocytes, while the mRNAs of other ADHs are either not detected (ADH1A and ADH1B) or present at very low levels (ADH1C and ADH4) (Nishimura and Naito, 2006). Likewise, the recent study by Bell et al. (2013a) in subcellular fractions of human B-cells found relatively high levels of the mRNA of ADH5 but not of the class I ADHs reported (Walsh et al., 2002) to be involved in ABC oxidation in human liver cytosol. Contrasting with the observed inhibition of oxidative ABC metabolism in human cytosolic incubations (Walsh et al., 2002), the ADH inhibitor 4-methylpyrazole (4MP) did not cause any reduction in the reactivity of ABC-specific T-cell clones