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Stability behaviour of antiretroviral drugs and their combinations. 4: Characterization of degradation products of tenofovir alafenamide fumarate and comparison of its degradation and stability behaviour with tenofovir disoproxil fumarate

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ABSTRACT

In this study, stress degradation behaviour of tenofovir alafenamide fumarate (TAF), a novel prodrug of tenofovir, was investigated and compared with currently used prodrug congener, tenofovir disoproxil fumarate (TDF), whose intrinsic stability was reported by us earlier [14]. Also, pH stability and gastrointestinal stability studies were conducted on both the drugs. High performance liquid chromatography (HPLC) analysis of stressed samples of TAF revealed formation of six degradation products (DPs) against twelve characterized earlier in the case of TDF (RSC Adv. 5(2015) 96117-96129). Like TDF, characterization of DPs of TAF was done by using sophisticated hyphenated liquid chromatography-high resolution mass spectrometry (LC-HRMS) and multistage mass spectrometry (MS^n) tools. pH-stability studies between pH 1.2–10 revealed greater stability of TAF, except in acidic conditions, where TAF was degraded extensively. Investigation of gastrointestinal stability in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and fed state simulated gastric fluid (FeSSGF) suggested that TAF must be administered in fed state, as the drug was practically stable in FeSSGF as compared to extensive loss at acidic pH and in SGF.

1. Introduction

Tenofovir is a Biopharmaceutical Classification System (BCS) Class III drug [1]. It has very low oral bioavailability, which is attributed to deprotonation of two hydroxyl groups of the drug that lead to two negative charges on the molecule. Due to its ionic state, the drug doesn't permeate across epithelial membrane at physiological pH. Thus tenofovir is supplied in its prodrug forms to increase its oral bioavailability, which is done by masking its free hydroxyl groups [2].

The first prodrug of tenofovir, i.e., tenofovir disoproxil fumarate (TDF) (9-[(*R*)-2-[[bis[[(isopropoxycarbonyl)oxy]methoxy]-phosphinyl]-methoxy]propyl]adenine fumarate) got its approval for clinical use for human immune deficiency virus (HIV) treatment in 2001, and for the hepatitis B virus (HBV) treatment in 2008. In 2015, a new prodrug of tenofovir, i.e., tenofovir alafenamide fumarate (TAF, propan-2-yl N-[(*S*)-({[(2*R*)-1-(6-amino-9H-purin-9-yl)propan-2-yl]-oxy}methyl)(phenoxy)phosphoryl]-L-alaninate, (2E)-but-2-enedioate (2:1)) was approved for clinical use [3]. The reason for introduction of new prodrug of tenofovir has been the requirement of high dose of 300 mg of TDF for eliciting the drug's action due to low bioavailability and quick hydrolysis in plasma. Practically, a very low amount of TDF reaches the target cells, where it again is slowly hydrolysed to the parent drug by serum and gut hydrolases (extracellular), carboxyesterases and phophodiesterases (intracellular). As shown in Fig. 1, TAF requires much lesser dose of 10 mg in the presence of a pharmacoenhancer or just 25 mg on its own, as it has much higher stability in plasma as compared to TDF. Therefore, it shows higher levels in lymphocytes, which are the primary site for its metabolism along with macrophages, hepatocytes, etc. [4]. Due to potent antiviral activity and its low dose, TAF even shows decreased adverse effects like nephrotoxicity and loss in bone mineral density [5–12].

Realizing the benefits of TAF over TDF, US FDA has very recently added following combination regimens in the Orange Book Cumulative Supplement of 5 May 2016 under approved drug products with therapeutic equivalent evaluations: Genvoya (cobicistat, elvitegravir, emtricitabine and TAF), Odefsey

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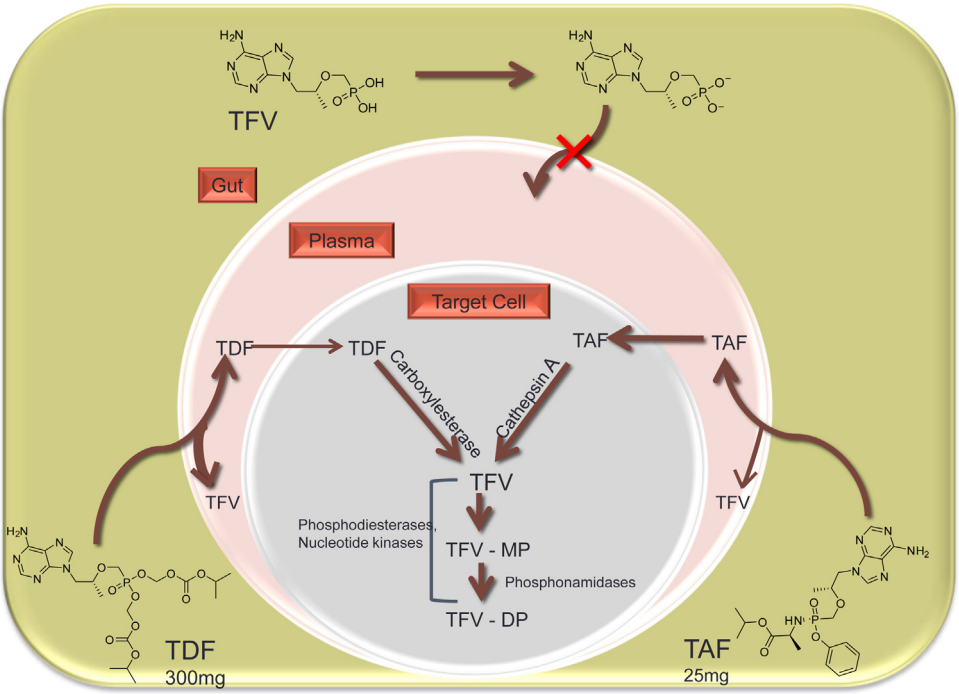

Fig. 1. Passage of tenofovir (TFV), tenofovir disoproxilfumarate (TDF) and tenofovir alafenamide fumarate (TAF) through membrane and their conversion to active metabolite tenofovir diphosphate (TFV-DP) through an intermediate tenofovir monophosphate (TFV-MP).

(emtricitabine, rilpivirine hydrochloride and TAF) and Descovy (emtricitabine and TAF) [13]. Thus TAF is likely to replace TDF for the treatment of HIV infection and even take over TDF in commerce. Therefore, it was of our interest to investigate degradation and stability profile of TAF in comparison to TDF, whose intrinsic stability profile was reported by us previously [14]. The study involved sophisticated mass spectrometry tools like liquid chromatography-high resolution mass spectrometry (LC-HRMS) and multistage mass spectrometry (MS^n) [15]. Interesting results were obtained, which are reported herein.

2. Experimental

2.1. Chemicals and reagents

TAF and TDF were obtained as gratis samples from Mylan Laboratories (Hyderabad, India). Sources of chemicals of analytical reagent grade were as follows: sodium hydroxide and sodium acetate (Ranbaxy Laboratories, SAS Nagar, India), hydrochloric acid (LOBA Chem. Pvt. Ltd., Mumbai, India), boric acid (Qualigens Fine Chemicals, Mumbai, India), hydrogen peroxide (H_2O_2) (Merck Specialities Private Limited, Mumbai, India), acetic acid (Central Drug House (P) Ltd., New Delhi, India), sodium chloride (s d Fine-Chem limited, Mumbai, India) and long-life, heat-treated and homogenized milk (UHT-milk) containing 3% fat (Amul taaza, Palanpur, India). HPLC grade methanol was purchased from Rankem (Maharashtra, India). Buffer salts and all other chemicals were of analytical reagent grade. Ultrapure water was obtained from ELGA water purification unit (Bucks, England).

2.2. Apparatus and equipments

Stability studies under accelerated conditions were carried out in humidity (KBF720, WTC Binder, Tuttlingen, Germany) chamber set at 40 °C/75% RH. The photostability chamber (KBWF 240, WTC Binder, Tuttlingen, Germany) was operated at 25 °C and at ambient humidity, and it was equipped with an illumination bank on inside top consisting of a combination of three UV (OSRAM L18W/73) and three white fluorescent (Philips, Trulite 18W/86) lamps, as recommended under option 2 by ICH guideline Q1B [16]. A Lux meter (model ELM 201, Escorp, New Delhi, India) and a near UV radiometer (model 206, PRC Krochmann GmbH, Berlin, Germany) were used to measure visible illumination and near UV energy, respectively. A Dri-Bath (Thermolyne, Dubuque, IA, USA) was used for solid state thermal stress studies.

pH/Ion analyzer (MA 235, Mettler Toledo, Schwerzenbach, Switzerland) was used to check pH of all the solutions. Other equipments used were sonicator (Power Sonic 510, Hwashin Technologies, Seoul, Korea), precision analytical balance (Denver Instruments, Gottingen, Germany) and digital shaking bath (SW 21, Julabo, Seelbach, Germany). Centrifuge and auto pipettes were from Eppendorf (Hamburg, Germany).

The separation of TAF and its degradation products (DPs) was achieved using LC-2010C HT liquid chromatograph (Shimadzu, Kyoto, Japan), equipped with a SPD-M20A prominence diode array detector. LC column used for the separation was Pursuit XRs (5 μ, C18, 250 × 4.6 mm i.d.) from Varian Inc., Lake Forest, CA, USA. For pH and gastrointestinal stability studies, the column used was Luna (5 μ, C18, 150 × 4.6 mm i.d.) procured from Phenomenex (Hyderabad, India).

LC-HRMS data were generated using LC-ESI-Q-TOF-MS, in which LC part consisted of 1100 series HPLC (Agilent Technologies, Waldbronn, Germany) comprising of an on-line degasser (G1379A), binary pump (G1312A), auto injector (G1313A), column oven (G1316A) and PDA detector (G1315B). The MS portion consisted of micrOTOF-Q spectrometer (Bruker Daltonics, Bremen, Germany). System control and data acquisition was done by Hystar software (version 3.1) from the same source. The calibration solution used was 5 mM sodium formate. The calibration was done with respect to accurate masses of sodium formate clusters having a general formula $Na(HCOONa)_n$ with the mass of m/z 90.9766 + increments of 67.9874 Da. Multistage (MS^n) experiments were carried out on LTQ-XL-MS 2.5.0 (Thermo, San Jose, CA, USA) mass spectrometer.