Minireviews

Factors Contributing to the Antiviral Effectiveness of Tenofovir

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ABSTRACT

Over 1 million people in the United States are living with human immunodeficiency virus (HIV), which may progress to AIDS. The use of antiviral therapy has successfully controlled the rate of viral growth in patients. Antiviral agents improve the quality of life and reduce the potential for spreading HIV; HIV is currently considered a chronic disease provided patients are compliant with their antiviral medications. Tenofovir is a nucleoside transcriptase inhibitor that prevents viral replication and is approved for treatment of HIV and chronic hepatitis B infection. Tenofovir is an antiretroviral drug used alone and in combination with other nucleoside reverse-transcriptase inhibitor agents to lower viral load in HIV patients. Tenofovir is administered as a prodrug to increase bioavailability. The prodrug forms of tenofovir are tenofovir

disoproxil fumarate, approved in 2001, and tenofovir alafenamide, approved in 2016. Tenofovir is extensively used in controlling HIV, as it is administered once daily, allowing for good compliance. This minireview discusses the impact of food, age, and drug transporters on tenofovir absorption and clearance. The changes in dosing that are needed in the presence of renal impairment, which is a common occurrence with HIV chronic disease progression, will also be discussed. The potential special conditions occurring with fixed-combination doses containing tenofovir will also be reviewed, including the use of cobicistat, a cytochrome P450 3A4 inhibitor. The short review also addresses some newer preparations using niosomes to improve tenofovir absorption and delivery to the target cells.

Introduction

Human immunodeficiency virus (HIV) is a virus that decimates the immune system. HIV targets a specific type of white blood cells known as T cells, T lymphocytes, or CD4 cells. A decline in CD4 cell count mediated by HIV leaves the body open to opportunistic infections. At one time, HIV rapidly progressed to AIDS and death, but advancements in highly active antiretroviral therapy have made HIV a chronic condition rather than a death sentence. Tenofovir (TFV), formerly known as 9-(R)-(2-phosphonomethoxypropyl)adenine, is a reverse-transcriptase inhibitor that has potent and selective inhibition of HIV and herpes viruses' reverse transcriptase. TFV is an acyclic nucleotide analog of adenosine 5' monophosphate that is phosphorylated intracellularly by adenylate kinase to its active form, TFV diphosphate (Fig. 1). TFV is effective in cases of nucleoside-resistant HIV infection, making it a first-line agent for treatment of this disease (Miller et al., 2001; Squires et al., 2003).

Despite its efficacy, poor bioavailability was a limiting factor in development of TFV as a clinical agent. Oral administration

of TFV had 18% bioavailability in beagles and 5.3% in monkeys (Shaw et al., 1997; Cundy et al., 1998). Research efforts subsequently focused to improve bioavailability by altering TFV formulation but maintain antiviral activity. Two methyl carbonate esters were added to form the prodrug TFV disoproxil fumarate (TDF), which demonstrated equivalent antiviral activity with bioavailability increased to 30.1% in beagles and 25% in humans (Shaw et al., 1997; Barditch-Crovo et al., 2001). TDF was approved in fall 2001 by the United States Food and Drug Administration (FDA) to treat chronic hepatitis B and HIV in conjunction with other agents (Gilead Sciences, 2001). There are several factors that alter TDF bioavailability and pharmacokinetics, including age, sex, and food intake. This article provides a review of TFV bioavailability as well as factors that have an impact on TFV pharmacokinetics in human and animal models.

Pharmacokinetics and Bioavailability of TDF in Patients

TDF is formulated into a single tablet for oral administration with a standard dose of 300 mg. TDF has a higher lipophilicity than TFV. The LogP, a measure of partition

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ABBREVIATIONS: AUC, area under the curve; BMD, bone mineral density; CI, confidence interval; CL_{CR}, creatinine clearance; COBI, cobicistat; EFV, efavirenz; EVG, elvitegravir; FDA, Food and Drug Administration; FTC, emtricitabine; HIV, human immunodeficiency virus; LPV, lopinavir; MRP, multidrug resistance-associated protein; PBMC, peripheral blood mononuclear cell; P-gP, P-glycoprotein efflux transporter; PrEP, pre-exposure prophylaxis; RPV, rilpivirine; t_{1/2}, half life; TAF, TFV alafenamide; TDF, TFV disoproxil fumarate; TDP, TFV diphosphate; TFV, tenofovir.

Fig. 1. Structure of deoxyadenosine 5' triphosphate (A) and tenofovir diphosphate (B).

coefficient between octanol and water, is 2.1 for TDF compared with -1.6 for TFV. The higher partition coefficient would enable increased intestinal drug absorption. As a result of higher lipophilicity, TDF has an oral bioavailability of 25% in HIV-1-infected patients (Barditch-Crovo et al., 2001). Following absorption, TDF is cleaved in the plasma by esterases (Fig. 2): first to a monoester intermediate, and then to TFV (Shaw et al., 1997; Choi et al., 2008). This hydrolysis is effective enough that TDF is not observed in systemic plasma samples.

After intestinal absorption and plasma hydrolysis, TFV is phosphorylated intracellularly by adenylate kinase to its active form, TFV diphosphate (TDP) (Fig. 3). As a nucleotide analog, TFV diphosphate competes with deoxyadenosine 5' triphosphate (Fig. 1) for incorporation into the growing DNA strand during HIV transcription and blocks the activity of reverse transcriptase, leading to elongation termination (Balzarini et al., 1993; Robbins et al., 1998).

Intravenous administration of 1 mg/kg TDF has a steady state volume of distribution of 813 ml/kg, indicating that tissue distribution is extensive and exceeds total body water. $C_{\max}$ of the same i.v. dose is approximately 2.7 $\mu\text{g/ml}$, and TFV serum concentration decreases in a biphasic manner (Deeks et al., 1998). The measured area under the curve (AUC) was measured AUC of 3.024 $\mu\text{g} \times \text{h/ml}$. The oral bioavailability of TDF was calculated by comparing the AUC for a 300 mg oral dose with the AUC for a 1 mg/kg i.v. dose; oral TDF has 25% bioavailability when compared with an i.v. dose (Barditch-Crovo et al., 2001).

The half life ($t_{1/2}$) for a single i.v. TDF injection was 7 hours compared with 12 hours for a single oral dose of 300 mg TDF

(Deeks et al., 1998; Barditch-Crovo et al., 2001; Hawkins et al., 2005). The intracellular $t_{1/2}$ of TDF is estimated to be 12–50 hours (Robbins et al., 1998). The pharmacologic antiviral activity of TDF and ultimately TFV is proportional to the intracellular concentration in target cells. A single oral dose of TDF to macaques provided almost eightfold higher intracellular levels of the active TFV diphosphate compared with a single s.c. injection of TFV (Durand-Gasselin et al., 2009). Additionally, oral TDF shows greater accumulation in peripheral blood mononuclear cells (PBMCs) when given as an oral prodrug rather than a s.c. injection of TFV to macaques (Durand-Gasselin et al., 2009). The bioavailability in macaques was 31%, which was similar to humans (Barditch-Crovo et al., 2001). These findings suggest that TDF first enhanced bioavailability, but also provided improved delivery to the target cells. The excellent uptake into cells allows for once-daily dosing. Once-daily dosing increases compliance, which will promote the therapeutic success that is important in controlling HIV as a chronic disease.

In general, differences in TDF pharmacokinetics are not apparent when comparing oral and i.v. routes of administration. Oral TDF has equivalent pharmacokinetics to i.v. TDF. Once $C_{\max}$ is reached, serum TFV concentrations decline in a biphasic manner. TFV is excreted 92% unchanged by the kidney and 4.5% unchanged in feces. Renal TFV clearance exceeds calculated creatinine clearances, indicating that TFV is actively secreted by the kidneys (Cundy et al., 1998; Barditch-Crovo et al., 2001; Durand-Gasselin et al., 2009). In vitro transport studies indicate that TFV is a substrate for the multidrug resistance-associated protein (MRP)4, an apical membrane efflux transporter (Ray et al., 2006). TFV was not a substrate for MRP2. These in vitro studies would suggest that active tubular secretion of TFV into the nephron lumen is mediated by MRP4.

Pharmacokinetics and Bioavailability of TDF in Patients with Renal Insufficiency

TDF pharmacokinetics and bioavailability differ in patients with renal insufficiency, which is an important consideration in individuals with HIV. The frequency of acute kidney injury is more common in HIV-infected individuals and can progress to end-stage renal disease. In 2005, it was estimated that in the United States, almost 3000 people had end-stage renal disease related to HIV (Wyatt et al., 2008). A decline in renal

Fig. 2. Conversion of tenofovir disoproxil fumarate to tenofovir by intestinal esterases.

Fig. 3. Intracellular activation of tenofovir to tenofovir diphosphate by adenylate kinase.

function is a frequent occurrence in HIV disease progression (Schwartz and Klotman, 1998; Wyatt et al., 2009). HIV-associated nephropathy primarily affects the glomerulus and is characterized by proteinuria, elevated serum creatinine and blood urea nitrogen, focal segmental glomerulosclerosis, and microcystic tubular dilatation. Renal lesions lead to the collapse of the glomeruli and rapid progression to kidney failure (Laurinavicius et al., 1999; Ray, 2012).

Although HIV-associated nephropathy occurs less frequently in patients whose HIV is well controlled with antiretroviral therapy (Fabian et al., 2013), renal insufficiency should be considered when using TDF. In a study conducted in patients with varying degrees of renal insufficiency, patients with moderate and severe renal impairment [noted as creatinine clearance (CL_{CR}) of 30–49 and <30 ml/min, respectively] had an AUC two- to sixfold that in patients with normal renal function. The increase in AUC was most likely due to a decrease in TFV clearance that was directly proportional to the observed decreases in CL_{CR} (Kearney et al., 2004).

Patients treated with TDF do have an increased incidence of renal impairment, and there is a positive correlation between increased TFV serum concentrations, duration of treatment with TDF, and incidence of renal toxicity (Verhelst et al., 2002; Fux et al., 2007; Ezinga et al., 2014; Quesada et al., 2015). TDF is associated with increased serum creatinine and plasma vitamin D binding, decreased free calcitriol levels and glomerular filtration rate, renal failure, and Fanconi syndrome (Hall et al., 2011; Del Palacio et al., 2012; Havens et al., 2013). Fanconi syndrome is a disorder of renal tubular function characterized by excess excretion of potassium, phosphate salts, protein, urate, and glucose. Clinical studies indicate that the mitochondria of the proximal tubules are the target of TFV-induced toxicity, as shown by enlarged and malformed mitochondria in electron microscopy cross sections (Côté et al., 2006; Woodward et al., 2009; Herlitz et al., 2010; Hall et al., 2011). Proximal tubular mitochondrial toxicity has also been described in mice and rats treated with TFV, as shown by disruptions in mitochondrial cristae and a reduction in mitochondrial DNA levels (Kohler et al., 2009; Lebrecht et al., 2009).

Changes in TDF administration are recommended for patients with decreased renal function to reduce plasma concentrations and to prevent worsening of renal damage. In patients with moderate impairment (CL_{CR} 30–49 ml/min), 300 mg dose

of TDF should be reduced in frequency to once every 48 hours instead of once daily. In patients with severe impairment (CL_{CR} 10–29 ml/min) or those who are on hemodialysis, the 300 mg dose should be administered every 72–96 hours or once every 7 days, respectively (Gilead Sciences, 2001).

Pharmacokinetics and Bioavailability of TDF in Fixed-Dose Combination Tablets

To simplify HIV treatment regimens and increase compliance, TDF is commonly paired with other antiretrovirals in single-dose combination tablet. The drugs currently combined with TDF in fixed-dose combinations are as follows: emtricitabine (FTC), efavirenz (EFV), rilpivirine (RPV), the integrase strand transfers inhibitor elvitegravir (EVG), and the CYP 3A4 inhibitor cobicistat (COBI). The most common formulations are as follows: TDF/FTC, TDF/FTC/EFV, TDF/FTC/RPV, and TDF/FTC/EVG/COBI. Each formulation includes TDF as a standard 300 mg dose. Bioequivalence is evaluated in combination tablet formulations by maintenance of pharmacokinetic parameters C_{max} , AUC, and $t_{1/2}$ within a confidence interval (CI) of 80%–125% of the reference formulation.

The first fixed-dose combination approved by the FDA for treatment of HIV-contained TDF/FTC/EFV. FTC, EFV, and TDF all have $t_{1/2}$ that are suitable for once per day dosing (FTC, 39 hours; EFV, 23 hours; TDF, 15–60 hours) (Barditch-Crovo et al., 2001; DiCenzo et al., 2003; Hawkins et al., 2005; Saag, 2006). When studied as a fixed-dose combination tablet, all pharmacokinetic parameters had geometric mean ratios close to 100% and 90% CI within the bioequivalence bounds of individual formulations, indicating that TDF, EFV, and FTC can be combined into a once-daily combination tablet (Mathias et al., 2007). The combination of TDF/FTC/EFV is FDA approved as a complete regimen for HIV treatment and can also be used in combination with other HIV medications (Gilead Sciences, 2006).

TDF/FTC/RPV combination provides two nucleos(t)ide reverse-transcriptase inhibitors (TDF/FTC) with a non-nucleoside reverse-transcriptase inhibitor, RPV, to treat HIV. Initial studies showed that addition of 25 mg RPV to a TDF/FTC treatment regimen showed equivalence in HIV suppression when compared with TDF/FTC + EFV regimens and had less adverse effects than EFV (Cohen et al., 2011; Molina et al., 2011).

RPV has a $t_{1/2}$ of approximately 50 hours, making it a suitable candidate for inclusion in a once-daily combination tablet (Cohen et al., 2013). In a bioequivalence study, all pharmacokinetic parameters of a TDF/FTC/RPV single-dose tablet demonstrated a 90% CI within the bioequivalence bounds of individual formulations, indicating that TDF, FTC, and RPV can be combined into a once-daily combination tablet. Although both TDF and FTC are approved to be taken with or without a meal, RPV bioavailability is reduced 40% in a fasted state alone and in a combination tablet. Therefore, TDF/FTC/RPV should be taken with food (Mathias et al., 2012).

TDF/FTC combination tablets also demonstrate bioequivalence for all tested pharmacokinetic parameters when compared with individual therapy (Blum et al., 2007). TDF/FTC is FDA approved to treat HIV-1 in conjunction with other therapies, but is not considered a complete regimen. TDF/FTC is also FDA approved for use in pre-exposure prophylaxis (PrEP) therapy to prevent the transmission of HIV-1 between sexual partners (Grant et al., 2010; Anderson et al., 2012; Baeten et al., 2012). The use of TFV-containing regimens for PrEP is discussed in further detail below.

Recently, COBI has been included in highly active antiretroviral therapy to treat HIV. Although it has no antiviral activity on its own, COBI is a pharmacoenhancer for drugs that are metabolized by cytochrome P450 3A enzymes (CYP3A). COBI is a potent CYP3A inhibitor, and is commonly used with protease and integrase inhibitors such as EVG in the treatment of HIV. In a fixed-dose combination study, administration of COBI/TDF/EVG combination tablets showed bioequivalence; however, TDF C_{max} was increased 30%, but AUC was largely unaffected (German et al., 2010). COBI may alter the $t_{1/2}$ of other drugs taken concurrently that are biotransformed by CYP3A as inhibition by COBI may slow biotransformation by CYP3A. COBI is a substrate for CYP3A, and treatment with agents that induce or inhibit CYP3A has the potential to alter the effects of COBI (Sherman et al., 2015).

This increase in C_{max} may be due to COBI's transient inhibition of P-glycoprotein efflux transporter (Pgp); TFV is a recognized substrate of the Pgp transporter (German et al., 2010; Lepist et al., 2012). It is worth noting that COBI inhibition of P-glycoprotein is weak, much like other protease inhibitors such as ritonavir (RTV); COBI, RTV, and other protease inhibitors do not inhibit efflux transporters at pharmacologically relevant concentrations in systemic circulation (Washington et al., 1998; Polli et al., 1999; van der Sandt et al., 2001; Ray et al., 2006; Cihlar et al., 2007) but may reach concentrations high enough to inhibit efflux transporters in the gut (Ray et al., 2006; Tong et al., 2007).

Effect of Food Intake on TDF Bioavailability

Taking TDF with food has shown to improve bioavailability. However, it does not appear that the type of meal matters, as no changes in pharmacokinetic parameters are seen with administration of a standard meal, high-fat meal, or protein-rich meal. In a study of healthy Japanese males, participants ingested a standard meal (11.4 g protein, 9.6 g fat, and 72.2 g carbohydrates) or a protein-rich drink (8.8 g protein, 8.8 g fat, and 34.3 g carbohydrates) prior to administration of a 300 mg dose of TDF. There was a 28% decrease in both AUC and C_{max} when TDF was administered in a fasted state compared with administration following a standard meal or a protein-rich drink.

Although C_{max} was slightly lower in the protein-drink group (554 ng/ml versus 613 ng/ml), AUC and $t_{1/2}$ were not different between meals, indicating that a standard meal and a protein-rich drink produce bioequivalent increases in TDF bioavailability over fasted administration (Shiomi et al., 2014).

Bioavailability of TDF was increased in one study using a very high-fat meal in uninfected males. Effects of a very high-fat meal were evaluated during phase I testing of TDF in healthy males. Participants ingested a high-fat meal (50% fat, 1000 kcal) 30 minutes prior to administration of TDF. TDF bioavailability in the fed group was increased 40% over the fasted group, and C_{max} was increased 14%. T_{max} also increased from 0.5 to 1 hour in a fasted state to 1.3–3 hours in a fed state, consistent with a slowing of gastric emptying following a meal (Barditch-Crovo et al., 2001; Kearney et al., 2004). This same group noted that a high-fat meal was not indicative of normal diet, and studied the effects of a light meal on TDF bioavailability and found no significant effect. According to its prescribing information, TDF can be administered with or without a meal (Gilead Sciences, 2001). It should be noted that certain TDF fixed-dose combination formulations such as TDF/FTC/RPV and TDF/FTC/EVG/COBI should be taken with food due to significant decreases in RPV (40%) and EVG (50%) bioavailability in a fasted state (German et al., 2010; Mathias et al., 2012; Shiomi et al., 2014).

Although TDF is intended to be cleaved by plasma esterases, it is susceptible to cleavage by intestinal carboxylesterases when administered by the oral route. It is hypothesized that this intestinal hydrolysis and efflux via Pgp are what limits TDF bioavailability in humans (Shaw et al., 1997; Yuan et al., 2001; van Gelder et al., 2002). Ester-rich fruit juices such as grapefruit, orange, cranberry, and concord grape have been shown to increase oral absorption of drugs such as lopinavir (LPV) and saquinavir through inhibition of Pgp, MRP2, and various cytochrome P450s (CYPs) (Bailey et al., 1998; Honda et al., 2004; Ravi et al., 2015). As TDF is a substrate of Pgp, studies were done to determine whether ester-rich fruit juices increase its bioavailability and intestinal absorption. Strawberry juice extract increased TDF intestinal absorption in Caco-2 cell lines to 67%, whereas grapefruit and cranberry juice extracts increased TDF bioavailability by 97% and 24% in an *in vivo* rat study (Van Gelder et al., 1999; Shailender et al., 2017). Additional investigation needs to be done to determine whether this effect is also seen in humans.

Age-Related Differences in TFV Bioavailability and Pharmacokinetics

TDF is FDA approved for use in the United States in children age 2 and older. As HIV is a chronic disease, it is important to determine the developmentally associated changes in pharmacokinetic parameters of antiretroviral therapy. Studies have been conducted to determine the pharmacokinetic profile of TDF administration in neonates, children/adolescents, and adults. TDF pharmacokinetics vary in children comparatively to adults, and these variables need to be considered when determining dosing. Initial pharmacokinetic studies were run following TDF's approval for use in adults. Hazra et al. (2004) determined the adult-equivalent dose in children to be $\sim 175 \text{ mg/m}^2$ based on calculations from initial pharmacokinetic studies in adults. In a cohort of

children ranging from 4 to 18 years of age given 175 mg TDF for 4 weeks, AUC and $C_{\max}$ were reported to be 34% and 27% lower than the equivalent dose in adults. The decrease in AUC may be explained by the observed 1.5 times faster clearance in children (Barditch-Crovo et al., 2001; Hazra et al., 2004).

Studies in which HIV-positive children were administered a full adult dose of TDF once daily showed similar pharmacokinetic changes observed in the initial pharmacokinetic study. Children and adolescents (defined as <25 years old) have lower plasma concentrations of TFV compared with adult patients, and clearance in children was 36% faster, which may contribute to the lower plasma concentration. The increase in clearance may be due to a larger relative kidney relative to body size as well as increased renal function (King et al., 2011; Baheti et al., 2013). Additionally, Baheti et al. (2013) reported that children have almost 50% higher intracellular concentrations of TDP following oral administration compared with adults. This phenomenon may be due to a lower effective concentration, EC_{50} , seen in children rather than adults for TFV (100 ng/ml versus 192 ng/ml, respectively).

Although no significant adverse effects were initially observed in studies using an adult dose, studies have also been conducted to determine effective weight-band TDF dosing in children due to concerns of bone density loss at different stages of bone dynamics. Longitudinal assessment of available data determined an overall incidence of bone mineral density (BMD) loss in children taking TDF at 24%–32%; this loss occurs within 6–12 months of initiation of treatment and then stabilizes (O'Brien et al., 2001; Jacobson et al., 2010; Puthanakit et al., 2012; Schtscherbyna et al., 2012; Aupibul et al., 2015).

A study conducted in children age 9–18 years also saw increased TFV clearance relative to adults, although clearance increased with body weight. Because of the differences in clearance, this model recommends prescribing TDF to children based on weight to achieve adult AUC: 150 mg at 20–30 kg body weight, 225 mg at 30–40 kg body weight, and the full adult dose of 300 mg at over 40 kg body weight (Bouazza et al., 2011); no mention of adverse effects was made in this study. Another study conducted based on weight-band dosing in children 3–17 years determined similar dosing parameters: 150 mg at 15–21 kg body weight, 225 mg at 22–33 kg body weight, and a full adult dose of 300 mg at >33 kg body weight. In this 96-week study, 28% of patients experienced a decrease in BMD by 24 weeks, at which point BMD remained constant (Aupibul et al., 2015).

TDF is most commonly administered in children as part of a treatment regimen also containing LPV and RTV, unlike adults who are commonly prescribed single-dose combination tablets (see previous section). It is relevant to note that LPV increases TDF AUC by 34% and serum concentration by 30% in adults (Barditch-Crovo et al., 2001; Kearney et al., 2004). This phenomenon is also seen in children, and is attributed to the observed 25% decrease in TDF clearance when coadministered with LPV (Bouazza et al., 2011; Aupibul et al., 2015). It is recommended that TDF dosing be adjusted in children when coadministered with LPV: 150 mg at 20–40 kg body weight, 225 mg at 40–50 kg body weight, and 300 mg at >55 kg body weight (Bouazza et al., 2011).

TDF is approved to treat HIV in children >2 years of age in combination with other medications. It is recommended that dosage be adjusted according to body weight in children <12 years of age (Gilead Sciences, 2001). Additional studies

need to be conducted on the observed interaction of TDF with LPV, as well as monitoring the incidence of bone density loss in children. Additional research is needed to evaluate the effect of age on the renal tubular transporters and potential impact on TFV clearance. TFV is a substrate for the efflux transporter MRP4. A study in rats reported that MRP4 expression developed slower than the basolateral membrane transporters, organic anion transporters 1 and 3 (Nomura et al., 2012). The developmental expression of MRP4 and other transporters is an area of limited information and may explain some of the differences related to TFV clearance with age.

TFV as a Prophylactic Agent: Sex-Related Differences in Pharmacokinetics

TDF is commonly used for antiretroviral PrEP in HIV-uninfected patients who engage in high-risk sexual encounters. Studies evaluating oral TDF/FTC PrEP efficacy show modest protection (76%) when taken twice per week and 75%–99% infection protection when taken every day. Studies using oral TDF 300 mg tablets as PrEP show a 67% reduction in HIV incidence, but high adherence was necessary (Anderson et al., 2012; Baeten et al., 2012). It should be noted that, although TFV pharmacokinetics are similar, there are documented differences in TDP intracellular concentrations in HIV-infected and uninfected patients; TDP concentrations are higher in HIV-infected patients (120 fmol/million cells) than in healthy patients (42 fmol/million cells) (Anderson et al., 2012; Baheti et al., 2013). Currently, TDF/FTC is the only FDA-approved PrEP regimen; it must be taken daily in conjunction with safer sex practices and HIV-status monitoring every 3 months. HIV-status monitoring is critical as HIV-1 resistance has been documented in cases of undetected infection when using TDF/FTC (Gilead Sciences, 2004).

To evaluate PrEP options for women, studies have been conducted to determine the efficacy of a topical 1% TDF gel formulation in the prevention of HIV infection. The original CAPRISA clinical trial showed modest protection, with an incidence of reduction ranging from 39% to 54% depending on adherence (Abdool Karim et al., 2010). TDP intracellular concentrations were not measured. A study of PrEP regimens of TDF, TDF/FTC, or 1% TDF vaginal gel in African women determined that there was no effect in the prevention of HIV transmission (Marrazzo et al., 2015); again, TDP concentration was not measured. Currently, 1% gel TDF formulation is not approved for PrEP, as efficacy has to date been shown to be moderate at best.

Recently, clinical studies were done to determine the differences in TFV-diphosphate distribution in healthy women using the TDF 1% gel, TDF 300 mg oral tablet, or both. TDP vaginal concentration was 130-fold higher in patients using the topical gel than oral dosing, but systemic TDP concentrations were 56-fold lower. Combination regimens of oral and topical TDF resulted in vaginal tissue concentrations threefold higher than in groups that used the oral formulation alone (Hendrix et al., 2013; Burns et al., 2015). Efficacy as a PrEP regimen was not directly measured in this study.

The extremely high vaginal concentrations of TDP may be due in part to hormonal regulation. Estradiol increases the amount of TDP present in isolated epithelial cells from the female reproductive tract, progesterone has no effect, and

estradiol–progesterone combination treatment negates the stimulatory effect of estradiol. In CD4 cells isolated from the female reproductive tract, progesterone alone and in combination with estradiol greatly reduced TDP concentration (Shen et al., 2014). These results suggest that hormonal regulation of TFV phosphorylation is dependent on cell type. It has been hypothesized that women experience a 7- to 10-day window of increased susceptibility to HIV infection due to immune suppression by sex hormones following ovulation (Wira and Fahey, 2008). The changes in TDF uptake and activation in the female reproductive tract may be due to the hormonal responses seen in previous studies.

It is worth noting that, whereas TDP concentrations vary in the female reproductive tract, administration of TDF with oral contraceptive pills did not change either TFV or oral contraceptive pill systemic levels (Kearney and Mathias, 2009). The observed responsiveness to sex hormones may be limited to tissues within the reproductive tract; additional study needs to be done in this area to fully determine the relationship between TDF pharmacokinetics and sex hormones.

TDF has also been examined in late pregnancy and labor to prevent vertical transmission of HIV. One study gave 300 mg TDF at the onset of labor, and to neonates within 12 hours of birth to infected mothers; a 13 mg/kg neonate dose produced intracellular TDP concentrations similar to adult levels (Hirt et al., 2011). However, plasma concentration remains low, as seen in children taking TDF, and there is a delay in the presence of TDP in neonatal PBMCs. There was no quantifiable TDP in fetal PBMCs. The delay in TFV phosphorylation could be due to differences in enzyme expression and activity in neonates versus children. Another study showed similar results: HIV-infected mothers were given a 600 mg dose of TDF, and neonates were given a 6 mg/kg daily dose, resulting in plasma concentrations similar to those seen in adults. Infant clearance was also equivalent to that seen in older children (Mirochnick et al., 2014).

It is important to note that TDF pharmacokinetics are slightly altered during pregnancy. During the third trimester, TDF AUC is 20% lower compared with postpartum levels. This transient difference may be directly attributable to increased weight and faster clearance during the last trimester of pregnancy (Best et al., 2015). Additional research needs to be done to determine changes in pharmacokinetic parameters during pregnancy, and how these changes affect vertical HIV transmission to children.

Alternative TFV Prodrug Formulations

Recently, TFV alafenamide (TAF) has undergone phase I–III testing and was approved to treat chronic hepatitis B in 2016. TAF has greater plasma stability, more effective cellular loading than TDF, and maintains a similar oral bioavailability at 17%. TAF has an EC_{50} of 0.005 μ M rather than 5 μ M seen with TDF, leading to significantly less plasma exposure, and by association lower systemic side effects (Lee et al., 2005; Sax et al., 2015; Buti et al., 2016). TAF underwent phase I–II testing in HIV patients, but did not make it to phase III due to the risk of HIV-1 resistance.

TAF has been approved to replace TDF in fixed-dose combination tablets to treat HIV following phase III trials. The combination product of TAF/FTC/COBI/EVG demonstrated 90% virologic success in both treatment-naïve and

treatment-experienced patients with lower incidence of adverse renal and bone effects when compared with TDF/FTC/COBI/EVG (Gilead Sciences, 2015b; Sax et al., 2015; Mills et al., 2016; Pozniak et al., 2016; Wohl et al., 2016). The combined preparation of TAF/FTC demonstrated 93% virologic success when compared with the combination of TDF/FTC, with no documented renal tubulopathy in either test group. It should be noted that whereas TDF/FTC is recommended as a PrEP regimen, TDF/FTC is not approved for PrEP regimen by the FDA as of May 2017 (Gilead Sciences, 2015a; Sax et al., 2015; Gallant et al., 2016; Mills et al., 2016). Although TDF is currently FDA approved for HIV-1 treatment in patients 2 years of age and older, TAF fixed-dose combinations are only indicated for patients greater than 12 years of age.

The use of liposomes and niosomes has been investigated to improve the bioavailability of TDF. When encapsulated in a liposome made of phospholipids and 7.5% cholesterol, TDF had an increased permeability rate across Caco-2 cells (3.71E-07 cm/s) compared with TDF alone (4.18E-07 cm/s) (Spinks et al., 2017). When encapsulated in a niosome created with sorbitan monoesterate in a 1:1 ratio, >99% of the TDF dose was released in vitro within 24 hours. In a subsequent in vivo study in rats, TDF AUC was increased twofold when encapsulated in a niosome compared with TDF alone; C_{max} , $T_{1/2}$, and T_{max} were also increased, possibly due to increased GI absorption and the release-retarding effects of niosomes (Azmin et al., 1985; Kamboj et al., 2014). No studies have yet been conducted to determine whether liposomes or niosomes are more efficient in improving TDF bioavailability or if encapsulation is effective in humans for the treatment of HIV using TDF.

Conclusions

TDF is the prodrug for TFV, which is a nucleotide analog reverse-transcriptase inhibitor that is very effective in the treatment of HIV and chronic hepatitis B. TDF has a favorable pharmacokinetic profile, allowing for once per day dosing, and its pharmacokinetics are largely unchanged when given in fixed-dose combination tablets. TDF has been shown to be effective as a prophylactic agent to prevent HIV-1 transmission, leading to site-specific formulations; this area of research remains highly active. TDF does have limited oral bioavailability, and research is active to determine whether bioavailability can be increased. More importantly, TDF improves intracellular concentration in PBMCs. Overall, TDF remains one of the first-line agents in the treatment of HIV and hepatitis B virus, providing once-daily dosing, which improves patient compliance.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Murphy, Valentovic.

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