Biowaiver Monographs for Immediate-Release Solid Oral Dosage Forms: Stavudine

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ABSTRACT: Literature data relevant to the decision to allow a waiver of *in vivo* bioequivalence (BE) testing for the approval of immediate-release (IR) solid oral dosage forms containing stavudine (d4T) are reviewed. According to Biopharmaceutics Classification System (BCS), d4T can be assigned to BCS class I. No problems with BE of IR d4T formulations containing different excipients and produced by different manufacturing methods have been reported and, hence, the risk of bioinequivalence caused by these factors appears to be low. Furthermore, d4T has a wide therapeutic index. It is concluded that a biowaiver is appropriate for IR solid oral dosage forms containing d4T as the single active pharmaceutical ingredient (API) provided that (a) the test product contains only excipients present in the IR d4T drug products that have been approved in a number of countries for the same dosage form, and (b) both test product and its comparator are either "very rapidly dissolving" or "rapidly dissolving" with similarity of dissolution profiles demonstrated at pH 1.2, 4.5, and 6.8. © 2011 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:10–16, 2012

Keywords: stavudine; absorption; Biopharmaceutics Classification System (BCS); permeability; regulatory science; solubility

INTRODUCTION

A biowaiver monograph based on literature data is presented on stavudine (d4T) with respect to its biopharmaceutical properties and the risk of waiving *in vivo* bioequivalence (BE) testing in the approval of new IR solid oral dosage forms containing d4T ("biowaiving"), including both reformulated products and new multisource drug products. This evaluation refers to drug products containing d4T as the only active pharmaceutical ingredient (API) and not any combination products. The purpose and scope of this series of monographs have been previously discussed.[1] Summarizing in few words, the aim is to evaluate all pertinent data available from literature sources for a given API to assess the risks associated with a biowaiver. For these purposes, risk is defined as the probability of an incorrect biowaiver decision, as well as the consequences of decision in terms of public health and individual patient risks. On the basis of these considerations, a recommendation can be made as to whether a biowaiver approval is advisable or not. This systematic approach to recommend or advise against a biowaiver decisions is referred to in a recently published World Health Organization (WHO) guideline.[2] These monographs do

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not intend to simply apply the guidance of WHO,[2] the US Food and Drug Administration (FDA),[3] and/or the European Medicine Agency (EMA),[4] but also aim at a critical evaluation of these and the regulatory documents of other countries. Biowaiver monographs have been already published for several APIs, also available online at www.fip.org/bcs.[5]

EXPERIMENTAL

Literature data were obtained from Web of Science, PubMed, and Micromedex databases up to December 2009. The keywords used for searching were d4T, intestine absorption, linear absorption, absolute bioavailability, bioequivalence, log *p*, solubility, permeability, and lipophilicity. Information was also obtained from regulatory documents published by WHO,[2] FDA,[3] and EMA.[4]

GENERAL CHARACTERISTICS

The structure of d4T,[6,7] as per International Nonproprietary Names, is shown in Figure 1.

Therapeutic Indication and Dose

Stavudine is a pyrimidine nucleoside antiretroviral agent with *in vitro* activity against human immunodeficiency virus (HIV) similar to zidovudine[8–10] and is applied for the treatment of HIV-1 infection as either monotherapy or in combination with other antivirals.[11] d4T inhibits HIV reverse transcriptase by competing with the natural substrate deoxythymidine triphosphate and its incorporation into viral DNA, causing termination of DNA elongation.[11] The phase I study reported by Browne et al.[12] started at 4 mg/(kg day) and the dose was escalated until a daily dose of 12 mg/(kg day) was reached. Little additional antiretroviral activity was gained by this dose escalation, but toxicity increased greatly. On a dosing schedule of every 12 h, activity was maintained and toxicity was lessened at doses as low as 0.5 mg/(kg day). Suboptimal antiviral effects were evident at doses of 0.25 mg/(kg day).[12,13] The recommended dose based on body weight is 40 mg twice daily for patients weighing at least 60 kg and 30 mg twice daily for patients weighing less than 60 kg. The recommended dose for newborns up to 13 days old is 0.5 mg/(kg dose), given every 12 h. The recommended dose for pediatric patients at least 14 days old and weighing less than 30 kg is 1 mg/(kg dose), given every 12 h. Pediatric patients weighing 30 kg or more should receive the recommended adult dosage.[11]

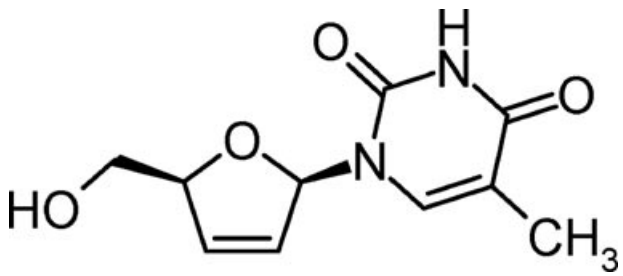

Figure 1. Structure of stavudine.

Therapeutic Index and Toxicity

Both preclinical and clinical studies have shown d4T to be less cytotoxic than zidovudine.[14,15] In clinical studies, d4T has shown to exert a significant antiviral effect with acceptable safety. The principal toxic effect is symptomatic peripheral sensory neuropathy, which is dose related.[12,16–19] Patients should be monitored for the development of neuropathy, which is usually manifested by numbness, tingling, or pain in feet or hands.[11] d4T-related peripheral neuropathy can be resolved by prompt withdrawal of the therapy. In some cases, symptoms may worsen temporarily following the discontinuation of therapy. If symptoms resolve completely, patients may tolerate resumption of treatment at one-half of the dose.[11] Patients with preexisting liver dysfunction have an increased frequency of liver function abnormalities including severe and potentially fatal hepatic adverse events, and should be monitored according to standard practice.[11] d4T levels used in treatment are generally 100-fold below those that are cytotoxic.[20] Experience with adults treated with 12–24 times the recommended daily dosage revealed no acute toxicity. Complications arising with chronic overdosage include the aforementioned peripheral sensory neuropathy and hepatic toxicity.[11]

CHEMICAL PROPERTIES

Solubility

The solubility in water was reported as 83 mg/mL at 23°C.[11] The pH–solubility profile of d4T at 37.0 ± 0.5°C was determined in 0.01 N HCl (78 mg/mL), pH 4.5 (101 mg/mL), and pH 6.8 (76 mg/mL);[21] no information about polymorphic form was reported.

Polymorphism

Polymorphic forms I, II, and III have been identified. Forms I and II are anhydrous; form III is hydrated and is pseudopolymorphic with forms I and II. The solubility of form II (106.8 mg/mL) in water at 25°C is higher than that of form I (88.8 mg/mL),[22] but polymorph dependent bioavailability (BA) has not been reported. Form I is the stable polymorph and is commercially available.[23]

Table 1. Excipients[‡] Present in Stavudine (d4T) IR Solid Oral Drug Products[§] with an MA[¶] in a Number of Countries[**], and the Minimal and Maximal Amount of the Excipient Present Pro Dosage Unit in Solid Oral Drug Products with an MA in the United States

Excipient	Drug Products Containing Excipient with an MA Granted by the Named Country	Range Present in Solid Oral Dosage Forms with an MA in the United States (mg)
Cellulose, microcrystalline	AU (1) BR (2) CA (3) EU (4) NZ (5) US (6–13)	4.6–1385[a]
Lactose	AU (1) BR (2) CA (3) EU (4) NZ (5) US (6–13)	23–1020[a]
Magnesium stearate	AU (1) BR (2) CA (3) EU (4) NZ (5) US (6–13)	0.15–401[a]
Sodium starch glycolate	AU (1) BR (2) CA (3) EU (4) NZ (5) US (6–13)	2–876[a]

(1) ZERIT®, capsules 15/20/30/40 mg; (2) ZERITAVIR, capsule 30/40mg; (3) PrZERIT, d4T capsules USP, 5/15/20/30/40 mg; (4) Zerit 15/20/30/40 mg hard capsule; (5) Zerit® d4T capsules 15/20/30/40mg; (6) d4T capsule 15/20/30/40mg [Aurobindo Pharma Limited]; (7) d4T capsule 15/20/30/40mg [Camber Pharmaceuticals, Inc.]; (8) d4T capsule 15/20/30/40mg [Greenstone LLC]; (9) d4T capsule 40mg [KAISER FOUNDATION HOSPITALS]; (10) d4T capsule 15/20/30/40mg [Mylan Pharmaceuticals Inc.]; (11) d4T capsule 40mg [State of Florida DOH Central Pharmacy]; (12) ZERIT (d4T) 20/30/40mg capsule, gelatin coated [State of Florida DOH Central Pharmacy]; (13) ZERIT (d4T) 15/20/30/40mg capsule, gelatin coated [E.R. Squibb & Sons, L.L.C.].

[‡]Excipients were excluded if it could be assumed that they are present only in the capsule shell; hence, colorants and/or printing ink are not included.

[§]Powders for oral solutions are excluded.

[¶]The approval of a drug product by the local regulatory authority. The terms drug approval and registration are also used.

[**]For abbreviations of the countries, see text.

[a]The upper range value reported is unusually high for solid oral dosage forms and the authors doubt its correctness.

Partition Coefficient

Log *p* and Clog *p* values of–0.47 and–0.73, respectively, were calculated using two different fragmentation methods that were based on atomic contributions to lipophilicity.[24] Other authors reported a log p (*n*-octanol/water) of 0.144 at 23°C.[11]

pKa

Stavudine is nonionized at physiological pH[25] and its pKa is 10.[26]

Available Dosage Forms' Strengths

The WHO Essential Medicines List[27] includes d4T IR capsules with strengths ranging from 15 to 30 mg. Capsules containing 15, 20, 30, and 40 mg commercially available (see Table 1).

PHARMACOKINETIC PROPERTIES

Absorption and BA

The pharmacokinetics of d4T is well described by a linear two-compartment open model with first-order absorption and elimination.[28] Plasma concentrations in patients with asymptomatic HIV infections reach a mean peak drug plasma concentration (C_{max}) of approximately 1.4 μg/mL within 1 h after oral administration of d4T (70 mg) as a single dose.[29] The mean BA of oral d4T in HIV-infected patients is over the range 86%–100% and is not affected by administration of food.[11,28–34] Also, the systemic exposure is the same regardless of capsule or solution administration.[11] Following administration of single doses of d4T ranging from 5 to 40 mg, C_{max} and area under the concentration–time curve (AUC) increase linearly in a dose-proportional manner.[35] Other authors reported linear pharmacokinetics for d4T over a range of 0.67–4.0 mg/kg.[33] Furthermore, there is no difference in oral BA between healthy volunteers and patients with severe hepatic impairment.[20]

Intestinal absorption of d4T in rats was studied *in situ* using a closed-loop method and *in vivo* using multiple sites of input method. Site dependency of absorption of d4T was investigated in three segments of rat intestine and, as a result, the transported amount into systemic circulation was greater in the upper intestinal tract (duodenum and jejunum) than in colon. The disappearance percent of d4T in the duodenum and jejunum was not significantly different. Also, the BA of d4T following three different routes (intraportal vein, intraduodenal, and intragastric) was higher than 90%. However, the mean resident time after dosing at the stomach site was longer than the duodenum site due to the effect of gastric emptying time.[36]

Permeability

The intestinal transport of d4T in rat and rabbit was characterized by *in situ* single-pass intestinal perfusion (SPIP) method and *in vitro* intestinal brush-border membrane vesicles (BBMV) method. The concentration dependent permeability behavior and the effect of inhibitors on the permeability of d4T in the SPIP method indicate that d4T is taken up by the small intestine of the rat by both passive and carrier-mediated mechanisms. The inhibition pattern of the carrier-mediated component of d4T permeability in the SPIP method by thymidine provides evidence that d4T shares N2 and possibly N_3 and/or facilitated non-sodium dependent pathways of absorption. Studies in rabbit BBMVs confirmed that d4T permeability was partially carrier-mediated.[37,38] Because nucleoside transporters typically have Michaelis-Menten constants for the uptake transporter (K_m) in the micromolar range, these systems are easily saturated at typical intestinal drug concentration and are, therefore, characterized as a high-affinity and low-capacity systems. So, at d4T therapeutic doses, these transporters are expected to be saturated and the impact of these kinetic characteristic on oral BA parameters

is likely to be minimal.[37] Indeed, the pharmacokinetics of d4T is linear over the therapeutic range.

The permeability of d4T was also investigated using a Caco-2 assay. Propranolol and mannitol served as high- and low-permeability reference standards and an apparent permeability coefficient (P_{app}) of d4T of $4.5 \pm 0.5 \times 10^{-6}$ cm/s was reported. In the same study, propranolol and mannitol showed P_{app} values of $19.2 \pm 0.4 \times 10^{-6}$ and $1.2 \pm 0.1 \times 10^{-6}$ cm/s, respectively; therefore, d4T was classified as a moderate-to-high-permeability API.[39] d4T is known to be hydrophilic, as characterized by a partition coefficient (Clog p) of–0.73.[24] Hydrophilic compounds usually use the paracellular rather than the transcellular pathway through intestinal membranes, as they lack lipophilic properties necessary to penetrate the cell membrane.[40,41] The paracellular pathway of the Caco-2 monolayer has been shown to be much more restrictive than rat or human small intestine, as reflected by the higher transepithelial electrical resistance measurements and lower permeability of hydrophilic marker compounds.[42–46] The restriction of the Caco-2 method to accurately reflect *in vivo* permeability of drugs that are absorbed paracellularly can explain the discrepancy between the results from BA and Caco-2 studies, analogous to the situation for sotalol.[47]

Distribution, Metabolism, and Elimination

Stavudine is widely distributed throughout the body, with a mean volume of distribution of 46 ± 21 L. Metabolism plays a limited role in the clearance of d4T in a mass balance study after an 80 mg dose of 14C–d4T to healthy subjects; approximately 95% of the total radioactivity was recovered in urine, of which 73.7% was due to the parent drug.[11] Other authors reported an urinary recovery of 39 ± 23% of the administered dose.[20] The mean terminal plasma elimination half-life is approximately 2.3 h following single oral doses. Mean renal clearance of the parent compound is approximately 272 mL/min, accounting for approximately 67% of the apparent oral clearance.[11,31]

DOSAGE PERFORMANCE

BE Studies

Two reports in the literature have demonstrated the BE of products containing d4T as the single API and Zerit® 40 mg (manufactured by Bristol-Myers Squib, NJ, USA) as the reference drug product.[48,49] In the first one, 40 healthy volunteers were enrolled and 90% confidence intervals (CIs) for log-transformed C_{max} and AUC_{0-t} were 93.9%–106.0% and 98.4%–101%, respectively. No dissolution test was performed. The composition of formulation was not reported.[48] In the second study, the BE was assessed by enrolling 24 healthy male subjects, and the CIs for log-transformed C_{max} and AUC_{0-t} were 90.25%–116.00% and 102.35%–110.11%, respectively; hence, test and reference products were bioequivalent with regard to both rate and extent of drug absorption despite the small but statistical significant difference in AUC between the test and reference. No dissolution testing was performed. The composition of formulation was not reported.[49] All the results meet the current BE criteria.

Excipients

Excipients present in IR d4T tablets with a marketing authorization (MA)* in Australia (AU),[50] the European Union (EU),[51]† Brazil (BR),[52] Canada (CA),[53] New Zealand (NZ),[54] and the United States (US)[55] are summarized in Table 1. In view of their MAs and national regulations, it can be inferred that the drug products listed in Table 1 successfully passed an *in vivo* BE study or clinical trial. Because one formulation will most probably be registered in several countries, these drug products correspond to a far lower number of formulations. Also, it cannot be taken for granted that every registered drug product has successfully met the current *in vivo* BE criteria.[56] Nevertheless, it seems safe to conclude that the risk of bioinequivalence caused by an excipient effect is low for excipients present in a large number of registered drug products when present in amounts not exceeding its normal use in IR tablets. Table 1 shows the range of excipients present in solid oral dosage forms with a MA in the United States.[57]

Dissolution

A dissolution method for d4T capsules is not included in present editions of the British Pharmacopoeia and the International Pharmacopoeia,[58] but the United States Pharmacopeia (USP) contains a dissolution test for d4T capsules: USP apparatus II (paddle); 75 rpm; medium—900 mL water at 37°C. The dissolution specification is "not less than 80% (Q) of the labeled amount of d4T dissolved in 30 min."[59]

* The approval of a drug product by the local regulatory authority. Also the terms: Drug Approval, and Registration, are used.

† Products having a MA in EU are authorized for use in Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Germany, Denmark, Estonia, Greece, Spain, Finland, France, Hungary, Ireland, Iceland, Italy, Liechtenstein, Lithuania, Luxembourg, Latvia, Malta, the Netherlands, Norway, Poland, Portugal, Romania, Sweden, Slovenia, Slovakia and the United Kingdom.

DISCUSSION

Solubility

According to the current regulatory guidances, an API is highly soluble if its dose/solubility ratio (*D*/S) is 250 mL or less in the pH range of 1.0–6.8[2,4] or 1.0–7.5[3] at 37°C, in which "dose" is to be understood as the highest dose strength[2,3] or the highest single dose administered.[4] d4T's D/S at pH 1.0–6.8 is around 0.5 mL, based on 40 mg of d4T as the highest dosage strength and also the highest single dose administered, so far less than the D/S cutoff for the "high-solubility" biowaiver criteria. Although there is no solubility data at pH 7.5, this experimental condition is overly conservative and there is a consensus that the pH range for the BCS should be narrowed to include only pH 1.0–6.8.[60] So, it can be concluded that d4T is highly soluble.

Permeability

Because its BA is over 85%[3,11,28,30–34] following oral administration, d4T is highly permeable according to WHO and EU BCS criteria,[2,4] but fails to consistently comply with the FDA requirement of 90% or more in all studies.[3] However, this FDA criteria is considered too conservative, and there is a consensus that the permeability class boundary should be lowered to 85%.[60] Data from Caco-2 studies, *in vivo* and *in situ* intestinal perfusion studies in animals support the classification of d4T as highly permeable.

BCS Classification

According to WHO and EU guidance, d4T can be assigned to BCS class I.[2,4] Other reports confirm this classification.[61–63] Although d4T does not fulfill the FDA requirements, there is a scientific consensus that these criteria are overly conservative.[60] On the contrary, d4T is a Biopharmaceutical Drug Disposition Classification System (BDDCS) class III API, as it does not present an extensive metabolism, but is mainly eliminated by renal excretion as an unaltered drug.[11] However, BDDCS was developed as a surrogate system for situations in which no BA data were available, which is not the case; BDDCS has not yet been recognized by regulatory authorities as an acceptable classification.[2–4]

Risks for Bioinequivalence Caused by Excipient and/or Manufacturing

No study directly investigating the influence of excipients on the absorption of d4T was identified. Also, in line with its BCS I classification, not one single report of bioinequivalence, nor a study not meeting the BE criteria was identified, indicating that product variations regarding commonly used excipients or in manufacturing process seem to be at low risk for d4T absorption.

Patient's Risk Associated with Bioinequivalence

Stavudine levels used in treatment are generally 100-fold below those that are cytotoxic.[20] Experience with adults treated with 12–24 times the recommended daily dosage revealed no acute toxicity.[11] Health Canada has published a guidance listing of drugs that commonly exhibit adverse effects at doses close to those required for therapeutic effect (e.g., "narrow therapeutic range drugs") and drugs for which the therapeutic use may result in dose or concentration dependent adverse effects that are persistent, irreversible or slowly reversible, and/or life threatening (e.g., "highly toxic drugs"). This list does not include d4T.[64]

CONCLUSION

A biowaiver for IR solid oral dosage forms containing d4T is scientifically justified, provided that (a) the test product contains only excipients present in IR d4T drug products that have been approved in a number of countries for the same dosage form, and (b) both the test and comparator dosage form are either very rapidly dissolving or rapidly dissolving with similarity of the dissolution profiles demonstrated at pH 1.2, 4.5, and 6.8.[2–4]

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