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Bioequivalence of dispersed stavudine: opened versus closed capsule dosing

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Abstract

Background—Stavudine, a nucleoside reverse transcriptase inhibitor, is used commonly to treat HIV-infected children in the developing world. The paediatric liquid formulation presents major logistical difficulties in rural and resource-limited areas, and prescribers are frequently forced to employ off-label 'opened capsule' dosing methods using the adult capsule. The South African Department of Health (DoH) has advised that caregivers should be instructed to disperse the contents of an adult capsule in 5 ml water and then withdraw the required dose using a syringe. The bioavailability of stavudine using the opened capsule dosing method has not previously been validated.

Methods—This was a randomized crossover pharmacokinetic study with each subject serving as his/her own control. A total of 28 healthy HIV-negative adult volunteers were randomized on a 1:1 basis to receive one of the two generic preparations. They were then further randomized to receive either intact or opened 30 mg capsules. After 1 week, those who initially received intact capsules, were given opened capsules and *vice versa*. The capsule dispersion technique used was identical to that prescribed by the DoH. Serial blood samples were collected and plasma stavudine concentrations were assayed by liquid chromatography tandem mass spectrometry. Stavudine pharmacokinetics were analysed using non-compartmental methods and formulations were compared using ANOVA.

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Disclosure statement

The authors declare no competing interests.

Results—Plasma drug exposure after stavudine administration as a solution using an opened capsule dosing method was found to be bioequivalent to intact capsule administration. This was true for both generics tested. Conclusions: The opened capsule dosing technique is bioequivalent to intact capsule dosing for stavudine in HIV-seronegative adults.

Introduction

Paediatric solutions present significant logistical difficulties in rural or resource-constrained settings. Despite its toxicity profile, stavudine is still commonly used to treat HIV-infected infants and children in the developing world. Paediatric stavudine solution (Zerit[®]; Bristol–Myers Squibb, Uxbridge, UK), available as a dry powder, must be reconstituted with a specific volume (202 ml) of distilled water. Once reconstituted, the solution must be transported by the caregiver in a cooler box, requires refrigeration at 4°C, and is then only stable for 1 month [1]. Refrigeration is often not possible in rural or resource-limited areas. The adult capsule formulation is stable at room temperature (<25°C) for 2 years [2], and is often prescribed in lieu of stavudine solution. The South African Department of Health (DoH) guidelines [3] advise that caregivers should be instructed to disperse the contents of an adult capsule in 5 ml water and then withdraw the required dose using a syringe. The bioavailability of stavudine using the ‘opened capsule’ dosing method has not previously been validated. In addition, as a number of generic alternatives are available and regional purchasing mechanisms may favour switching suppliers, it is important to determine whether the bioavailability using the opened capsule method varies between the various generic stavudine preparations available on public tender.

The US Food and Drug Administration (FDA) considers two products bioequivalent if the 90% CI of the log transformed maximum concentration (C_{max}) and area under the curve (AUC) of the test formulation in the fasting state is between 80% and 125% of the reference formulation [4]. A formal pharmacokinetic (PK) study is the ideal for determining this. However, the large number of blood draws required for a formal PK study, are impractical in small children. Healthy adult volunteers are an acceptable surrogate for bioequivalence studies of generic medicines, including antiretrovirals [5–7]. This principle spares children much unnecessary pain, and is supported by the FDA [4,8].

This study aimed to compare the bioavailability of stavudine administered as a solution using an opened capsule dosing method with the same dose given as an intact capsule in healthy adult volunteers. In addition, this study aimed to compare the bioequivalence of the two most commonly used generic stavudine capsule preparations available in South Africa: Stavir[®] (Cipla Life Sciences, Mumbai, India) and Aspen Stavudine[®] (Aspen Pharmacare, Port Elizabeth, South Africa).

Methods

Study design

This was a randomized crossover study with each subject serving as his/her own control. After screening, 28 healthy HIV-negative adult volunteers were randomized on a 1:1 basis to receive one of the two generic preparations. They were then further randomized to receive

either intact or opened 30 mg capsules. After 1 week, those who initially received intact capsules were given opened capsules and *vice versa* (see Additional file 1). Literature-based variability in exposure (mean $\pm$ SD AUC 2,568 $\pm$ 454 ng•h/ml) for stavudine was accounted for in calculation of sample size for the study. A sample size of 28 (14 per arm) would have a power of 0.99 to detect a 30% difference in AUC of formulations, or a power of 0.85 to detect a 20%, at an α -value of 0.05.

This study received approval from the ethics committee of Stellenbosch University and was conducted in accordance with the Declaration of Helsinki.

Study procedures

Participants were fasted from 6 h prior until 2 h after the dose was given. The dispersion technique used was identical to that prescribed by the DoH [3]: each capsule was opened by the pharmacist, mixed into 5 ml water and agitated until completely dispersed. The mixture was immediately swallowed by the participant, under direct supervision. Stavudine PK blood samples were collected at the following times: predose, 15 min, 30 min, 45 min, 60 min, 90 min, 2 h, 4 h, 6 h and 8 h after dosing. The reason for only sampling to 8 h relates to the very short half-life of stavudine (approximately 1 h). With absorption nearly complete by 1 h, <1% of peak plasma drug level is present in the bloodstream after 8 h. Samples were processed on site and frozen within 8 h of collection. Samples remained frozen at -70°C until analysis.

Assay method

Plasma stavudine concentrations were assayed by liquid chromatography tandem mass spectrometry using a validated method on an API 4000 LC mass spectrometer (Applied Biosystems, Foster City, CA, USA). The mobile phase consisted of gradient of acetonitrile and 0.5% glacial acetic acid. Chromatography was performed on a Phenomenex Synergi Fusion C18 Column (Phenomenex, Torrance, CA, USA) maintained at 25°C . 2-Deoxythymidine was used as an internal standard. For each participant, all PK samples were processed in a single batch. A quantity of 50 μl of each sample was precipitated with acetonitrile containing the internal standard, centrifuged and 5 μl of the supernatant injected onto the column. Standard curves in the range 0.02–6 $\mu\text{g/ml}$ and appropriate quality control samples were run with each batch. The lower limit of quantification was 20 ng/ml. Inter- and intra-day coefficients of variation were <9% for all quality control samples.

Data analysis

The PK analysis was performed using non-compartmental methods and ANOVA bioequivalence analysis using WinNonlin version 5.3 (Pharsight, Mountain View, CA, USA). This included a 90% CI of the ratio of log transformed parameters (C_{max} and $\text{AUC}_{0-\text{infinity}}$), as per FDA guidance on bioequivalence studies [4]. $\text{AUC}_{0-\text{infinity}}$ was calculated using the linear/log trapezoidal rule. The maximum observed concentration was taken as C_{max} . If that concentration was not unique, then the first observed value was used in the AUC determination.

Results

Baseline characteristics of participants were well matched with an overall median age of 37.5 years (range 22–61; Additional file 2). Plasma drug exposure after stavudine administration as a solution using an opened capsule dosing method was bioequivalent to intact capsule administration. This was true for both generics tested, that is, Aspen Stavudine[®] and Cipla Stavir[®] (Table 1). The median ratio of AUC_{0–8} to AUC_{0–infinity} was 0.947, confirming that sampling time was sufficiently long, and that AUC_{0–8} is a reasonable estimation of AUC_{0–infinity}.

For Aspen Stavudine[®], there was no statistically significant difference in the PK parameters (time to maximum concentration [T_{max}], C_{max} and AUC) or the mean concentration–time profiles of the opened capsule versus intact capsule dosing methods (Figure 1 and Additional file 3). For Cipla Stavir[®], the T_{max} after intact capsule dosing was significantly later than after opened capsule dosing (0.87 h versus 0.53 h; *P*=0.0078; Additional file 4). This difference is clearly visible in the superimposed mean concentration–time profiles of the two dosing methods (Figure 2).

Discussion

Our data shows that administration of stavudine as a solution using an opened capsule dosing method is bioequivalent to intact capsule dosing. This was true for both generics tested (Aspen Stavudine[®] and Cipla Stavir[®]). Although still within the prescribed PK parameters, Cipla Stavir[®] capsules, when dispersed in water, follow a different PK curve to that of intact Cipla Stavir[®] capsules. Therefore Cipla Stavir[®] may not be an ideal product when an opened capsule dosing method is prescribed. This finding is in line with our previous study of the physical characteristics of generic stavudine capsules, which found a significant delay in the dispersability of Cipla Stavir[®] capsule contents compared with Bristol–Myers Squibb Zerit[®] capsules and to Aspen Stavudine[®] capsules [9]. Although the opened capsule dosing method has been prescribed by the South African DoH since 2004, none of the three stavudine capsule patient information leaflets condone the use of an opened capsule dosing technique [1,2,10], and it remains an off-label dosing method. In conclusion, the off-label opened capsule dosing method is bioequivalent to intact capsule dosing for stavudine. A suitable product should be supplied when this method is prescribed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Plasma concentration–time profiles after opened and intact capsule dosing for Aspen Stavudine®

Mean plasma concentrations are shown; error bars reflect the SEM. Aspen Stavudine® (Aspen Pharmacare, Port Elizabeth, South Africa).

Figure 2. Plasma concentration–time profiles after opened and intact capsule dosing for Cipla Stavir®. Mean plasma concentrations are shown; error bars reflect the SEM. Stavir® (Cipla Life Sciences, Mumbai, India).

Table 1

Bioequivalence of stavudine administered as a solution using an ‘opened capsule’ dosing method, compared to the same dose given as an intact capsule

Pharmacokinetic index	Opened capsule, mean (±SD)	Reference formulation (intact capsule), mean (±SD)	Geometric least squares mean ratio (90% CI)
$C_{\max}$			
Aspen Stavudine®, ng/ml	739 (256)	787 (255)	105% (90–123)
Cipla Stavir®, ng/ml	782 (229)	706 (138)	103% (88–121)
AUC _{0-infinity}			
Aspen Stavudine®, ng•h/ml	1,601 (534)	1,787 (467)	112% (102–122)
Cipla Stavir®, ng•h/ml	1,540 (226)	1,768 (369)	94% (90–99)

Aspen Stavudine® (Aspen Pharmacare, Port Elizabeth, South Africa). Stavir® (Cipla Life Sciences, Mumbai, India). AUC, area under the curve; $C_{\max}$, maximum concentration.