Plasma pharmacokinetics of once- versus twice-daily lamivudine and abacavir: simplification of combination treatment in HIV-1-infected children (PENTA-13)

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Background: There are few data on plasma and intracellular pharmacokinetics (PK) of once-daily (q24h) nucleoside analogues in HIV-infected children.
Methods: Children aged 2–13 years receiving combination treatment containing lamivudine (3TC) (4 mg/kg) and/or abacavir (ABC) (8 mg/kg) twice daily (q12h) were included in this single-arm, open-label, crossover study. Intensive plasma PK sampling was performed at steady state, after which children switched to q24h dosing and PK sampling was repeated 4 weeks later. Daily area under the curve (AUC_{0-24}) and peak level (C_{max}) of q24h and q12h regimens were compared by geometric mean ratios (GMRs) with 90% confidence intervals (CIs). Children were followed for 24 weeks to evaluate safety and virological response.
Results: 24 children were enrolled, of whom 20 [median age (range) 5.6 (2.1–12.8) years] had evaluable PK data for 3TC (*n*=19) and/or ABC (*n*=14). GMRs of 3TC and ABC AUC_{0-24} and C_{max} q24h versus q12h significantly exceeded 1.0. GMRs were not significantly different between children aged 2–6 versus 6–13 years old (*P*>0.08). Of note, 3TC C_{max} values for both q12h and q24h were significantly lower in children aged 2–6 versus 6–13 years old. No child discontinued due to adverse events. At baseline, 16 out of 20 children had a viral load <100 copies/ml compared with 17 out of 19 at week 24.
Conclusion: AUC_{0-24} and C_{max} of both 3TC and ABC q24h were not inferior to q12h dosing in children. Insufficient results were obtained concerning intracellular levels of the active triphosphate moieties of both agents. Virological data did not indicate a marked difference in antiviral activity between q12h and q24h regimens.

Introduction

Treatment of HIV-1 infection with highly active antiretroviral (HAART) regimens in children is complicated by several factors, including adherence to medication. This is related to pill burden or volume of liquid of the drugs, their palatability, the complexity of medication schedules and the degree of interference with a child or carer's daily activities [1,2]. Decreasing the frequency of medication intake during a day offers the possibility of increased convenience and the likelihood of enhancing adherence to HAART in HIV-1-infected children.

The nucleoside analogues lamivudine and abacavir are commonly used as part of HAART in children. Whilst pharmacokinetics (PK) and efficacy of these drugs administered once daily (q24h) in adults have shown promising results [3–6], no data are yet available on their q24h use in HIV-1-infected children.

The objective of this study was to compare the plasma and intracellular PK of q24h with twice daily (q12h) lamivudine and abacavir in HIV-1-infected children aged between 2 and 13 years old. A second objective was to evaluate if there were age-related differences in the PK of lamivudine and abacavir between younger (≥2 to 6 years old) and older children (>6 to <13 years old).

Materials and methods

This was a within-child, two-period, crossover, open-label PK study conducted in two centres. HIV-1-infected

children between 2 and 13 years old who used q12h lamivudine and/or abacavir as part of their HAART regimen were eligible for inclusion. In a subpopulation of patients, analysis of intracellular levels of lamivudine and abacavir was also performed.

Study population

To compare PK parameters between younger and older children, enrolment was performed 1:1 in age strata of ≥2 to 6 years and >6 to <13 years old. Children had to be receiving three- or four-drug HAART containing lamivudine and/or abacavir, and in a clinically stable condition for more than 6 months, with an expectation of staying on their current regimen for at least a further 3 months. Being virologically stable was defined as having HIV-1 RNA below the lower limit of quantification (<400 copies/ml), or low (between 400 and 20 000 copies/ml) with the last two measurements taken at least 3 months apart being either both <1000 copies/ml or with not more than a 0.7 log difference. Absolute CD4+ cell count had to be stable or rising prior to study entry and not be expected to fall within the next 6 months. Carers and children, where appropriate, had to be able and willing to give informed consent.

Children were excluded if they had intercurrent illnesses or used concomitant therapy, with the exception of *Pneumocystis carinii* pneumonia prophylaxis. Moreover, renal or liver function abnormalities of grade 3 or greater (NIH classification) were considered exclusion criteria.

Medication

Doses of study medication were 4 mg/kg q12h for lamivudine and/or 8 mg/kg q12h for abacavir. The same daily doses were maintained after crossover to 8 mg/kg q24h for lamivudine and/or 16 mg/kg q24h for abacavir. Daily adult doses (that is, 300 mg for lamivudine and 600 mg for abacavir) were not exceeded. Lamivudine was prescribed in tablets of 150 or 300 mg, or oral liquid formulation containing lamivudine at 10 mg/ml. Abacavir was prescribed in tablets of 300 mg or oral liquid formulation containing abacavir at 20 mg/ml. The same formulation was used for daily use as has been used for twice-daily medication. For doses of lamivudine and abacavir, a difference of <25% in the total daily dose between the q12h and q24h doses was accepted due to problems in combining tablets and liquid formulations to achieve the correct dose.

PK sampling

Serial blood samples to obtain PK profiles of lamivudine and/or abacavir of q12h and q24h regimens were taken on two occasions from all the patients. The first sampling was performed at steady state on q12h regimens, 4 weeks after screening. Subsequently, children crossed over to q24h lamivudine and/or abacavir and PK sampling was repeated at week 8. The procedure of PK sampling was as follows. In the morning, children were admitted to the day care unit of their hospital; ingestion of medication was directly observed by the study nurse and children were allowed to take medication with or without food. Blood samples of 2 ml were drawn at time points 0 (pre-dose), 1, 2, 3, 4, 6, 8 and 12 h post-ingestion of medication for q12h regimens and at time points 0 (pre-dose), 1, 2, 3, 4, 6, 8 and 24 h for q24h regimens. Within 24 h of collection, blood samples were centrifuged and plasma was stored at –20°C. Plasma concentrations of lamivudine and abacavir were determined by a validated high-performance liquid chromatography assay with UV detection at a wavelength of 260 nm. In brief, a sample volume of 500 μl was pretreated by solid phase extraction on a Waters Oasis MAX® extraction cartridge (Waters Instruments, Inc., Minneapolis, MN, USA). Eluate was evaporated under nitrogen at 37°C, dissolved in 0.2 ml of a mixture of 95:5 water (Baker Analyzed™ HPLC; JT Baker, Phillipsburg NJ, USA): acetonitrile (Acetonitrile Super Gradient; LabScan, Dublin, Ireland), and injected on a 150×4.6 mm Symmetry Shield RP18 3.5 μm analytical column with a 3.9×20 mm Symmetry Shield RP18 3.5 μm guard column (Waters Instruments, Inc.). Mobile phase consisted of acetonitrile/acetate buffer of pH 4.6. Elution was performed using a gradient. Lower limits of quantification were 0.050 mg/l for lamivudine and 0.015 mg/l for abacavir. Accuracy was 92–98% for lamivudine and 97–100% for abacavir. Within-day variability was 1.4–2.3% for lamivudine and 1.1–1.9% for abacavir. Between-day variability was 0.66–2.2% for lamivudine and 0.16–2.3% for abacavir.

Measurements of intracellular drug triphosphates

Children weighing >35 kg were enrolled into a substudy where trough intracellular concentrations of lamivudine triphosphate (3TC-TP) and carbovir triphosphate (CBV-TP; the active moiety of abacavir) were measured at baseline (q12h) and at week 4 (q24h). Intracellular levels of 3TC-TP and CBV-TP were measured as previously described [7]. Briefly, peripheral blood mononuclear cells were separated from 10 ml whole blood by density cushion centrifugation, washed and counted, and then two extractions were carried out (the first in 60% methanol overnight, followed by perchloric acid). Quantitation of intracellular 3TC-TP and CBV-TP was carried out using an enzymatic assay based on inhibition of HIV reverse transcriptase, as measured by reduction in incorporation of radiolabelled deoxynucleoside triphosphates

into a synthetic template primer. Owing to the very limited amount of blood available from children, there was insufficient material for multiple, repeated analyses (although samples were analysed in duplicate). However, we have previously estimated the inter- and intra-assay variability for CBV-TP to be 5–15% and 11%, respectively, and the inter- and intra-individual variability of 3TC-TP assay to be 10–18% and 15%, respectively [7]. Concentrations of drug triphosphates are expressed in pmol/10^6 cells.

PK parameters of lamivudine and abacavir

PK parameters of lamivudine and abacavir were calculated using non-compartmental methods using Microsoft Excel 2000® [8]. The peak level (C_{max}) and trough level at the 12-h or 24-h time points (C_{min}) were determined visually from the plasma PK curve. Area under the plasma concentration–time curves 0–12 h ($AUC_{0–12}$) and 0–24 h ($AUC_{0–24}$) were calculated using the trapezoidal rule. To compare q12h with q24h regimens, estimates of daily AUC ($AUC_{0–24}$) were made ($AUC_{0–24} = 2 \cdot AUC_{0–12}$) in the q12h regimen. Relative apparent oral clearance (Cl/kg·F) was calculated as dose (mg)/AUC·body weight (kg).

Statistics

Statistical calculations were performed using SPSS v10.0 (SPSS, Inc., Chicago, IL, USA). To establish bioequivalence between the q12h and q24h regimens, a total of 16 PK sets of q12h and q24h results was anticipated for lamivudine and the same number for abacavir. Geometric means of all PK parameters were calculated. In the case of undetectable C_{min}, AUC was calculated until the time point with the last detectable drug level and extrapolated to infinity. For comparison of q12h with q24h regimens for each patient, ratios of $AUC_{0–24}$ and C_{max} and Cl/F·kg q24h versus q12h were calculated. Geometric mean ratios (GMRs) with 90% CI were calculated after log-transformation of within-patient ratios. A GMR with 90% CI falling entirely within 0.80–1.25 was considered as bioequivalence.

Data were compared with published historical controls on steady-state PKs: for lamivudine, adults on 150 mg q12h or 300 mg q24h [6,9,10] and for abacavir adults on 300 mg q12h and children using 8 mg/kg abacavir [11,12]. PK parameters and GMRs of PK parameters for q24h versus q12h regimens were compared between children of ≥2 to 6 years versus >6 to <13 years old using a *t* test for independent samples on log-transformed values. The Fisher's Exact test was used to compare the number of cases of C_{min} below the lower limit of quantification in younger versus older children. A two-sided *P*-value <0.05 was considered statistically significant.

Results

A total of 24 HIV-1-infected children underwent both sessions of PK sampling. Of these children, four were excluded from data analysis: one child on lamivudine and abacavir, because the morning dose had already been administered prior to the start of PK sampling on the first sampling day; two children on lamivudine because the q12h and q24h doses differed by more than 25% (in one child on lamivudine and abacavir, the data for abacavir were also excluded for the same reason); and one child on lamivudine because an incorrect q12h lamivudine dose had inadvertently been administered. Baseline patient characteristics of the remaining 20 children overall and per drug group are given in Table 1.

Table 1. Baseline patient characteristics overall and in patients using lamivudine or abacavir

	Overall (*n*=20)	Patients using 3TC (*n*=19)	Patients using ABC (*n*=14)
Age, years	5.6 (2.1–12.8)	5.8 (2.1–12.8)	5.1 (2.1–12.8)
Gender, male/female	10/10	9/10	6/8
Body weight, kg	22.5 (12.5–60.5)	21.3 (12.5–60.5)	19.3 (13.7–60.5)
Antiretroviral therapy used (*n*)	AZT, 3TC, ABC, NVP (6)	AZT, 3TC, ABC, NVP (6)	AZT, 3TC, ABC, NVP (6)
	AZT, 3TC, NVP (2)	AZT, 3TC, NVP (2)	AZT, 3TC, ABC (1)
	AZT, 3TC, ABC (2)*	AZT, 3TC, ABC (2)	AZT, ABC, EFV (1)
	AZT, ABC, EFV (1)	AZT, 3TC, NFV (1)	3TC, ABC, EFV (5)
	3TC, ABC, EFV (5)	3TC, TDF, EFV (1)	3TC, ABC, LPV/r (1)
	3TC, TDF, EFV (1)	3TC, ABC, EFV (5)	
	3TC, D4T, NVP (1)	3TC, ABC, LPV/r (1)	
	3TC, AZT, NFV (1)	3TC, D4T, NVP (1)	
	3TC, ABC, LPV/r (1)		
Other co-medication (*n*)	Amoxicillin/clavulanic acid (1)	Amoxicillin/clavulanic acid (1)	Amoxicillin/clavulanic acid (1)

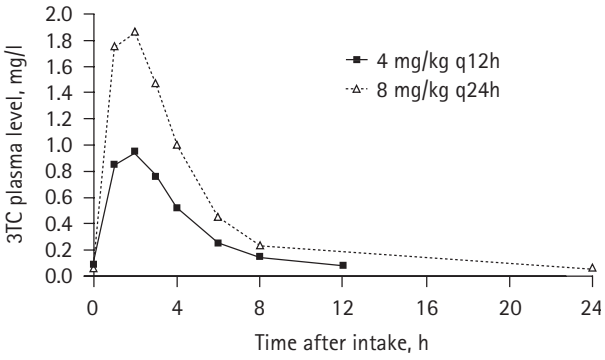
Data are presented as medians (interquartile range). *One patient using 3TC/ABC with non-evaluable ABC data. 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; D4T, stavudine; EFV, efavirenz; LPV/r, lopinavir/ritonavir; NFV, nelfinavir; NVP, nevirapine; TDF, tenofovir.

In 19 children, the PK profiles of lamivudine were evaluated. Median doses [interquartile range (IQR)] of lamivudine were 4.1 (4.0–4.3) mg/kg q12h and 8.2 (7.8–8.4) mg/kg q24h.

PK parameters of lamivudine per dosing regimen and those of historical controls in adults and children are given in Table 2. The q24h regimen resulted in a higher geometric mean AUC_{0-24} and an approximately twofold higher geometric mean C_{max} for q24h than for lamivudine q12h (Figure 1); both differences were statistically significant. GMRs of AUC_{0-24} differed significantly from 1.0, which precluded bioequivalence between the q24h and q12h regimen. Cl/F·kg was significantly lower for q24h versus q12h lamivudine.

In the children who were ≥2 to 6 years old (n=10), plasma levels of lamivudine were lower than in the children aged >6 to <13 years (n=9) (Table 3). For the q24h regimen, C_{max} was significantly lower (P=0.01) and there was a trend towards lower AUC_{0-24} (P=0.12). Similar results were observed for the q12h regimen (P=0.03 for C_{max} and P=0.05 for AUC_{0-12h}, respectively). Elimination half-lives of lamivudine were not significantly different between the two age groups. C_{min} was not different between children aged ≥2 to 6 and >6 to <13 years. For lamivudine q24h, C_{min} was <0.050 mg/l in five out of 10 younger children versus three out of nine older children (P=0.65). Using lamivudine q12h, C_{min} was <0.050 mg/l (P=1.00) in two out of 10 younger children versus one out of nine older children. Meanwhile, GMRs for the q24h regimen versus the q12h regimen of lamivudine were not significantly different between younger and older children: GMRs in younger and older children were 1.17 and 1.06 for AUC_{0-24}, 1.84 versus 1.96 for C_{max} and 0.85 versus 0.93 for Cl/F·kg, respectively (all P values >0.30).

Figure 1. Plasma pharmacokinetics of lamivudine (3TC)



q12h, twice daily; q24h, once daily.

Abacavir

PK data of abacavir were evaluable in 14 children. The median doses (IQR) of q12h abacavir were

Table 2. Pharmacokinetic parameters of lamivudine and abacavir, both dosed q12h and q24h

	Current study			Historical controls in adults	
Lamivudine					
PK parameter	4 mg/kg q12h GM (90% CI) (n=19)	8 mg/kg q24h GM (90% CI) (n=19)	GMR (90% CI) q24h versus q12h (n=19)	Lamivudine 150 mg q12h [9] (n=12) (mean ±SD)	Lamivudine 300 mg q24h [9] (n=12) (mean ±SD)
AUC_{0-24}, mg/l·h	8.88 (7.67–10.28)	9.80 (8.64–11.12)	1.12 (1.03–1.21)	17.09 ±6.46	16.64 ±4.15
C_{max}, mg/l	1.11 (0.96–1.29)	2.09 (1.80–2.42)	1.90 (1.67–2.16)	2.08 ±0.82	3.46 ±0.85
C_{min}, mg/l, median (range)	0.067 (<0.050–0.153)	0.050 (<0.050–0.076)	N/A	0.33 ±0.22	0.15 ±0.087
Cl/F*kg, l/h·kg	0.90 (0.78–1.04)	0.80 (0.70–0.92)	0.89 (0.82–0.96)	N/A	N/A
	Current study			Historical control adults	Historical control children
Abacavir					
PK parameter	8 mg/kg q12h GM (90% CI) (n=14)	16 mg/kg q24h GM (90% CI) (n=14)	GMR (90% CI) q24h versus q12h (n=14)	Abacavir 300 mg q12h [11] (n=20) GM (95% CI)	Abacavir 8 mg/kg q12h [12] mean (%CV) (n=45)
AUC_{0-24}, mg/l·h	9.91 (8.26–11.89)	13.37 (11.80–15.16)	1.35 (1.19–1.54)	11.6 (10.34–13.0)	9.8 (47)
C_{max}, mg/l	2.14 (1.79–2.56)	4.80 (4.04–5.71)	2.25 (1.83–2.77)	2.89 (2.55–3.27)	3.71 (37)
C_{min}, mg/l, median (range)	0.025 (<0.015–0.070)	<0.015 (<0.015–0.046)	N/A	N/A	N/A
Cl/F·kg, l/h·kg	1.58 (1.30–1.93)	1.16 (1.01–1.34)	0.73 (0.64–0.84)	N/A	N/A

q12h, twice daily; q24h, once daily; N/A, not applicable; SD, standard deviation; PK, pharmacokinetic; CV, coefficient of variation; GM, geometric mean; GMR, geometric mean ratio.

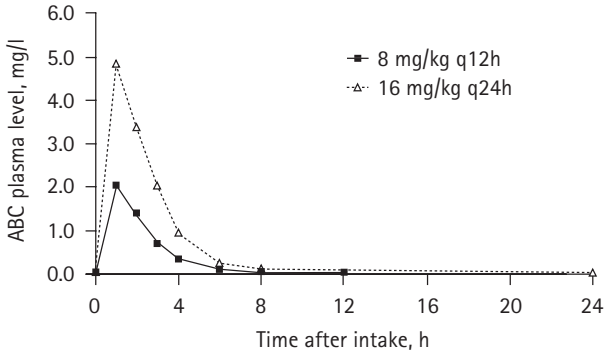
8.1 (7.8–8.5) mg/kg q12h and 16.4 (15.4–16.8) mg/kg q24h. PK parameters of abacavir are given in Table 2.

GMR of AUC_{0-24} slightly, but significantly exceeded 1.0, precluding bioequivalence between the q24h and q12h regimen. C_{max} of abacavir q24h was more than twice the value and significantly higher than C_{max} of abacavir q12h (Figure 2). Similar to lamivudine, significantly higher AUC_{0-24} and C_{max} values for abacavir q24h were reflected by a significantly decreased Cl/F·kg for this regimen.

AUC_{0-24}, C_{max} and Cl/F·kg in children ≥2 to 6 years old (*n*=9) were not significantly different in children aged >6 to <13 years (*n*=5) (*P* values >0.12 and >0.50 for q24h and q12h regimens, respectively; Table 3). However, C_{min} of abacavir q24h tended to be lower in younger children, since all nine children of the younger age group had a C_{min} of abacavir of <0.015 mg/l, while this was the case in only two out of the five older children (*P*=0.03). For the q12h regimen, this difference was not present: one out of nine younger children versus one out of five older children had a C_{min} <0.015 mg/l (*P*=0.60).

GMRs of AUC_{0-24}, C_{max} and Cl/F·kg for the q24h regimen versus the q12h regimen of abacavir were not significantly different between younger and older children; GMRs in younger and older children were 1.46 and 1.17 for AUC_{0-24}, 2.61 versus 1.72 for C_{max} and 0.67 versus 0.85 for Cl/F·kg (all *P* values >0.08).

Figure 2. Plasma pharmacokinetics of abacavir



q12h, twice daily; q24h, once daily. ABC, abacavir.

Intracellular levels

Due to technical and logistical problems, paired trough intracellular levels of lamivudine triphosphate (3TC-TP) and carbovir triphosphate (CBV-TP) were only available in two and one subject, respectively. For

Table 3. Lamivudine and abacavir pharmacokinetic parameters in children >2–6 years compared with >6 to <13 years old (geometric means (GM) (90% confidence interval) and *P* values for differences between GM

	Lamivudine 4 mg/kg q12h			Lamivudine 8 mg/kg q24h		
Parameter	Age ≥2 to 6 years (*n*=10)	Age >6 to <13 years (*n*=9)	*P* value	Age ≥2 to 6 years (*n*=10)	Age >6 to <13 years (*n*=9)	*P* value
Body weight*, kg	16.0 (12.5–29.3)	26.1 (21.3–60.5)	N/A	16.1 (13.5–28.6)	25.8 (22.2–61.3)	N/A
Dose*, mg/kg	4.1 (3.6–4.4)	4.0 (2.5–4.9)	0.54	8.3 (7.4–8.5)	7.9 (4.9–9.3)	0.65
AUC_{0-24}, mg/l·h	7.60 (6.12–9.45)	10.55 (8.82–12.63)	0.05	8.80 (7.43–10.43)	11.04 (9.06–13.45)	0.12
C_{max}, mg/l	0.94 (0.78–1.13)	1.34 (1.08–1.67)	0.03†	1.72 (1.48–1.99)	2.59 (2.04–3.28)	0.01†
C_{min}*, mg/l	0.068 (<0.050–0.15)	0.067 (<0.050–0.11)	N/A	0.050 (<0.050–0.076)	0.061 (<0.050–0.074)	N/A
Cl/F·kg, l/h·kg	1.09 (0.89–1.34)	0.73 (0.63–0.85)	0.13	0.92 (0.78–1.08)	0.69 (0.55–0.87)	0.07

	Abacavir 8 mg/kg q12h			Abacavir 16 mg/kg q24h		
Parameter	Age ≥2 to 6 years (*n*=9)	Age >6 to <13 years (*n*=5)	*P* value	Age ≥2 to 6 years (*n*=9)	Age >6 to <13 years (*n*=5)	*P* value
Body weight*, kg	16.7 (13.7–23.9)	26.1 (23.7–60.5)	N/A	16.8 (14.1–24.1)	25.8 (24.2–61.3)	N/A
Dose*, mg/kg	8.4 (8.0–8.8)	7.6 (5.0–8.4)	0.03†	16.6 (15.6–16.9)	14.9 (9.8–17.1)	0.18
AUC_{0-24}, mg/l·h	9.27 (7.06–12.18)	11.17 (8.76–14.24)	0.41	13.55 (11.19–16.42)	13.06 (10.91–15.63)	0.81
C_{max}, mg/l	1.94 (1.50–2.51)	2.54 (2.00–3.22)	0.22	5.07 (3.92–6.56)	4.36 (3.39–5.60)	0.48
C_{min}*, mg/l	0.027 (<0.015–0.040)	0.022 (<0.015–0.070)	N/A	<0.015 (<0.015–<0.015)	0.016 (<0.015–0.046)	N/A
Cl/F·kg, l/h·kg	1.80 (1.37–2.36)	1.26 (0.96–1.64)	0.13	1.21 (1.00–1.47)	1.08 (0.81–1.44)	0.51

*Median (range); †statistically significant. q12h, twice daily; q24h, once daily; N/A, not available or not done.

lamivudine, individual values of 3TC-TP at week 0 (q12h) versus week 4 (q24h) were 3.41 versus 12.23 pmol/10^6 cells in one patient; and 5.63 versus 3.20 pmol/10^6 cells in the second patient. For abacavir, individual levels of CBV-TP at week 0 (q12h) versus week 4 (q24h) were 0.20 versus 0.45 pmol/10^6 cells in one child.

Safety

None of the 20 evaluable children discontinued the once-daily treatment during the 24 weeks of follow-up. One grade III neutropaenia occurred at week 12, but this resolved before week 24 without any change in (doses of) study medication.

At baseline, 16 out of the 20 children had a viral load below the detection limit of 100 copies/ml; at the end of the follow-up period, this was the case in 17 out of the 19 children with a week 24 viral load measurement. The one child who missed the week 24 viral load measurement had undetectable viral load measurement throughout the study period.

Discussion

For both lamivudine and abacavir, GMRs of AUC_{0-24} of q24h versus q12h regimens were significantly higher than 1.0. Whereas the q24h and q12h regimens were not bioequivalent, these data suggest non-inferiority of the q24h regimens of lamivudine and abacavir with regard to the corresponding q12h regimens. This finding was independent of age in children within the two evaluated age strata of ≥2 to 6 years and >6 to <13 years. However, of note, for both the q12h and q24h regimens of lamivudine, younger children tended to have lower plasma levels than older children.

Q24h dosing of antiretroviral drugs simplifies medication schedules and may therefore be desirable for both HIV-1-infected adults and children. Although attractive, the feasibility of many q24h antiretroviral regimens remains to be explored, especially in children. For both lamivudine and abacavir, the active, triphosphorylated moieties have a longer *in vivo* intracellular half-life than their non-phosphorylated forms in plasma, which support the possibility of q24h use of these drugs [6,7,13]. Currently, the quantification of intracellular levels of nucleoside analogues requires more complicated sample handling than is needed for the determination of plasma levels of these drugs. In addition, a larger sample volume is needed, which may be difficult to obtain from certain patients, for example, young children. It was our intention to study the intracellular PKs of both lamivudine and abacavir in a subset of (older) children at week 0 (on the q12h regimen) and at week 4 (q24h regimen). Due to technical and logistical problems, we have only been able to measure intracellular levels of the active moiety of abacavir, CBV-TP, in one child and the intracellular levels of the active moiety of lamivudine, 3TC-TP, in two children. This highlights difficulties in studying intracellular PKs of nucleoside reverse transcriptase inhibitors in children. While these few data do not allow us to draw any conclusions, it was reassuring to note that intracellular 3TC-TP and CBV-TP concentrations in the few children studied were in the range of values observed for adults [14,15].

However, data do exist for limited numbers of adult subjects on the intracellular PKs of lamivudine and abacavir. For lamivudine, intracellular triphosphate concentrations have been correlated with virological outcome [16,17]. In healthy volunteers, regimens of 150 mg q12h and 300 mg q24h lamivudine have been found equivalent, not only in terms of plasma levels, but also intracellular triphosphate levels, which has been explained by the apparently saturable, rate-limiting conversion from lamivudine diphosphate to the active triphosphate [6,9,17]. This mechanism would result in intracellular accumulation of lamivudine diphosphate and the possibility of continued formation of triphosphate after lamivudine has been eliminated from the plasma compartment [17]. Efficacy of q24h lamivudine in HIV-1-infected adult patients has been evaluated in non-comparative studies, which showed favourable results [18,19]. In addition, three comparative studies in HIV-1-infected adults have shown no difference between the efficacy of the q24h versus q12h regimen [3,4,20]. In contrast to lamivudine, for abacavir, significant correlations (although mostly weak) were found between abacavir plasma AUC, C_{max} and C_{min} and HIV-1 viral load decline in adult patients [11,21]. Moreover, after both a 150-mg and 600-mg abacavir dose in HIV-1-infected adults, the triphosphorylated anabolite of abacavir (carbovir) has been shown to persist intracellularly for at least 24 h at levels above the *in vitro* inhibitory constant for antiretroviral activity of carbovir [13,22]. Interim results of an ongoing comparative study of abacavir q24h and q12h in HIV-1-infected adults have shown no difference between efficacy of the q24h versus the q12h regimen [3,5].

Several other findings deserve special attention. Firstly, the safety and antiviral activity of the q24h regimen appear to be similar to those of the q12h regimen, although these findings will obviously need to be confirmed in a larger, comparative trial. Secondly, for lamivudine, plasma levels in our juvenile patients were approximately 50% lower than seen in a comparative PK study of lamivudine q24h versus q12h in adults [9]. However, the data showed similarity with other historical controls of lamivudine q12h in adults (data not shown) [10,23]. Of interest, the currently recommended

dose of 4 mg/kg q12h lamivudine in children approximates to twice the licensed adult dose of 150 mg q12h (~2 mg/kg body weight) and was selected to compensate for the approximately 50% lower plasma levels in children under 12 years old, than those in adults using 150 mg q12h lamivudine [10]. However, the hypothesis that 4 mg/kg q12h lamivudine would result in plasma levels in children under 12 years old being similar to those in adults using lamivudine 150 mg q12h has, to the knowledge of the authors, not been previously confirmed in published data.

Another important issue might be age-related differences in PKs of lamivudine and abacavir. Indeed, in our study, a trend for lower plasma levels of lamivudine was found in younger children. As discussed above, lower levels of lamivudine have been reported in children under 12 years old, but no differences have previously been reported between children of ≥2 to 6 versus >6 to <13 years old [10]. Our data, however, suggest that such age-related differences may exist. According to our data, children in the age group of ≥2 to 6 years old possibly need higher doses of lamivudine to reach plasma levels similar to adults.

The PKs of abacavir were similar to steady-state data in adults and children using abacavir q12h and $AUC_{0–24}$, C_{max} and Cl/F·kg were not significantly different between younger and older children. Although this correlates with earlier data suggesting the absence of age-related differences in the PKs of abacavir q12h in children, in our data, a C_{min} of abacavir below the lower limit of quantification occurred at a higher rate in younger children using the q24h regimen than in older children [24].

It should be noted that our study did not include children under 2 years of age. This may be a challenging patient population due to, for example, fluctuating gastrointestinal absorption rate and slower renal clearance (the latter being the principal route of elimination for both lamivudine and abacavir metabolites) in very young children [10,25,26]. Therefore, the findings presented here cannot simply be extrapolated to children younger than 2 years old, and need additional investigation.

The slight but significant increase that was found between the $AUC_{0–24}$ of q24h and q12h regimens of lamivudine, in addition to the approximately two times higher C_{max} for the q24h regimen, suggest dose-proportionality of lamivudine PKs within the investigated dose range. This is in accordance with earlier findings in adults and children [10,27,28]. In the present study, PKs of abacavir tended to be more than dose-proportional for $AUC_{0–24}$ and C_{max}. In both adults and children, more than dose-proportional PKs have been reported with escalating single doses of abacavir, but not in studies of steady-state PKs [11] [21,24,29]. The most likely explanation for more than dose-proportional PKs of abacavir seems to be saturation of first-pass metabolism of abacavir, since the more than dose-proportional increases were observed for C_{max} and $AUC_{0–24}$, but not C_{min} [11].

In conclusion, our results suggest that in HIV-1-infected children ≥2 to <13 years old, the plasma pharmacokinetics of both lamivudine and abacavir given in a q24h regimen are non-inferior to the corresponding q12h regimens. Based on these plasma PK data, the q24h regimens of abacavir and lamivudine should be further explored in comparative efficacy and safety studies versus q12h regimens in HIV-1-infected children aged ≥2 to <13 years. Such studies should preferably also incorporate formal comparisons of adherence between such once- and twice-daily dosing regimens. The tendency for lower plasma levels of lamivudine in children ≥2 to 6 years old compared with older children poses the question as to whether higher doses of lamivudine should be administered to younger children.

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