Relative Bioavailability of Enalapril Administered as Orodispersible Minitablets in Healthy Adults

Clinical Pharmacology
in Drug Development
2019, 0(0) 1–11
© 2019, The American College of
Clinical Pharmacology
DOI: 10.1002/cpdd.728

Anne Van Hecken¹, Björn B. Burckhardt², Feras Khalil², Jan de Hoon¹,
Ingrid Klingmann⁵, Marissa Herbots¹, Stephanie Laeer², Florian B. Lagler⁴,
and Jörg Breitskreutz³

Abstract

The angiotensin-converting enzyme inhibitor enalapril is commonly used to treat chronic heart failure in children. Because some children are unable to swallow capsules or tablets, a new, age-appropriate, orodispersible minitab (ODMT) containing 1 mg of enalapril was developed within the EU-funded LENA (Labeling of Enalapril from Neonates up to Adolescents) consortium. In order to support the clinical evaluation of this new formulation in children, a relative bioavailability study was performed in healthy adults, comparing the bioavailability of enalapril in the ODMT with that of a reference product (RP) Renitec, a registered standard enalapril tablet formulation. In this open-label, randomized 3-way crossover study, 24 healthy subjects received a 10-mg enalapril dose administered as (1) 2 × 5-mg tablets of the RP swallowed with water, (2) 10 × 1-mg ODMT swallowed with water, and (3) 10 × 1 mg ODMT dispersed on the tongue. When the relative bioavailability of the ODMT formulation swallowed with water was compared with that of the RP, the estimated 90% CIs for the ratio of area under the concentration-time curve ($AUC_{0-\infty}$) and/or peak concentration (C_{max}) of enalapril were 92.34% to 106.49% and 91.28% to 115.72%, respectively, which are within the accepted bioequivalence limits of 80% to 125%. Following dispersion of the ODMT in the mouth, a slightly higher C_{max} for enalapril was observed as compared with the RP with an upper 90%CI of 127.57%, slightly exceeding the bioequivalence limit. Taken together, it was demonstrated that the method of administration of the ODMT, swallowed or dispersed, did not significantly affect the bioavailability of enalapril.

Keywords

relative bioavailability, enalapril, ODMT, chronic heart failure

Children are often treated as small adults, not as the special patients they are. Regulations exist to encourage the development of drugs and formulations for children. However, off-patent drugs are often administered to children without appropriate formulations or systematic clinical investigations. Therefore, the Paediatric Use Marketing Authorisation has been designed as an incentive for off-patent medicinal products offering 10 years of data protection. It is a dedicated marketing authorization covering the indication(s) and appropriate formulation(s) for medicines developed exclusively for use in the pediatric population.

The European Medicines Agency (EMA) Paediatric Committee has prioritized the unmet needs for the cardiovascular off-patent drugs in the European pediatric population.¹ Enalapril is an angiotensin-converting enzyme inhibitor commonly used for cardiac failure in children of all ages including the age group under

1 year of age, although it is not labeled for any condition in patients <20 kg in European countries.^{2–5} The pharmacopoeial form of enalapril is its maleate salt. Current problems with the use of enalapril in children include no appropriate formulation and insufficient

¹Center for Clinical Pharmacology, University Hospitals of Leuven, Leuven, Belgium

²Institute of Clinical Pharmacy and Pharmacotherapy, Heinrich Heine University, Düsseldorf, Germany

³Ethicare GmbH, Haltern am See, Germany

⁴Paracelsus Medical University, Salzburg, Austria

⁵Pharmaplex bvba, Wezembeek-Oppem, Belgium

Submitted for publication 18 March 2019; accepted 15 July 2019.

Corresponding Author:

Anne Van Hecken, Center for Clinical Pharmacology, University Hospitals of Leuven, 3000 Leuven, Belgium
(e-mail: anne.vanhecken@uzleuven.be)

stability of the liquid-soluble formulation for young pediatric patients, insufficient pediatric pharmacokinetic (PK) data for any formulation, insufficient safety data in neonates, infants, and young children, and limited pharmacodynamic and efficacy data for pediatric cardiological indications.⁶

The aims of the LENA (Labeling of Enalapril from Neonates up to Adolescents) project are to address these shortcomings and to provide a basis for a future pediatric use marketing authorization of enalapril maleate by developing an age-appropriate orodispersible minitab (ODMT) suitable for all pediatric subsets.⁷ For this new galenic formulation, PK data in adults have to be provided in order to compare the bioavailability of enalapril in the new galenic formulation with that of the existing standard formulation, a conventional immediate-release tablet. This relative bioavailability is necessary in order to define the need for adjusting the enalapril doses to be used in the pediatric clinical studies. Because enalapril undergoes rapid conversion to the active compound enalaprilat, it also needs to be investigated whether the PK of the enalapril ODMT is affected by swallowing of the minitab with water versus dispersion in the oral cavity.

There is comprehensive information available on the PK behavior of the standard marketed enalapril tablets in adults. Enalapril is a lipid-soluble prodrug that is rapidly and well absorbed (60-70%) from the gastrointestinal tract, reaching peak serum concentrations about 1 hour after oral drug administration. Following absorption, enalapril is extensively and rapidly hydrolyzed in the liver by carboxylesterase to the active metabolite enalaprilat, a potent angiotensin-converting enzyme inhibitor. Peak concentrations of enalaprilat occur about 4 hours after an oral dose of an enalapril tablet. Elimination of enalapril occurs mainly by metabolic hydrolysis to enalaprilat with an elimination half-life of about 1 to 2 hours. Unchanged enalapril and enalaprilat are predominantly excreted in the urine with a total urinary recovery of approximately 60% after oral administration of enalapril maleate. The elimination of enalaprilat is polyphasic, with an initial phase that reflects renal filtration (elimination half-life 2 to 6 hours), followed by a prolonged phase (elimination half-life 30 to 35 hours), reflecting high-affinity binding to plasma angiotensin-converting enzyme. In the elimination phase, enalaprilat is the only detectable metabolite. The PK of enalapril and enalaprilat do not differ significantly between fasted and fed conditions.⁸⁻¹¹

Although there have been various papers on ODMTs since the first report on this promising new drug formulation,¹² no PK data for other newly developed ODMTs have been published so far. From other rapidly disintegrating drug formulations such as lyophilizates

Table 1. Summary of Subject Characteristics

Age, y	
Mean	27.7
Median	24.4
Range	22.1-47.2
Race, n (%)	
White	24 (100)
Gender, n (%)	
Female	11 (45.8)
Male	13 (54.2)
BMI, kg/m ²	
Mean	22.7
Median	22.2
Range	18.6-28.8

BMI indicates body mass index.

for oral use^{13,14} and orodispersible films,^{15,16} it is well known that some active pharmaceutical ingredients (APIs) may be at least partially absorbed via the oromucosal pathway. Other APIs are poorly absorbed from the oral cavity but can be swallowed when dissolved in the saliva and then absorbed from the intestine.

The primary objectives of this study were to assess the relative bioavailability of a single 10-mg dose of enalapril administered as 10 1.0-mg ODMTs taken (1) with water and (2) dispersed in the oral cavity versus (3) 2 × 5-mg tablets of the RP taken with water. Whether the PK of enalapril is affected when the ODMT is entirely swallowed with water versus dispersed in the oral cavity, general safety, and tolerability including local tolerability and palatability of enalapril administered as ODMT were all assessed as secondary objectives.

Methods

Subjects

The study involved 24 healthy nonsmokers, consisting of 11 nonpregnant, nonbreastfeeding female and 13 male volunteers between 18 and 55 years old with body mass index between 18.5 and 30 kg/m². Baseline demographics of the participants are shown in Table 1.

The general health condition of subjects was confirmed by medical history evaluation, blood pressure (BP) assessment, physical examination, 12-lead ECG, and clinical laboratory assessment (serum chemistry, hematology, and urinalysis). Women of childbearing potential had to agree to practice a highly effective method of birth control from screening at least until the last study visit.

Throughout the study, chronic intake of prescription or nonprescription medication (including vitamins and herbal supplements) other than the study drug was prohibited, except for acetaminophen and hormonal contraception.

The use of acetaminophen was allowed up to 2 g per day for the treatment of headache or other pain. If a subject had to use medication during the study, continuation of the study was allowed if according to the investigator the concomitant medication was not relevant within the context of the trial.

Study Design and Procedures

This has been a single-center, open-label, randomized 3-way crossover, 3-treatment, 3-period study.

The study was approved by the independent Ethics Committee of the University Hospitals KU Leuven and was performed in the Center of Clinical Pharmacology within this institution. All participants provided written informed consent, and the study was conducted in accordance with the International Council on Harmonisation Tripartite Guideline on Good Clinical Practice. All subjects attended a screening visit within 21 days before receiving the first dose of the study drug. On inclusion, each eligible subject received a randomization number (from 001 to 024) and was assigned to a treatment sequence according to a randomization list, generated for a balanced crossover design so that each formulation

- was administered once per subject
- occurred the same number of times in each period
- was preceded equally often by each of the other formulations

After an overnight fast, the study subjects were administered a 10-mg enalapril maleate oral dose on 3 occasions, separated by at least 1 week of washout, as follows:

- Treatment A: 2 × 5-mg tablets of the RP swallowed with 240 mL of water
- Treatment B: 10 × 1-mg ODMT, swallowed with 240 mL of water
- Treatment C: 10 × 1-mg ODMT, dispersed on the tongue following wetting of the mouth with 20 mL of water, followed by administration of 220 mL of water at 1 hour postdose

Consumption of water was allowed as desired except for 1 hour before dosing until 2 hours after treatment, except for treatment C, where at 1 hour postdose, 220 mL of water was administered according to the study protocol. The duration of the study was approximately 6 weeks including prestudy and poststudy evaluations. The first subject first visit was on August 4, 2014, and the last subject last visit was September 15, 2014.

Study Assessments

Following all treatments, blood samples were obtained for the determination of enalapril and enalaprilat at predose and at 10, 20, 30, 45 minutes and 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 24, 36, and 48 hours postdose.

Safety was monitored by clinical assessments of spontaneously reported adverse events and by repeated measurements of vital signs, physical examinations, 12-lead ECGs, and standard laboratory safety tests (hematology and chemistry) at regular time points after dosing.

The palatability of the ODMT formulation was evaluated by a Palatability and Taste Questionnaire completed immediately after intake and at 10 minutes after the ODMT administration.

Bioanalyses

Fifty microliters of each collected serum sample underwent sample purification according to a tailored in-house protocol utilizing Oasis 96-well MAX solid-phase extraction plates (10 mg, 1 mL). Purified samples were diluted 1:2, and a volume of 10 µL was injected for the bioanalytical analyses using high-performance liquid chromatography coupled with a tandem mass spectrometer (LC 10, Shimadzu, Kyoto, Japan, and API 2000 mass spectrometer, AB Sciex, Redwood City, California). Chromatographic separation was carried out on an XBridge BEH C18 3.5 µm column (3.0 mm x 150 mm; Waters Corp, Milford, Massachusetts) applying a gradient of methanol and water (both acidified with 0.1% formic acid and 2 mM ammonium formate) within a run time of 7 minutes. For quantification, the ion transitions 377.2 to 234.2 m/z for enalapril, 349.1 to 206.1 m/z for enalaprilat, and 425.3 to 351.2 m/z for benazepril (internal standard) were used. The bioanalytical method was validated in accordance with EMA and Food and Drug Administration (FDA) bioanalytical method validation guidance using European Pharmacopoeia Reference Standards of enalapril and enalaprilat.^{17,18} Linearity of the bioanalytical method was established from 0.2 to 200 ng/mL enalapril and from 0.18 to 180 ng/mL enalaprilat. The method was further characterized by an intrarun precision ranging from 2.2% to 5.0% for enalapril and 4.9% to 18.0% for enalaprilat. The interrune precision, expressed as coefficient of variation, varied between 3.0% and 10.8% for enalapril and 10.9% to 18.6% for enalaprilat. Mean accuracy results of the quality control samples were likewise within the guideline requirements of ±15% at all concentration levels except the lower limit of quantification (±20%). The Internal standard normalized matrix effect was less than 15% among 7 different sources and according

to EMA guidelines. All analytes were fully recovered from the sorbent of the mixed-mode anion exchanger, resulting in a process efficiency of 66% to 93%.

ODMTs and Reference Product

ODMT containing 1.0 mg enalapril maleate were used in this study. The production, packaging, and labeling were performed under Good Manufacturing Practice conditions by NextPharma (Waltrop, Germany) under the supervision of the sponsor, Ethicare GmbH (Haltern am See, Germany).

As reference product (RP), tablets containing 5 mg of enalapril maleate (MSD Belgium, Brussels, Belgium) were used.

PK and Statistical Analyses

The PK and statistical analyses of the data were performed using Phoenix WinNonlin version 6.3 (Certara, Princeton, New Jersey).

The pharmacokinetic parameters were calculated for both enalapril and enalaprilat following each of the administered treatments (A, B, and C) from the individual serum concentrations versus actual time profiles by a noncompartmental analysis according to the following methods:

- The linear trapezoidal method was used to calculate the area under the serum concentration-time curve from time 0 to the last quantifiable observed concentration (AUC_{0-t_z}).
- The AUC_{0-t_z} was extrapolated to infinity ($AUC_{0-\infty}$) by adding the terminal area calculated as C_{last}/λ_z , where C_{last} is the last quantifiable observed concentration.
- C_{max} was obtained directly from the concentration-versus-time data as the maximum observed concentration in a profile.
- t_{max} was the time at which C_{max} is observed. If 2 or more concentration values were equal to C_{max} , the time of the first concentration was chosen.
- λ_z was estimated at the terminal phase by linear regression after log-transformation of the concentrations using at least 3 points.
- Half-life was calculated as $\ln(2)/\lambda_z$.

To compare and evaluate the relative bioavailability of enalapril and enalaprilat after the single oral administration of treatments A, B, and C, the geometric mean ratios of each pair and the 90% CIs of these ratios were calculated for AUC_{0-t_z} , $AUC_{0-\infty}$, and C_{max} .

An ANOVA was performed on the natural logarithmically transformed C_{max} , AUC_{0-t_z} , and $AUC_{0-\infty}$ values and included treatment, period, and sequence as fixed effects as well as subject nested within sequence

as random effect. The obtained CI limits were back-transformed via antilog and presented.

Observed t_{max} times were evaluated separately and independently for each of the group comparisons of interest based on the Mann-Whitney U-test.

Results

Twenty-three subjects successfully completed all 3 periods of the study. One subject had a predose positive drug screen for tetrahydrocannabinol in study period 2 and therefore did not receive treatment A (the RP); this subject was dosed in periods 1 and 3 and received the 2 ODMT treatments according to the randomization schedule.

Pharmacokinetics

Mean ($\pm$ SD) serum concentration-time profiles of enalapril and enalaprilat following treatment with enalapril 10 mg administered as the RP (A), 10×1 mg ODMT swallowed with water (B), and 10×1 mg ODMT dispersed in the mouth (C) are presented in Figures 1 and 2, respectively. Analytical results for enalapril and enalaprilat that were below the lower limit of quantification were reported as 0 and as such included in the calculations of the means. Following all 3 treatments, enalapril concentrations were detectable up to approximately 6 hours after the dose administration, which reflects the fast drug elimination and the rapid conversion of enalapril to its active metabolite enalaprilat (Figure 1). Figure 2 shows that the serum concentration-time profiles for the active metabolite enalaprilat were comparable for all treatments.

Tables 2 and 3 provide the descriptive statistics for the primary PK parameters of enalapril and enalaprilat following the administration of a single oral dose of 10 mg enalapril given as the RP (A), ODMT swallowed (B), and ODMT dispersed in the mouth (C).

The first quantifiable enalapril and enalaprilat concentrations were observed between 0.17 and 1.0 hour postdose for enalapril and between 0.17 and 1.5 hours for enalaprilat following all treatments. The mean ($\pm$ SD) C_{max} of enalapril was 61.20 ± 20.09 ng/mL at 0.88 ± 0.30 hours following the ODMT when swallowed, 68.00 ± 24.70 ng/mL at 1.02 ± 0.36 hours following the ODMT dispersed in the mouth versus 61.10 ± 23.02 ng/mL at 1.25 ± 0.90 hours following the standard enalapril formulation.

Regarding the time of the peak exposure (t_{max}), there were slight differences among the 3 treatments as the mean ($\pm$ SD) t_{max} values for enalapril were found to be 1.25 hours (± 0.90), 0.88 hours (± 0.30), and 1.02 hours (± 0.36) following treatments A, B, and C, respectively. However, this difference was not statistically significant

Figure 1. Mean ($\pm$ SD) serum concentration-time curves for enalapril following 10 mg of enalapril provided as tablets (treatment A), orodispersible minitables swallowed (treatment B), and dispersed in the mouth (treatment C).

Figure 2. Mean ($\pm$ SD) serum concentration-time curves for enalaprilat following 10 mg of enalapril provided as tablets (treatment A), orodispersible minitables swallowed (treatment B), and dispersed in the mouth (treatment C).

except for the comparison between ODMT test formulation when swallowed and the RP (P -value = .0478, Mann-Whitney U-test), indicating a relatively faster drug absorption for the ODMTs compared with the reference formulation. It should be noted that in 1 subject in the reference group (subject 5, treatment A), the measured profile showed a double-peak curve and an exceptionally late $t_{\max}$ value of enalapril ($t_{\max}$ of

4.55 hours). Delayed gastric emptying might be a reason for this late time point. With this value excluded, the mean $t_{\max}$ value of the reference formulation amounted to 1.10 hours with a SD of 0.55 hours, and the difference between groups was no longer significant (P -value = .075, Mann-Whitney U-test). However, no clear reason was identified that clarifies the obtained profile in this subject.

Table 2. Summary Statistics of Enalapril PK Parameters Following 10 mg of Enalapril Provided as Tablets (A), ODMT Swallowed (B), and Dispersed in the Mouth (C)

Treatment Parameter	A (RP)				B (ODMT Swallowed)				C (ODMT Dispersed)			
	C _{max} (ng/mL)	t _{max} (h)	AUC _{0-t} (h·ng/mL)	AUC _{0-∞} (h·ng/mL)	C _{max} (ng/mL)	t _{max} (h)	AUC _{0-t} (h·ng/mL)	AUC _{0-∞} (h·ng/mL)	C _{max} (ng/mL)	t _{max} (h)	AUC _{0-t} (h·ng/mL)	AUC _{0-∞} (h·ng/mL)
N	23	23	23	23	24	24	24	24	24	24	24	24
Mean	61.10	1.25	99.69	100.44	61.20	0.88	97.06	97.79	68.00	1.02	103.84	104.66
SD	23.02	0.90	37.17	37.25	20.09	0.30	32.47	32.61	24.70	0.36	36.71	36.80
CV%	37.67	71.58	37.29	37.09	32.82	34.74	33.45	33.35	36.33	35.56	35.35	35.16
Min	21.98	0.50	44.25	44.96	27.23	0.50	38.22	38.74	34.81	0.50	51.99	52.58
Median	57.43	1.00	101.22	101.71	61.64	0.75	96.16	96.72	60.83	1.00	95.38	96.26
Max	117.96	4.55	184.31	185.26	120.24	2.00	188.32	189.46	128.16	2.07	190.83	191.64
GM	56.84	1.08	92.94	93.71	58.15	0.84	91.90	92.63	64.11	0.97	97.95	98.77
CV% GM	41.60	54.89	40.57	40.33	34.00	29.65	35.54	35.41	35.78	33.50	36.06	35.90

AUC_{0-t} indicates area under the curve to the last quantifiable concentration; AUC_{0-∞}, AUC extrapolated to infinity; C_{max}, maximum serum concentration; CV%, coefficient of variation; GM, geometric mean; Max, maximum; Min, minimum; N, number; ODMT, oral dispersible minitabets; PK, pharmacokinetic; RP, reference product; t_{max}, time to maximum concentration.

Table 3. Summary Statistics of Enalaprilat PK Parameters Following 10 mg of Enalapril Provided as Tablets (A), ODMT Swallowed (B), or Dispersed in the Mouth (C)

Treatment Parameter	A (RP)				B (ODMT Swallowed)				C (ODMT Dispersed)			
	C _{max} (ng/mL)	t _{max} (h)	AUC _{0-t} (h·ng/mL)	AUC _{0-∞} (h·ng/mL)	C _{max} (ng/mL)	t _{max} (h)	AUC _{0-t} (h·ng/mL)	AUC _{0-∞} (h·ng/mL)	C _{max} (ng/mL)	t _{max} (h)	AUC _{0-t} (h·ng/mL)	AUC _{0-∞} (h·ng/mL)
N	23	23	23	23	24	24	24	24	24	24	24	24
Mean	48.15	3.72	390.88	407.32	45.98	3.65	377.80	395.60	49.74	3.63	401.01	419.94
SD	18.51	0.75	95.94	97.98	18.62	0.85	85.97	87.97	18.50	0.96	89.60	92.31
CV%	38.45	20.22	24.54	24.05	40.50	23.40	22.76	22.24	37.19	26.44	22.34	21.98
Min	24.83	2.50	191.62	206.69	19.64	2.00	220.58	233.29	20.38	2.00	207.84	217.81
Median	42.95	3.50	411.72	420.23	42.12	3.75	363.72	387.95	50.15	3.50	396.46	414.16
Max	82.86	6.00	582.37	600.92	83.20	5.00	538.68	564.43	93.37	6.00	552.93	571.49
GM	44.82	3.65	379.05	395.57	42.49	3.54	368.28	386.03	46.51	3.50	390.41	409.20
CV% GM	40.46	19.51	26.38	25.66	42.64	26.69	23.62	23.16	39.29	27.18	24.81	24.38

AUC_{0-t} indicates area under the curve to the last quantifiable concentration; AUC_{0-∞}, AUC extrapolated to infinity; C_{max}, maximum serum concentration; CV%, coefficient of variation; GM, geometric mean; Max, maximum; Min, minimum; N, number; ODMT, oral dispersible minitabets; PK, pharmacokinetic; RP, reference product; t_{max}, time to maximum concentration.

Enalapril was rapidly eliminated from plasma following all treatments with a half-life of 0.75 hour (± 0.09) following the RP, 0.77 hour (± 0.11) following the ODMT swallowed, and 0.77 hour (± 0.21) following ODMT dispersed.

Relative Bioavailability

The ratio of the primary parameters C_{max}, AUC_{0-t}, and AUC_{0-∞} for enalapril after the RP versus ODMT and ODMT swallowed versus ODMT dispersed are visualized in Figure 3. When comparing the test formulation (10 × 1-mg ODMT swallowed with water, treatment B) to the reference formulation (2 × 5-mg tablets of the RP, treatment A) with regard to relative bioavailability, the estimated 90% CIs for the ratio of AUC_{0-tz}, AUC_{0-∞}, and C_{max} of enalapril were

92.32-106.58%, 92.34-106.49%, and 91.28-115.72%, respectively, which were all within the bioequivalence limits of 80% to 125%. These results indicated that 10 × 1-mg ODMT, when swallowed whole (treatment B), showed the same bioavailability as 2 × 5-mg tablets of the RP (treatment A).

Comparison of the ODMTs administered as 10 × 1-mg minitabets dispersed on the tongue (treatment C) with the reference formulation (treatment A) indicated the estimated 90% CIs for the ratio of AUC_{0-tz}, AUC_{0-∞}, and C_{max} of enalapril to be 98.40-113.59%, 98.46-113.56%, and 100.63-127.57%, respectively, with the 90% upper CI for C_{max} slightly exceeding the bioequivalence upper limit. However, comparison of enalaprilat (the active metabolite of enalapril) in these 2 treatments, showed the C_{max} and the AUC_{0-tz} to be

Figure 3. Ratio with 90% CIs of the test formulations B (ODMT swallowed) and C (ODMT dispersed) versus the reference product (A) for enalapril primary PK parameters. $AUC_{0-\infty}$ indicates area under the curve from 0 to infinity; AUC_{0-tz} , area under the curve from 0 to the last quantifiable concentration; C_{max} , peak concentration.

Table 4. Assessment of Relative Bioavailability for Enalapril by ANOVA Following 10 mg of Enalapril Provided as RP Tablets (A), ODMT Either Swallowed (B) or Dispersed in the Mouth (C)

Parameter	Units	Treatment	Reference	Ref LSM	Test LSM	Ratio (%) Test/Ref	90%CI Lower	90%CI Upper
AUC_{0-tz}	h·ng/mL	B	A	4.53	4.52	99.19	92.32	106.58
	h·ng/mL	C	A	4.53	4.58	105.72	98.40	113.59
	h·ng/mL	C	B	4.52	4.58	106.58	99.31	114.38
$AUC_{0-\infty}$	h·ng/mL	B	A	4.54	4.53	99.16	92.34	106.49
	h·ng/mL	C	A	4.54	4.59	105.74	98.46	113.56
	h·ng/mL	C	B	4.53	4.59	106.63	99.41	114.38
C_{max}	ng/mL	B	A	4.04	4.06	102.78	91.28	115.72
	ng/mL	C	A	4.04	4.16	113.31	100.63	127.57
	ng/mL	C	B	4.06	4.16	110.24	98.09	123.90

AUC_{0-tz} indicates area under the curve to the last quantifiable concentration; $AUC_{0-\infty}$, AUC extrapolated to infinity; C_{max} , maximum serum concentration; LSM, least-squares mean; ODMT, orodispersible minitabets.

in the range of 80% to 125% with C_{max} of 104.33% (90%CI 92.26-117.99%) and AUC_{0-tz} 103.28 (90%CI 95.07-112.19%).

Finally, when the ODMT dispersed on the tongue (treatment C) was compared with the ODMT swallowed as a whole (treatment B), the estimated 90% CIs for the ratio of AUC_{0-tz} , $AUC_{0-\infty}$, and C_{max} of enalapril were all in a range of 80% to 125%, indicating similar rates and extents of drug absorption following both ways of administration of the ODMT (Tables 4 and 5).

Safety

No serious adverse events were reported, and no subject was discontinued due to an adverse event. Sixteen of the 24 subjects reported a total of 32 postdose adverse events, 25 of which were considered drug related (9 following the RP, 8 following ODMT swallowed, and 8 following ODMT dispersed). The most frequently

reported adverse events were headache (reported 5 times following the RP, and 4 and 2 times following ODMT swallowed and dispersed, respectively), and postural dizziness (2 times following each treatment). These adverse events, as well as the mild blurred vision reported by 1 subject following the RP and the mild nausea and diarrhea reported once following the RP and ODMT swallowed, respectively, were in line with the most common adverse events as listed in the summary of product characteristics of the RP. All the adverse events were mild in intensity except for 1 moderate headache following the RP, and all adverse events were resolved by the end of the study. As could be expected following administration of 10 mg enalapril, mild decreases in systolic and diastolic blood pressure occurred after all treatments. The mean maximum decreases were 5.6, 5.5, and 7.3 mm Hg for systolic BP, and 6.9, 5.0, and 8.3 mm Hg for the diastolic BP following treatments A, B, and C, respectively. There were no clinically

Table 5. Assessment of Relative Bioavailability for Enalaprilat by ANOVA Following 10 mg of Enalapril Provided as RP tablets (A), ODMT Either Swallowed (B) or Dispersed in the Mouth (C)

Parameter	Units	Treatment	Reference	Ref. LSM	Test LSM	Ratio (%) Test/Ref	90%CI Lower	90%CI Upper
AUC _{0-tz}	h·ng/mL	B	A	5.93	5.91	97.43	89.69	105.83
	h·ng/mL	C	A	5.93	5.97	103.28	95.07	112.19
	h·ng/mL	C	B	5.91	5.97	106.01	97.71	115.01
AUC _{0-∞}	h·ng/mL	B	A	5.98	5.96	97.73	89.96	106.18
	h·ng/mL	C	A	5.98	6.01	103.60	95.36	112.55
	h·ng/mL	C	B	5.96	6.01	106.00	97.69	115.02
C _{max}	ng/mL	B	A	3.80	3.75	95.30	84.27	107.77
	ng/mL	C	A	3.80	3.84	104.33	92.26	117.99
	ng/mL	C	B	3.75	3.84	109.48	96.99	123.57

AUC_{0-tz} indicates area under the curve to the last quantifiable concentration; AUC_{0-∞}, AUC extrapolated to infinity; C_{max}, maximum serum concentration; LSM, least-squares mean; ODMT, orodispersible minitabets.

significant laboratory adverse events that were considered related to the study medication.

Oral Palatability of the ODMT

Results of the palatability questionnaire revealed that following treatment C (ODMT dispersed on the tongue) all 24 subjects experienced the occurrence of 1 or more taste sensations, whereas only 9 of the 24 subjects reported the occurrence of a taste following treatment B (swallowing of the ODMT). The most dominant taste following both treatments was bitterness, reported by 23 of 24 subjects following treatment C and 7 of 24 subjects following treatment B. The bitterness was mild in intensity following treatment B (median score 2/10) and mild to moderate (median score 6/10) following treatment C. In combination with the bitter taste, 15 of 24 subjects who received the ODMT dispersed on the tongue reported both a mild astringent taste and a mild sour taste. The astringent taste was not reported by the subjects following swallowing of the ODMT; however, a sour taste was reported by 3 subjects.

The ODMT did not cause any irritation in the mouth except in 1 subject following both ODMT treatments, and mild numbness was reported by 3 subjects following treatment C and by 1 subject following treatment B.

The ODMT, when dispersed on the tongue (treatment C), dissolved easily in the mouth with a mean rating score of 2/10 (0/10 is “very easily”) and could easily be swallowed (treatment B) with a median score of 0/10.

Besides the mild to moderate bitter taste of the ODMT, especially when the ODMT were dispersed on the tongue, the ODMT were generally well tolerated.

Discussion

This study was designed to evaluate the PK comparability of enalapril in a newly developed orodispersible

minitabets formulation to commercially available enalapril tablets. The results of the study demonstrated that enalapril ODMTs (10 × 1 mg) and 2 × 5-mg tablets of the RP, both swallowed with water, were pharmacokinetically comparable and are therefore therapeutically interchangeable. The geometric mean ratios of AUC_{0-tz}, AUC_{0-∞}, and C_{max} for subjects administered enalapril ODMTs with water compared with subjects treated with enalapril RP were 99.19%, 99.16%, and 102.78% for enalapril. The 90%CIs of these point estimates were within the 80% to 125% range, and therefore, the results meet the EMA bioequivalence criteria.¹⁹ Also, the active metabolite enalaprilat demonstrated the bioequivalence criteria in treatment A compared with treatment B. On the other hand, when the ODMT dispersed was compared with the RP, the estimated 90%CI for the ratio of C_{max} was 100.63% to 127.57%, slightly exceeding the upper bioequivalence limit. However, point estimates for geometric means for AUC_{0-tz}, AUC_{0-∞}, and C_{max} following ODMTs dispersed versus swallowed were comparable, and 90%CIs completely fit into the range of 80% to 125%. The results indicate that enalapril from ODMTs is not absorbed via buccal mucosa because time to peak exposure between dispersed and swallowed whole ODMTs were not different. These results further indicate that, when the ODMTs were dispersed in the mouth, the absorption occurs only once the dispersed ODMT content in the saliva arrives in the stomach. If the dispersed ODMTs arrive in the stomach, a slightly higher extent of enalapril absorption was observed in comparison with the RP and swallowed ODMTs.

In summary, the small (2 mm in diameter and height) orodispersible minitabets can reliably substitute for the common tablet formulation. This demonstrates that ODMTs might be an advantage for special populations having difficulties swallowing conventional tablets such

as neonates, infants, and young children as well as elderly people.

Several studies have been published investigating enalapril and enalaprilat after oral administration of 10 mg enalapril in healthy volunteers.^{20–23} Reasons for the substantial variation observed in obtained PK parameters among different studies (geometric means of AUC_{0-tz} , $AUC_{0-\infty}$, and C_{max} and their 90% CIs) might be differences in the applied study designs such as sampling time points, duration of sampling, demographics of the study population, and method of detection of the drug concentrations. Moreover, alterations in PK parameters might be caused by the human matrix used and its sample preparation. In comparison to the pivotal studies of the RP, serum was analyzed in the study here presented, whereas other authors determined plasma concentrations of enalapril and its active metabolite enalaprilat. However, all drug concentrations in the presented study were determined by a validated bioanalytical assay in compliance with EMA and US FDA guidance on bioanalytical method validation. The applied liquid chromatography-tandem mass spectrometry methods were accurate, precise, and not affected by a matrix effect.²⁴

A recent publication using a comparable study design and method of detection to this study demonstrated maximum plasma concentrations of enalapril and enalaprilat, following 10 mg of a standard enalapril tablet, to be 58 ng/mL and 41 ng/mL, which are similar to 61 ng/mL and 48 ng/mL in this study.²³ Time to maximum concentration and $AUC_{0-\infty}$ were also similar. This demonstrates that enalapril ODMTs are pharmacokinetically similar to the RP and potentially also to other enalapril products when investigated with a similar study design.

In another recent publication based on the same clinical study as described in this article and using the same serum concentration data, a model-dependent PK analysis was performed in contrast with the non-compartmental method as requested by EMA bioequivalence guidelines. The model-dependent analysis indicated that the rate and extent of absorption and elimination were not different between the ODMT formulation and the RP, concluding that the relative bioavailability of enalapril was not different between the formulations.²⁵

The overall safety profile of enalapril from ODMTs was acceptable and similar to that from enalapril tablets. All the adverse events were mild in intensity except for 1 moderate headache following administration of the RP. All adverse events were resolved by the end of the study. This is consistent to other reports about enalapril administered in a dose of 10 mg to healthy volunteers.

EMA's definition of acceptability comprises the attributes taste, palatability, and swallowability.²⁶ The taste of the new ODMT formulation of enalapril was rated by the adult volunteers as acceptable. It was well tolerated despite a sensed mild bitter and sour taste. The investigations on palatability showed a mild astringency, especially when the ODMTs were kept for a longer period on the tongue (treatment C). The swallowability of conventional 2-mm minitables, without the attribute "orodispersible," has been demonstrated by a series of studies for neonates,²⁷ infants, toddlers, and preschool children.²⁸ In these studies the minitables showed equal or higher acceptance than an age-appropriate volume of sweet syrup. In summary, the new ODMT formulation meets all criteria of today's understanding of acceptance in young children.²⁹ The resulting acceptability must be confirmed in future pediatric patients.

Conclusion

The current study was designed to evaluate the relative availability of enalapril in a newly developed ODMT formulation for future use in infants and children. This relative bioavailability study in adults, comparing the bioavailability of enalapril in the new galenic formulation with that of the existing standard formulation, was necessary in order to evaluate whether enalapril doses from ODMTs in the planned pediatric clinical studies should be adjusted.

The results indicated that the ODMT formulation, when swallowed with water, fell within the bioequivalence criteria limits when compared with the RP. Following dispersion in the mouth, a slightly higher C_{max} for enalapril was observed as compared with the reference product. To address this slightly higher C_{max} of enalapril under dispersing conditions, the pediatric studies foresaw treatment start with a low dose of enalapril ODMT and individually adapted titration under blood pressure and heart rate control. Finally, it was demonstrated that the method of administration of the ODMT, swallowed or dispersed, did not significantly affect the bioavailability of enalapril.

Apart from a transient mild to moderate bitter taste, especially when the ODMTs were dispersed on the tongue, the new formulation containing 1.0 mg of enalapril per orodispersible minitab was generally well tolerated and should show high acceptance in the pediatric population.

On the basis of these results it can be concluded that a dose adjustment of enalapril in future clinical investigations with the ODMT formulation administered to newborns, infants, and toddlers, children, and adolescents (from birth to 12 years of age) is not indicated.

Moreover, it can be expected that the means by which the ODMTs are administered, either swallowed whole with water or dispersed in the mouth, will not significantly affect the bioavailability of enalapril in children.

Acknowledgments

We acknowledge the following colleagues from the LENA consortium who also participated in the LENA project for performing the relative bioavailability study, including Dr Wolfgang Wiedey, Ethicare GmbH, Haltern am See, Germany; Lucie Spatenkova, Pharmaplex bvba, Wezembeek-Oppem, Belgium; Dr Angelika Moder, Paracelsus Medical University, Salzburg, Austria.

The project LENA is funded by a European Union Seventh Framework Program (FP7/2007-2013) under the grant agreement no. 602,295.

Conflicts of Interest

The authors report no conflicts of interest associated with this work.

References

1. Thabet Y, Slavkova M, Breitkreutz J. 10 years EU regulation of pediatric medicines—impact on cardiovascular drug formulations. *Expert Opin Drug Deliv*. 2018;15(3):261-270.
2. Wells T, Frame V, Soffer B, et al; Enalapril Pediatric Hypertension Collaborative Study Group. A double-blind, placebo-controlled, dose-response study of the effectiveness and safety of enalapril for children with hypertension. *J Clin Pharmacol*. 2002;42(8):870-880.
3. Grenier MA, Fioravanti J, Truesdell SC, Mendelsohn AM, Vermilion RP, Lipshultz SE. Angiotensin-converting enzyme inhibitor therapy for ventricular dysfunction in infants, children and adolescents: a review. *Prog Pediatr Cardiol*. 2000;12(1):91-111.
4. Frenneaux M, Stewart RA, Newman CM, Hallidie-Smith KA. Enalapril for severe heart failure in infancy. *Arch Dis Child*. 1989;64(2):219-223.
5. Sluysmans T, Styns-Cailteux M, Tremouroux-Wattiez M, et al. Intravenous enalaprilat and oral enalapril in congestive heart failure secondary to ventricular septal defect in infancy. *Am J Cardiol*. 1992;70(9):959-962.
6. Hurtado Rph HJ. Pediatric oral formulations: a continual challenge. *Int J Pharm Compd*. 2007;11(1):17-19.
7. Walsh J. Reflection on the pharmaceutical formulation challenges associated with a Paediatric Investigation Plan for an off-patent drug. *AAPS PharmSciTech*. 2017;18(2):250-256.
8. MacFadyen RJ, Meredith PA, Elliott HL. Enalapril clinical pharmacokinetics and pharmacokinetic-pharmacodynamic relationships. *Clin Pharmacokinet*. 1993;25:274-282.
9. Zisaki A, Miskovic L, Hatzimanikatis V. Antihypertensive drugs metabolism: an update to pharmacokinetic profiles and computational approaches. *Curr Pharm Des*. 2015;21:806-822.
10. Gomez HJ, Cirillo VJ, Irvin JD. Enalapril: a review of human pharmacology. *Drugs*. 1985;30:13-24.
11. Verbeeck RK, Kanfer I, Löbenberg R, et al. Biowaiver monographs for immediate-release solid oral dosage forms: enalapril. *J Pharm Sci*. 2017;106:1933-1943.
12. Stoltenberg I, Breitkreutz J. Orally disintegrating mini-tablets (ODMTs)—a novel solid oral dosage form for paediatric use. *Eur J Pharm Biopharm*. 2011;78(3):462-469.
13. Seager H. Drug-delivery products and the Zydis fast-dissolving dosage form. *J Pharm Pharmacol*. 1998;50(4):375-382.
14. Clarke A, Brewer F, Johnson ES, et al. A new formulation of selegiline: improved bioavailability and selectivity for MAO-B inhibition. *J Neural Transm*. 2003;110(11):1241-1255.
15. Borges AF, Silva C, Coelho JFJ, Simões S. Oral films: current status and future perspectives II—intellectual property, technologies and market needs. *J Control Rel*. 2015;206:108-121.
16. Hoffmann EM, Breitenbach A, Breitkreutz J. Expert opinion on drug delivery, advances in orodispersible films for drug delivery. *Expert Opin Drug Deliv*. 2011;8(3):299-316.
17. European Medicines Agency (EMA), Committee for Medicinal Products for Human Use (CHMP). Guideline on bioanalytical method validation. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf. Accessed August 1, 2019.
18. US Center for Drug Evaluation and Research Food and Drug Administration (FDA). Bioanalytical method validation guidance for industry. <https://www.fda.gov/media/70858/download>. Accessed August 1, 2019.
19. European Medicines Agency (EMA), Committee for Medicinal Products for Human Use (CHMP). Guideline on the investigation of bioequivalence. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf. Accessed August 1, 2019.
20. Davies RO, Gomez HJ, Irvin JD, Walker JF. An overview of the clinical pharmacology of enalapril. *Br J Clin Pharmacol*. 1984;18(Suppl 2):215S-229S.
21. Gu Q, Chen X, Zhong D, Wang Y. Simultaneous determination of enalapril and enalaprilat in human plasma by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2004;813(1-2):337-432.
22. Lu S, Jiang K, Qin F, Lu X, Li F. Simultaneous quantification of enalapril and enalaprilat in human plasma by high-performance liquid chromatography-tandem mass

- spectrometry and its application in a pharmacokinetic study. *J Pharm Biomed Anal.* 2009;49(1):163-167.
23. Moffett BS, DiSanto AR, Espinosa O, Hou J, Colabuono P. Bioequivalence of enalapril oral solution for treatment of pediatric hypertension and enalapril tablets. *Clin Pharmacol Drug Dev.* 2014;3(6):493-498.
 24. Burckhardt BB, Laeer S. Sample preparation and extraction in small sample volumes suitable for pediatric clinical studies: challenges, advances, and experiences of a bioanalytical HPLC-MS/MS method validation using enalapril and enalaprilat. *Int J Anal Chem.* 2015;Article ID 796249.
 25. Faisal M, Cawello W, Burckhardt B, Laeer S. Model-dependent pharmacokinetic analysis of enalapril administered to healthy adult volunteers using orodispersible minitabets for use in paediatrics. *Paediatrics.* 2018;18:117-126.
 26. Kozarewicz P. Regulatory perspectives on acceptability testing of dosage forms in children. *Int J Pharm.* 2014;469:245-248.
 27. Klingmann V, Seitz A, Meissner T, Breitzkreutz J, Moeltner A, Bosse HM. Acceptability of uncoated mini-tablets in neonates—a randomized controlled trial. *J Pediatr.* 2015;167:893-896.
 28. Klingmann V, Spomer N, Lerch C, et al. Favorable acceptance of mini-tablets compared with syrup: a randomized controlled trial in infants and preschool children. *J Pediatr.* 2013;163:1728-1732.
 29. Blume J, Ruano AL, Wang S, Jackson DJ, Tylleskär T, Strand LI. Oral medicine acceptance in infants and toddlers: measurement properties of the caregiver-administered children's acceptance tool (CareCAT). *BMC Paediatrics.* 2018;18:117-126.