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Pharmacokinetic Assessment of an Oral Enalapril Suspension for Use in Children

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ABSTRACT: The angiotensin-converting enzyme (ACE) inhibitor enalapril is commonly used to treat pediatric hypertension. Because some children are unable to swallow tablets or require doses less than the lowest available enalapril tablet, an enalapril suspension was developed. This study examined the relative bioavailability of enalapril suspension (10 mg) (S) compared with 10-mg marketed VASOTEC™ tablets (T) in 16 healthy adult subjects. The geometric mean ratio (S/T) estimate of urinary recovery of free enalaprilat, the active moiety, was 0.92 (90% confidence interval (CI): 0.80, 1.07). Urinary recovery data indicate that approximately 50% of the dose was absorbed (50% recovered in urine as enalapril plus enalaprilat) with about 30% of the dose recovered as free enalaprilat for both S and T. The geometric mean ratios (S/T) of serum AUC and C_{max} were 1.01 (90% CI: 0.90, 1.13) and 0.98 (90% CI: 0.83, 1.16), respectively. Suspension T_{max} was slightly shorter (0.5 h) than that for tablet, but this difference is not clinically significant. Both formulations were well tolerated and there were no clinically significant adverse experiences. We conclude that the bioavailability of enalapril oral suspension 10-mg is similar to that of VASOTEC™ 10-mg tablet. Instructions for compounding enalapril are provided. Copyright © 2000 John Wiley & Sons, Ltd.

Key words: enalapril; hypertension; pediatric; pharmacokinetics; suspension

Introduction

Angiotensin-converting enzyme (ACE) inhibitors are widely used in the treatment of pediatric hypertension and have been found to be particularly effective treatments for hypertension in infants. ACE inhibitors are currently the principal agents for antihypertensive therapy in children both because of their effectiveness and their beneficial influence on cardiac and renal function and peripheral vasculature [1]. The ACE inhibitor enalapril, introduced into clinical practice in 1984, has been used in pediatric and adolescent age groups and is recommended for use in children by the National High Blood Pressure Working Group on Hypertension Control in Children and Adolescents [2].

Drug administration to pediatric patients presents a number of challenges. Many drugs that are commonly used in children and infants have no labeling for pediatric use [3]. In most cases, there is an absence of commercially available formulations suitable for use in pediatric patients and a lack of data to support the stability of extemporaneously prepared formulations [4]. Liquid formulations are needed to allow accurate dosing based on body weight. In addition, liquids are necessary for children who are unable to swallow solid dosage forms and for children with dysphagia [4]. To allow treatment

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of younger pediatric patients who are either unable to swallow tablets or who require doses less than that of the lowest available tablet, 2.5 mg, a liquid preparation of enalapril was developed. This study was designed to assess the bioavailability of the enalapril suspension, dosed as 10 mL of a 1 mg/mL formulation, relative to the 10-mg marketed VASOTEC™ tablets.

Enalapril is well absorbed and is converted by hydrolysis in the liver to the active drug enalaprilat [5,6]. The primary route of elimination of enalaprilat is urinary excretion; 92–96% of an intravenous dose of enalaprilat is recovered in urine unchanged [7]. The serum concentration profile of enalaprilat exhibits a prolonged terminal phase, representing a small fraction of the administered dose of enalapril that has bound to ACE. The amount bound does not increase with dose, indicating a saturable site of binding. Due to this saturable binding of enalaprilat to ACE, the AUC of serum enalaprilat does not increase proportionally with dose. However, since enalaprilat is excreted unchanged in urine, urinary excretion is dose proportional [7] and directly reflects bioavailability. Therefore, the bioavailability of enalapril following administration of enalapril suspension (10 mg) and 10-mg marketed VASOTEC™ tablets was assessed by comparing urinary recovery of enalaprilat. Total absorption of enalapril was estimated using the cumulative urinary excretion of enalapril and enalaprilat. Serum AUC and C_{max} of enalaprilat were used to gauge relative serum exposure of the two formulations. A similar approach has been used recently to assess the bioavailability of another formulation of enalapril [8]. The study dose of 10 mg (0.15 mg/kg in a 70-kg adult) is within the usual dosage range of 10–40 mg for hypertensive patients and equal to the pediatric starting dose recommended by the National High Blood Pressure Working Group on Hypertension Control in Children and Adolescents [2].

Methods

Study Design

This open-label, randomized, two-period, crossover study in healthy volunteers was conducted to determine the relative bioavailability of single doses of enalapril suspension and VASOTEC™ marketed tablets. The study involved 16 subjects, ten male and six female (mean age 31.5 years, range 18–44 years). All subjects were nonsmokers within 20% of ideal body weight and judged to be in good health based on medical history, physical examination and routine laboratory data including negative pregnancy testing for women of reproductive potential. Informed consent was obtained from all subjects. Each subject received one dose of each of two treatments according to a randomized allocation schedule. The treatments were 10 mL of 1 mg/mL enalapril suspension (treatment S) and 10-mg marketed VASOTEC™ tablet (treatment T). Following an overnight fast, subjects received the designated treatment at approximately 08:00 h with 240 mL of water. To maintain urine output, approximately 1 cup of water was given to each subject every 2 h for 12 h after dosing. Subjects remained fasted until 4 h postdose, when a light meal was provided. There was a minimum 7-day washout between treatment periods.

Blood samples for serum enalaprilat assay were collected predose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 18, 24, 36, 48 and 72 h postdose. Urine samples were collected predose and at 0–2, 2–4, 4–8, 8–12, 12–24, 24–36, 36–48 and 48–72 h postdose for assay of free and total enalaprilat concentrations.

Preparation of Suspension

Enalapril suspension was prepared at the study unit according to the following procedure. Ten milliliters of BICITRA™ (ALZA Corporation, Mountain View, CA) were added to a PET bottle containing two tablets of enalapril 20-mg and the bottle was shaken manually for at least 5 min. The contents stood at room temperature for about 20 min, after which 30 mL of Ora-Sweet SF™ (Paddock Laboratories, Minneapolis, MN) was added. The resulting mixture was shaken for about 2 min. The concentration of enalapril in the suspension was about 1 mg/mL. Aliquots of suspension were retained for assay of enalapril potency.

Pharmacokinetic Measurements

Serum and urine samples were stored at $-20°C$ until assayed. Concentrations of enalaprilat in serum and urine were determined by radioimmunoassay [9]. The lower quantification limits for enalaprilat in serum and urine were 0.4 and 20 ng/mL, respectively. Within-study CV% of quality control samples ranged from 5 to 10%. The quality controls used in the analysis of samples met our criteria for acceptance, which is $\pm 20\%$ of the nominal value. Total concentrations of enalaprilat in serum and urine were determined following incubation with freshly prepared rat liver homogenate, which converts enalapril to enalaprilat. Throughout this report, 'free' enalaprilat in serum or urine refers to intact enalaprilat. Total enalaprilat refers to the sum of intact enalaprilat plus enalaprilat resulting from *ex vivo* hydrolysis of enalapril.

To assess the bioavailability of S relative to T, urinary recovery of free and total enalaprilat was calculated from 0- to 72-h urine collection. The percentage of enalapril dose excreted in urine recovered as free or total enalaprilat was calculated from urinary recovery of free or total enalaprilat and actual assayed potency (expressed as enalapril maleate—10.09 mg for tablet; individual assayed values for suspension, range 9.87–10.5 mg) of the formulations, after appropriate correction for the difference in molecular weight between enalapril maleate and enalaprilat. Serum enalaprilat $AUC_{0-72\ h}$, C_{max} and T_{max} were used to assess relative serum exposure of S and T. Serum $AUC_{0-72\ h}$ was calculated using the linear-log trapezoidal method. Serum concentrations of enalapril were estimated as the difference between total and free serum enalaprilat corrected for the different molecular weights of enalapril and anhydrous enalaprilat.

Safety

Adverse experiences were monitored throughout the study and were defined as any unfavorable and unintended changes in the structure, function or chemistry of the body, or worsening of a pre-existing condition. Subjects were questioned regarding any adverse experiences and reported experiences were graded in intensity as mild, moderate, or severe. The investigator evaluated all adverse experiences as to their severity, duration, outcome, and relation to study medication.

Statistical Analysis

Natural log-transformed serum $AUC_{0-72\ h}$, C_{max}, and urinary recovery of free and total enalaprilat were evaluated using a normal theory ANOVA for a two-treatment, two-period, two-treatment-sequence, crossover design with a single blocking factor, gender. Initially a 'full model' was explored which included interaction terms for gender-by-sequence, gender-by-period and gender-by-formulation [10]. None of the three interaction terms were of concern, so their corresponding sums of squares and degrees of freedom were pooled with the appropriate error terms. The resulting 'reduced model' contained between subject factors of gender, sequence, and the between-subject error term of subject within gender-by-sequence. Within-subject factors were period, formulation and within-subject error. The 90% confidence intervals (CI) for the true ratios (S/T) of the two formulations were calculated using the mean square error from the ANOVA and exponentiating the results from the log scale (difference in mean logs and 90% CI) back to the original scales.

To evaluate T_{max}, distribution-free methodologies were used following a similar strategy. Specifically, the true difference $(S-T)$ in T_{max} between the formulations was estimated using the Hodges–Lehmann estimator and the corresponding Moses confidence interval based upon the exact null distribution, including ties, of the Wilcoxon rank sum test comparing within-subject linear combinations of the data between the two treatment sequences [10–12].

Sample size of 16 was determined *a priori* from an assessment of the variability of log transformed enalapril $AUC_{0-72\ h}$, C_{max}, and urinary recovery data on file for healthy male subjects.

Results

Following administration of S and T, serum concentrations of the prodrug enalapril peaked at

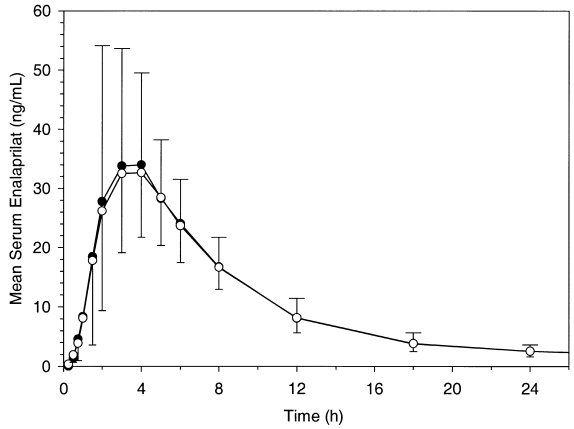

Figure 1. Mean (±S.D.) serum concentrations (ng/mL) of free enalaprilat following administration of enalapril suspension 10 mg (○) and 10-mg marketed VASOTEC™ tablet (●, wide error bar caps) in healthy adult volunteers. Note: off-scale error bars were omitted

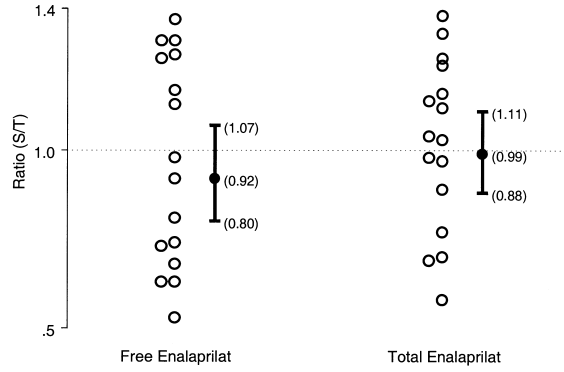

Figure 2. Individual ratios (S/T) of 0–72 h urinary recovery of free and total enalaprilat. Geometric mean estimates and 90% CI are indicated

approximately 45 min postdose and declined rapidly to less than 1 ng/mL by 6 h postdose (data not shown). Mean serum enalaprilat concentration profiles were similar for S and T, as illustrated in Figure 1. Enalaprilat pharmacokinetic parameters for both formulations are summarized in Table 1. Individual urinary recovery ratios (S/T) for free and total enalaprilat ranged from 0.53 to 1.37 and 0.58 to 1.38, respectively, as shown in Figure 2. The geometric mean ratio (S/T) estimate and the corresponding 90% CI for the true value of free enalaprilat recovered in urine were 0.92 and (0.80, 1.07), respectively, demonstrating that the two formulations have similar bioavailability. Figure 3 gives individual ratios (S/T) of serum $AUC_{0-72\ h}$ and C_{max}. As this figure indicates, the two formulations have similar serum exposures, with geometric mean ratio (S/T) estimates and 90% CI of 1.01 (0.90, 1.13) and 0.98 (0.83, 1.16) for $AUC_{0-72\ h}$ and C_{max}, respectively. There was a slight difference (S − T) in T_{max} observed between the two formulations of −0.5 h with a 90% CI of (−1.0, 0.0) (Figure 4).

Absorption of enalapril, as assessed by 72-h urinary recovery of total enalaprilat, was approximately 50% following both treatments, with approximately 30% of the dose recovered in urine as free enalaprilat. Accordingly, conversion of enalapril to enalaprilat was about 60%. Cumulative mean urinary excretion of free and

Table 1. Summary pharmacokinetic parameters of enalaprilat following administration of enalapril suspension 10 mg (S) and marketed 10-mg VASOTEC™ tablet (T) in healthy adult volunteers

Parameter	Mean (S.D.)		Geometric mean		Geometric mean ratio (90% CI)
	S	T	S	T	S/T
$AUC_{0-72\ h}$ (ng · h/mL)	333.28 (75.44)	337.00 (107.25)	330.8	327.8	1.01 (0.90, 1.13)
C_{max} (ng/mL)	33.93 (11.91)	37.40 (23.22)	33.8	34.4	0.98 (0.83, 1.16)
T_{max} (h)	3.44 (1.03)	3.94 (1.18)	3.0[a]	4.0[a]	−0.5 (−1.0, 0.0)[b]
Urinary recovery (0–72 h)					
Free enalaprilat (% dose)	27.93 (7.68)	30.20 (9.35)	27.1	29.4	0.92 (0.80, 1.07)
Total enalaprilat (% dose)	48.73 (9.15)	49.86 (12.30)	47.8	48.4	0.99 (0.88, 1.11)

[a] Median.
[b] Hodges–Lehmann difference (90% CI).
$AUC_{0-72\ h}$, C_{max} and percentage dose recovered in urine were potency adjusted.

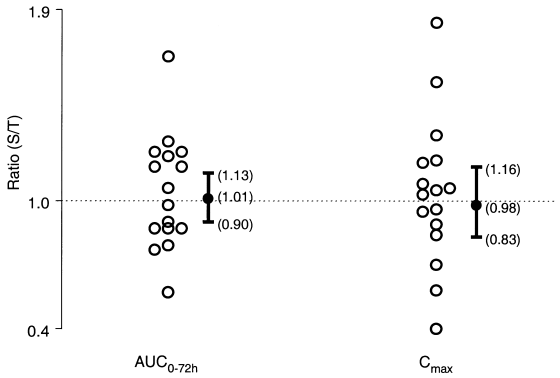

Figure 3. Individual ratios (S/T) of serum enalaprilat $AUC_{0-72\ h}$ and C_{max}. Geometric mean estimates and 90% CI are indicated

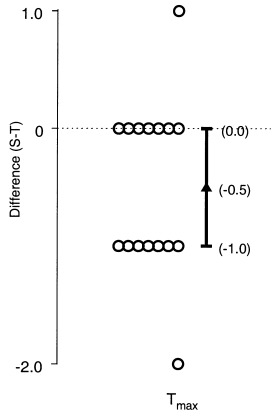

Figure 4. Individual differences (S − T) in serum enalaprilat T_{max}. Hodges–Lehmann difference and 90% CI are indicated

total enalaprilat following administration of S and T is shown in Figure 5.

Both men and women participated in this study and no statistically significant gender differences in pharmacokinetic parameters were observed with the exception of C_{max}. On average, C_{max} was 45% greater ($p = 0.06$) in women than in men, but this difference is not clinically important. This gender effect was reasonably consistent for both formulations and as such, there was no suggestion of a gender-by-formulation interaction.

Two subjects reported mild clinical adverse experiences. One subject experienced pain of short duration at the intravenous catheter site. This adverse experience was not considered to be drug related. Unscheduled menses occurred

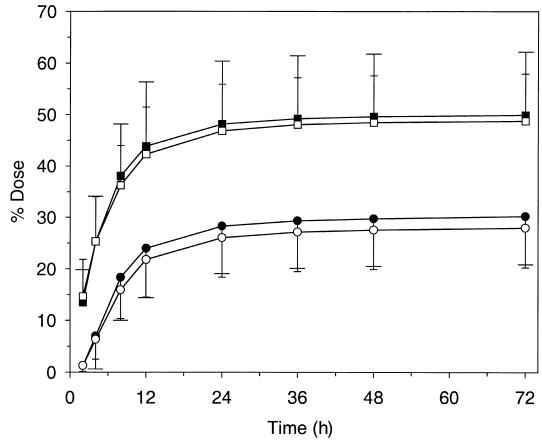

Figure 5. Mean (±S.D.) percentage dose free (circles) and total (squares) enalaprilat excreted in urine following administration of enalapril suspension (10 mg) (○, □) and 10-mg marketed VASOTEC™ tablet (●, ■, wide error bar caps)

in one subject and lasted 6 days. This adverse event was considered to be possibly drug related. There were no serious adverse experiences and no subject discontinued because of a clinical adverse experience.

Discussion

This open-label, two-period, crossover study in healthy adult volunteers demonstrated, based on cumulative urinary excretion of enalaprilat, that enalapril suspension and marketed VASOTEC™ tablets are similarly bioavailable. Comparison of serum AUC and C_{max} indicates that enalapril suspension 10 mg has a serum exposure similar to that observed following administration of marketed 10-mg VASOTEC™ tablet. Enalapril suspension had a slightly shorter T_{max} than VASOTEC™ tablet. Blood pressure response requires binding of enalaprilat to ACE and subsequent inhibition of the enzyme. There is no direct link between serum concentrations and blood pressure response due to slow dissociation. As such, with multiple dosing, a slight change in T_{max} would not be important. Enalapril suspension was generally well tolerated.

The liquid preparation used in this study is referred to as a suspension because the tablet excipients are not fully dissolved; however, the

active ingredient, enalapril maleate, is in solution. Similar preparations of enalapril have been described [13,14]. This suspension was designed to address specific objectives such as ease and reproducibility of preparation for the pharmacy (mean $\pm$ S.D. suspension concentration of enalapril achieved in this study was 1.01 ± 0.02 mg/mL, range 0.99–1.05 mg/mL), ease of dosing, protection from microbial contamination, stability to support the suspension shelf-life, and acceptable taste for the patient. In this study, there were no adverse experiences related to formulation taste. Stability data (not shown) demonstrated acceptable enalapril maleate pediatric suspension stability, based on USP regulatory specifications, for 4 weeks at 5°C/ambient relative humidity (RH). Stability was monitored at 5°C/ambient RH with samples tested at 1, 2 and 4 weeks. To support 24-h room temperature excursions, stability was also monitored at 25°C/35% RH for 1 week. There was no difference in stability behavior due to repeated bottle opening.

An enalapril suspension preparation with documented stability and known bioavailability is now available and has been used in clinical studies in children and infants with hypertension [15–17]. This enalapril suspension provides for greater ease and individualization of dosing in pediatric patients. In addition, the similarity of enalapril suspension to marketed tablets will provide chronically treated pediatric patients with the flexibility to change formulations over time.

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