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ORIGINAL ARTICLE

Effect of CES1 genetic variation on enalapril steady-state pharmacokinetics and pharmacodynamics in healthy subjects

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Aims: Enalapril is a prodrug and needs to be activated by carboxylesterase 1 (CES1). A previous in vitro study demonstrated the CES1 genetic variant, G143E (rs71647871), significantly impaired enalapril activation. Two previous clinical studies examined the impact of G143E on single-dose enalapril PK (10 mg); however, the results were inconclusive. A prospective, multi-dose, pharmacokinetics and pharmacodynamics (PK/PD) study was conducted to determine the impact of the CES1 G143E variant on enalapril steady-state PK and PD in healthy volunteers.

Methods: Study participants were stratified to G143E non-carriers ($n = 15$) and G143E carriers ($n = 6$). All the carriers were G143E heterozygotes. Study subjects received enalapril 10 mg daily for seven consecutive days prior to a 72 hour PK/PD study. Plasma concentrations of enalapril and its active metabolite enalaprilat were quantified by an established liquid chromatography–tandem mass spectrometry (LC–MS/MS) method.

Results: The CES1 G143E carriers had 30.9% lower enalaprilat C_{max} ($P = 0.03$) compared to the non-carriers (38.01 *vs*. 55.01 ng/mL). The carrier group had 27.5% lower $AUC_{0-\infty}$ ($P = 0.02$) of plasma enalaprilat compared to the non-carriers (374.29 *vs*. 515.91 ng*h/mL). The carriers also had a 32.3% lower enalaprilat-to-enalapril $AUC_{0-\infty}$ ratio ($P = 0.003$) relative to the non-carriers. The average maximum reduction of systolic blood pressure in the non-carrier group was approximately 12.4% at the end of the study compared to the baseline ($P = 0.001$). No statistically significant blood pressure reduction was observed in the G143E carriers.

Conclusions: The CES1 loss-of-function G143E variant significantly impaired enalapril activation and its systolic blood pressure-lowering effect in healthy volunteers.

KEYWORDS
angiotensin-converting enzyme (ACE) inhibitors, carboxylesterase 1 (CES1), enalapril, pharmacogenetics, pharmacokinetics

Principal investigator statement: The authors confirm that the Principal Investigator for this paper is Hao-Jie Zhu and that he had direct clinical responsibility for patients.

ClinicalTrials.gov Identifier: NCT03051282

1 | INTRODUCTION

Enalapril is an angiotensin-converting enzyme inhibitor (ACEI), and ACEI is considered to be the first-line therapy for hypertension, heart failure and chronic kidney disease (CKD).[1–3] More than 5 million enalapril prescriptions were dispensed in the US in 2018.[4] Enalapril is a prodrug and needs to be enzymatically biotransformed in vivo to its active metabolite enalaprilat to produce its intended pharmacological effect.[5] Prodrugs (e.g., enalapril) are often designed to overcome the low bioavailability associated with the low cellular permeability of these hydrophilic compounds. In the case of enalapril, the carboxylic acid functional group was masked using an ester-prodrug design, and the ester bond needs to be cleaved by the hepatic hydrolase carboxylesterase 1 (CES1) to release its active metabolite enalaprilat (Figure 1).[6,7] Currently, eight out of ten ACEIs approved by the Food and Drug Administration (FDA) are ester prodrugs, and these prodrugs are all activated by CES1.[5] Two widely perceived assumptions behind the ester-prodrug design are (1) prodrugs are activated via unspecific esterases in the body, and (2) the interindividual variability in activating a prodrug is clinically insignificant. However, a previous study showed that enalapril can only be efficiently activated by CES1, but not other hydrolases.[6] In addition, significant interindividual variability in the activation of enalapril and other ester prodrugs has been consistently observed in the clinic,[8,9] suggesting that genetic variants of prodrug activating enzymes (e.g., CES1) could be a critical factor contributing to the variability in the pharmacokinetics (PK) and pharmacodynamics (PD) of many prodrugs.

CES1 is a major phase I drug-metabolizing enzyme,[10–12] responsible for hydrolysing a wide range of drugs, pesticides, environmental pollutants and endogenous compounds.[5] Among numerous CES1 genetic polymorphisms identified so far, the CES1 nonsynonymous variant G143E (rs71647871) is the only loss-of-function variant with the demonstrated clinical importance.[13] The minor allele frequencies (MAF) of this variant range from 0% to 4% in different populations.[6,7,12,13–16] A previous prospective clinical trial with methylphenidate (CES1 substrate, non-prodrug) has shown that G143E heterozygous carriers ($n = 6$) had 152.4% higher exposure to methylphenidate compare to non-carriers ($n = 16$) ($P < 0.0001$).[17] Other prospective PK studies with oseltamivir and clopidogrel (CES1 substrates) have shown a similar result, where healthy volunteers with G143E polymorphism had significantly altered C_{max} and AUC of the CES1 substrate drugs.[18–20]

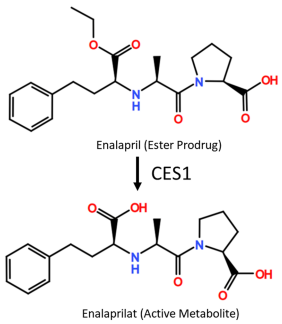

FIGURE 1 CES1-mediated enalapril activation

What is already known about this subject

- CES1 is an important drug-metabolizing enzyme, responsible for the metabolism of a wide range of drugs (especially ester-prodrugs), pesticides, environmental pollutants and endogenous compounds.
- Enalapril is an ester-prodrug, and the previous in vitro study showed that enalapril is selectively activated by CES1.
- The CES1 genetic variant G143E (rs71647871) impairs enalapril activation in vitro.
- Two previous clinical studies examined the impact of G143E on enalapril PK (10 mg single dose) in healthy volunteers; however, the results were inconclusive.

What this study adds

- This is the first multi-dose clinical study examining the impact of G143E on the steady-state PK and PD of enalapril.
- The CES1 G143E carriers had significantly less enalaprilat (enalapril active metabolite) exposure compared to the non-carriers.

Previous studies have suggested enalapril monotherapy often resulted in inadequate response in patients with hypertension.[21] The interindividual variability in response to enalapril therapy is particularly concerning when treating heart failure or renal disease because there are no biomarkers such as blood pressure for monitoring the efficacy of enalapril in these patient populations. An in vitro study demonstrated that the catalytic activity of *CES1* G143E on enalapril activation was completely lost in cells transfected with the variant, suggesting G143E might be associated with the interindividual variability in response to enalapril treatment.[6,22] Two previous clinical studies examined the impact of G143E on enalapril PK in healthy volunteers treated with a single dose of enalapril (10 mg); however, the results were inconclusive.[23,24] One study with Danish healthy volunteers (six carriers and 16 non-carriers) showed no significant differences in enalaprilat PK between the carriers and the non-carriers ($P > 0.05$).[23] The other study in Finnish subjects showed a 20% decrease for the enalaprilat $AUC_{0-\infty}$ ($P = 0.049$) in G143E carriers ($n = 10$) compared to non-carriers ($n = 12$).[24] It is worth noting that long-term enalapril treatment is required in clinical practice, and it remains unexplored whether CES1 genetic variants could affect the steady-state PK and PD of enalapril. Several studies showed that steady-state PK parameters could be different from single-dose PK parameters.[25,26] In the present study, we conducted a multiple-dose PK

study in healthy subjects to determine the impact of the G143E variant on enalapril steady-state PK and its blood pressure-lowering effect.

2 | METHODS

2.1 | Materials

Enalapril, enalapril-d5, and enalaprilat were purchased from Cayman Chemical (Ann Arbor, Michigan, USA), and enalaprilat-d5 was purchased from Toronto Research Chemicals (Toronto, Canada). Blank human plasma was obtained from Innovative Research (Novi, Michigan, USA). Taq polymerase was obtained from New England Biolabs (Ipswich, MA, USA). All other chemicals and agents were of the highest analytical grade commercially available. Enalapril tablets (Vasotec®) from Merck with the same lot number were given to all participants to minimize the source of variability.

2.2 | Study design

A multi-dose enalapril PK and PD study was conducted at Michigan Clinical Research Unit, Ann Arbor, MI. All participants signed a University of Michigan Institutional Review Board (IRB) approved informed consent prior to participation (NCT03051282). The two arms in the study were the G143E carrier group and non-carrier group based on their CES1 genotype. Participants took enalapril 10 mg with 240 mL room-temperature water for 7 consecutive days, and participants were instructed to fast 1 hour before and after the drug administrations to avoid potential food effects on drug absorption. Participants fasted starting 10 p.m. the night before the PK study, and a 72-hour PK study was initiated on the seventh day at 8 a.m. Ten mL of blood was collected at the baseline (Day 1), immediately prior to the seventh dose of enalapril (Day 7, 0 h), and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24, 48 and 72 hours post-dosing (Figure 2). Blood samples were centrifuged at 2000*g* for 10 minutes at 4 °C, and the plasma samples were collected, labelled and stored at −80 °C until analysis.

For the PD markers, resting systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate were measured at the baseline (Day 1, prior to taking the first dose of medication) and during the PK study (Day 7); the PD markers were measured immediately prior to each blood draws during the PK study. The average maximum BP reduction was calculated by subtracting the lowest BP measured from Day 7 from the baseline BP (Day 1).

2.3 | Study participants

The major inclusion criteria were healthy volunteers aged between 18 and 55 years old. The major exclusion criteria were volunteers with any pre-existing condition, concurrent medication (including prescription and over-the-counter medications, herbal/vitamin supplements and oral contraceptives), tobacco use, and/or excessive alcohol consumption. The detailed inclusion and exclusion criteria are listed in

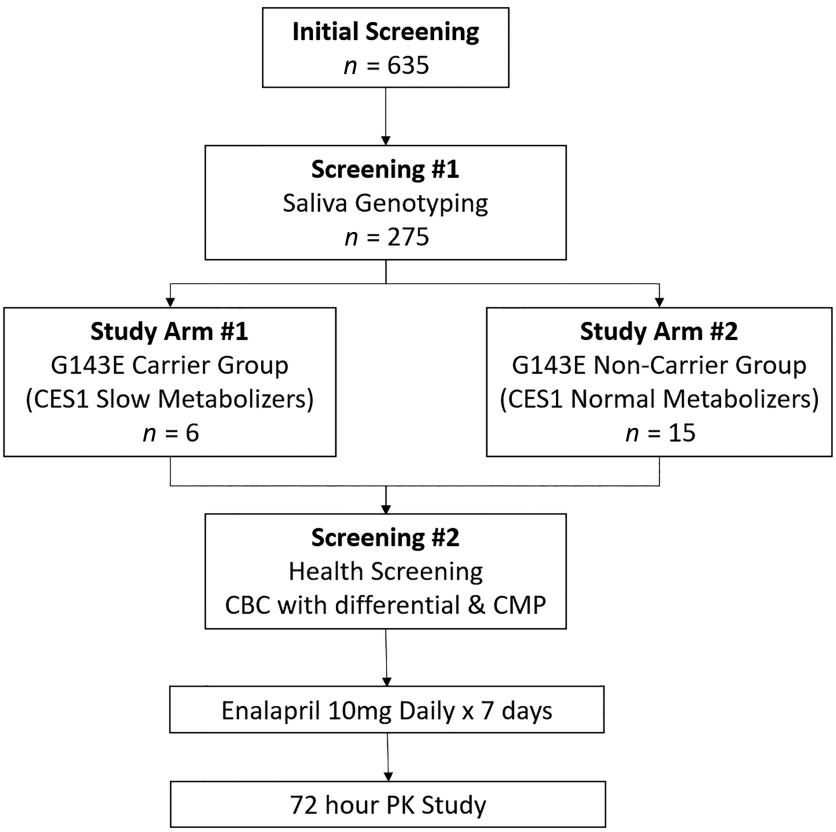

FIGURE 2 Study design. A total of 635 subjects were initially screened but only 275 of them met the inclusion/exclusion criteria. 275 subjects were invited to the screening visit #1 to sign the informed consent form and to provide the saliva sample for the CES1 genetic testing. Based on their CES1 G143E genotypes, subjects were stratified into the G143E carrier group ($n = 6$) and the G143E non-carrier group ($n = 15$). All participants in both groups completed the physical assessment and routine laboratory test to ensure their kidney and liver functions are normal. All participants took 10 mg of enalapril for 7 consecutive days, and a 72-hour PK study was conducted on the seventh day

Table 1. All participants completed the physical assessment and routine laboratory tests (complete blood count (CBC) with differential and comprehensive metabolic panel (CMP)). The urine pregnancy test was done on all female participants. Baseline characteristics were matched between the G143E carrier ($n = 6$) and the non-carrier groups ($n = 15$) (Table 2). The sample size of 21 (six carriers and 15 non-carriers) provided approximately 80% power to detect the clinically meaningful AUC difference (30%) between G143E carriers and non-carriers at a 0.05 significance level when a standard deviation of 25% is assumed for each group.[19,20,23,24]

2.4 | Genotyping procedure

A total of 275 healthy volunteers provided saliva samples for genotyping. Pure Link Genomic DNA Mini Kits (Life Technology, Austin, TX, USA) were used to extract DNA from saliva samples. The extracted DNA was genotyped using the genotyping method we published previously.[6] All participants in the G143E carrier group ($n = 6$) had a 143G/E genotype (i.e., G143E heterozygous carrier), and all participants in the non-carrier group ($n = 15$) had 143G/G genotype (i.e., wild type) (Figure S1 in the Supporting Information).

TABLE 1 Inclusion and exclusion criteria of the study

Inclusion criteria
• Healthy volunteers between the ages of 18–55 years old.
• Normal clinical laboratory values of CBC and CMP.
Exclusion criteria
• Volunteers with any pre-existing medication condition (including pregnancy) were excluded as it might interfere with enalapril absorption, distribution, metabolism or excretion.
• Volunteers with any concurrent medication (including prescription and over-the-counter medications, birth control, herbal/vitamin supplements and oral contraceptives), tobacco smokers, and excessive alcohol (>3 drink/day) users were excluded to avoid drug–drug interaction.
• No subjects weighing under 50 kg were selected.
• Subjects expressing inability to conform to dietary restrictions required for the study. Dietary restrictions were (1) abstaining from alcohol and grapefruit-containing product starting 1 week prior to the study till the end, (2) fasting 1 hour before and after medication administration, and (3) fasting overnight a day before the 72-hour PK study.
• Asian descents were excluded as MAF of CES1 G143E is approximately 0% in the Asian population.

2.5 | Plasma enalapril and enalaprilat quantification by LC–MS/MS

Enalapril and enalaprilat plasma concentrations were determined using an established liquid chromatography–tandem mass spectrometry (LC–MS/MS) method [Her, submitted]. Briefly, 150 μL of plasma was prepared by mixing 30 μL trichloroacetic acid (TCA) 30% (w/v) containing the internal standards enalapril-d5 and enalaprilat-d5. The mixture was vortexed for 5 minutes and centrifuged for 10 minutes, and the supernatant was injected into an LC–MS/MS for the enalapril and enalaprilat quantification. The lower limit of quantification (LLOQ) was 0.5 ng/mL for both enalapril and enalaprilat. Accuracy and precision results met the requirements in the FDA bioanalytical method validation guidance ranging from 2.1% to 9.6% for precision and from 96.9% to 114.2% for accuracy.[27] A parallel reaction-monitoring method was utilized to acquire the product ions of all four target precursors at m/z of 377.2 (enalapril), 349.2 (enalaprilat), 382.2 (enalapril-d5) and 354.2 (enalaprilat-d5). The assay was validated in accordance with the FDA Bioanalytical Method Validation Guidance for Industry.[28]

2.6 | Data analysis

LC–MS/MS data were analysed using the Skyline software (version 20.1.0.76, University of Washington, Seattle, WA). The profile of time–plasma concentrations of enalapril and enalaprilat were plotted using ggplot2 (R package) (Figure 3); enalaprilat concentrations *vs.* time were plotted on a semi-log scale (Figure 4). The PK parameters of enalapril and enalaprilat including peak concentration (C_{max}), area under the plasma concentration–time curve from 0 h

TABLE 2 Baseline characteristics of study participants

	Carriers ($n = 6$)	Non-carriers ($n = 15$)	*P*-value
Age	23.1 ± 2.7	25.0 ± 3.5	0.2
Sex (F/M)[a]	1/5	2/13	1.0
BMI[b]	25.1 ± 4.0	23.0 ± 2.8	0.2
CrCl[c]	111.6 ± 13.8	122.7 ± 24.2	0.3
Race or ethnic group			
White	6	15	1.0

[a]F indicates female; M indicates male.
[b]BMI, body mass index, is calculated as the body mass divided by the square of body height.
[c]CrCL, creatinine clearance is calculated using Cockcroft–Gault formula with participant's actual body weight.
Statistical differences in baseline characteristics between the carrier and the non-carrier group were evaluated using the two-tail student *t*-test.

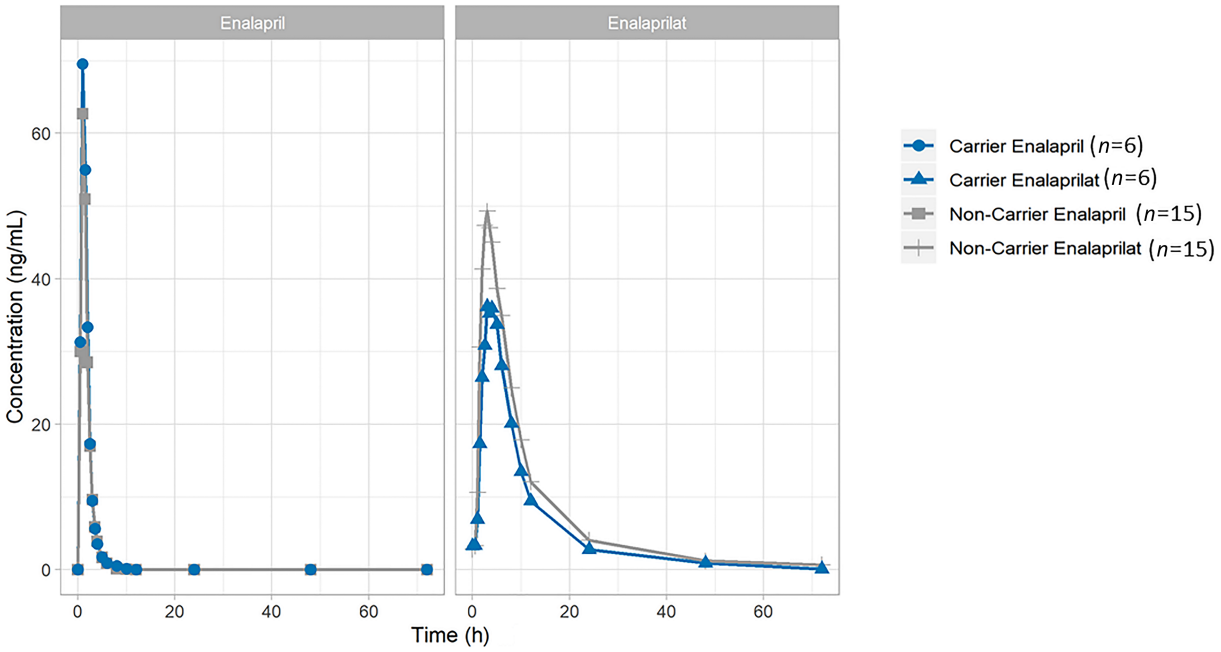

FIGURE 3 The profiles of the time–plasma concentrations of enalapril (left) and enalaprilat (right). Left panel shows enalapril concentrations (ng/mL) over time (hour), and right panel shows enalaprilat (active metabolite) concentrations (ng/mL) over time (hour). G143E carriers (i.e., CES1 slow metabolizers) are represented in blue, and G143E non-carriers (i.e., CES1 normal metabolizers) are represented in gray. Enalapril concentrations were slightly higher in the carrier group compared with the non-carrier group. Enalaprilat concentrations were significantly lower in the carrier group compared to the non-carrier group

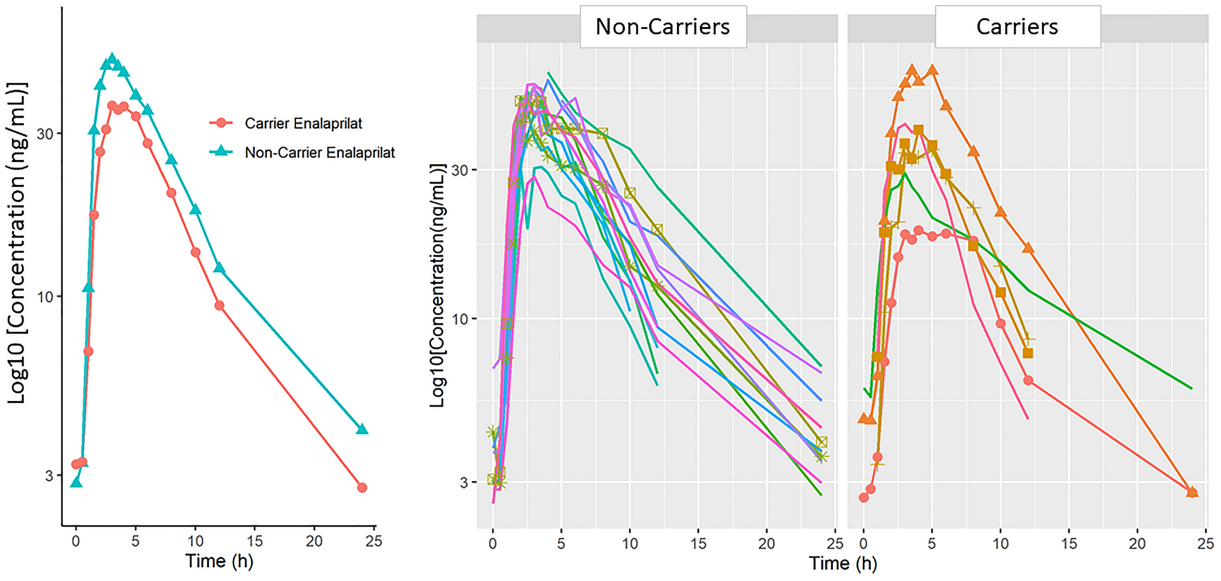

FIGURE 4 Enalaprilat concentrations (ng/mL) *vs*. time (hour) were plotted on a semi-log scale (left panel). Red represents the average enalaprilat concentrations in the G143E carrier group (i.e., CES1 slow metabolizers), and blue represents the averages in the G143E non-carrier group (i.e., CES1 normal metabolizers). The right panel shows individual variability of enalaprilat plasma concentrations with each color presenting an individual participant

to ∞ h ($AUC_{0-\infty}$), half-life ($t_{1/2}$), and clearance (CL) were estimated by non-compartmental analysis (NCA) using the R package PKNCA version 0.9.2. Statistical differences of PK and PD parameters between *CES1* G143E genotypes were evaluated using the one-tail student *t*-test. A *P*-value less than 0.05 was considered statistically significant

2.7 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[29]

3 | RESULTS

Baseline characteristics were matched between the G143E carrier ($n = 6$) and the non-carrier groups ($n = 15$) to avoid potential confounding factors (e.g., age, sex, race, renal and liver functions)[14] (Table 2). Previous studies have suggested that CES1 expression is higher in females than in males,[30] and African Americans tend to respond less to ACE inhibitors due to the downregulated renin-angiotensin-aldosterone system (RAAS) pathway.[31] To avoid those potential confounding factors, age, sex, race and renal functions were matched between the two study arms.

Compared to the previously reported G143E MAF (3.7%) for the white population,[18] our study showed the MAF of 2%. Again, all our study participants were Caucasian due to the geographical position. The genotypes did not deviate from the Hardy–Weinberg equilibrium. We have identified nine G143E heterozygotes from initial screening ($n = 275$). Two participants declined to continue the study, and one participant was excluded due to the age limit (the participant turned 56 before proceeding to screening visit #2) (Figure 2).

3.1 | Effect of CES1 G143E on enalapril pharmacokinetics

The CES1 G143E carrier group had 30.9% lower enalaprilat C_{max} ($P = 0.03$) and 27.5% lower $AUC_{0-\infty}$ ($P = 0.027$) compared to the non-carrier group (Figure 5). The carrier group also had 30.7% higher T_{max} ($P = 0.01$) of enalaprilat compared to the non-carrier group (Table 3). Even though statistically insignificant, the carrier group had 2.2% higher enalapril C_{max} and 9.1% higher $AUC_{0-\infty}$ compared to the non-carrier group. The elimination half-life and T_{max} of enalapril did not significantly differ between the two genotype groups.

Overall, the carrier group had a 32.3% lower enalaprilat-to-enalapril $AUC_{0-\infty}$ ratio ($P = 0.003$) compared to the non-carrier group (Figure 5). Noticeable interindividual variability in PK parameters was observed in both carrier and non-carrier groups. In non-carriers, the coefficient of variance (CV%) of the $AUC_{0-\infty}$ was 31.1% for enalapril and 29.1% for enalaprilat. In carriers, the CV% of the $AUC_{0-\infty}$ was 32.0% for enalapril and 28.0% for enalaprilat.

3.2 | Effect of CES1 G143E on enalapril pharmacodynamics

To minimize the potential confounding effects caused by the baseline variability in BP and heart rate among the individuals, the post-treatment BP and heart rate were normalized to the corresponding baseline values. The average maximum reduction of SBP in the non-carrier group was approximately 12.4% at the end of the study compared to the baseline ($P = 0.001$). There was no statistically significant SBP reduction observed in the G143E carriers ($P > 0.05$) (Figure 6). In addition, there was a statistically significant difference in the average maximum reduction of SBP between the non-carrier and the carrier groups ($P = 0.016$, non-carriers: 14.6 ± 13.13 mmHg *vs.* carriers: −1.0 ± 10.68 mmHg). DBP and heart rate did not differ significantly from the baseline in both groups ($P > 0.05$). Mean SBP reductions were found to be correlated with enalaprilat plasma concentrations.

4 | DISCUSSION

Numerous CES1 genetic polymorphisms have been investigated for their potential impact on CES1 function, and the G143E variant is the

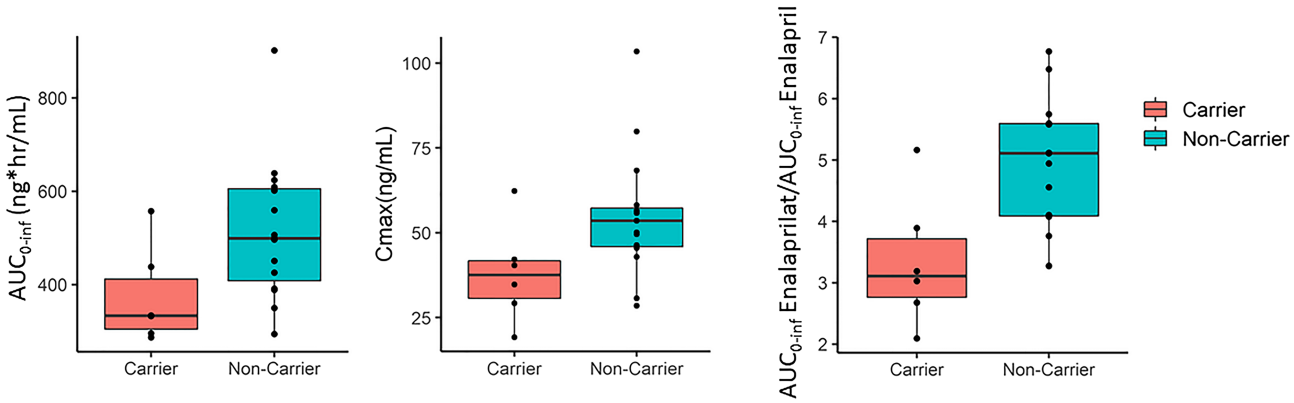

FIGURE 5 Major PK parameters comparison between the CES1 G143E carrier and non-carrier groups. The CES1 G143E carrier group (red) had 27.5% lower $AUC_{0-\infty}$ ($P = 0.027$) and 30.9% lower C_{max} ($P = 0.03$) of enalaprilat compared to the non-carrier group (blue). The carrier group (red) also had a 32.3% lower enalaprilat-to-enalapril $AUC_{0-\infty}$ ratio ($P = 0.003$) compared to the non-carrier group (blue)

TABLE 3 Summary of PK parameters

	Non-carriers				Carriers				Average comparison (carrier/non-carrier)	90% CI of average comparison	*P*-value
Enalapril	**Average**		**Sd**	**CV (%)**	**Average**		**Sd**	**CV (%)**			
AUC_{0-72h} (ng*hr/mL)	106.5	±	33.1	31.1	115.8	±	36.2	31.3	1.09	0.85–1.42	0.29
$AUC_{0-\infty}$ (ng*hr/mL)	108.0	±	33.6	31.1	117.8	±	37.7	32.0	1.09	0.84–1.43	0.28
C_{max} (ng/mL)	68.0	±	22.2	32.7	69.5	±	20.0	28.7	1.02	0.80–1.34	0.44
T_{max} (h), median (range)	1.0 (0.5–1.5)			22.3	1.0 (1.0–1.0)			0.00	0.99	0.89–1.11	0.36
$t_{1/2}$ (h)	1.3	±	1.4	105.7	2.1	±	2.1	101.5	1.35	0.62–2.93	0.18
Enalaprilat											
AUC_{0-72h} (ng*hr/mL)	502.0	±	151.3	30.1	363.2	±	104.6	28.8	0.72	0.58–0.92	0.03[a]
$AUC_{0-\infty}$ (ng*hr/mL)	515.9	±	150.0	29.1	374.3	±	104.7	28.0	0.73	0.58–0.92	0.02[a]
C_{max} (ng/mL)	55.0	±	18.6	33.7	38.0	±	14.5	38.2	0.69	0.49–0.96	0.03[a]
T_{max} (h), median (range)	2.9 (1.5–4.0)			23.3	3.8 (3.0–5.0)			1.3	1.32	1.10–1.59	0.01[a]
$t_{1/2}$ (h)	16.5	±	6.3	38.1	10.9	±	4.9	44.9	0.66	0.46–0.95	0.03[a]
AUC/AUC_{0-72h} (Enalaprilat/Enalapril)	4.9	±	1.1	21.6	3.3	±	1.0	29.4	0.67	0.52–0.85	0.002[a]
$AUC/AUC_{0-\infty}$ (Enalaprilat/Enalapril)	4.9	±	1.1	21.9	3.3	±	1.1	32.0	0.68	0.51–0.87	0.003[a]

Statistical differences of PK and PD parameters between *CES1* G143E genotypes were evaluated using the one-tail student *t*-test; geometric mean and 90% CIs were included.

[a]indicates the statistically significant difference between the G143E non-carrier (i.e., CES1 normal metabolizers) and the G143E carrier group (i.e., CES1 slow metabolizers).

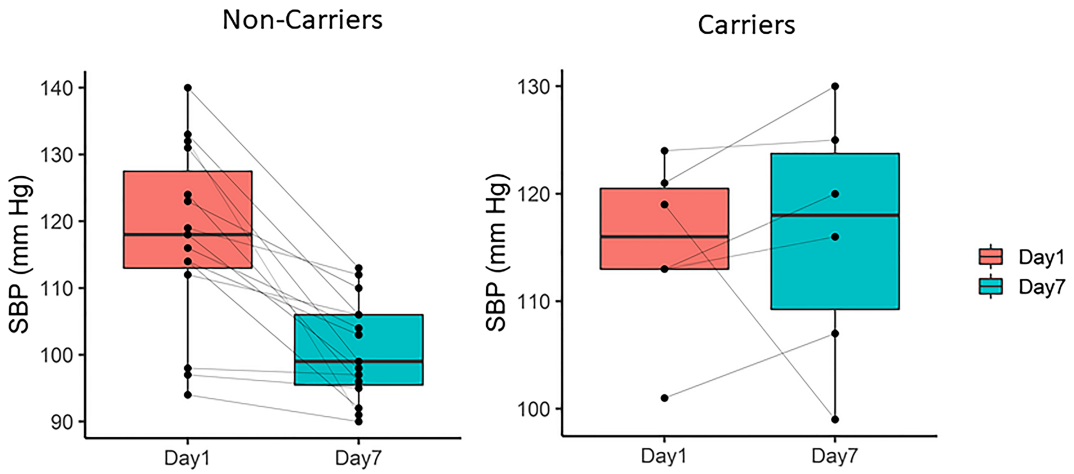

FIGURE 6 Blood pressure-lowering effect of enalapril in the CES1 G143E carriers and non-carriers.The paired sample *t*-test was performed to examine the difference between the baseline SBP and the SBP at the end of the study on each study arm. The non-carrier group had approximately 12.4% lower SBP at the end of the study compared to the baseline ($P = 0.001$). There was no statistically significant blood pressure reduction observed in the G143E carriers

only one that has been shown consistently to significantly affect both PK and PD of CES1 substrate drugs.[5] A previous in vitro study showed enalapril is selectively activated by hepatic CES1, and CES1 G143E genetic variant completely impairs enalapril activation.[6] In this multi-dose healthy volunteer enalapril PK study, we demonstrated that CES1 G143E carriers had significantly lower enalaprilat exposure compared to the non-carriers. We also observed an appropriate trend of an increased plasma concentration of the prodrug enalapril in the carriers, although the differences did not reach the level of statistical significance. In addition, the blood pressure-lowering effect was only

observed in the non-carrier group, which is consistent with the higher plasma concentrations of enalaprilat observed in the non-carrier group. Previous literature reported healthy volunteers responded less to the blood pressure-lowering medication (e.g., enalapril) compared to patients with hypertension due to the downregulated renin-angiotensin-aldosterone system (RAAS) pathway.[32,33] In clinical practice, the impact of G143E on the therapeutic effect of enalapril could be more pronounced in a patient population.

Two previous clinical studies examined the impact of G143E on single-dose enalapril PK, and the results were inconclusive.[23,24] One study reported that the mean enalaprilat $AUC_{0-\infty}$ in the carriers ($n = 6$) was 6% lower than that in the non-carriers ($n = 16$) after study subjects administered a single dose of 10 mg enalapril orally; however, the difference was statistically insignificant. It is worth noting that, in addition to G143E (six variant carriers), several other CES1 variations were included in the analysis (e.g., 15 individuals with different gene copy variations and 16 controls).[23] Another single dose (10 mg enalapril) PK study in Finnish subjects (10 G143E carriers and 12 non-carriers) showed a modest 20% decrease for the enalaprilat $AUC_{0-\infty}$ in the G143E carriers compared to the non-carriers with a borderline significance ($P = 0.049$).[24] As a comparison, in the present multi-dose enalapril PK/PD study, despite the smaller sample size ($n = 6$ G143E carriers), a 27.5% reduction in enalaprilat $AUC_{0-\infty}$ was observed in the G143E carriers (Figure 5). The underlying mechanism of the greater effect of G143E in this multiple-dose study relative to the previous single-dose trials remains elusive. We speculate that the impact of G143E on reducing enalaprilat formation may have accumulated following each dose, and the maximum effect was achieved after the PK reached a steady-state. Overall, this finding indicates that the CES1 G143E variant may have a more significant impact on the steady-state PK; our results (steady-state PK) are more indicative of the effects of this variant on real-world patient populations. However, it should be noted that, given the small sample size and large interindividual PK variabilities in both the present investigation and the two previous single-dose studies, the observed differences might not be statistically significant.

In line with previous reports, significant intragroup variability of enalapril PK was observed. Even though CES1 genetic polymorphisms are an important factor contributing to CES1 variability, it is worth noting that all CES1 genetic variants identified to date can only explain a small portion of interindividual variability of the CES1 function.[5] Considering the low CES1 G143E MAF (0–4%), it can therefore be assumed that CES1 G143E variation can only explain a limited portion of enalapril response variation in clinical practice. Previous in vitro studies observed a marked variability of CES1 protein expression in human liver samples.[6,34] Considering G143E only affects the catalytic efficiency of CES1 without altering its expression,[18,30,35] the main source of interindividual variability in the current study may have resulted from the different expression levels of CES1 in individual participants. Therefore, reliable CES1 biomarkers capable of predicting hepatic CES1 expression could be used to further improve the therapeutic outcomes of enalapril and other CES1 substrate drugs. As an excretory protein, CES1 can be released into the blood from tissues with high levels of CES1 expression (i.e., the liver). Indeed, we recently detected CES1 protein in human plasma using a highly sensitive LC–MS/MS proteomics assay.[36] Although plasma CES1 is insignificant for drug metabolism due to its extremely low plasma concentration, there is potential for plasma CES1 protein to be served as a biomarker to predict the PK and PD of enalapril and other CES1 substrate drugs. Alternatively, plasma exosomes are extracellular vesicles and contain functional proteins and nucleic acids derived from cells of different origins. A recent study showed high correlations between exosomal mRNA expressions and hepatic protein levels for several hepatic drug-metabolizing enzymes,[37] but CES1 was not included in the study. It is plausible that CES1 genetic variants and plasma and exosomal CES1 could be used as complemental biomarkers allowing for the prediction of a large portion of CES1 variability and the development of an individualized pharmacotherapy strategy to improve the effectiveness and safety of drugs metabolized by CES1.

The main limitation of this study is its small sample size, which was mainly due to the low MAF of the G143E variant. In particular, given this small sample size and that the study was done in healthy volunteers, the blood pressure-lowering effect needs to be further evaluated to better understand the true effect, especially in a patient population. Importantly, we were not able to evaluate the effect of homozygous G143E on enalapril activation as all participants in the carrier group were G143E heterozygotes. It can be hypothesized that the magnitude of difference in PK and PD parameters between non-carries and homozygous G143E carriers may be even greater than what was observed with the G143E heterozygotes group.

This multi-dose enalapril healthy volunteer PK study demonstrated that the CES1 G143E variant significantly reduced enalapril activation and its blood pressure-lowering effect in healthy volunteers. Assuming enalapril follows linear kinetics,[38–40] a 27.5% reduction in enalapril AUC might require a 38.3% increase in enalapril dose in G143E carriers. This is especially important for the treatment of heart failure or CKD as there is no biomarker (such as blood pressure) to adjust the dose or to switch the medication. Future studies are warranted to investigate the effects of the G143E variant on the activation and clinical outcomes of enalapril and other ACEI prodrugs in patients with hypertension, heart failure, and CKD.

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COMPETING INTERESTS

All authors have declared no conflicts of interest in relation to this study.

CONTRIBUTORS

L.H. and H-J.Z. wrote the manuscript; H-J.Z., A.H.W. and B.E.B. designed the research; L.H., X.W., H.C., S.J., J.S. and L.S. performed the research; L.H. analysed the data.

DATA AVAILABILITY STATEMENT

The data that support the findings of the study will be shared upon reasonable request. All participants signed a University of Michigan Institutional Review Board (IRB) approved informed consent prior to participation (NCT03051282).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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