Impact of carboxylesterase 1 genetic polymorphism on trandolapril activation in human liver and the pharmacokinetics and pharmacodynamics in healthy volunteers

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Abstract
Trandolapril, an angiotensin-converting enzyme inhibitor prodrug, needs to be activated by carboxylesterase 1 (CES1) in the liver to exert its intended therapeutic effect. A previous in vitro study demonstrated that the CES1 genetic variant G143E (rs71647871) abolished CES1-mediated trandolapril activation in cells transfected with the variant. This study aimed to determine the effect of the G143E variant on trandolapril activation in human livers and the pharmacokinetics (PKs) and pharmacodynamics (PDs) in human subjects. We performed an in vitro incubation study to assess trandolapril activation in human livers (5 G143E heterozygotes and 97 noncarriers) and conducted a single-dose (1 mg) PK and PD study of trandolapril in healthy volunteers (8 G143E heterozygotes and 11 noncarriers). The incubation study revealed that the mean trandolapril activation rate in G143E heterozygous livers was 42% of those not carrying the variant ($p = 0.0015$). The clinical study showed that, relative to noncarriers, G143E carriers exhibited 20% and 15% decreases, respectively, in the peak concentration ($C_{\text{max}}$) and area under the curve from 0 to 72 h ($AUC_{0-72}$) of the active metabolite trandolaprilat, although the differences were not statistically significant. Additionally, the average maximum reductions of systolic blood pressure and diastolic blood pressure in carriers were ~ 22% and 23% less than in noncarriers, respectively, but the differences did not reach a statistically significant level. In summary, the CES1 G143E variant markedly impaired trandolapril activation in the human liver under the in vitro incubation conditions; however, this variant had only a modest impact on the PK and PD of trandolapril in healthy human subjects.

Abbreviations: ACE, Angiotensin-converting enzyme; AUC, Area under curve; bpm, Beat per minute; CES1, Carboxylesterase 1; CL, Clearance; CV%, Coefficient of variance; CE, Collision energy; DP, Declustering potential; DBP, Diastolic blood pressure; DDI, Drug-drug interactions; $t_{1/2}$, Half-life; IRB, Institutional Review Board; IS, Internal standards; $C_{\text{intrinsic}}$, Intrinsic clearance; LC, Liquid chromatography; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; LLOQ, Lower limit of quantification; $V_{\text{max}}$, Maximum Velocity; $K_{\text{m}}$, Michalis-Menten constant; MCRU, Michigan Clinical Research Unit; MAF, Minor allele frequency; NCA, Non-compartmental analysis; PRM, Parallel reaction monitoring; $C_{\text{max}}$, Peak Concentration; PD, Pharmacodynamics; PK, Pharmacokinetics; QC, Quality control; SPE, Solid phase extraction; SD, Standard deviation; SBP, Systolic blood pressure; TOF-MS, Time-of-flight Mass Scan.

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INTRODUCTION

Trandolapril is an angiotensin-converting enzyme (ACE) inhibitor that has been used for the treatment of hypertension, heart failure postmyocardial infarction, and left-ventricular dysfunction postmyocardial infarction for over 2 decades. Trandolapril has a favorable pharmacological profile compared with other ACE inhibitors, as it exhibits a long duration of action and high ACE inhibition potency. 

Retrospective reviews have suggested that the outcomes of trandolapril therapy are highly variable among individuals. 

Although some genetic and nongenetic factors were found to be associated with the interindividual variability in responses to trandolapril treatment, a large part of the variability remains unexplained. Moreover, due to the lack of reliable predictors of drug response, the current clinical management of ACE inhibitors is largely based on a targeted population dosing approach. Thus, there is a critical need to identify factors contributing to interindividual variability in trandolapril treatment in order to optimize the clinical use of trandolapril.

Like many other ACE inhibitors, trandolapril is formulated as a prodrug to improve the otherwise poor bioavailability, and it requires activation by carboxylesterase 1 (CES1) in the liver. This activation process is crucial for successful trandolapril therapy because its active metabolite (i.e., trandolaprilat) is approximately eight times more potent at inhibiting ACE than the parent compound trandolapril. CES1 is the most abundant drug-metabolizing enzyme in the human liver, contributing to 80%–95% of total hepatic hydrolytic activity. Significant interindividual variability in CES1 expression and activity has been reported, which is attributed to both genetic and nongenetic regulators.

In vitro studies showed that the CES1 nonsynonymous variant G143E (rs71647871) was a loss-of-function variant for the hydrolysis of various CES1 substrates. Several clinical studies also reported a significant impact of CES1 G143E on the pharmacokinetics (PKs) of the CES1 substrate medications methylphenidate, clopidogrel, and oseltamivir. However, the G143E variant showed a negligible effect on the PKs of enalapril and quinapril, although enalapril hydrolysis was markedly impaired by the variant in vitro. Given the apparent substrate-dependent effect of CES1 G143E and the discrepancy between the in vitro and in vivo findings, it is difficult to draw conclusions concerning the clinical impact of this CES1 variant on its substrate drugs based solely on in vitro discoveries.

An in vitro study has demonstrated that the catalytic activity of CES1 G143E on trandolapril activation was abolished in cells transfected with the variant. In the present study, we first conducted an in vitro incubation study to examine the impact of CES1 G143E on trandolapril activation in human livers. We then evaluated the effect of this variant on the PK and PD of a single dose of trandolapril in healthy volunteers.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Our study demonstrated that CES1 G143E significantly impaired trandolapril activation in human livers. However, the effect of this variant on the PK and PD of a single dose of trandolapril in healthy volunteers was modest.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This study revealed an in vitro/in vivo discrepancy regarding the effect size of CES1 G143E variant on trandolapril activation. Further studies are needed to determine the clinical impact of CES1 variants in patients treated with ACE inhibitor prodrugs.

MATERIALS AND METHODS

Materials

Trandolapril and trandolaprilat were purchased from Cayman Chemical. Trandolapril-phenyl-d5 and trandolaprilat-phenyl-d5 were products of Toronto Research Chemicals. Blank human
plasma was obtained from Innovative Research. Water Oasis HLB columns were purchased from Waters Corporation. Taq polymerase was obtained from New England Biolabs. All other chemicals and agents were of the highest analytical grade commercially available.

A total of 102 individual normal human liver samples were obtained from XenoTech LLC and the Cooperative Human Tissue Network. The donors comprised 46 men and 56 women with ages ranging from 22 to 81 years, and included 92 Whites, 6 African Americans, 2 Hispanics, and 2 classified as other.

Subjects

After signing a written informed consent approved by the University of Michigan Institutional Review Board, a total of 311 healthy volunteers provided saliva samples for CES1 G143E genotype screening. The minor allele frequency (MAF) of CES1 G143E was reported as 3.7%, 4.3%, 2%, and 0% in White, Hispanic, African American, and Asian populations, respectively; accordingly, Asians were excluded from the initial genotype screening. To avoid potential confounding effect by age, sex, renal function, and drug-drug interactions, baseline characteristics were matched between the G143E carriers and noncarrier controls. Subjects on any prescription or over-the-counter medications, herbal/ vitamin supplement, or oral contraceptives were excluded; tobacco smokers and the excessive alcohol users were also excluded. Detailed inclusion and exclusion criteria are listed in Table S1. Of the 311 genotyped volunteers, 8 subjects with the CES1 143G/E genotype (i.e., G143E heterozygotes) and 12 with the CES1 143G/G genotype (i.e., wild type) were included in the PK/PD study. The health of these 20 participants was confirmed by medical history review, physical examination, and routine laboratory tests. Following the health evaluation, subjects completed a 1 mg single oral dose trandolapril PK/PD study at the Michigan Clinical Research Unit (MCRU). Of note, a G143E noncarrier who was the only African American in the entire study population was excluded from data analysis because we were unable to recruit any African American carriers to account for the known differences in response to ACE inhibitor treatment between Whites and African Americans. The characteristics of the study participants are shown in Table S2.

CES1 genotyping

Pure Link Genomic DNA Mini Kits (Life Technology) were used to extract total genomic DNA from human liver tissues and saliva samples. The extracted DNA was genotyped for the G143E variant using the genotyping method we published previously.

Trandolapril hydrolysis in human liver s9 fractions

Human liver s9 factions (HLS9) were prepared, and an in vitro incubation study was performed to measure trandolapril hydrolysis (activation), as described in previous publications. Briefly, human liver tissues were homogenized on ice and centrifuged at 9000 × g at 4°C for 20 min. Following removal of the floating lipid layer, the supernatant was centrifuged one more time under the same conditions. The resulting supernatant was then transferred to a clean tube and diluted to 2 mg protein/ml as the HLS9 stock solution. The samples were stored at −80°C until use. CES1 activity on trandolapril hydrolysis (activation) in HLS9 was evaluated by the incubation of 200 µM trandolapril with 0.2 mg/ml HLS9 at 37°C for 10 min. Following the incubation, a fourfold volume of acetonitrile containing the internal standard simvastatin acid (20 ng/ml) was added to terminate the reaction. Samples were then thoroughly vortexed and centrifuged at 17,000 × g at 4°C for 20 min. The supernatant was collected for the analysis of trandolaprilat concentration by an established liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay.

Trandolapril PK/PD study

Study participants abstained from alcohol and grapefruit containing products beginning 1 week prior to the initiation of the PK study and continuing until the end of the investigation. Subjects were admitted to the MCRU at ~ 8:00 a.m. after an overnight fast. A single dose of trandolapril (1 mg) was orally administered with 240 ml of room-temperature water at 8:30 a.m. Subjects remained fasting until 12:30 p.m. (i.e., 4 h after trandolapril administration) to avoid potential food effects on drug absorption. A standardized lunch was provided ~ 4 h after dosing. Time points for blood collection were immediately prior to the dose of trandolapril (0 h), and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24, 48, and 72 h postdosing. Ten ml blood was collected in a heparin-containing blood collection tube. Plasma samples were obtained via centrifugation at 2000 × g for 10 min at 4°C, and the samples were labeled and stored at −80°C until analysis. Resting systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate were measured prior to dosing and at 0.5, 1, 1.5, 2, 4, 6, 8, 10, and 12 h postdosing administration as PD markers. All blood samples and vital signs were obtained by a registered nurse in the MCRU.

PK sample preparation

Trandolapril-phenyl-d5 and trandolaprilat-phenyl-d5 were respectively used as the internal standards (IS) for the
quantifications of trandolapril and trandolaprilat. The calibration curves consisted of trandolapril and trandolaprilat at concentrations of 10, 30, 100, 300, and 1000 pg/ml. Quality control (QC) samples containing both analytes were prepared at concentrations of 30 pg/ml (low), 300 pg/ml (medium), and 1000 pg/ml (high). A solid-phase extraction method was used to extract analytes from plasma samples. Briefly, 200 µL of plasma sample was mixed with 20 µl of IS working solution and 800 µl of 0.1% formic acid. The final concentrations of trandolapril-phenyl-d5 and trandolaprilat-phenyl-d5 were 200 pg/ml and 2 ng/ml, respectively. Following centrifugation at 17,000 × g for 2 min followed by solid-phase extraction on Waters Oasis HLB columns in accordance with the manufacturer’s instructions. The eluent was vacuum dried in a SpeedVac SPD1010 concentrator (Thermo Scientific) and reconstituted in 100 µl of 0.1% formic acid. 

The bioanalytical method for trandolapril and trandolaprilat was validated for specificity, linearity, the lower limit of quantification (LLOQ), precision, accuracy, recovery, and stability based on the US Food and Drug Administration (FDA) Bioanalytical Method Validation Guidance for Industry. No significant interference peaks were observed in blank plasma (Figure S1). The assay showed excellent linearity (r² > 0.999) over the concentration range of 10–3000 pg/ml and an LLOQ of 10 pg/ml for both trandolapril and trandolaprilat (Figure S2). The precision, accuracy, recovery, matrix effect, and stability were evaluated by analyzing QC samples at three concentrations (30, 300, and 1000 pg/ml) with five replicates each. The overall precision and accuracy were within the ranges of 3.8–13.3% and 82.7–111.1%, respectively, for both analytes (Table S4). The recovery and matrix effect for both analytes ranged from 84.5% to 110.2% and from 83.7% to 106.5%, respectively (Table S5). Both trandolapril and trandolaprilat were stable after the processed samples were kept in an autosampler at 4°C for 48 h or after 3 successive freeze (−20°C) and thaw cycles of the QC samples (Table S6).

Trandolapril and trandolaprilat quantification by LC-MS/MS

The concentrations of trandolapril and trandolaprilat in plasma were determined on a TripleTOF 5600 mass spectrometer (Sciex) coupled with an Eksigent 2D plus LC system (Eksigent Technologies). Analytes were separated via a trap-elute configuration, which includes a trapping column (ChromXP C18-CL, 120 Å, 5 mm, 0.3 mm cartridge; Eksigent Technologies) and an analytical column (ChromXP C18-CL, 120 Å, 150 × 0.3 mm, 5 µm; Eksigent Technologies, Dublin, CA). The mobile phase consisted of water with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B). Samples were loaded on the trapping column with 100% mobile phase A delivered at a flow rate of 15 µl/min for 3 min, and the analytes were separated on the analytical column at a flow rate of 5 µl/min with the gradient described in Table S3. A blank injection (water) was included between sample injections to prevent sample carryover. The mass spectrometer was operated in a parallel reaction monitoring (PRM) positive ion mode with an ion spray voltage floating at 5500 V, ion source gas one at 10 psi, ion source gas two at 30 psi, curtain gas at 25 psi, and source temperature at 450°C. The PRM acquisition consisted of a 250 ms time-of-flight MS scan from 140–650 Da and subsequent MS/MS scans from 100 to 250 Da for all 4 target precursors at m/z of 403.23 (trandolapril), 431.27 (trandolapril), 408.26 (trandolapril-phenyl-d5), and 436.30 (trandolapril-phenyl-d5).

Bioanalytical method validation

The bioanalytical method for trandolapril and trandolaprilat was validated for specificity, linearity, the lower limit of detection (LLOD), and stability. The limit of quantification (LOQ), precision, accuracy, recovery, and stability based on the US Food and Drug Administration (FDA) Bioanalytical Method Validation Guidance for Industry. No significant interference peaks were observed in blank plasma (Figure S1). The assay showed excellent linearity (r² > 0.999) over the concentration range of 10–3000 pg/ml and an LLOQ of 10 pg/ml for both trandolapril and trandolaprilat (Figure S2). The precision, accuracy, recovery, matrix effect, and stability were evaluated by analyzing QC samples at three concentrations (30, 300, and 1000 pg/ml) with five replicates each. The overall precision and accuracy were within the ranges of 3.8–13.3% and 82.7–111.1%, respectively, for both analytes (Table S4). 

RESULTS

Impact of CES1 G143E on trandolapril activation in human livers

Five of the 102 human liver samples were found to be CES1 G143E heterozygotes. The impact of the CES1 G143E variant on trandolapril activation was assessed by comparing the formation rates of the active metabolite trandolaprilat between those 5 G143E carriers and the remaining 97 noncarriers after incubation of trandolapril with the HLS9 samples. The mean trandolaprilat formation rate in the G143E carrier group (G/E; 483.6 ± 40.6 pmol/min/mg protein) was 42% of that in the noncarrier group (G/G; 1241.0 ± 699.3 pmol/min/mg protein) (p = 0.0015; Figure 1).
Effect of CES1 G143E on trandolapril pharmacokinetics

Baseline characteristics of the G/E and G/G groups were comparable (Table S2). None of these baseline characteristics showed a significant impact on trandolapril PKs according to the multiple linear regression analysis (Table S7). Concentration-time profiles of trandolapril and trandolaprilat in the two groups are illustrated in Figure 2. PK parameters for trandolapril, including $C_{\text{max}}$, $AUC_{0-72\text{h}}$ (Figure 3a), $t_{1/2}$, and CL (Table 1) did not significantly differ between carriers and noncarriers. For the active metabolite trandolaprilat, the $C_{\text{max}}$ and $AUC_{0-72\text{h}}$ of the G143E carrier group were, respectively, 20% and 15% lower than those of the noncarrier group. However, the differences did not reach a statistically significant level ($p = 0.17$ for $C_{\text{max}}$ and $p = 0.33$ for $AUC_{0-72\text{h}}$, Figure 3b). Additionally, neither the trandolaprilat to trandolapril $C_{\text{max}}$ ratios nor the trandolaprilat to trandolapril $AUC_{0-72\text{h}}$ ratios showed a significant difference between the two groups ($C_{\text{max}}$ trandolaprilat to trandolapril ratios: $1.84 \pm 0.79$ [G/G], $1.65 \pm 1.24$ [G/E]).
PK parameters of a single oral dose of trandolapril (1 mg) in healthy subjects with different CES1 G143E genotypes

| PK parameters          | CES1 143G/G |     | CES1 143G/E |     | P value |
|------------------------|-------------|-----|-------------|-----|---------|
|                        | Means       | SD  | CV%         | Means | SD     | CV%     |
| Trandolapril           |             |     |             |       |        |         |
| C<sub>max</sub>, ng/ml | 0.85        | 0.55| 64.7        | 0.86  | 0.48   | 55.8    |
| AUC<sub>0–72 h</sub>, ng × h/ml | 1.27 | 0.56 | 44.1     | 1.33  | 0.82   | 61.7    |
| t<sub>1/2</sub> h     | 6.55        | 2.60| 39.7        | 6.52  | 5.31   | 81.4    |
| CL, L/h               | 893.90      | 308.23| 34.5       | 1027.58 | 564.44 | 54.9    |
| Trandolaprilat        |             |     |             |       |        |         |
| C<sub>max</sub>, ng/ml | 1.27        | 0.41| 32.3        | 1.02  | 0.29   | 28.4    |
| AUC<sub>0-72 h</sub>, ng × h/ml | 44.95 | 13.91| 30.9      | 38.37 | 14.15  | 36.9    |
| t<sub>1/2</sub> h     | 65.96       | 36.59| 55.5       | 93.46 | 27.98  | 29.9    |
| CL, L/h               | 12.20       | 4.15 | 34.0       | 9.96  | 2.79   | 28.0    |
| Trandolaprilat/trandolapril |         |     |             |       |        |         |
| C<sub>max</sub>, ng/ml | 1.84        | 0.79| 42.9        | 1.65  | 1.24   | 75.2    |
| AUC<sub>0-72 h</sub>, ng × h/ml | 40.76 | 18.19| 44.6      | 41.87 | 30.64  | 73.2    |
| t<sub>1/2</sub> h     | 11.67       | 8.35 | 71.6       | 31.39 | 25.69  | 81.8    |
| CL, L/h               | 0.01        | 0.01 | 100.0      | 0.01  | 0.01   | 100.0   |

Abbreviations: AUC<sub>0–72 h</sub>, area under the curve from 0 to 72 h; CES1, carboxylesterase 1; CL, clearance; C<sub>max</sub>, peak concentration; CV%, coefficient of variance; PK, pharmacokinetic; t<sub>1/2</sub>, terminal half-life.

Effect of CES1 G143E on trandolapril pharmacodynamics

PD parameters consisted of the resting SBP/DBP and heart rate, measured predose (baseline) and postdose. No significant difference was found in baseline PD parameters between the two groups (Table 2), but interindividual variabilities in blood pressure (BP) and heart rate were evident (CV%: 6.3%–21.3%). To minimize potential confounding effects of this PD baseline variability, BP, and heart rate were normalized to the corresponding baseline values. Upon analysis, mean SBP and DBP were found to be inversely correlated with trandolaprilat plasma concentration (Figure 4d). In the noncarrier group, maximal reduction of SBP and DBP were achieved 6–8 h after dosing (Figure 4), corresponding to the trandolaprilat time of maximum plasma concentration (T<sub>max</sub>; 6.7 h). The BP-reducing effect of trandolapril was less appreciable in G143E carriers relative to noncarriers (Figure 4a,b). The maximum reductions of SBP and DBP in the carrier group were 78.6% and 80.0%, respectively, of those in the noncarrier group, although the differences were not statistically significant (SBP, p = 0.54; DBP, p = 0.26; Table 2 and Figure 5). There were no significant changes in heart rate after trandolapril administration in either the noncarrier or carrier group (Figures 4c and 5).

DISCUSSION

The clinical relevance of CES1 genetic polymorphisms has been well-documented for various CES1 substrate medications. A previous in vitro study has demonstrated that trandolapril is selectively activated by CES1 in the human liver, and the CES1 variants, G143E and D260fs, completely impaired trandolapril activation in s9 fractions prepared from cells transfected with the variants. In the present study, we first evaluated the effect of CES1 G143E on trandolapril activation in human livers. Consistent with the previous in vitro HLS9 incubation study on other ACE inhibitor prodrugs, the mean trandolapril activation rate in the G143E heterozygous human livers was reduced to ~42%...
of that in the livers without the variant \((p = 0.0015)\). We further conducted a trandolapril PK study in healthy volunteers and observed a much smaller effect of the G143E variant on trandolapril activation. The C\text{max} and AUC\text{0–72 h} of trandolapril were decreased by 20% and 15%, respectively, in the G143E carrier group \((n = 8)\) relative to the noncarrier control \((n = 11; \text{Figure 3 and Table 1})\); however, the differences were not statistically significant. We did not report the AUC from zero to infinity \((\text{AUC}_0-\infty)\) because the extrapolated AUC\text{72–\infty} exceeded 50% of AUC\text{0–\infty}, indicating a potentially unreliable estimation. The PK parameters obtained in this study were comparable to those reported in previous trandolapril healthy volunteer PK studies.\(^{35,36}\) The PD outcomes were in good agreement with the PK findings. The BP-lowering effect of trandolapril was greater in noncarriers than carriers, although the difference did not reach a statistically significant level. Consistent with previous studies,\(^{35,37}\) the maximum BP reduction in noncarriers occurred at 6–8 h after dosing, which corresponds to the T\text{max} of trandolapril.

The CES1 G143E variant was originally identified in a study subject who displayed a markedly abnormal PK profile in a methylphenidate PK study.\(^{20}\) Subsequent in vitro functional studies showed that the G143E was a loss-of-function variant for all tested CES1 substrate drugs without altering CES1 protein expression.\(^{11,12,15,18,19,21,22}\) However, the clinical impact of the G143E variant differed significantly among drugs metabolized by CES1. For instance, this single-nucleotide polymorphism (SNP) significantly altered the PKs and PDs of the CES1 substrate medications methylphenidate,\(^{20,24}\) oseltamivir,\(^{22,25}\) and clopidogrel\(^{21,26}\); however, it had much less potent effect on the activation of the ACE inhibitor prodrugs enalapril,\(^{28}\) quinapril,\(^{27}\) and trandolapril, as we reported in this investigation. Of note, these studies utilized healthy volunteers with similar study designs and sample sizes. Specifically, Stage et al. examined the impact of CES1 G143E on methylphenidate and enalapril in the same Danish volunteers \((6 \text{ carriers and 16 noncarriers})\). Interestingly, the mean AUC\text{0–\infty} of methylphenidate was found to be 152.4% higher in G143E carriers than in controls \((p < 0.0001)\), whereas there was no significant difference between the two groups for enalapril PK parameters.\(^{28}\) Likewise, Tarkiainen et al. performed CES1 G143E pharmacogenetic studies of clopidogrel and two ACE inhibitor prodrugs
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In the same Finnish subjects (carriers = 10 and noncarriers = 12) and revealed similar substrate dependency of the G143E variant’s effects on the metabolism of these drugs. Specifically, they reported a 153% decrease of the clopidogrel carboxylic acid to clopidogrel AUC0-∞ ratios in carriers (p = 0.009), 26 but only a modest 20% decrease for the enalaprilat AUC0-∞ (p = 0.049) and no significant impact on quinapril PKs. 27 In line with these previous reports, our study also suggests that ACE inhibitor prodrugs might be less susceptible to the effect of CES1 G143E in vivo, although in vitro human liver incubation studies consistently showed an ~50% reduction of the metabolism of all tested CES1 substrates in CES1 G143E heterozygotes. 11,32,38–40 The mechanisms underlying the apparent substrate-dependent effect of the G143E remains unexplored. We speculate that the catalytic properties of CES1 on different substrates and differences in the disposition of these drugs in the liver might contribute to the discordant effects of the G143E variant on the PKs of CES1 substrates. In-depth research into this phenomenon could help investigators better predict the in vivo effect of CES1 genetic variants on a specific substrate drug using in vitro findings.

In addition to the G143E, several other common CES1 genetic variants have been studied in the context of their impacts on the metabolism and efficacy of ACE inhibitor prodrugs; however, the findings were largely inconsistent. For instance, −816 A>C (rs378161), an SNP located in the promoter region of CES1P1 VAR, was associated with changes in

FIGURE 4 Mean systolic blood pressure (SBP), (a), diastolic blood pressure (DBP), (b), heart rate (c) in G143E noncarriers (G/G, n = 11, open blue circle) and G143E carriers (G/E, n = 8, red squares), and a representation combining trandolaprilat plasma concentrations (red dots), SBP (green square), DBP (blue triangle) and heart rate (grey inverted triangle) in G143E noncarriers (d) from 0 to 12 h after the oral administration of a single dose of 1 mg trandolapril

FIGURE 5 The maximum reduction of systolic blood pressure (SBP; left y-axis), diastolic blood pressure (DBP; left y-axis), and heart rate (right y-axis) after a single oral dose of trandolapril (1 mg) in G143E noncarriers (G/G, green) and carriers (G/E, red). The means were indicated by horizontal bars in each group (enalapril and quinapril) in the same Finnish subjects (carriers = 10 and noncarriers = 12) and revealed similar substrate dependency of the G143E variant’s effects on the metabolism of these drugs. Specifically, they reported a 153% decrease of the clopidogrel carboxylic acid to clopidogrel AUC0-∞ ratios in carriers (p = 0.009), 26 but only a modest 20% decrease for the enalaprilat AUC0-∞ (p = 0.049) and no significant impact on quinapril PKs. 27 In line with these previous reports, our study also suggests that ACE inhibitor prodrugs might be less susceptible to the effect of CES1 G143E in vivo, although in vitro human liver incubation studies consistently showed an ~50% reduction of the metabolism of all tested CES1 substrates in CES1 G143E heterozygotes. 11,32,38–40 The mechanisms underlying the apparent substrate-dependent effect of the G143E remains unexplored. We speculate that the catalytic properties of CES1 on different substrates and differences in the disposition of these drugs in the liver might contribute to the discordant effects of the G143E variant on the PKs of CES1 substrates. In-depth research into this phenomenon could help investigators better predict the in vivo effect of CES1 genetic variants on a specific substrate drug using in vitro findings.

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the PDs of imidapril\(^4^1\) and clopidogrel\(^4^2\); these were believed to be consequences of its upregulating effect on the expression of \(CES1P1\) \(^VAR\).\(^5^3\) \(CES1P1\) \(^VAR\) is a CES1-expressing variant of the pseudogene \(CES1P1\) and contributes ~2% of the total hepatic CES1 expression.\(^4^4\) However, a retrospective pharmacogenetic analysis of the International Verapamil SR Trandolapril study found no significant association between the \(-816\) \(A>C\) genotype and trandolapril efficacy.\(^3^2\) A follow-up in vitro study further confirmed the lack of association between this variant and CES1 protein expression or trandolapril activation in human liver.\(^3^2\) Furthermore, an in vitro functional study examined several \(CES1\) genetic polymorphisms, including \(-75\) \(G>T\) (rs3815583), \(S75N\) (rs2307240), \(CES1\) copy variants (i.e., \(CES1/CES1\) \(^VAR\) and \(CES1P1/CES1P1\) \(^VAR\)), and \(CES1\) diplotype, but did not identify any significant associations of these variants with ACE inhibitor prodrug activation and CES1 protein expression in the human liver.\(^1^1\) Thus, these common \(CES1\) variants were unlikely to affect trandolapril activation in vivo.

The relatively small sample size is a major limitation of this clinical study, which is mainly due to the low MAF of the G143E variant. In addition, none of the carriers in the study were G143E homozygous. Thus, we were unable to evaluate the effect of the G143E homozygous genotype on the PKs and PDs of trandolapril. Moreover, findings from a single-dose healthy volunteer study are not necessarily applicable to patient outcomes. Indeed, the BP-lowering effect of trandolapril in healthy volunteers is usually not as significant as in patients with hypertension.\(^4^5\) Hence, a larger difference in trandolapril PK and PD between the G143E genotypes might be observed in patients with hypertension receiving maintenance doses of trandolapril.

In summary, our in vitro incubation study demonstrated that the \(CES1\) G143E variant significantly reduced trandolapril activation in the human liver. However, the effect of this variant on the PKs and PDs of a single dose of trandolapril in healthy volunteers was modest, and the PK and PD differences between the G143E genotypes did not reach a statistically significant level with such a small sample size (11 noncarriers and 8 carriers). Thus, the clinical significance of this variant in patients treated with trandolapril and other ACE inhibitor prodrugs warrants further investigation. Finally, along with previous reports,\(^2^3^–^2^6\) our results suggest that the G143E variant may affect the PKs of CES1 substrate medications in a substrate-dependent manner. A better understanding of this substrate-dependent effect could help with extrapolating findings from in vitro \(CES1\) pharmacogenetics experiments to potential clinical consequences on a substrate-specific basis.

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**CONFLICT OF INTEREST**
The authors declared no competing interests for this work.

**AUTHOR CONTRIBUTIONS**
X.W., L.H., A.H.W., B.E.B., and H-J.Z. wrote the manuscript. H-J.Z., A.H.W., and B.E.B. designed the research. X.W., L.H., J.S., and J.X. performed the research. X.W. analyzed the data.

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