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Effect of Low-Dose Vonoprazan on Hepatic Cytochrome P450 Activity By 13C-Aminopyrine Breath Test in Healthy Subjects

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Background: Proton pump inhibitors (PPIs) affect hepatic drug metabolism via cytochrome P450 (CYP) enzymes, but the impact of potassium-competitive acid blockers (P-CABs), such as vonoprazan (VPZ), is not fully understood. This study aimed to evaluate whether maintenance-dose VPZ influences hepatic CYP activity using the 13C-aminopyrine breath test (13C-ABT).

Material/Methods: Twenty healthy subjects aged 18 years or older were recruited. Each participant underwent 13C-ABT under 2 conditions: without treatment (control) and after 8 days of 10 mg VPZ once daily. After oral administration of 100 mg 13C-aminopyrine, breath samples were collected before dosing and every 15-30 minutes for 3 hours. The delta-over-baseline (DOB) ratio, reflecting $^{13}CO_2/^{12}CO_2$, was measured using infrared spectroscopy.

Results: Nineteen participants (median age 24 years; 10 men, 9 women) completed the study. In the control condition, DOB values increased from 15 minutes after dosing and reached a peak at 49.7 ± 35.8 minutes. The VPZ condition showed no significant differences in the DOB curve compared to the control condition ($P = 0.316$). The area under the DOB curve over 3 hours was comparable between conditions, with a geometric mean ratio of 1.09 (90% confidence interval 0.97-1.23). Peak values and time to peak were also similar between 2 conditions.

Conclusions: Administration of 10 mg of VPZ did not significantly affect hepatic CYP activity as assessed by the 13C-ABT, suggesting that VPZ at a maintenance dose has minimal effect on hepatic drug metabolism and drug–drug interactions.

Clinical Trial Registration: Japan Registry of Clinical Trials (jRCT): jRCTs041220044

Keywords: Breath Tests • Aminopyrine • Cytochrome P-450 Enzyme System • Drug Interactions • Healthy Volunteers • Gastric Acid • Sodium-Potassium-Exchanging ATPase

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Introduction

Vonoprazan (VPZ), a potassium-competitive acid blocker (P-CAB), has become widely prescribed in recent years due to its more potent and sustained acid suppression compared with proton pump inhibitors (PPIs) [1-3]. Its rapid and stable suppression of gastric acid has led to its widespread use in the treatment of acid-related diseases and in the prevention of gastrointestinal bleeding in patients receiving antithrombotic therapy [4-6]. With long-term use, these agents can interact with medications used for treatment of comorbid conditions [7].

PPIs have been reported to reduce the absorption and systemic exposure of several drugs, including gefitinib, erlotinib [8], and itraconazole [9]. In contrast, drug interactions of P-CABs have not been fully elucidated and remain less well characterized. Such interactions may occur through pharmacokinetic mechanisms—alterations in absorption, distribution, metabolism, or excretion—or pharmacodynamic mechanisms, particularly those involving modulation of cytochrome P450 (CYP) enzymes [10,11].

Although in vitro data can be used to predict in vivo drug interactions, accurate prediction is often complicated by factors such as the specific interaction sites, competitive inhibition, and the presence of active metabolites [12,13]. VPZ has been reported to inhibit multiple CYP enzymes, including CYP3A4, CYP2C9, CYP2D6, and CYP2B6, suggesting a potential for drug–drug interactions [14]. In the U.S. prescribing information, VPZ is described as a substrate of CYP3A and as exhibiting time-dependent inhibitory effects on CYP2B6, CYP2C19, and CYP3A4/5 in vitro. In clinical studies, administration of VPZ 20 mg twice daily nearly doubled the exposure to midazolam [15]. Accordingly, the product label classifies VPZ as a weak inhibitor of CYP3A and CYP2C19, and recommends monitoring or dose adjustment when co-administered with sensitive substrates of CYP3A or CYP2C19, as well as avoiding concomitant use with strong CYP3A inducers [16]. This underscores the importance of confirming isoform-specific interactions using index substrates or modeling approaches. However, the extent of this inhibition varies depending on experimental conditions, and it remains unclear whether VPZ affects CYP activity at the low doses typically used in long-term clinical settings.

We previously demonstrated that the 13C-aminopyrine breath test (13C-ABT) is a non-invasive tool that reflects hepatic drug-metabolizing activity, mediated by multiple CYP enzymes, including CYP2C19, CYP1A2, CYP3A4, and CYP2C9 [17]. Based on its ability to assess overall hepatic CYP function, 13C-ABT is considered useful for evaluating metabolic capacity in the context of drug–drug interactions [18,19]. Given this mechanistic scope, we hypothesized that vonoprazan can influence hepatic CYP-mediated metabolism. Therefore, in this study, we investigated whether the maintenance dose of VPZ affects hepatic CYP activity as assessed by the 13C-ABT.

Material and Methods

Ethics Statement

This prospective study was conducted at Hamamatsu University School of Medicine in accordance with the principle of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board (IRB approval number: CRB4180008) and adhered to the Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects. The study was registered with the Japan Registry of Clinical Trials (jRCT ID: jRCTs041220044). Written informed consent was obtained from all participants.

Subjects

Between April 2022 and October 2024, 20 healthy adult volunteers were recruited for this study. Inclusion criteria were as follows: participants aged 18 years or older, able to abstain from alcohol and smoking during the study medication period and a washout period of 14 to 28 days, and capable of providing written informed consent.

At enrollment, all participants underwent blood testing to assess complete blood count and biochemical parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), total bilirubin, albumin, creatinine (Cre), and blood urea nitrogen (BUN).

Exclusion criteria included: history of gastric ulcers, myocardial infarction, or severe liver disease (defined as AST or ALT greater than 3 times the upper limit of normal); severe renal impairment (Cre > 1.5 mg/dL); other serious systemic illnesses; history of abdominal surgery; continuous use of medications during the study period; known hypersensitivity to the study drug; pregnancy or plans to conceive during the study period; and any condition deemed unsuitable by the principal or sub-investigators.

One subject was excluded due to protocol deviation, resulting in a final analysis cohort of 19 participants. The participant who discontinued was unable to attend the test due to an unavoidable personal matter on the scheduled day and was therefore withdrawn. A second 8-day dosing period was not anticipated in the study protocol.

A formal sample size calculation was not performed because this was an exploratory, within-subject controlled study. The

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sample size was determined based on prior 13C-ABT studies [17,18,20] and feasibility considerations. Given the paired within-subject design, which provides greater statistical efficiency, we aimed to include at least 15 participants per condition. A total of 20 participants were enrolled, of whom 19 were included in the final analysis.

Study Protocol

All participants first underwent the 13C-ABT as a control following oral administration of 100 mg of 13C-aminopyrine dissolved in 100 mL of water. After a washout period of at least 14 days, participants received vonoprazan (VPZ) 10 mg once daily after dinner for 8 consecutive days, corresponding to the clinically used maintenance dose for acid-related disorders. Considering the elimination half-life of vonoprazan (approximately 7-9 hours) and previous pharmacokinetic studies demonstrating that a steady state is reached within several days without further time-dependent during longer dosing periods [21], an 8-day administration period was selected to ensure steady-state exposure before the breath test. The 13C-ABT was repeated on day 8 of VPZ administration.

The 13C-ABT is a method for assessing hepatic oxidative metabolic capacity by orally administering 13C-labeled aminopyrine and measuring the amount of labeled carbon dioxide exhaled in breath samples. It enables non-invasive evaluation of hepatic microsomal enzyme activity, particularly cytochrome P450–mediated demethylation, and is regarded as a useful in vivo indicator of overall hepatic CYP activity. It was conducted using a protocol similar to that previously reported in our study, with further details described below.

13C-ABT

Participants were instructed to abstain from alcohol, smoking, and medications during the study medication period and a washout period of 14 to 28 days prior to each 13C-ABT session. They were monitored daily for their adherence via email for 8 days, and they recorded their medication intake in a diary, which was submitted on the final day. Participants were instructed to consume an appropriate lunch at 1: 00 PM on the test day and to refrain from eating dinner until completion of the breath test. In the evening, 100 mg of 13C-aminopyrine dissolved in 100 mL of water and the study drug (VPZ) were co-administered with an additional 200 mL of water, and the breath test was performed.

Breath samples were collected using a dedicated collection device immediately before administration (baseline) and at 15, 30, 45, 60, 75, 90, 105, 120, 150, and 180 minutes after administration (11 time points in total). The carbon isotope ratio ($^{13}CO_2/^{12}CO_2$) in exhaled CO_2 was measured using an infrared spectrometer (POCone Plus; Otsuka Pharmaceutical Co., Ltd.). It was calibrated before each use with certified gas standards. The assay's lower limit of quantification (LLOQ) for DOB was 0.1‰, with intra-assay CV < 5%. Reproducibility was with a standard deviation of ≤ 0.3‰. Measurement accuracy was within 0.3‰ (for Δ13C values < 20‰) and 2% (for Δ13C values ≥ 20‰). Duplicate measurements were performed, and quality control procedures followed manufacturer guidelines to ensure analytical robustness. The isotope ratio measured by the analyzer was converted to delta-over-baseline (DOB) values and subsequently expressed as%dose/h using the standard ABT calculation formula. DOB values (%dose/h) represent the change in the $^{13}CO_2/^{12}CO_2$ ratio relative to baseline. For inferential statistical analyses, baseline-adjusted values were used.

Endpoints of This Study

The primary endpoint was the change in the time course of DOB values in breath samples, with or without the administration of VPZ. Secondary endpoints included the time to reach the maximum DOB value, the maximum DOB value itself, and the area under the curve (AUC) of DOB. Additionally, any adverse effects associated with VPZ administration were assessed as secondary outcomes.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan). Categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate.

This study employed a within-subject repeated-measures design, in which each participant underwent both control and VPZ condition. For inferential analysis, DOB values were baseline-adjusted by subtracting the pre-administration value (0min) from each subsequent measurement within the same condition. These baseline-adjusted values were used for statistical comparisons and geometric mean ratio (GMR) calculations, whereas the actual values are presented for descriptive purposes only.

To evaluate the effect of VPZ compared with the control condition over time, repeated-measures analyses with two-within-subject conditions was performed. Paired comparisons of DOB values between the control and VPZ conditions at each time point were conducted using the Wilcoxon signed-rank test; These analyses were considered exploratory, and no correction for multiple comparisons was applied.

The area under the curve (AUC) of DOB values was calculated using the linear trapezoidal method on the baseline-adjusted

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values. The maximum value (Cmax) and time to peak (Tmax) were directly extracted from the observed data points.

All calculations were performed using SPSS and validated using EZR, a graphical interface for R. Differences in AUC between conditions were evaluated using the Wilcoxon signed-rank test. A two-sided *P* value of < 0.05 was considered statistically significant.

Results

Characteristics of the Subjects Enrolled in This Study

A total of 19 subjects completed the study. The median age was 24 years (range: 19-43), with 10 males and 9 females. The median body mass index (BMI) was 20.7. Blood test results indicated no abnormalities in liver or renal function among the participants (**Table 1**). All participants completed the study protocol and medication adherence was confirmed by daily email monitoring and medication diaries. No adverse events occurred during the study. Vital signs and blood chemistry parameters remained within normal ranges for all participants. A summary of safety data is provided in **Table 2**.

Table 1. Characteristics of the subjects enrolled in this study.

Number of subjects	19
Age, median (range), years	24 (19-43)
Sex, male/female	10/9
Height, cm	165.2 ± 7.2
Body weight, kg	58.4 ± 9.1
BMI, kg/m^2	21.3 ± 2.4
WBC, /μL	5604 ± 1260
RBC, ×10^4/μL	486 ± 38
Hb, g/dL	14.1 ± 1.1
Plt, ×10^4/μL	27.0 ± 5.9
AST, U/L	20.7 ± 5.8
ALT, U/L	18.1 ± 9.6
LD, U/L	179 ± 89
ALP, U/L	70.4 ± 17.9
T. Bil, mg/dL	0.77 ± 0.38
BUN, mg/dL	11.7 ± 2.9
Cre, mg/dL	0.71 ± 0.11

Data are expressed as mean ± SD. BMI, body mass index; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PLT, platelets; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LD, lactate dehydrogenase; ALP, alkaline phosphatase; T.Bil, total bilirubin; BUN, Blood urea nitrogen; Cre, creatinine.

Metabolism of 13C-Aminopyrine in Exhaled Air Reflecting CYP Activity

Changes in the carbon isotope ratio of carbon dioxide ($^{13}CO_2/^{12}CO_2$) in breath samples following ingestion of 100 mg of 13C-aminopyrine are presented in **Table 3** as DOB values, which reflect hepatic drug metabolism mediated by CYP enzyme activity. All data were adjusted for baseline values from each participant's pre-administration breath sample. For inferential statistical analyses, baseline-adjusted values were used.

DOB values increased from 15 minutes after dosing and peaked at 45 minutes. The mean time to reach the maximum DOB value was 49.7 ± 35.8 minutes. The maximum DOB value was 6.46 ± 2.15%dose/h, and the area under the DOB curve from 0 to 3 hours (AUC_{0-3h}) was 14.57 ± 4.26%dose/h (**Table 3**). Tmax did not differ between the control and VPZ conditions.

To evaluate the potential impact of VPZ on hepatic drug metabolism, we next compared the time course of DOB values between the control and the condition receiving daily administration of 10 mg of VPZ.

Comparison of Time Course of DOB of 13C-Aminopyrine Between the Control and the 10 mg VPZ Condition

To assess whether daily administration of low-dose VPZ (10 mg/day) affects CYP-mediated hepatic metabolism of aminopyrine, 13C-ABT was performed on the 8th day of VPZ administration. The time course of DOB values in the control and VPZ conditions is shown in **Figure 1A**. In both conditions, DOB increased from 15 minutes after administration, peaked, and then declined steadily over the 180-minutes observation period.

The repeated-measures ANOVA revealed a significant main effect of time ($F(10, 180) = 116.52$, $p < 0.001$), indicating changes in breath test values over time. However, there was no significant main effect of treatment condition ($F(1, 18) = 1.06$, $P = 0.316$) and no significant interaction between treatment and time ($F(10, 180) = 0.66$, $P = 0.760$), suggesting that the overall metabolic response pattern over time was not significantly different between the control and VPZ conditions (**Figure 1A**). Paired tests were used to examine the significance of differences between the 2 conditions at each time point, but no significant differences were found at any time. Similarly, there were no significant differences in the maximum DOB values or the time to peak DOB (**Figure 1B**).

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Table 2. Adverse events of the subjects enrolled in this study.

Laboratory test	Control	VPZ (Day8)	ΔVPZ–Control
WBC, ×10³/μL	5.60 ± 1.26	6.13 ± 1.61	0.53 ± 1.43
RBC, ×10⁶/μL	4.86 ± 0.38	4.93 ± 0.43	0.08 ± 0.23
Hb, g/dL	14.1 ± 1.1	14.3 ± 1.3	0.2 ± 0.9
Plt, ×10⁴/μL	27.0 ± 5.9	27.4 ± 6.5	0.4 ± 6.5
AST, U/L	20.7 ± 5.8	21.5 ± 7.6	0.74 ± 9.8
ALT, U/L	18.1 ± 9.6	21.3 ± 10.9	3.2 ± 8.8
LD, U/L	179 ± 89	165 ± 33	-14 ± 87
ALP, U/L	70.4 ± 17.9	73.7 ± 19.9	3.2 ± 10.0
T. Bil, mg/dL	0.77 ± 0.38	0.68 ± 0.38	-0.09 ± 0.20
BUN, mg/dL	11.7 ± 2.9	12.6 ± 2.8	0.8 ± 2.6
Cre, mg/dL	0.71 ± 0.11	0.73 ± 0.09	0.02 ± 0.07
AE Category	**Control (n = 19)**	**VPZ (Day 8, n = 19)**	**AE**
Anaphylaxis	0	0	None
Gastrointestinal symptoms	0	0	None
Skin symptoms	0	0	None
Others	0	0	None

Data are expressed as mean ± SD. VPZ, vonoprazan; AE, adverse events; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PLT, platelets; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LD, lactate dehydrogenase; ALP, alkaline phosphatase; T.Bil, total bilirubin; BUN, Blood urea nitrogen; Cre, creatinine.

Table 3. Time course and summary metrics of the 13C-aminopyrine breath test under the control condition.

	Actual value	Adjusted value for baseline
DOB, 0 min, %dose/h	-0.41 ± 0.21	0
DOB, 15 min, %dose/h	3.07 ± 2.07	3.48 ± 2.10
DOB, 30 min, %dose/h	5.55 ± 2.52	5.96 ± 2.54
DOB, 45 min, %dose/h	5.47 ± 2.04	5.88 ± 2.07
DOB, 60 min, %dose/h	5.25 ± 1.69	5.66 ± 1.71
DOB, 75 min, %dose/h	5.12 ± 1.59	5.53 ± 1.61
DOB, 90 min, %dose/h	4.95 ± 1.41	5.36 ± 1.44
DOB, 105 min, %dose/h	4.76 ± 1.47	5.17 ± 1.49
DOB, 120 min, %dose/h	4.59 ± 1.44	5.00 ± 1.46
DOB, 150 min, %dose/h	4.24 ± 1.23	4.65 ± 1.25
DOB, 180 min, %dose/h	3.99 ± 1.23	4.41 ± 1.26
Maximum value of DOB, %dose/h	6.05 ± 2.11	6.46 ± 2.15
Time to maximum value of DOB, min	49.74 ± 35.78	49.74 ± 35.78
AUC 0-3h of DOB, %dose/h	13.33 ± 4.26	14.57 ± 4.26

DOB, delta-over-baseline; AUC, areas under the curve. Actual values and baseline-adjusted values are presented. Inferential statistical analyses were performed using baseline-adjusted values.

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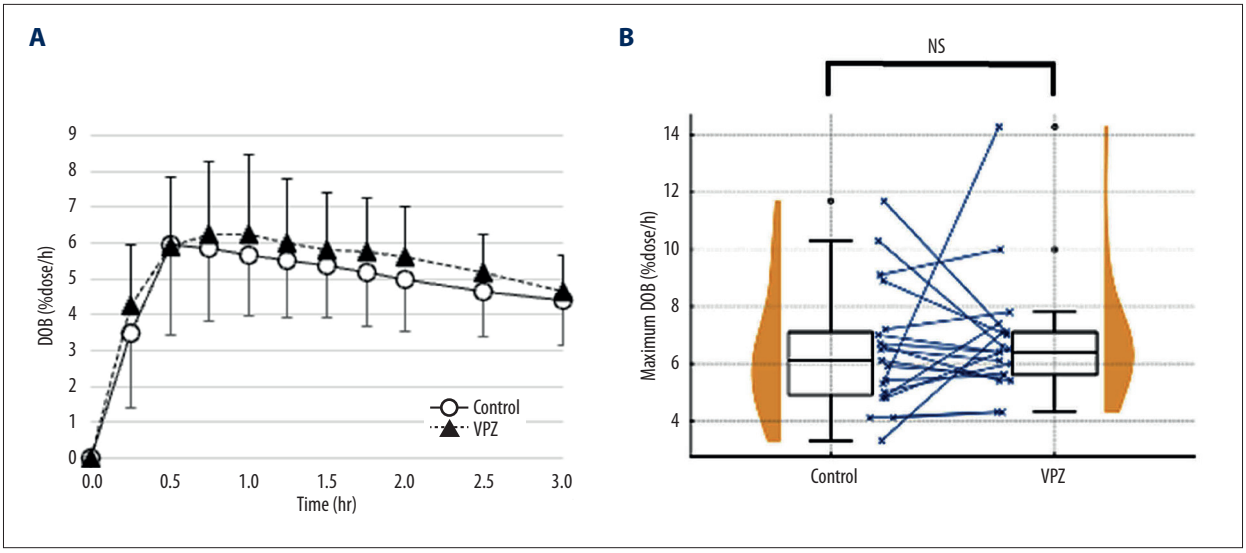

Figure 1. Comparison of delta-over-baseline (DOB) of 13C-aminopyrine between control and VPZ. (**A**) Comparison of time course changes of DOB between control and VPZ. Bars indicate standard deviation (SD). For inferential statistical analyses, baseline-adjusted values were used. The repeated-measures ANOVA revealed a significant main effect of time ($F(10, 180) = 116.52$, $P < 0.001$), whereas there was no significant main effect of treatment condition ($F(1, 18) = 1.06$, $p = 0.316$) and no significant interaction between treatment and time ($F(10, 180) = 0.66$, $P = 0.760$). (**B**) Comparison of the maximum DOB value between control and VPZ. Paired raincloud plot showing the maximum value of DOB in each subject under control and VPZ conditions (n = 19). Half-violins (orange) depict the distribution density, overlaid with box-and-whisker plots (black) and individual paired data points (blue ×) connected by lines. Statistical significance was evaluated using the Wilcoxon signed-rank test. No significant difference was observed between 2 conditions ($P = 0.922$). NS, not significant.

Comparison of AUC of 13C-Aminopyrine Between the Control and the VPZ Condition

To further evaluate the effect of VPZ on hepatic CYP activity, the area under the curve $(AUC)_{0-3h}$ of DOB values was compared between 2 conditions. The median AUC_{0-3h} was 13.5%dose/h in the control condition and 15.0%dose/h in the VPZ condition. There was no statistically significant difference in AUC_{0-3h} between the 2 conditions (**Figure 2A**). The geometric mean ratio (GMR) for AUC_{0-3h} between the VPZ and control conditions was 1.09 (90% CI: 0.97-1.23), indicating no significant change in hepatic metabolic activity (**Table 4**).

To explore whether potential effects might vary over specific time intervals, the AUC was also calculated separately for the 0-1 hour, 1-2 hour, and 2-3 hour periods. However, no significant differences in AUC were observed between the control and VPZ conditions for any of these time intervals (**Figure 2B-2D**).

Because sex-related differences have been reported in the expression of hepatic drug-metabolizing enzymes [22], we performed subgroup analyses based on gender. In an exploratory sex-stratified analysis, an increase in DOB AUC after VPZ administration was observed in female subjects (Wilcoxon signed-rank test, $W = 5$, $P = 0.039$), whereas no significant difference was observed in male subjects ($P = 0.846$) (**Figure 3**).

Discussion

This prospective controlled study investigated, for the first time, whether a maintenance dose of vonoprazan (VPZ) affects hepatic drug metabolism in humans using the 13C-ABT. While previous in vitro and animal studies have demonstrated that VPZ can inhibit multiple cytochrome P450 (CYP) isoforms, particularly CYP3A4, CYP2C9, CYP2B6, and CYP2D6 [23], our results showed that daily administration of 10 mg VPZ did not significantly affect aminopyrine metabolism in healthy volunteers. These findings support the safety of low-dose VPZ for hepatic drug metabolism and drug–drug interactions in clinical use.

VPZ, the first potassium-competitive acid blocker (P-CAB), is approved in Japan at 40 mg/day for *Helicobacter pylori* eradication and 20 mg/day for initial treatment of peptic ulcer disease and reflux esophagitis [24]. For maintenance therapy and prophylaxis of NSAID- or aspirin-induced ulcers, 10 mg/day dose of VPZ is commonly used [14]. In vitro study, VPZ is primarily metabolized by CYP3A4, with minor contributions from CYP2C19, CYP2B6, CYP2D6, and SULT2A1 [14].

Although animal studies have shown that VPZ inhibits CYP-mediated metabolism and can interact with CYP3A4 inhibitors such as voriconazole and poziotinib [25,26], the clinical relevance of these findings in humans remains uncertain. Some clinical studies report a reduction in the antiplatelet effect of

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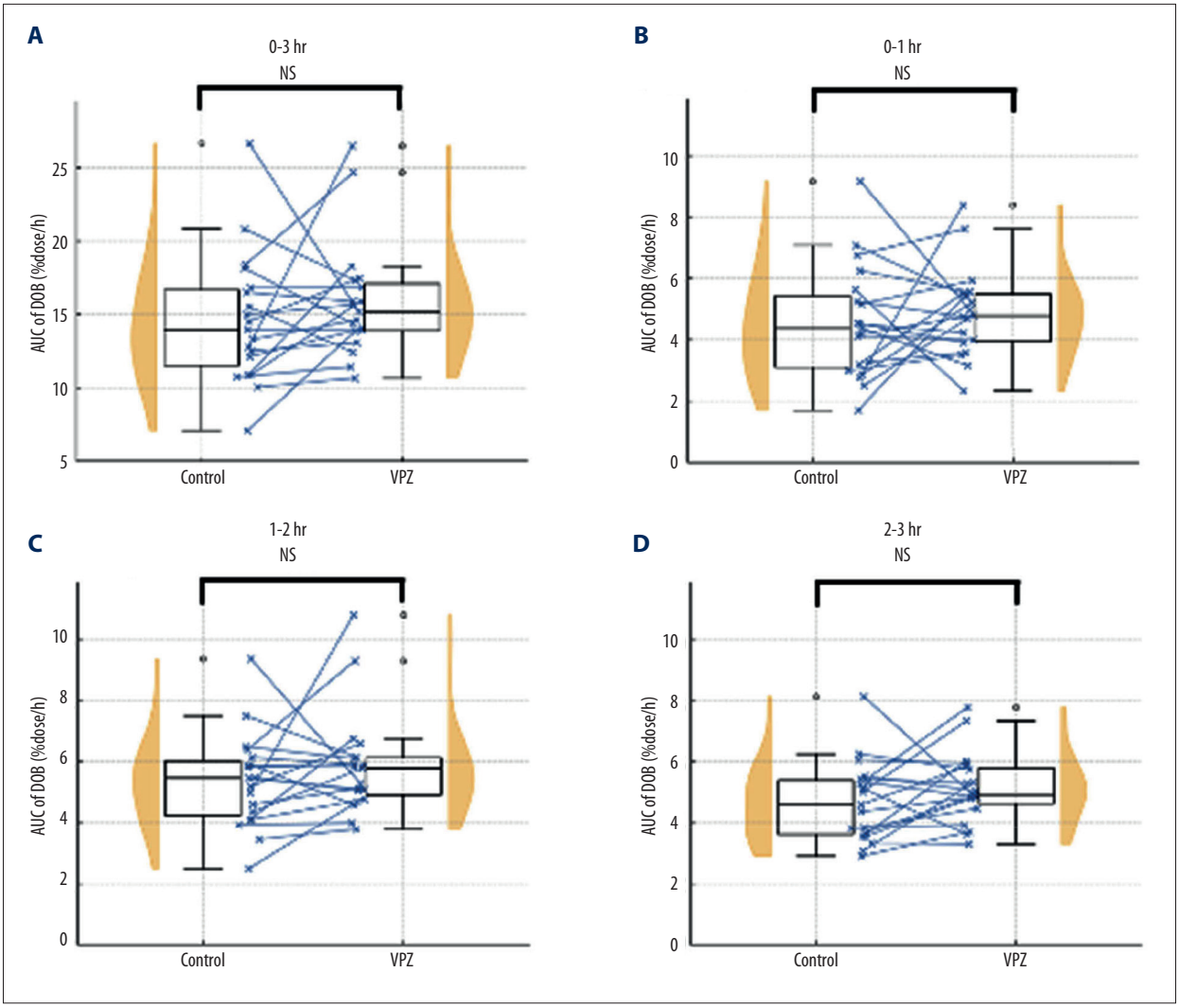

Figure 2. Comparison of area under the curve (AUC) of DOB between control and VPZ conditions at each time interval. Paired raincloud plots illustrating [^{13}C]-ABT cumulative recovery (AUC of DOB,%dose/h) under control and VPZ conditions across different time intervals. (**A**) 0-3 h, (**B**) 0-1 h, (**C**) 1-2 h, (**D**) 2-3 h. Each panel combines half-violin distributions (orange), central boxplots (black), and paired data (blue × and connecting lines) for visualization of distribution and individual changes. All comparisons were performed using the Wilcoxon signed-rank test (n = 19). No significant difference was observed between 2 conditions. NS, not significant.

prasugrel with VPZ compared to PPIs [27], while others show no significant change in tacrolimus levels [28] or prasugrel activity [29]. These discrepancies suggest that the in vitro inhibitory potential of VPZ may not translate directly to clinically significant interactions at maintenance doses.

In our prior study using the 13C-ABT, we demonstrated that PPI-induced changes in CYP activity could vary according to CYP2C19 genetic polymorphisms, confirming that aminopyrine metabolism is predominantly mediated by CYP2C19 and is sensitive to its inhibition [17]. Given that CYP enzymes, particularly the 5 major isoforms, are involved in the metabolism of approximately 90% of clinically used drugs, a comprehensive assessment of VPZ's impact on hepatic metabolism is important. The 13C-ABT serves this role by evaluating N-demethylation of aminopyrine—a reaction primarily catalyzed by CYP2C19 and other CYP450 isoforms—and thus provides an integrated measure of hepatic CYP monooxygenase activity.

The present study evaluated the effect of maintenance-dose vonoprazan (10 mg/day) on hepatic CYP-mediated metabolism using the 13C-ABT. This dosing regimen reflects routine clinical maintenance therapy for acid-related disorders. Because vonoprazan reaches steady-state concentrations within several days, the 8-day administration period used in this study was expected to be sufficient to detect potential modulation of overall hepatic CYP activity.

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Table 4. Comparison of 13C-aminopyrine breath test parameters between control and vonoprazan conditions.

	Control (Mean ± SD)	Vonoprazan (Mean ± SD)	GMR (VPZ/Control)	90% CI
Maximum value of DOB (%dose/h)	6.46 ± 2.15	6.81 ± 2.16	1.05	0.93-1.18
AUC $_{0-3h}$ of DOB (%dose/h)	14.57 ± 4.26	15.90 ± 3.86	1.09	0.97-1.23
AUC $_{0-1h}$ of DOB (%dose/h)	4.54 ± 1.81	4.89 ± 1.41	1.08	0.91-1.29
AUC $_{1-2h}$ of DOB (%dose/h)	5.35 ± 1.50	5.87 ± 1.64	1.10	0.96-1.26
AUC $_{2-3h}$ of DOB (%dose/h)	4.68 ± 1.28	5.14 ± 1.13	1.10	0.96-1.27

Note: GMR and 90% CI were calculated based on log-transformed data and then back-transformed. GMR > 1 indicates higher value with VPZ.

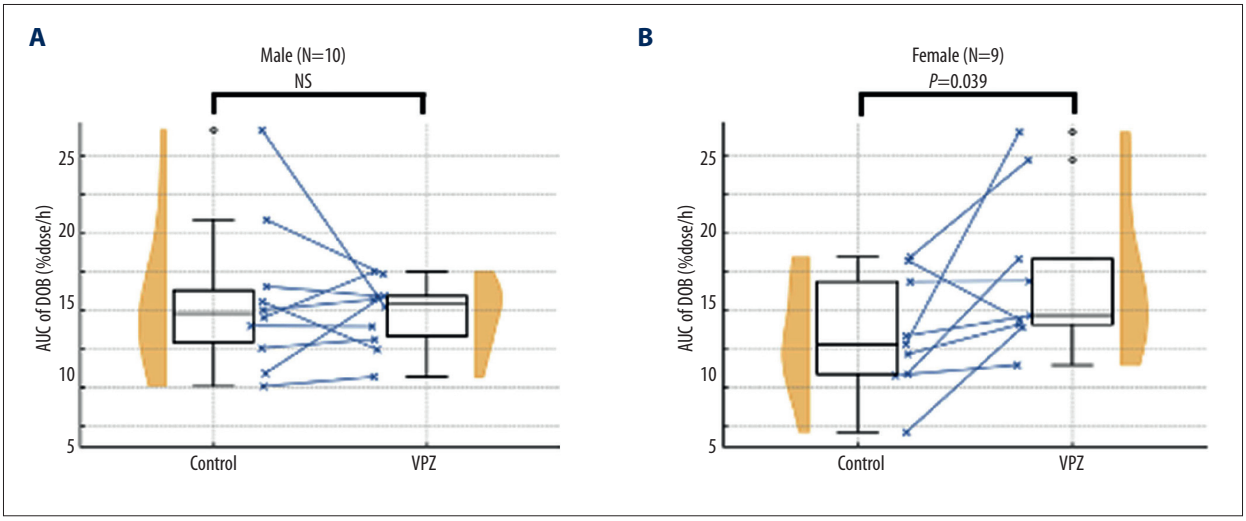

Figure 3. Comparison of area under the curve (AUC) 0-3h of DOB between control and VPZ conditions by sex. AUC of DOB (%dose/h) stratified by gender (Male: n = 10; Female: n = 9). In female subjects, VPZ administration was associated with a statistically significant increase in DOB AUC (Wilcoxon signed-rank test, W = 5, *P* = 0.039). No significant difference was observed in males (*P* = 0.846). Each panel combines half-violin distributions (orange), central boxplots (black), and paired data (blue × and connecting lines) for visualization of distribution and individual changes. NS, not significant.

In this study, changes in the $^{13}CO_2/^{12}CO_2$ ratio in exhaled breath after ingestion of 100 mg of 13C-aminopyrine were measured in the absence of VPZ, serving as a control to reflect hepatic drug metabolism mediated by CYP enzyme activity. DOB values increased from 15 minutes after administration, with a mean time to peak (Tmax) of 49.7 ± 35.8 minutes. These findings are consistent with those of our previous study on the effects of PPIs using 13C-ABT, as well as with other published reports [17,20]. The reproducibility and responsiveness of the 13C-ABT in this study were considered adequate for the comprehensive assessment of hepatic CYP activity.

The effects of low-dose VPZ (10 mg/day) were evaluated using multiple parameters, including the time course of DOB, maximum DOB value, time to peak DOB, and AUC. Across all of these measures, no significant differences were observed between the VPZ and control conditions, indicating no detectable impact of VPZ on aminopyrine metabolism. In addition, AUC analysis stratified by hourly intervals revealed no significant changes. These results suggest that the CYP inhibitory effect of VPZ at a maintenance dose of 10 mg/day is minimal and unlikely to cause clinically relevant drug interactions in healthy individuals. This provides valuable evidence supporting the hepatic safety of VPZ in routine clinical practice.

Although 13C-ABT reflects global hepatic CYP activity, it does not isolate isoform-specific contributions (e.g., CYP3A4 or CYP2B6). Since aminopyrine has been reported to be metabolized by multiple CYP isoforms [30], the absence of an effect of VPZ on DOB values in this study may reflect either a true lack of inhibition or limited sensitivity of the ABT method to detect minor CYP isoform inhibition. The primary objective of

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this study was to comprehensively confirm the effects on CYP metabolism using a safe and simple aminopyrine breath test. However, to clarify these remaining issues, further studies using index substrates and modeling approaches, as outlined in the ICH M12 guideline [31] (eg, midazolam for CYP3A4, bupropion for CYP2B6), are warranted. Additionally, the use of the erythromycin breath test (EBT), a specifically developed CYP3A4 probe, may compensate for the potentially low sensitivity of ABT alone to CYP3A4 inhibition. Future studies may consider using both ABT and EBT in parallel to more precisely analyze isoform-specific interactions.

An exploratory sex-stratified analysis suggested a higher DOB AUC after VPZ administration in female subjects, whereas no significant difference was observed in males. These findings should be interpreted cautiously and considered hypothesis-generating. Prior studies have reported sex-dependent variation in CYP3A4 and CYP2C19 expression, which may partly explain the observed differences [32,33]. Further investigation in larger and more diverse populations is needed to confirm the findings and explore underlying mechanisms.

This study has several limitations. First, it was a single-center study with a relatively small sample size. The sample size was determined based on previous ABT-based studies assessing PPI effects on CYP activity. While sufficient to detect moderate changes, we acknowledge that the study may have been underpowered to detect subtle effects, and small or modest changes in hepatic metabolism cannot be excluded. Second, because the study population consisted of young, healthy Japanese adults, the findings may not be generalizable to other ethnic groups, elderly patients, or individuals with liver disease, comorbidities or polypharmacy; therefore, caution is warranted when extrapolating these results, particularly in light of potential age-related changes in hepatic metabolism [34], and further studies in older populations are needed. Third, only a single dose level of VPZ (10 mg/day) was evaluated. Without data on higher doses, we were unable to assess potential dose-dependent effects, which might have further supported the relative safety of low-dose VPZ. Fourth, due to technical limitations, we did not measure the plasma concentrations of VPZ or aminopyrine over time. As a result, interindividual differences in gastrointestinal absorption may have introduced bias into the results.

Despite these limitations, few studies have evaluated the effect of low-dose VPZ on CYP activity in humans. To our knowledge, this is the first study to assess potential drug interactions comprehensively using the 13C-ABT, offering novel insights into the hepatic safety profile of VPZ. Further large-scale clinical studies are warranted to confirm these findings and address the remaining questions.

Conclusions

In this prospective controlled study, a maintenance dose of vonoprazan (10 mg daily) did not significantly affect hepatic drug metabolism as assessed by the 13C–aminopyrine breath test in healthy volunteers. These findings suggest that low-dose vonoprazan has minimal effect on hepatic metabolism and a low potential for clinically relevant drug–drug interactions. Further studies are warranted to confirm these results and extend them to broader patient populations.

Patient Consent

Written informed consent was obtained from all participants.

The study protocol was approved by the Certified Clinical Research Review Board of Hamamatsu University School of Medicine.

Data Availability Statement

The data are not publicly available due to privacy or ethical restrictions.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

Abbreviations

PPI, proton pump inhibitor; **P-CAB,** potassium-competitive acid blockers; **VPZ,** vonoprazan; **CYP,** cytochrome P450; **DOB,** delta-over-baseline; **AUC,** area under the curve; **ABT**, aminopyrine breath test; **AST,** aspartate aminotransferase; **ALT,** alanine aminotransferase; **γ-GTP**, γ-glutamyl transpeptidase; **ALP,** alkaline phosphatase; **Cre,** creatinine; **BUN,** blood urea nitrogen; **BMI,** body mass index.

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