



Report

Effects of Phenobarbital and Dosing Time on The Plasma Concentration and Pharmacological Activity of Tadalafil in Mice

Hiroshi Kawai,^{a,*} Naoki Takeda,^b Naoko Kojima,^c Reiko Iwadate,^a Naomi Kudo,^a and Atsushi Mitsumoto^d

^aFaculty of Pharmacy and Pharmaceutical Sciences, Josai University; 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan; ^bDepartment of Pharmacy, Higashimatsuyama Municipal Hospital; 2392 Matsuyama, Higashimatsuyama, Saitama 355-0005, Japan; ^cDepartment of Pharmacy, Saitama Medical University Hospital; 38 Morohongo, Moroyama, Iruma, Saitama 350-0495, Japan; ^dFaculty of Pharmaceutical Sciences, Josai International University; 1 Gumyo, Togane, Chiba 283-8555, Japan

Received February 28, 2025; Accepted April 22, 2025

Tadalafil is a potent selective phosphodiesterase 5 inhibitor used to treat pulmonary arterial hypertension. As tadalafil is a substrate of CYP3A, coadministration with a CYP inducer or inhibitor may affect the pharmacokinetics and pharmacological activity of tadalafil. Additionally, CYP expression is regulated by biological clocks and its activity fluctuates diurnally. Moreover, tadalafil's dosing time may also affect its pharmacokinetics. Therefore, this study aimed to investigate the effects of phenobarbital and tadalafil dosing time on the plasma concentration and pharmacological activity of tadalafil. Adult male ICR mice were treated with phenobarbital for 5 days, followed by oral administration of tadalafil perorally. Phenobarbital pretreatment decreased plasma tadalafil and pulmonary cGMP levels in a dose-dependent manner. In mice without phenobarbital pretreatment, the plasma tadalafil concentration tended to be higher when administered in the morning than in the evening. In contrast, plasma tadalafil concentration was higher when administered in the evening than in the morning in mice pretreated with phenobarbital. Similarly, pulmonary cGMP levels were higher when tadalafil was administered in the evening than in the morning in mice pretreated with phenobarbital. Notably, these effects on metabolism and pharmacological activity were observed at clinically relevant doses. Conclusively, these results indicate that the coadministration of phenobarbital and tadalafil may suppress the plasma levels and pharmacological activities of tadalafil. Additionally, dosing time should be carefully considered to maximize the efficacy of tadalafil.

Key words tadalafil, phenobarbital, drug interaction, circadian rhythm, pulmonary arterial hypertension

INTRODUCTION

Tadalafil is a potent selective phosphodiesterase (PDE)-5 inhibitor clinically used to treat erectile dysfunction and pulmonary arterial hypertension (PAH).¹⁻³⁾ PAH is characterized by progressive pulmonary vascular remodeling and increased pulmonary vascular resistance. Tadalafil inhibits PDE5, which plays an important role in cGMP degradation in pulmonary arterioles. PDE5 inhibition further increases the cGMP levels, resulting in the vasodilation of pulmonary arterioles. An animal study showed that tadalafil (0.5–10 mg/kg) increases the pulmonary cGMP levels and improves the pulmonary hemodynamics and survival rate using a PAH rat model.⁴⁾

As tadalafil is metabolized by cytochrome P450 (CYP)-3A4, co-administration of drugs increasing or decreasing CYP3A4 activity possibly affects its activity. Notably, several CYP3A4 inhibitors/inducers, such as ketoconazole (inhibitor) and rifampin (inducer), exhibit pharmacokinetic interactions

with tadalafil.⁵⁻⁷⁾ Phenobarbital is a potent CYP3A inducer, possibly decreasing the plasma tadalafil concentrations. However, specific interactions between phenobarbital and tadalafil remain unknown. Tadalafil is used to treat both adults and children with PAH, with sedative drugs also used in some cases. Therefore, determination of the specific effect phenobarbital on tadalafil activity is clinically important.

Circadian clock regulates drug metabolism and disposition by controlling various biological functions in different organs, including the liver.^{8,9)} Human CYP3A4 mainly corresponds to CYP3A11 in mice and exhibits circadian expression patterns. CYP-metabolized drugs exhibit diurnal fluctuations in pharmacokinetics and pharmacodynamics.¹⁰⁻¹²⁾ Moreover, various CYP3A substrates exhibit dosing time-dependent plasma concentration changes and toxicity in mice.^{11,12)} As tadalafil is a CYP3A substrate, plasma concentration and pharmacological activity of tadalafil are possibly affected by its dosing time.

In this study, we aimed to investigate the effects of pheno-

*To whom correspondence should be addressed. e-mail: hkawai@josai.ac.jp



© 2025 Author(s) BPB Reports applies the Creative Commons Attribution (CCBY) license to works we published. The license was developed to facilitate open access - namely, free immediate access to, and unrestricted reuse of, original works to all types. Under this license, authors agree to make articles legally available for reuse, without permissions of fees, for virtually any purpose. Anyone may copy, distribute, or reuse these articles, as long as the author and original source are properly cited. <https://creativecommons.org/licenses/by/4.0/>

barbital and its dosing time on the plasma concentration and pharmacological activity of tadalafil. We examined the effects of phenobarbital pretreatment on plasma tadalafil and pulmonary cGMP levels. Additionally, specific impacts of dosing time were analyzed by administering tadalafil in the morning and evening.

MATERIALS AND METHODS

Chemicals Midazolam was purchased from Sandoz (Tokyo, Japan). Medetomidine and butorphanol were purchased from Meiji Animal Health (Tokyo, Japan). Tadalafil was purchased from CombiBlocks (San Diego, CA, USA). Loratadine was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). Ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA) was purchased from Dojindo (Kumamoto, Japan). A Cyclic GMP ELISA kit was purchased from Cayman Chemical (Ann Arbor, MI, USA). A BCA protein assay kit was purchased from Takara (Shiga, Japan). PVDF membrane was purchased from Millipore (Bedford, MA, USA). Anti CYP3A4 antibody (13384) was purchased from Cell Signaling Technology (Danvers, MA, USA). Anti β -actin antibody (010-27841), protease inhibitor cocktail and other chemicals were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

Animals Six-week-old male ICR mice were obtained from Japan SLC, Inc. (Shizuoka, Japan). The mice were maintained in an air-conditioned room at $22 \pm 2^\circ\text{C}$ under a 12 h/12 h light/dark cycle (lights on at 07:00 h, Zeitgeber time [ZT] 0), and had free access to food and water. The time of day was expressed as ZT, which was defined in terms of hours after light onset at 07:00 h. All animal experiments were approved by the Institutional Animal Care and Use Committee of Josai University (April, 2020 – March, 2024; approval number: JU20080, JU21077, JU22066, JU23056), and performed in accordance with the guidelines for animal experiments of Josai University and the Japanese Ministry of Education, Culture, Sports, Science and Technology.

Experimental Schedule Mice were intraperitoneally administered phenobarbital (10 or 100 mg/kg/day) or saline once daily in the morning (ZT1–ZT4) for 5 days. A day after the final administration of phenobarbital, mice were orally administered tadalafil (0.3, 1, or 3 mg/kg) or saline at ZT1 or ZT13. The dosing time was determined according to previous studies that detected chronopharmacological effects of CYP3A substrates.^{11,12)} To analyze liver CYP3A11, mice without tadalafil administration were anesthetized with a mixture of midazolam, medetomidine, and butorphanol at the next day of the final administration of phenobarbital, and the liver was dissected out after perfusion with phosphate-buffered saline (PBS). To analyze plasma tadalafil concentration and pulmonary cGMP content, mice were sacrificed by cervical dislocation 1 h after tadalafil administration. Trunk blood was collected in a plastic tube pre-wetted with EDTA, and the lungs were immediately removed. The sampling time was set at the time point close to the $t_{\max}$ of tadalafil. For pharmacokinetic analysis, submandibular blood was collected at 0.5, 1, 1.5, 2, 4, and 8 h after tadalafil administration. Blood samples were centrifuged at $1,200 \times g$ for 20 min to obtain the plasma. Liver, plasma and lung samples were stored at -80°C until analysis. All the data were obtained from two or more independent experiments.

Measurement of Plasma Tadalafil Concentrations Sam-

ple preparation and HPLC were conducted using the modified method for the analysis of human and rat plasma.^{13,14)} Briefly, 100 ng of loratadine (internal standard) was added to 30 μL of plasma. The sample was alkalized with 1/10 volume of 1 M NaOH and extracted with ethyl acetate. The extract was dried at 40°C under N_2 , and the resultant residue was dissolved in 30 μL of an aqueous solution of 12 mM of triethylamine and 20 mM of orthophosphoric acid with acetonitrile (66/34), sieved using a 0.45 μm filter, and subjected to HPLC.

Tadalafil and loratadine were quantified using a Shimadzu LC10 HPLC-UV system (Shimadzu, Kyoto, Japan) coupled with a Kinetex C18 column (100 mm $\times$ 3.0 mm, 2.6 μm ; Phenomenex, Torrance, CA, USA) with a guard column (Security Guard; Phenomenex, Torrance, CA, USA). The mobile phase consisted of an aqueous solution of 12 mM of triethylamine and 20 mM of orthophosphoric acid in acetonitrile (66/34). Analysis was performed at a flow rate of 0.2 mL/min at room temperature. The detection wavelength was set at 254 nm.

Measurement of Pulmonary cGMP Contents Pulmonary cGMP levels were measured using a cGMP ELISA kit (Cayman Chemical, Ann Arbor, Michigan, USA), according to the manufacturer's instructions. Briefly, lung samples were homogenized in 10 volumes of 5% trichloroacetic acid and centrifuged at $1,500 g$ for 10 min. The supernatant was washed three times with diethyl ether, and the residual diethyl ether was removed from the aqueous layer by heating at 70°C for 5 min. A portion of the resultant solution was incubated with antibodies against cGMP and acetylcholine esterase-linked cGMP in a well pre-coated with anti-rabbit IgG for 18 h at 4°C . After five washes with wash buffer, antibody reaction was detected using Ellman's reagent for 90 min at room temperature. The absorbance was measured at 405 nm to determine the cGMP level in the sample solution, and the amount of cGMP in the tissue (pmol/g tissue) was calculated.

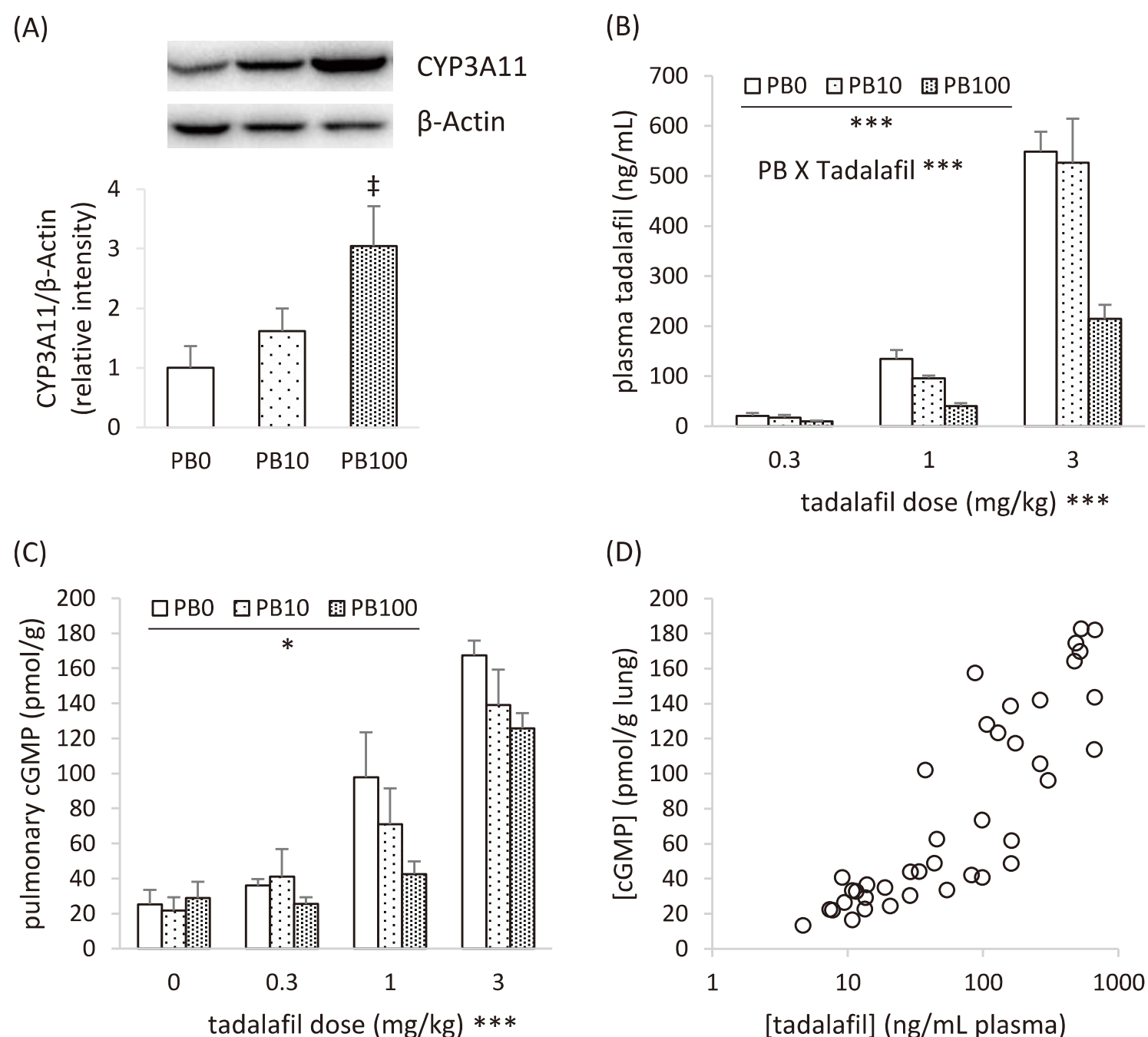
Western Blotting The livers were homogenized in 5 volumes of lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 1 mM phenylmethylsulfonyl fluoride, 2 $\mu\text{L}/\text{mL}$ of protease inhibitor cocktail). After centrifugation at $10,000 \times g$ for 10 min at 4°C , the supernatant was used for analysis. The protein contents of the sample were determined using the bicinchoninic acid assay kit (Takara, Shiga, Japan). The SDS protein samples (10 μg total protein) were subjected to 10% SDS-polyacrylamide gel electrophoresis, followed by the transfer onto PVDF membranes. The membrane was incubated with 5% skim milk in PBS containing 0.1% Tween-20 for 1 h at room temperature, primary antibody (diluted at the ratio of 1:1,000 for CYP3A11; 1:10,000 for β -actin) overnight at 4°C , and horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. The protein bands were visualized using ChimiDoc imaging system (Bio-Rad, Hercules, CA, USA) after adding ECL prime reagent (Cytiva, Tokyo, Japan).

Statistical Analysis Data are expressed as means $\pm$ standard error of the mean (SEM). The effects of phenobarbital, tadalafil, and dosing time on plasma tadalafil and pulmonary cGMP levels were analyzed using multi-way ANOVA (Table 1, Figs. 1 and 3). The time course of plasma tadalafil concentration was analyzed using repeated-measures ANOVA (Fig. 2). The Huynh–Feldt ϵ correction was used to evaluate F ratios for repeated measures. Pairwise comparison between groups was performed by Dunnett's test (Fig. 1A). Correla-

Table 1. Pharmacokinetic parameters.

	ZT1			ZT13		
	PB0	PB10	PB100	PB0	PB10	PB100
AUC_{0-8h} (ng·h/mL) ***	627.8 ± 46.6	380.9 ± 26.5	117.6 ± 21.6	536.0 ± 65.4	410.7 ± 55.2	153.4 ± 17.0
C_{max} (ng/mL) ***	170.7 ± 13.7	119.3 ± 9.9	55.2 ± 10.0	162.1 ± 20.7	154.4 ± 16.6	68.6 ± 7.5
t_{max} (h)	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
$t_{1/2}$ (h) †††	3.3 ± 0.7	2.9 ± 1.5	2.7 ± 0.4	2.0 ± 0.6	1.8 ± 0.7	1.8 ± 0.4

*** $p < 0.001$ in main effect of PB, ††† $p < 0.001$ in main effect of dosing time by ANOVA.

**Fig. 1.** Effects of Phenobarbital on Plasma Concentration and Pharmacological Effect of Tadalafil.

(A) Protein contents of CYP3A11 in the liver. Each column represents the mean + SEM ($n = 3$). (B) Plasma tadalafil and (C) pulmonary cGMP levels in mice pretreated with phenobarbital (0, 10, and 100 mg/kg: PB0, PB10, and PB100, respectively). Each column represents the mean + SEM ($n = 4-6$). (D) Correlation between plasma tadalafil and pulmonary cGMP levels. Each plot represents the results of measurement in each mouse. Log [tadalafil] and [cGMP] shows correlation with monotonic increase (MIC = 0.777, MAS = 0.049). ‡ $p < 0.05$ vs PB0 by Dunnett's test. * $p < 0.05$, *** $p < 0.001$ in the main effect or interaction using ANOVA.

tion between factors was analyzed by maximal information coefficient (MIC) and maximum asymmetry score (MAS) (Fig. 1D). Statistical significance was set at $p < 0.05$. All statistical analyses were performed using the R program (version 4.1.2; The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS AND DISCUSSION

Effects of Phenobarbital Pretreatment on Plasma Tadalafil and Pulmonary cGMP Levels

Fig. 1A shows the

CYP3A11 protein levels in the liver of mice treated with different concentrations of phenobarbital (0, 10, and 100 mg/kg: PB0, PB10, and PB100, respectively) for five days. CYP3A11 levels were elevated in PB100-treated mice ($p = 0.046$). Fig. 1B–D shows the plasma tadalafil and pulmonary cGMP levels in mice treated with phenobarbital for five days and then with tadalafil (0.3, 1, and 3 mg/kg) the next day. Two-way ANOVA revealed the main effects of both tadalafil ($F[2,31] = 154.63$; $p < 0.001$) and phenobarbital ($F[2,31] = 19.98$; $p < 0.001$) and their interactions ($F[4,31] = 9.74$; $p < 0.001$) on the plasma tadalafil concentrations (Fig. 1B). Similarly, main effects of tadalafil ($F[3,40] = 51.14$; $p < 0.001$)

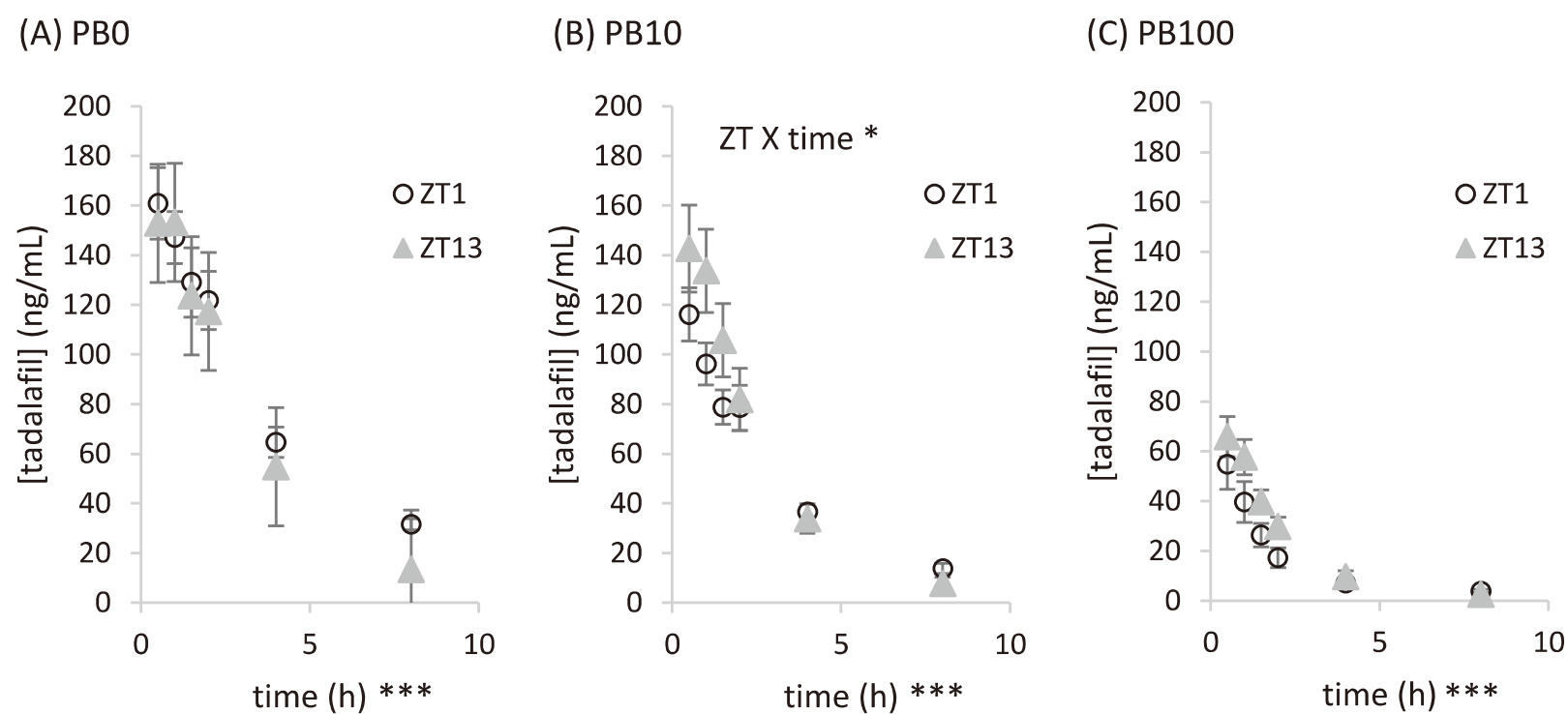


Fig. 2. Time Course of Plasma Tadalafil after Administration at ZT1 and ZT13 in Mice with or without Phenobarbital Pretreatment.

Plasma tadalafil concentrations after tadalafil administration at ZT1 or ZT13 in mice pretreated with phenobarbital at 0 mg/kg (PBO, A), 10 mg/kg (PB10, B), or 100 mg/kg (PB100, C). Each plot represents the mean $\pm$ standard error of the mean (SEM; $n = 6-8$). * $p < 0.05$, *** $p < 0.001$ in the main effect or interaction at each PB level using ANOVA.

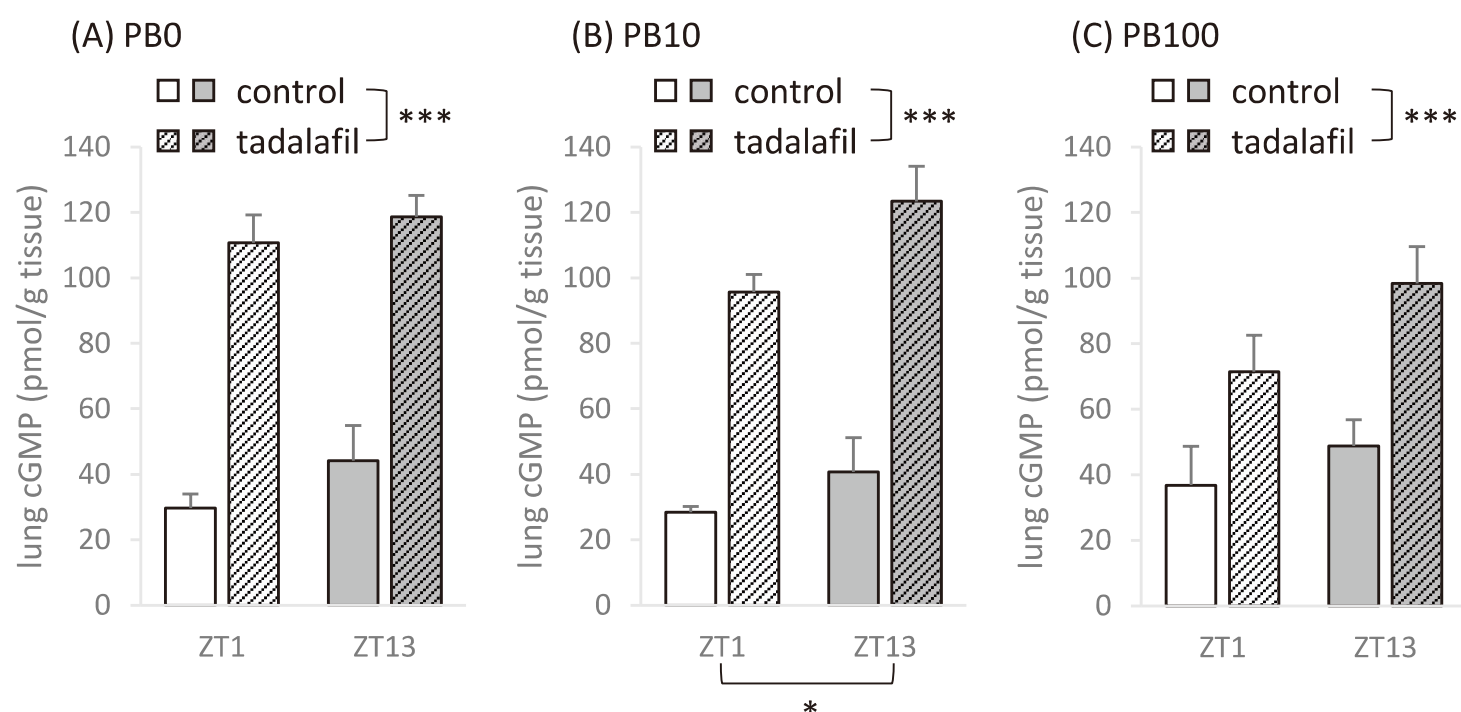


Fig. 3. Effects of Phenobarbital and Dosing Time on Pulmonary cGMP Induction by Tadalafil.

Pulmonary cGMP levels after tadalafil administration at ZT1 or ZT13 in mice pretreated with phenobarbital at 0 mg/kg (PBO, A), 10 mg/kg (PB10, B), or 100 mg/kg (PB100, C). Each column represents the mean $\pm$ standard error of the mean (SEM; $n = 6-7$). * $p < 0.05$, *** $p < 0.001$ in the main effect at each PB level using ANOVA.

and phenobarbital ($F[2,40] = 4.50$; $p = 0.025$) on pulmonary cGMP levels were observed (Fig. 1C). At the examined doses, pulmonary cGMP levels were correlated with the plasma tadalafil levels ($MIC = 0.777$; $MAS = 0.049$; Fig. 1D).

Tadalafil is predominantly metabolized by hepatic CYP3A4 in humans and CYP3A11 in mice to a catechol form that is further metabolized to a methylcatechol form and glucuronide conjugate.⁵⁻⁷ Given that these metabolites are not pharmacologically active at circulating concentrations, the pharmacological activity of tadalafil depends on its concentration.⁵ In the present study, we found that phenobarbital reduced plasma tadalafil and pulmonary cGMP levels in a dose-dependent manner. Pretreatment with phenobarbital might induce CYP3A, which metabolizes tadalafil, resulting in a reduction in the plasma concentration and pharmacological effects of

tadalafil.

A study reported that chronic tadalafil treatment induced cGMP in the lungs, reduced the mean pulmonary artery pressure, and increased the survival rate of rats with monocrotaline-induced PAH.⁴ Specifically, tadalafil (0.5 mg/kg) increased the pulmonary cGMP levels to approximately 120 pmol/g tissue and reduced the mean pulmonary artery pressure by approximately 20%.⁴ These reports are consistent with our findings on the pulmonary cGMP levels in mice, suggesting that phenobarbital pretreatment-induced decrease in cGMP levels affects the pulmonary artery pressure and therapeutic effects of tadalafil against PAH.

Previous studies have demonstrated the pharmacological effects of tadalafil at a clinically relevant dose of 1 mg/kg;^{15,16} therefore, 1 mg/kg tadalafil dose was selected for

further experiments in this study.

Effects of Dosing Time on Plasma Tadalafil and Pulmonary cGMP Levels Next, we investigated the effects of dosing time on the pharmacokinetics and pharmacological effects of tadalafil in the lungs of mice. Mice pretreated with phenobarbital (0, 10, and 100 mg/kg/day for five days) were orally administered tadalafil (1 mg/kg) at ZT1 and ZT13 and plasma tadalafil and pulmonary cGMP levels were determined. Plasma tadalafil concentrations are shown in Fig. 2. Three-way repeated measures ANOVA revealed the main effects of phenobarbital treatment ($F[2,38] = 44.66$; $p < 0.001$) and time course ($F[5,190] = 173.29$; $p < 0.001$; $\varepsilon = 0.47$) and interactions between phenobarbital treatment and time course ($F[10,190] = 9.87$; $p < 0.001$; $\varepsilon = 0.47$) and dosing time and time course ($F[5,190] = 3.17$; $p = 0.039$; $\varepsilon = 0.47$). Notably, interactions between dosing time and time course were observed with PB10 ($F[5,70] = 3.00$; $p = 0.043$; $\varepsilon = 0.58$). At 1 h after tadalafil administration, plasma tadalafil level at ZT1 was 28% lower than that at ZT13 in PB10-treated mice ($F[1,14] = 3.98$; $p = 0.066$).

Overall, phenobarbital treatment decreased the plasma tadalafil levels, and plasma tadalafil levels were lower at ZT1 than at ZT13. In this study, we used 1 mg/kg tadalafil, relevant to the clinical dose used in children.^{5,15,16} Low doses of phenobarbital used in this study (10 mg/kg) are also relevant for clinical use.^{17,18} Our results suggest that combined treatment with phenobarbital and tadalafil induces CYP expression and decreases the plasma tadalafil concentrations in clinical settings.

Table 1 presents the pharmacokinetic parameters of tadalafil measured using the data shown in Fig. 2. Two-way ANOVA revealed that phenobarbital treatment decreased the area under the concentration-time curve from 0 to 8 h (AUC_{0-8h} ; $F[2,38] = 53.48$; $p < 0.001$) and maximum concentration (C_{max} ; $F[2,38] = 29.06$; $p < 0.001$) of tadalafil in a dose-dependent manner. Additionally, dosing time affected the half-life ($t_{1/2}$; $F[1,38] = 20.73$; $p < 0.001$) of tadalafil, with the ZT13 group exhibiting shorter $t_{1/2}$ values than the ZT1 group at all phenobarbital doses. Notably, phenobarbital reduced the AUC and C_{max} values, but not the $t_{1/2}$ values. These changes are consistent with previous reports on the pharmacokinetics of tadalafil and related drugs. A previous study showed that the co-administration of the CYP3A inducer, bosentan, significantly reduced the C_{max} , but not $t_{1/2}$, of tadalafil.⁷ Another study reported that the effects of CYP inhibitors on the pharmacokinetics of sildenafil, a CYP3A substrate, were more pronounced on AUC and C_{max} than on $t_{1/2}$,⁶ suggesting that CYP3A primarily affects the first-pass metabolism rather than the systemic clearance of the drug.⁶ We hypothesized that an increase in CYP3A levels reduces the plasma tadalafil levels and that phenobarbital possibly decreases the absorption rate or increases the elimination rate of tadalafil. Further studies should investigate the effects of phenobarbital on the absorption, distribution, and elimination of tadalafil to clarify its action mechanisms and specific impacts on drug interactions.

Plasma tadalafil levels were higher at ZT1 than at ZT13 in mice without phenobarbital treatment although the difference was not significant; whereas, the opposite pattern was observed in phenobarbital-treated mice. Phenobarbital treatment might alter the peak time of plasma concentration, which may be attributed to the effects of phenobarbital on the circadian clock. Phenobarbital affects the expression of core clock

genes such as *Clock*, *Bmal1*, and *Pers* and alters their expression rhythm.¹⁹ Phenobarbital phase advanced the circadian rhythms of physiological and behavioral activities, such as body temperature and locomotor activity.¹⁹⁻²¹ Moreover, there was a significant difference in the peak time of theophylline clearance between phenobarbital-treated and non-treated rats, suggesting that phenobarbital may cause a phase shift in drug metabolism.²² Phenobarbital-induced alteration in the circadian rhythm of drug metabolism may affect the peak time of plasma tadalafil concentration and pharmacological activity.

Fig. 3 shows the pulmonary cGMP levels in mice treated with phenobarbital and tadalafil. Three-way ANOVA revealed the main effects of tadalafil treatment ($F[1,69] = 161.88$; $p < 0.001$), dosing time ($F[1,69] = 10.97$; $p = 0.001$), and interactions between phenobarbital and tadalafil treatments ($F[2,69] = 5.12$; $p = 0.008$). Tadalafil increased the cGMP levels at all tested phenobarbital doses (PB0: $F[1,69] = 77.38$ [$p < 0.001$], PB10: $F[1,69] = 69.04$ [$p < 0.001$], and PB100: $F[1,69] = 23.67$ [$p < 0.001$]). However, phenobarbital reduced the cGMP levels in tadalafil-treated mice ($F[2,69] = 6.69$; $p = 0.002$). Dosing time affected the cGMP levels with PB10 ($F[1,22] = 7.03$; $p = 0.015$). Overall, effects of tadalafil on pulmonary cGMP levels showed diurnal fluctuations in phenobarbital-treated mice. These findings highlight the importance of optimal dosing time in enhancing the therapeutic effects of tadalafil.

As shown in Fig. 2B and Fig. 3B, plasma tadalafil and pulmonary cGMP levels were higher at ZT13 than at ZT1 in phenobarbital-treated mice, suggesting that the dosing time-dependent plasma tadalafil concentrations causes the chronopharmacological effects of tadalafil on cGMP. We also speculated another possibility that the circadian rhythm of NO/cGMP system would have influences on the chronopharmacological effects of tadalafil. NO/cGMP system contributes to the circadian regulation of blood pressure, and several factors such as soluble guanylyl cyclase activity and endothelial NO synthase level show diurnal fluctuation in rats.^{23,24} Phosphodiesterase 5 expression also shows diurnal fluctuation in testicular Leydig cells.²⁵ The diurnal fluctuation of cGMP production by guanylyl cyclase and cGMP degradation by phosphodiesterase might affect the pharmacological effect of tadalafil on cGMP level. Further studies should be necessary to elucidate the precise mechanism of the chronopharmacological effects of tadalafil.

In conclusion, this study revealed that phenobarbital treatment decreased the plasma concentration and pharmacological activity of tadalafil. Moreover, dosing time affected the pharmacokinetics and pharmacological activity of tadalafil, especially in phenobarbital-treated mice. These effects were observed at clinically relevant doses, suggesting that phenobarbital co-administration reduces and tadalafil dosing time impacts the therapeutic efficacy of tadalafil.

Conflict of interest The authors declare no conflict of interest.

REFERENCES

- 1) Barnes H, Brown Z, Burns A, Williams T. Phosphodiesterase 5 inhibitors for pulmonary hypertension. *Cochrane Database Syst. Rev.*, **1**, CD012621 (2019).
- 2) Henrie AM, Nawarskas JJ, Anderson JR. Clinical utility of tadalafil in the treatment of pulmonary arterial hypertension: an evidence-based review. *Core Evid.*, **10**, 99–109 (2015).

- 3) Christiansen D, Porter S, Hurlburt L, Weiss A, Granton J, Wentlandt K. Pulmonary arterial hypertension: A palliative medicine review of the disease, Its Therapies, and Drug Interactions. *J. Pain Symptom Manage.*, **59**, 932–943 (2020).
- 4) Sawamura F, Kato M, Fujita K, Nakazawa T, Beardsworth A. Tadalafil, a long-acting inhibitor of PDE5, improves pulmonary hemodynamics and survival rate of monocrotaline-induced pulmonary artery hypertension in rats. *J. Pharmacol. Sci.*, **111**, 235–243 (2009).
- 5) Eli Lilly and Co. Adcirca (tadalafil) prescribing information. Available at <https://pi.lilly.com/us/adcirca-pi.pdf>. Accessed December 11, 2023.
- 6) Gupta M, Kovar A, Meibohm B. The clinical pharmacokinetics of phosphodiesterase-5 inhibitors for erectile dysfunction. *J. Clin. Pharmacol.*, **45**, 987–1003 (2005).
- 7) Wrishko RE, Dingemanse J, Yu A, Darstein C, Phillips DL, Mitchell MI. Pharmacokinetic interaction between tadalafil and bosentan in healthy male subjects. *J. Clin. Pharmacol.*, **48**, 610–618 (2008).
- 8) Koyanagi S. Chrono-pharmaceutical approaches to optimize dosing regimens based on the circadian clock machinery. *Biol. Pharm. Bull.*, **44**, 1577–1584 (2021).
- 9) Dong D, Yang D, Lin L, Wang S, Wu B. Circadian rhythm in pharmacokinetics and its relevance to chronotherapy. *Biochem. Pharmacol.*, **178**, 114045 (2020).
- 10) Dallmann R, Okyar A, Lévi F. Dosing-time makes the poison: circadian regulation and pharmacotherapy. *Trends Mol. Med.*, **22**, 430–445 (2016).
- 11) Guo L, Zhang L, Xu H, Yu P, Wang Z, Lu D, Chen M, Wu B. Diurnal hepatic CYP3A11 contributes to chronotoxicity of the pyrrolizidine alkaloid retrorsine in mice. *Xenobiotica*, **51**, 1019–1028 (2021).
- 12) Zhou Z, Lin Y, Gao L, Yang Z, Wang S, Wu B. Cyp3a11 metabolism-based chronotoxicity of brucine in mice. *Toxicol. Lett.*, **313**, 188–195 (2019).
- 13) Shakya AK, Abu-awwad AN, Arafat TA, Melhim M. Validated liquid chromatographic-ultraviolet method for the quantitation of tadalafil in human plasma using liquid-liquid extraction. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **852**, 403–408 (2007).
- 14) Cheng CL, Chou CH. Determination of tadalafil in small volumes of plasma by high-performance liquid chromatography with UV detection. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **822**, 278–284 (2005).
- 15) Kohno H, Ichida F, Hirono K, Ozawa S, Yoshimura N, Nakamura T, Akita C, Ishida K, Taguchi M. Plasma concentrations of tadalafil in children with pulmonary arterial hypertension. *Ther. Drug Monit.*, **36**, 576–583 (2014).
- 16) Ivy D, Bonnet D, Berger RMF, Meyer GMB, Baygani S, Li B; LVHV Study Group. Efficacy and safety of tadalafil in a pediatric population with pulmonary arterial hypertension: phase 3 randomized, double-blind placebo-controlled study. *Pulm. Circ.*, **11**, 20458940211024955 (2021).
- 17) Favié LMA, Groenendaal F, van den Broek MPH, Rademaker CMA, de Haan TR, van Straaten HLM, Dijk PH, van Heijst A, Simons SHP, Dijkman KP, Rijken M, Zonnenberg IA, Cools F, Zecic A, van der Lee JH, Nuytemans DHGM, van Bel F, Egberts TCG, Huitema ADR; on behalf of the PharmaCool study group. Phenobarbital, midazolam pharmacokinetics, effectiveness, and drug-drug interaction in asphyxiated neonates undergoing therapeutic hypothermia. *Neonatology*, **116**, 154–162 (2019).
- 18) Moffett BS, Weingarten MM, Galati M, Placencia JL, Rodman EA, Riviello JJ, Kayyal SY. Phenobarbital population pharmacokinetics across the pediatric age spectrum. *Epilepsia*, **59**, 1327–1333 (2018).
- 19) Legan SJ, Donoghue KM, Franklin KM, Duncan MJ. Phenobarbital blockade of the preovulatory luteinizing hormone surge: association with phase-advanced circadian clock and altered suprachiasmatic nucleus Period1 gene expression. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **296**, R1620–R1630 (2009).
- 20) Peraino C, Ehret CF, Groh KR, Meinert JC, D’Arcy-Gomez G. Phenobarbital effects on weight gain and circadian cycling of food intake and body temperature. *Proc. Soc. Exp. Biol. Med.*, **165**, 473–479 (1980).
- 21) Nishikawa J, Kast A, Albert H. Circadian rhythm of the liver of male rats dosed with phenobarbital--I. Organ weight, cellular structures, glycogen contents and mitotic activity. *Chronobiol. Int.*, **4**, 161–173 (1987).
- 22) Zeruesenay D, Siegmund W, Franke G, Zschiesche M. Circadian time-dependent kinetics of theophylline and its modulation by phenobarbital pretreatment in rats. *Chronobiol. Int.*, **10**, 81–86 (1993).
- 23) Witte K, Schnecko A, Zuther P, Lemmer B. Contribution of the nitric oxide-guanylyl cyclase system to circadian regulation of blood pressure in normotensive Wistar-Kyoto rats. *Cardiovasc. Res.*, **30**, 682–688 (1995).
- 24) Denniff M, Turrell HE, Vanezis A, Rodrigo GC. The time-of-day variation in vascular smooth muscle contractility depends on a nitric oxide signalling pathway. *J. Mol. Cell. Cardiol.*, **66**, 133–140 (2014).
- 25) Baburski AZ, Sokanovic SJ, Andric SA, Kostic TS. Aging has the opposite effect on cAMP and cGMP circadian variations in rat Leydig cells. *J. Comp. Physiol. B*, **187**, 613–623 (2017).