



Effects of $1\alpha,25$ -dihydroxyvitamin D_3 on the pharmacokinetics and biodistribution of ergothioneine, an endogenous organic cation/carnitine transporter 1 substrate, in rats

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Abstract

Purpose This study aimed to investigate the effects of $1\alpha,25$ -dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) on the expression levels of organic cation/carnitine transporter 1 (OCTN1) as well as the pharmacokinetics and biodistribution of ergothioneine, an OCTN1 substrate, in rats.

Methods Rats pretreated with $1,25(OH)_2D_3$ (2.56 nmol/kg/day) for four days were administered ergothioneine (2 mg/kg) intravenously. The expression levels of rat OCTN1 (rOCTN1) in organs were determined using real-time quantitative polymerase chain reaction. Ergothioneine levels in plasma, urine, and organs (with and without intravenous injection of exogenous ergothioneine) were determined using liquid chromatography-tandem mass spectrometry.

Results $1,25(OH)_2D_3$ pretreatment resulted in a significant decrease in rOCTN1 mRNA expression levels in the kidney and brain, a significant increase in basal plasma levels of ergothioneine (from 48 h), and a significant decrease in the tissue-plasma partition coefficient (K_p) in all tissues (except the heart and lungs) and the basal urine levels of ergothioneine. After intravenous administration, the pharmacokinetic profiles of ergothioneine were consistent with the basal levels of endogenous ergothioneine, with an increase in AUC_∞ by 85%, a significant decrease in total clearance by 49%, and a decrease in V_{ss} by 32% in $1,25(OH)_2D_3$ -treated rats. The K_p value and urinary recovery of ergothioneine also decreased in the $1,25(OH)_2D_3$ -treated group.

Conclusion This study showed the effects of $1,25(OH)_2D_3$ on the expression and function of rOCTN1 by investigating the interaction between $1,25(OH)_2D_3$ and ergothioneine. Dose adjustment and possible changes in bioavailability should be considered before the co-administration of vitamin D or its active forms and OCTN1 substrates.

Keywords $1\alpha,25$ -Dihydroxyvitamin D_3 · Pharmacokinetics · Biodistribution · Ergothioneine · OCTN1 substrate

Introduction

Vitamin D is a fat-soluble vitamin that plays a vital role in calcium absorption and bone maturation (Robien et al. 2013; Choi et al. 2020). It participates in cell proliferation/differentiation and the modulation of inflammatory response pathways (Guillot et al. 2010; Wang et al. 2012). Moreover, vitamin D is used in the treatment of several autoimmune

diseases (Schoindre et al. 2011). $1\alpha,25$ -dihydroxyvitamin D_3 ($1,25(OH)_2D_3$, also known as calcitriol) is the biologically active form of vitamin D, which functions as a ligand of the vitamin D receptor (VDR) (Haussler et al. 1998). After binding to the VDR, it activates the transcriptional regulation of target genes, thereby regulating the expression levels of several transporters, receptors, and metabolic enzymes (Wang et al. 2012). The direct upregulation or downregulation of many genes via VDR activation demonstrates that VDR plays a crucial role in the biological actions of vitamin D (Uitterlinden et al. 2002; Wang et al. 2012).

Vitamin D, or the active form of vitamin D ($1,25(OH)_2D_3$), is widely used as a therapeutic drug and/or supplement due to its beneficial effects. Therefore, their interaction with other drugs has received considerable research attention. Vitamin D can interact with statins, such

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as atorvastatin, lovastatin, and fluvastatin, via the CYP3A4 enzyme (Dobs et al. 1991; Montagnani et al. 1994; Choi et al. 2020). Similarly, rifampin, isoniazid, tacrolimus, and phenobarbital interfere with the vitamin D metabolic processes (Falkiewicz et al. 2006; Nnoaham and Clarke 2008). The combination of thiazides and vitamin D supplementation increases the risk of hypercalcemia, especially in elderly individuals or patients with hyperparathyroidism (Hathcock et al. 2007). Notably, $1,25(\text{OH})_2\text{D}_3$ regulates the function and regulation of metabolic enzymes and drug transporters, ultimately affecting the pharmacokinetics of specific substrates for several metabolic enzymes and drug transporters (Choi et al. 2020). For example, $1,25(\text{OH})_2\text{D}_3$ administration results in a decrease in rat kidney organic anion transporters (rOAT1/OAT3) expression, leading to a dramatic decrease in renal clearance of cefadroxil and cefdinir (Kim et al. 2014). Our recent study also demonstrated that $1,25(\text{OH})_2\text{D}_3$ downregulates the mRNA and protein expression of Cyp2b1 and Cyp2c11 in rats, which consequently alters the metabolic function and systemic pharmacokinetics of bupropion and tolbutamide (substrates of Cyp2b1 and Cyp2c11, respectively) (Doan et al. 2020). In another recent study of our group, $1,25(\text{OH})_2\text{D}_3$ regulated the expression of rat organic cation transporters (rOCT) and rat multidrug and toxin extrusion proteins (rMATE), which was evidenced by a decrease in renal and non-renal (metabolic) clearance of procainamide hydrochloride (an organic cation transporter substrate) and a decrease in the renal clearance of the metabolite N-acetyl procainamide (Balla et al. 2021).

Organic cation/carnitine transporter 1 (OCTN1), also called the ergothioneine transporter (Gründemann et al. 2005), is a member of the SLC22 family, which is strongly expressed in the rat liver (Koepsell and Endou 2004; Pangeni et al. 2021). OCTN1 transports zwitterions and organic cations in both directions in the kidney and contributes to their active secretion and reabsorption (Volk 2014; Ivanyuk et al. 2017). A specific endogenous substrate of OCTN1 is ergothioneine, a natural antioxidant compound extracted from mushrooms, fungi, and cyanobacteria (Halliwell et al. 2016; Nakamichi et al. 2016; Ames 2018). Because ergothioneine does not pass freely through the cell membrane, tissues that lack OCTN1 do not accumulate ergothioneine, whereas those with high expression of OCTN1 can take up ergothioneine at high concentration levels and retain it for a long time (Gründemann et al. 2005). OCTN1-mediated transport is involved in the disposition of several clinically essential drugs in the body. For example, it results in neurotoxicity caused by oxaliplatin (Jong et al. 2011). Moreover, co-administration of ergothioneine ameliorated oxaliplatin-induced peripheral neuropathy in rats by reducing its accumulation via OCTN1 (Nishida et al. 2018). OCTN1 and its transport mechanism have been implicated in many treatments,

especially those using OCTN1 substrates as a therapeutic agent (Nakamichi et al. 2012; Yang et al. 2016). However, information on OCTN1 based drug disposition or drug–drug interactions (DDIs) remains limited.

Alterations in the expression levels of drug transporters are pivotal in affecting the pharmacokinetics and pharmacodynamics of drug substrates (Evers et al. 2018). Various studies have been conducted to evaluate drug transporters, their correlation with DDIs, and adverse drug reactions due to their impact on pharmacodynamic and safety profiles in patients. Considering that $1,25(\text{OH})_2\text{D}_3$ and VDR are involved in various therapeutic drugs and OCTN1 has a crucial role in the drug delivery process, investigating the possible involvement of $1,25(\text{OH})_2\text{D}_3$ in the regulation of OCTN1 expression is required. Therefore, in this study, we investigated the effects of $1,25(\text{OH})_2\text{D}_3$ on rat OCTN1 (rOCTN1) expression levels and ergothioneine pharmacokinetics and biodistribution.

Materials and methods

Materials and reagents

$1,25(\text{OH})_2\text{D}_3$, l-ergothioneine, cefdinir, and corn oil were purchased from Sigma-Aldrich (St. Louis, MO, USA). High-performance liquid chromatography (HPLC) grade acetonitrile and formic acid were obtained from Honeywell Burdick and Jackson (Muskegon, MI, USA) and Sigma-Aldrich, respectively. All other analytical grade reagents were used without further purification.

$1,25(\text{OH})_2\text{D}_3$ pretreatment in rats

Male Sprague Dawley rats (7–8 weeks old, 220–280 g; Nara Bio Tech., South Korea) were acclimated to laboratory conditions and provided ad libitum access to food and water and maintained under 12-h/12-h light/dark cycles for one week. The treatment of $1,25(\text{OH})_2\text{D}_3$ (2.56 nmol/kg/day) was performed as previously reported (Chow et al. 2009, 2010; Maeng et al. 2011; Doan et al. 2020; Balla et al. 2021). Rats were randomly divided into the control and $1,25(\text{OH})_2\text{D}_3$ -treated groups. The injection vehicle was a mixture of ethanol in filtered corn oil for the control group or a mixture of ethanol solution of $1,25(\text{OH})_2\text{D}_3$ in filtered corn oil (equivalent to 2.56 nmol/mL of $1,25(\text{OH})_2\text{D}_3$) for the treatment groups. Rats in the two groups were injected intraperitoneally (1 mL/kg) for four consecutive days at the same time every day. Pharmacokinetic studies were performed on the fifth day (24 h after the last dose of $1,25(\text{OH})_2\text{D}_3$). Rats were weighed daily and monitored throughout the experiment.

Real-time quantitative polymerase chain reaction (RT-qPCR)

The expression levels of rOCTN1 in the liver, kidney, spleen, heart, lung, muscle, and brain were determined using RT-qPCR by normalizing its level with the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Control and 1,25(OH)₂D₃-treated rats were intraperitoneally injected with a mixture of anesthetic and muscle relaxant (Zoletil®, Vibrac, TX, USA, and Rompun®, Bayer, Germany) 24 h after the last dose of 1,25(OH)₂D₃ pretreatment and then sacrificed for tissue harvesting. The collected tissues were immediately immersed in liquid nitrogen and stored at – 80 °C until use. Total RNA was extracted from 100 mg of tissue homogenate using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). A Nanodrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used to measure total RNA concentration at 260–280 nm. The first-strand cDNA was synthesized from 1 µg total RNA using a Prime-Script 1st strand cDNA synthesis kit (Takara Bio, Shiga, Japan) with the following thermal conditions: incubating at 37 °C for 15 min, inactivating enzymes at 85 °C for 5 s, and cooling to 4 °C. The synthesized cDNA was subsequently used for qPCR assays using the SYBR® Premix Ex Taq II kit (Takara Bio) on an Agilent Mx3005 (Agilent, Boblingen, Germany). According to the manufacturer's procedure and a slight modification to optimize the thermal conditions for target genes, the amplification and detection of reaction mixtures were evaluated using the MxPro-Mx3005 software with the following thermal conditions: 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 56 °C for 30 s, followed by the melting curve. The experiment was carried out using the comparative quantification mode, and the delta-delta method (2^{-ΔΔC_t}) for relative mRNA quantification was used to calculate the fold expression (Maeng et al. 2012; Doan et al. 2020; Balla et al. 2021). The qPCR primer pairs used were as follows: rGAPDH forward primer 5'-CGCTGGTGCTGAGTATGTCG-3'; rGAPDH reverse primer 5'-CTGTGGTCA TGAGCCCTTCC-3'; rOCTN1 forward primer 5'-AGCATT TGTCCTGGGAACAG-3'; rOCTN1 reverse primer 5'-ACT CAGGGATGAACCACCAG-3'.

Determination of the basal level of endogenous ergothioneine and in vivo pharmacokinetic study

Control and 1,25(OH)₂D₃-treated rats without ergothioneine administration were used to determine the mean basal level of ergothioneine in the plasma, urine, and tissues during the entire experiment. Blood samples were collected 30 min before 1,25(OH)₂D₃ administration on four consecutive days and at the same time on the fifth and sixth day. At each sampling time, 0.3 mL of blood was collected from the tail vein and centrifuged at 14,000 rpm for 15 min at 4 °C to obtain

plasma. Urine samples were collected within 24 h from the fifth to the sixth day. Various tissues, including the liver, kidney, spleen, heart, lungs, brain, and muscle were collected on the sixth day. The samples were stored at – 20 °C until analysis.

The pharmacokinetic study of exogenous ergothioneine administration was performed in rats after four consecutive days of pretreatment. Rats were anesthetized with intraperitoneal injections (20 mg/kg) of Zoletil® and Rompun®. The femoral vein and artery were cannulated with polyethylene tubing (Clay Adams, NJ, USA) filled with heparinized saline (20 IU/mL) for drug administration and blood sampling, respectively. An aqueous solution of ergothioneine (2 mg/mL) was administered intravenously to rats of both 1,25(OH)₂D₃-treated and control groups at a dose of 2 mg/kg (1 mL/kg). Blood samples were collected at 0 (basal endogenous level), 1, 5, 15, 30, 60, 90, 120, 240, 360, 480, and 1,440 min after drug administration and equal volumes of 20 IU/mL heparinized normal saline were injected to compensate for fluid loss. The collected blood was immediately centrifuged for 15 min (14,000 rpm, 4 °C), and the obtained plasma was stored at – 20 °C until analysis (Maeng et al. 2019; Doan et al. 2020).

Concurrently, urine was collected for 24 h at three intervals of 0–4, 4–8, and 8–24 h. The urine was then weighed and stored at – 80 °C until analysis (Jin et al. 2014). After the last sampling time point, the rats were immediately euthanized, and several organs (liver, kidney, spleen, heart, lungs, brain, and muscle) were excised. Tissues were rinsed with normal saline to remove surplus blood before weighing and transferred into homogenate collection tubes, which were placed on ice. Tissues were stored at – 80 °C after their wet weights were measured (Maeng et al. 2007; Doan et al. 2020). The pretreatment and sample collection processes for animal experiments are depicted in Fig. 1.

Sample preparation for analysis

Standard working stock solutions of ergothioneine in acetonitrile were prepared by the serial dilution of the master stock solution in distilled water (1 mg/mL). For the internal standard (IS), the master stock of cefdinir in dimethyl sulfoxide (1 mg/mL) was diluted with acetonitrile to obtain a working IS solution (100 ng/mL). All stock and working standard solutions were stored at – 20 °C until use.

Standards for plasma, urine, and tissue samples were prepared by spiking 10 µL of working stock solutions to 90 µL of 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Considering the presence of endogenous ergothioneine in blank plasma, urine, and tissue samples, BSA was used instead of these matrices. BSA has not only similar components with the body fluid matrices but also good repeatability and accuracy for analysis of endogenous

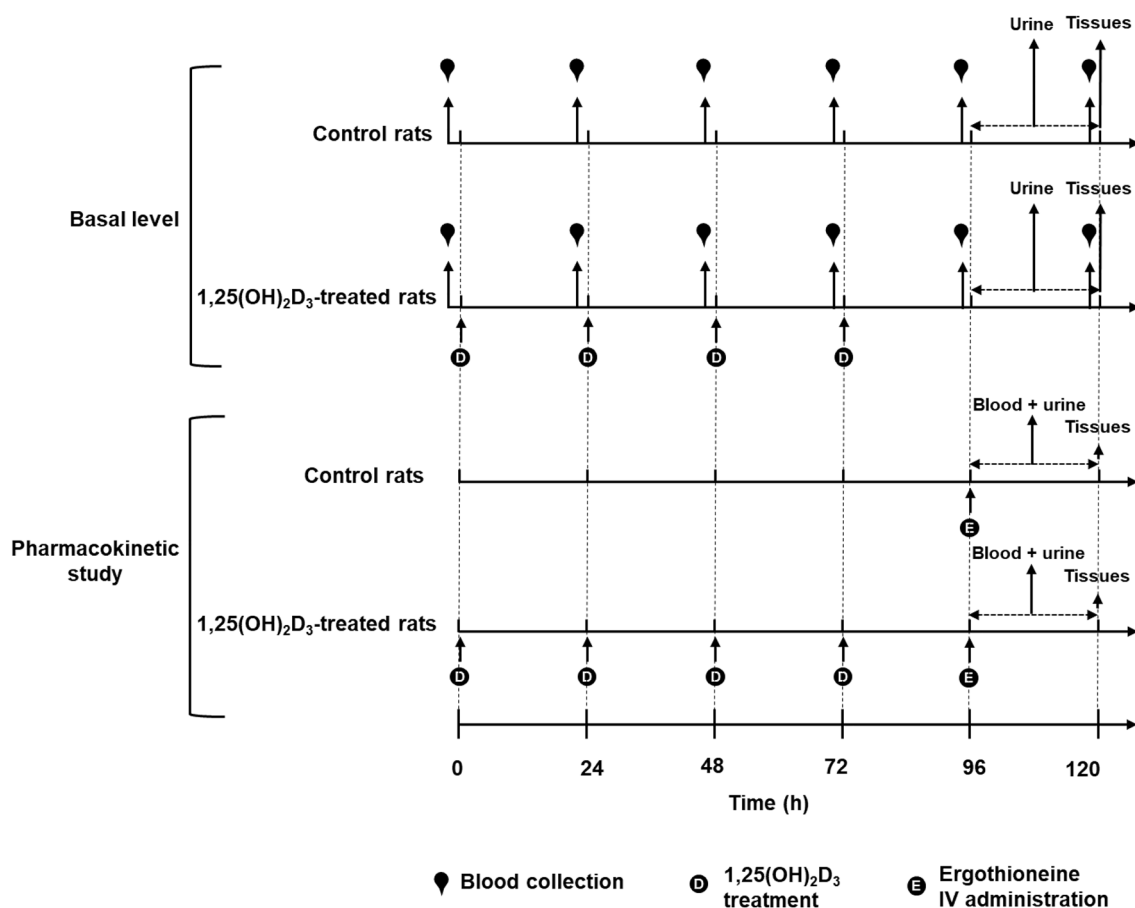


Fig. 1 Experimental schedule of the basal level determination and pharmacokinetic studies in the control and 1,25(OH)₂D₃-treated rats

compounds (Shen et al. 2018). In our preliminary study, samples prepared with 1% BSA in PBS solution also showed a good linearity, which was better than that from samples prepared with water or PBS. Therefore, this solution was used to prepare the standards and dilute all biological samples (plasma, tissues, and urine) with appropriate dilution factors prior to analysis. The final concentrations of ergothioneine for calibration curve were 2, 5, 10, 50, 100, 500, 1000, 5000, and 10,000 ng/mL. A total of 200 μ L of the IS solution was added to each sample, and the mixture was vortexed for 1 min and then centrifuged at 14,000 rpm for 15 min at 4 $^{\circ}$ C. The supernatant (200 μ L) was collected for LC–MS/MS analysis (Jin et al. 2014; Kim et al. 2014).

For basal ergothioneine determination, plasma was not diluted, whereas urine was diluted tenfold with 1% BSA. Tissue samples were homogenized in a two-fold volume of PBS and then diluted with 1% BSA (50-fold for liver and kidney; 20-fold for spleen, heart, lungs, and muscle; and tenfold for brain). For the pharmacokinetic study, plasma and urine were diluted 50 and 100 times with 1% BSA, respectively. Tissue samples were homogenized in a two-fold volume of PBS and then diluted with 1% BSA (1,000-fold for

the liver and kidney; 500-fold for the spleen; and 100-fold for the lungs, heart, brain, and muscle). A total of 100 μ L of the diluted sample (plasma, urine, or tissue) was mixed with 200 μ L of the IS solution, followed by vortex mixing and centrifugation (14,000 rpm, 15 min, 4 $^{\circ}$ C) to collect the supernatant for LC–MS/MS analysis as mentioned above.

Quantitative analysis of ergothioneine

Ergothioneine was quantified using an LC–MS/MS system consisting of an electrospray tandem triple quadrupole mass spectrometer (Agilent 6490 QQQ) with an ESI⁺ Agilent Jet Stream ion source coupled with a 1290 Infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA). A SynergiTM 4 μ m polar-RP 80A column (150 \times 2.0 mm, 4 μ m, Phenomenex, Torrance, CA, USA) conjugated with a guard column (SecurityGuardTM, 4.0 \times 3.0 mm, Phenomenex, Torrance, CA, USA) was used to separate ergothioneine and IS from the biological matrix. The mobile phase comprised 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). Sample separation was conducted using a flow rate of 0.2 mL/min and an 18-min gradient condition (0 min: 5% B, 5–9 min: 35%

B, 9.1–18 min: 5% B). The retention time for ergothioneine and cefdinir was 2.5 and 11.0 min, respectively. The injection volume for each sample was 2 μ L. Temperatures of the column and the autosampler were 25 and 4 $^{\circ}$ C, respectively. Mass spectrometry was conducted in the positive mode using an electrospray ionization source. Simultaneously, multiple reaction monitoring (MRM) transitions of m/z 230.2 \rightarrow 127.1 and m/z 396.2 \rightarrow 227.1 were applied for ergothioneine and IS, respectively. The Mass Hunter software (version A.06.00, Agilent Technology) was used for data acquisition and processing. The capillary voltage was set to 3000 V, and the gas temperature was maintained at 350 $^{\circ}$ C. The nitrogen sheath gas pressure for nebulizing the sample was set at 50 psi, and the gas flow was set at 12 L/min.

To quantify ergothioneine levels in pharmacokinetic samples, calibration curves were established by plotting the peak area ratios of ergothioneine to IS versus ergothioneine concentration using weighted ($1/x$) least-squares linear regression analysis. The method was validated for selectivity and linearity as previously described (Yoon et al. 2020; Nguyen et al. 2021). The results shown in Supplemental Figs. S1 and S2 indicate that the selectivity of the method is adequate for ergothioneine analysis and that the LC–MS/MS response is directly proportional to the concentration of ergothioneine in plasma and tissues. The calibration curves for ergothioneine ranged from 2 to 10,000 ng/mL. All correlation coefficient (R) of the calibration curves were ≥ 0.990 , indicating the good linearity of the analysis method. The mean accuracy of individual calibrators was $\leq 15\%$ for each calibration. The lower limit of quantitation (LLOQ), defined as the lowest concentration that could be determined with precision and accuracy of $\pm 20\%$, was 2 ng/mL.

Data analysis

Pharmacokinetic parameters, including total area under the plasma concentration versus time curve from time zero to infinity (AUC_{∞}), total body clearance (CL), apparent distribution volume at the steady state (V_{ss}), and mean residence time (MRT) were determined using non-compartmental analysis with WinNonlin® 8.3 (Pharsight Co., Mountain View, CA, USA).

Differences were considered statistically significant at $p < 0.05$, as determined by using the two-tailed Student's t -test between unpaired average values for control and treated groups. Results are presented as the means \pm standard deviations (SDs).

Results and discussion

The effects of $1,25(OH)_2D_3$ treatment on the pharmacokinetics of several drugs, such as procainamide (substrate of rOCT and rMATE), cefdinir, cefadroxil (substrates of rOAT1 and rOAT3), and buspirone (substrate of CYP3A4), in rats have been reported in the literature (Kim et al. 2014; Jeong et al. 2019; Maeng et al. 2019). OCTN1 is well known for its influential role in the secretion, reabsorption, and tissue distribution of drugs; however, there is limited information on the clinical relevance of this transporter to a specific substrate, ADME, and DDIs (Evans and Fornasini 2003; Koepsell and Endou 2004). With its vital role in the pharmacokinetics of various drugs, alterations in the expression level of OCTN1 might lead to DDIs when its substrates are administered simultaneously with vitamin D analogs (Chakraborti 2011). Therefore, it is worth investigating the effect of $1,25(OH)_2D_3$ treatment on the pharmacokinetics and biodistribution of ergothioneine, a well-known OCTN1 substrate.

Effect of $1\alpha,25$ -dihydroxyvitamin D_3 on rOCTN1 mRNA expression levels in representative organs

Alterations in the mRNA expression levels of several transporters, such as rMDR1, rOAT1, rOAT3, rOCT2, and rMATE, by $1,25(OH)_2D_3$ treatment have been reported in vivo in previous studies (Chow et al. 2009, 2010; Kim et al. 2014, 2020; Jeong et al. 2019). In the present study, the qPCR results (Fig. 2) revealed a significant decrease in

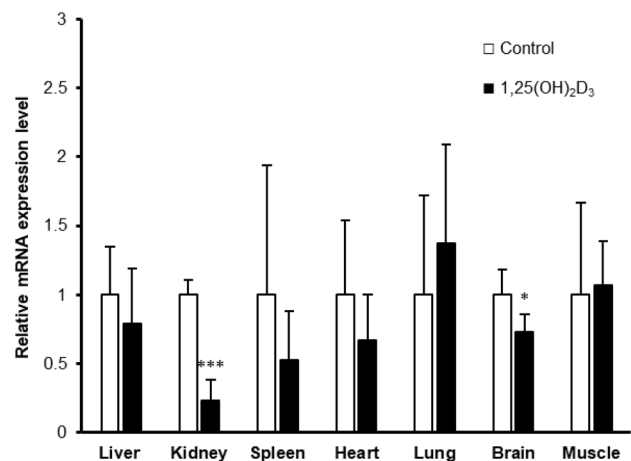


Fig. 2 Effect of $1,25(OH)_2D_3$ on the mRNA expression levels of OCTN1 in different organs of the controls (open bars) and $1,25(OH)_2D_3$ -treated (closed bars) rats. The mRNA expression levels were normalized to those of rat GAPDH. Data are presented as the means \pm SDs ($n = 3$). * $p < 0.05$ and *** $p < 0.001$ compared to the control group

the mRNA expression level of kidney and brain rOCTN1 in 1,25(OH)₂D₃-treated rats (by 77% and 27% compared to those in the control rats, respectively), indicating that treatment with 1,25(OH)₂D₃ led to the downregulation of rOCTN1 expression. Although a decreasing trend was observed in the mRNA levels of rOCTN1, there were no significant changes in other tissues, such as the liver, spleen, and heart, likely due to the large variation. In addition, our RT-qPCR data were in agreement with previous reports in which OCTN1 was shown to be more strongly expressed in the kidney and brain than in other organs in rat (Slitt et al. 2002) and human (Cheah and Halliwell 2012).

Effects of 1 α ,25-dihydroxyvitamin D₃ on the basal level of endogenous ergothioneine in plasma, urine, and tissues

The ergothioneine basal level in plasma, which served as the endogenous level (Fig. 3a), was similar in control and 1,25(OH)₂D₃-treated rats on the first and the second days. However, the level of endogenous ergothioneine in the 1,25(OH)₂D₃-treated group significantly increased from the third day to the sixth day of pretreatment, indicating that 1,25(OH)₂D₃ exerted an apparent effect on the downregulation of rOCTN1 expression approximately 48 h after the first injection. In addition, the cumulative amount of ergothioneine excreted in urine for 24 h in the 1,25(OH)₂D₃-treated group was 1880 ng, approximately twofold lower than that in the control group (3858 ng) (Fig. 3b). The significantly decreased amount of ergothioneine excreted in the urine of the treated rats could be likely due to the reduction in active secretion by rOCTN1 in the kidney. In addition, the amount of ergothioneine recovered in urine was much lower than the amount of ergothioneine accumulated in all tissues. Previous studies have reported that the excretion of ergothioneine occurs at a very slow rate under normal conditions (Tang et al. 2018; Pochini et al. 2019).

All tissues from the two groups contained the highest amounts of endogenous ergothioneine in the liver, followed by the kidney, spleen, heart, lung, muscle, and brain (Fig. 4). After pretreatment with 1,25(OH)₂D₃, the tissue-plasma partition coefficient (K_p) of basal endogenous ergothioneine significantly decreased by 52.4, 66.2, 64.2, 44.3, and 46.7% in the liver, kidneys, spleen, brain, and muscle, respectively (Table 1). The highest K_p value in the liver was in agreement with the strong expression of rOCTN1 in the rat liver (Koepsell and Endou 2004).

In the kidney, rOCTN1 is only located on the apical side of the proximal tubule cell and acts as a bifunctional transporter for both active secretion and reabsorption of ergothioneine (Koepsell and Endou 2004; Tang et al. 2018). When rOCTN1 expression is downregulated by 1,25(OH)₂D₃, the ergothioneine amount in the urine may affect the

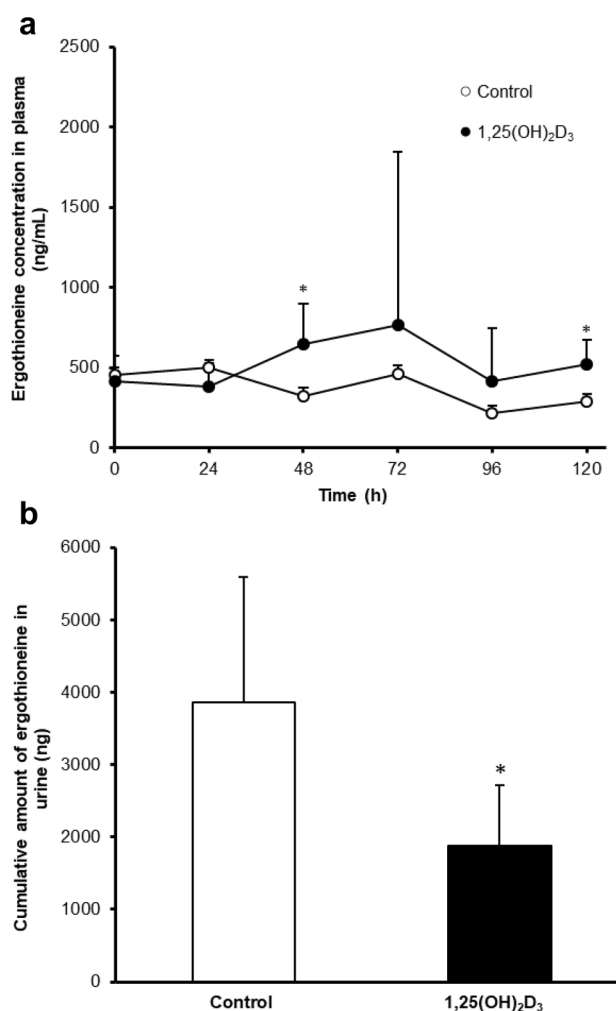


Fig. 3 **a** Basal levels of endogenous ergothioneine in the plasma of control (open circle) and 1,25(OH)₂D₃-treated (closed circle) rats. **b** Cumulative amount of endogenous ergothioneine in the urine of control and 1,25(OH)₂D₃-treated rats. Data are presented as the means \pm SDs (control, $n = 5$; 1,25(OH)₂D₃, $n = 6$). * $p < 0.05$ compared to the control group

ergothioneine level in the kidneys via both active secretion and reabsorption. It is hypothesized that the decreased K_p in the kidneys of the 1,25(OH)₂D₃-treated rats is likely due to the increased plasma level of ergothioneine and decreased reabsorption of ergothioneine from the urine. However, considering that the observed urinary recovery of ergothioneine was relatively low, other mechanisms or influx transporters may be involved. However, the transporter that plays a role in ergothioneine accumulation in the kidney remains unidentified (Pochini et al. 2019), which is required further study.

In other tissues, rOCTN1 is located in the basolateral, but not the apical membrane, playing the role of influx transport for ergothioneine from the blood to the tissue. The reduction in rOCTN1 expression and/or activity in the 1,25(OH)₂D₃-treated group may have decreased the accumulation of

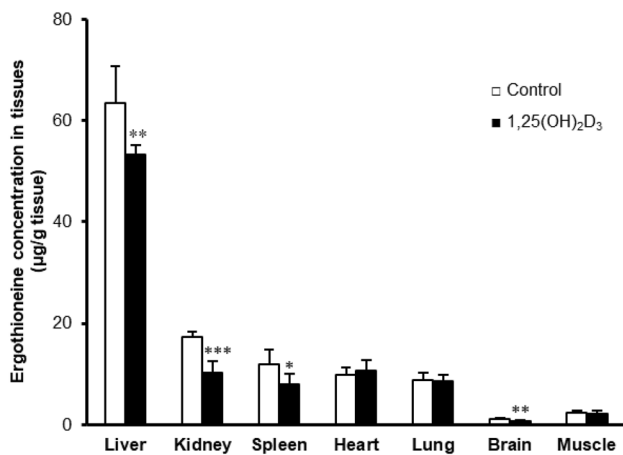


Fig. 4 Basal levels of endogenous ergothioneine in different organs of controls (open bars) and 1,25(OH)₂D₃-treated (closed bars) rats. Data are presented as the means \pm SDs (control, n=5; 1,25(OH)₂D₃, n=6). *p<0.05, **p<0.01, and ***p<0.001 compared to the control group

Table 1 Tissue partition coefficient (K_p) of the representative organs for the basal endogenous ergothioneine levels in the control and 1,25(OH)₂D₃-treated rats (means \pm SDs)

Organs	Control (n=5)	1,25(OH) ₂ D ₃ (n=6)
Liver	224.4 \pm 53.9	106.7 \pm 62.3**
Kidney	60.85 \pm 10.93	20.56 \pm 11.88***
Spleen	42.50 \pm 14.19	15.22 \pm 7.87**
Heart	34.59 \pm 7.01	22.25 \pm 15.27
Lung	30.67 \pm 6.68	17.75 \pm 11.47
Brain	3.585 \pm 0.447	1.587 \pm 0.929**
Muscle	8.385 \pm 1.141	4.471 \pm 3.279*

*p<0.05, **p<0.01, and ***p<0.001 compared to the control group

ergothioneine in these tissues (Pochini et al. 2019). Interestingly, rOCTN1 is absent in the blood–brain barrier (BBB) but is strongly expressed in the brain and plays an important role in protecting neuronal cells from oxidative stress by scavenging free radicals (Nakamichi et al. 2016; Nishida et al. 2018; Tang et al. 2018; Pochini et al. 2019). Therefore, ergothioneine may cross the BBB and accumulate in brain tissue via other transporters. Based on the information in the literature, the vital transporter could be OCTN2, which was reported to be expressed in the BBB and transport ergothioneine, even with low affinity (Ingoglia et al. 2016). The reduction of ergothioneine accumulation in the brain of 1,25(OH)₂D₃-treated rats could be attributed to the lower function of rOCTN1 in the brain and/or the obstacle to carrying ergothioneine through the BBB by rOCTN2. The cooperation of rOCTN1 and rOCTN2 may play a crucial role in the transport and interaction of many drugs targeting the

brain. The effects of 1,25(OH)₂D₃ on rOCTN2 should be investigated in future studies.

Effects of 1 α ,25-dihydroxyvitamin D₃ on the pharmacokinetic profiles, tissue distribution, and urinary excretion of ergothioneine

To the best of our knowledge, the intravenous pharmacokinetic profile of ergothioneine has not yet been reported in the literature. In this study, we investigated the pharmacokinetic profile of an intravenous bolus of exogenous ergothioneine in cases of emergency ergothioneine requirements. The plasma concentration–time profiles of ergothioneine after intravenous administration of 2 mg/kg to the control and 1,25(OH)₂D₃-treated rats (Fig. 5a) revealed significantly higher ergothioneine plasma levels in the 1,25(OH)₂D₃-treated group than in the control group. The calculated pharmacokinetic parameters (Table 2) showed that the AUC _{∞} of ergothioneine increased by 85%, likely due to the significant decrease in the total clearance of the 1,25(OH)₂D₃-treated group by 49%. Moreover, the V_{ss} of ergothioneine in 1,25(OH)₂D₃-treated rats was remarkably decreased by approximately 32% compared to that in the control, indicating a lower tissue distribution of ergothioneine due to the decrease in rOCTN1 activity in 1,25(OH)₂D₃-treated rats. In addition, after pretreatment with 1,25(OH)₂D₃, the weight gain of 1,25(OH)₂D₃-treated rats was significantly lower than that of control rats, which is consistent with the results of previous studies (Jeong et al. 2019; Maeng et al. 2019). Loss of appetite is a common symptom of vitamin D intoxication, which can be attributed to a reduction in weight gain in rats treated with 1,25(OH)₂D₃ (Çağlar and Tuğçe Çağlar 2021).

The effect of 1,25(OH)₂D₃ treatment on exogenous ergothioneine levels was fairly consistent with that of basal levels, with increased plasma level and decreased tissue distribution of ergothioneine in the treated group. In a previous study, the exogenous ergothioneine levels in OCTN1 knockout (mOCTN1^{-/-}) mice after oral administration decreased in most representative tissues and significantly increased in plasma as compared to those in wild-type mice (Kato et al. 2010). The similar trends in ergothioneine levels in plasma and tissues between mOCTN1^{-/-} mice and 1,25(OH)₂D₃-treated rats strongly suggest that 1,25(OH)₂D₃ treatment decreases rOCTN1 activity in vivo.

A significant decline in the urinary recovery of ergothioneine was observed (Fig. 5b). Although rats were administered exogenous ergothioneine, the urinary recovery of ergothioneine within 24 h in both control and 1,25(OH)₂D₃-treated rats was still negligible (3.89% and 1.08%, respectively). These data were consistent with the urinary recovery of ergothioneine in humans (Cheah et al. 2017) and mice

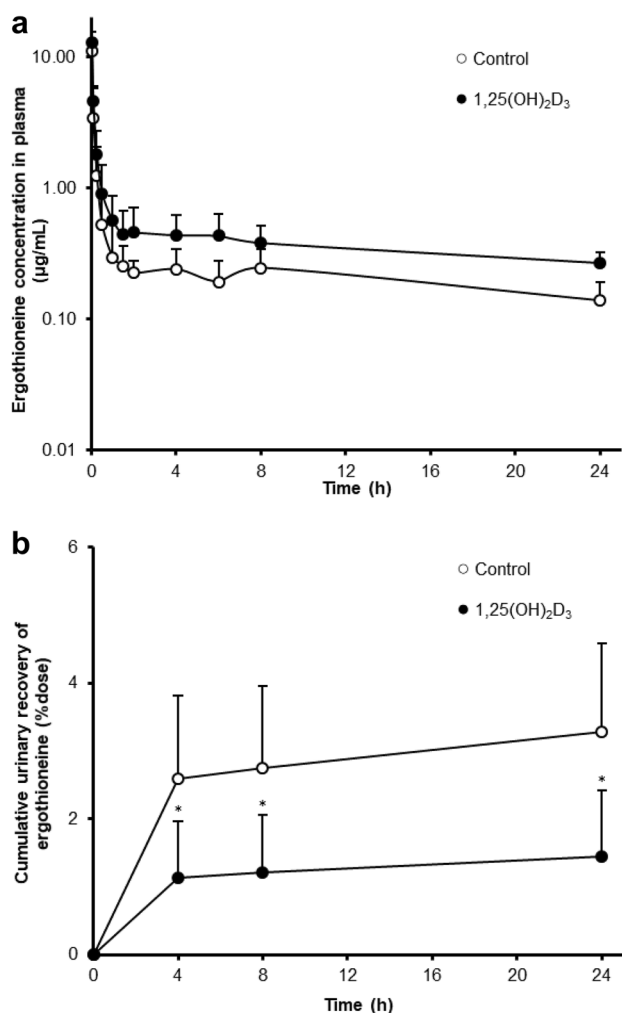


Fig. 5 **a** Plasma concentration–time profiles of ergothioneine after 2 mg/kg IV administration to the control (open circle) and 1,25(OH)₂D₃-treated (closed circle) rats. **b** Cumulative urine recovery of ergothioneine after IV administration to the control (open circles) and 1,25(OH)₂D₃-treated (closed circles) rats. Data are presented as the means ± SDs (control, n = 7; 1,25(OH)₂D₃, n = 6). *p < 0.05 compared to the control group

Table 2 Pharmacokinetic parameters of ergothioneine after 2 mg/kg IV administration to the control and 1,25(OH)₂D₃-treated rats (means ± SDs)

Parameter	Control (n = 7)	1,25(OH) ₂ D ₃ (n = 6)
Initial body weight (g) ^a	323.0 ± 20.8	323.7 ± 21.1
Final body weight (g) ^b	347.6 ± 24.7	287.7 ± 19.4 ***
AUC _∞ (µg × min/mL)	647.5 ± 327.5	1198 ± 457*
CL (mL/min/kg)	3.729 ± 1.734	1.894 ± 0.733*
MRT (h)	27.11 ± 17.31	33.50 ± 20.07
V _{ss} (mL/kg)	4820 ± 1291	3298 ± 959*

^aMeasured before pretreatment of 1,25(OH)₂D₃

^bMeasured before IV administration of ergothioneine

*p < 0.05 and ***p < 0.001 compared to the control group

(Kato et al. 2010), which was reported to be relatively low (< 4%) after oral administration.

The tissue concentrations and K_p of ergothioneine in the liver, kidney, spleen, heart, lung, brain, and muscle are shown in Fig. 6a and Table 3, respectively. Ergothioneine was predominantly distributed in the liver and kidneys. There were significant reductions in the tissue distribution of ergothioneine in all tissues of the 1,25(OH)₂D₃-treated rats. The K_p of ergothioneine in the liver, kidney, spleen, heart, lungs, brain, and muscle of 1,25(OH)₂D₃-treated rats decreased by 41.8%, 60.1%, 69.0%, 50.8%, 32.7%, 39.4%, and 47.3%, respectively, compared to that of the control rats. In addition, when the K_p of exogenous and endogenous (basal) ergothioneine was compared (Fig. 6b), the kidney showed the greatest fold change (3.13- and 3.70-fold

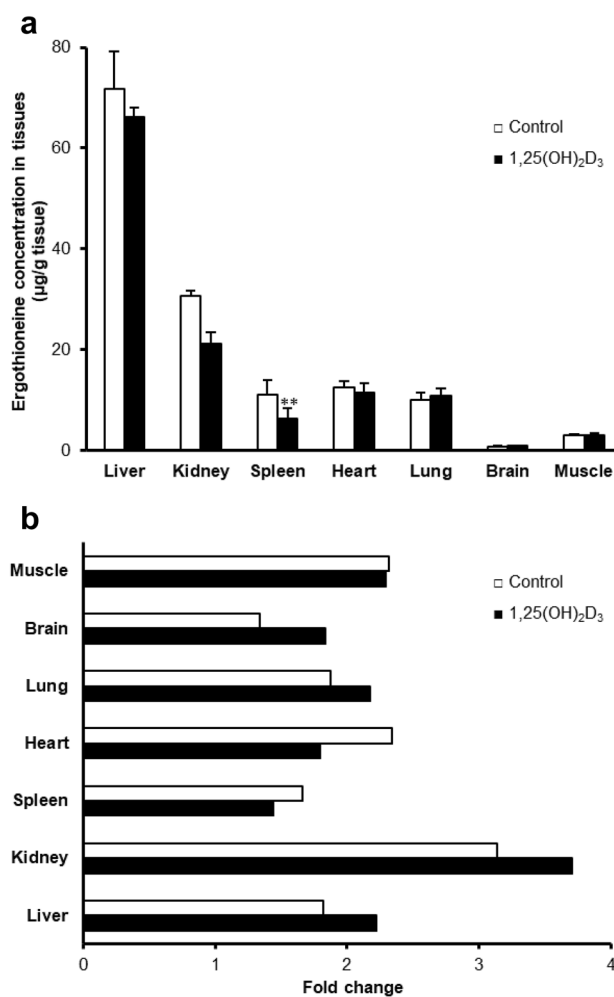


Fig. 6 **a** Amount of ergothioneine accumulated in different organs of controls (open bars) and 1,25(OH)₂D₃-treated (closed bars) rats after IV administration of ergothioneine. Data are presented as the means ± SDs (control, n = 5; 1,25(OH)₂D₃, n = 6). **p < 0.01 compared to the control group. **b** Fold change of K_p (K_p of ergothioneine after exogenous administration versus K_p from endogenous basal level for each tissue)

Table 3 Tissue partition coefficient (K_p) of the representative organs for IV administration of exogenous ergothioneine in the control and 1,25(OH) $_2$ D $_3$ -treated rats (means \pm SDs)

Organs	Control (n = 5)	1,25(OH) $_2$ D $_3$ (n = 6)
Liver	407.8 \pm 113.9	237.3 \pm 82.8*
Kidney	190.7 \pm 59.4	76.12 \pm 47.89**
Spleen	70.56 \pm 17.46	21.89 \pm 2.34***
Heart	81.04 \pm 37.23	39.88 \pm 10.04*
Lung	57.37 \pm 14.60	38.61 \pm 8.48*
Brain	4.799 \pm 1.199	2.909 \pm 0.448**
Muscle	19.43 \pm 8.95	10.25 \pm 3.90*

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared to the control group

in the control and treated groups, respectively). Similar to our results, a previous study found the highest basal ergothioneine levels in the liver and kidneys of wild-type mice (Tang et al. 2018). The authors also reported an increase in ergothioneine concentrations in all organs, with the greatest fold change in the kidney after oral administration of ergothioneine.

Recently, ergothioneine has been applied for prevention of various diseases (i.e., neurological disorders, cardiovascular disorders, and ocular disorders), concomitant application for cancer therapies, and cosmeceuticals (Borodina et al. 2020; Cheah and Halliwell 2021). However, there have been no reports of symptoms caused by ergothioneine deficiency. Ergothioneine has antioxidant functions in tissues that are highly exposed to reactive oxygen species. Considering that many neurological diseases are caused by oxidative stress, ergothioneine transport mediated by OCTN1 is linked to antioxidant effect (Nakamichi and Kato 2017). Another study determined the possible role of OCTN1–ergothioneine axis and the relationship between alterations in kidney function and ergothioneine intestinal absorption via OCTN1 (Shinozaki et al. 2017). Therefore, our current data contribute additional evidence for the pivotal role of rOCTN1 in the kidney and brain and the potential interaction of rOCTN1 substrates with vitamin D or the active form of vitamin D. Thus, the moderation of vitamin D in the treatment or supplementation should be carefully considered, particularly when OCTN1 substrates (such as oxaliplatin, ipratropium, metformin, gabapentin, etc.) are co-administered.

Recently, with the emergence of coronavirus disease 2019 (COVID-19), both vitamin D and ergothioneine have been proposed as potential agents for COVID-19 management. Habitual use of vitamin D supplements is related to a lower risk of COVID-19 (Ma et al. 2021). The role of vitamin D in the prevention and potential implications of COVID-19 has also been reported (Ilie et al. 2020; Laird et al. 2020; Razdan et al. 2020; Ghareeb et al. 2021; Walrand 2021). In addition, ergothioneine has been suggested to be beneficial

for alleviating symptoms and improving the prognostic outcomes of COVID-19 patients (Cheah and Halliwell 2021). These effects are due to its cytoprotective property as a super antioxidant (especially in the lungs, the primary target tissue of the coronavirus) and its role in inflammation (Laurenza et al. 2008; Repine and Elkins 2012; Stoffels et al. 2017). In this study, we found that the accumulation of ergothioneine in the organs significantly decreased after the treatment of vitamin D active form. Collectively, vitamin D dosage and the addition of ergothioneine should be considered in careful moderation. Further studies are needed to elucidate the underlying mechanisms of vitamin D and ergothioneine from the perspective of the prevention and treatment of COVID-19.

Conclusion

This is the first study to investigate the interaction between 1,25(OH) $_2$ D $_3$ and ergothioneine, a well-known substrate of rOCTN1 transporter, by observing its biodistribution and pharmacokinetics in rats. We found that 1,25(OH) $_2$ D $_3$ inhibited the rOCTN1 gene expression and functional activity, leading to increased ergothioneine levels in the blood and decreased ergothioneine accumulation in rat tissues. This research area is gaining increasing attention owing to the widespread combination of vitamin D and various drugs for therapeutic purposes, which requires further human studies when vitamin D and OCTN1-targeted medicines are co-administered.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40005-022-00563-1>.

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Conflict of interest All authors (D.-K. Vo, T.-T.-L. Nguyen, and H.-J. Maeng) declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

Research involved in human and animal rights Animal studies were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Gachon University (date of approval: May 25, 2017; approval number: GIACUC-R2017011).

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