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# Metabolites of lansoprazole inhibit CFTR-mediated $Cl^-$ transport and retard cyst progression

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**ABSTRACT**: Polycystic kidney disease (PKD) is a genetic disorder that results in renal cyst formation and cyst enlargement. PKD causes abnormal cell proliferation and fluid secretion in the cyst which results in a loss of normal function and eventually leads to renal failure. A previous study reported the beneficial effect of lansoprazole, a proton pump inhibitor, on cyst progression *in vitro* and renal cyst progression in PKD rats. The present study investigated whether the major metabolites of lansoprazole, lansoprazole sulfide, and lansoprazole sulfone affected the progression of microcysts derived from the Madin Darby canine kidney (MDCK) cells. The results showed that treatment of the MDCK cells with lansoprazole sulfide or lansoprazole sulfone increased the expression of sterol regulatory element-binding protein-1c (SREBP-1c), a target protein of the liver X receptor. Lansoprazole sulfide or lansoprazole sulfone significantly inhibited cystic fibrosis transmembrane conductance regulator (CFTR)-mediated $Cl^-$ secretion compared with vehicle-treating cells. However, they did not affect calcium-activated chloride channel (CaCC)-mediated $Cl^-$ secretion. Incubation of MDCK-derived cysts with lansoprazole sulfide or lansoprazole sulfone for 3 and 6 days led to a decrease in the growth of the cysts without affecting the viability of the cells. In addition, lansoprazole sulfide significantly reduced the number of forskolin-induced cysts, but lansoprazole sulfone had no effect. The inhibitory effect of lansoprazole sulfide on cyst formation was related to reduced cell proliferation. This finding is the first *in vitro* evidence supporting that the metabolites of lansoprazole could inhibit $Cl^-$ secretion and suppress cyst progression of the renal collecting duct cells.

**KEYWORDS**: lansoprazole, CFTR, kidney, cell proliferation, polycystic kidney disease

## INTRODUCTION

Polycystic kidney disease (PKD) is a hereditary disorder characterized by multiple fluid-filled cysts occupying renal parenchyma. These PKD cysts cause a loss of normal function and eventually lead to renal failure [1, 2]. Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations of PKD1 and/or PKD2 genes encoding polycystin-1 (PC-1) and polycystin-2 (PC-2) proteins, respectively [3]. However, autosomal recessive polycystic kidney disease (ARPKD) is caused by the mutation of polycystic kidney and hepatic disease 1 (PKHD1) encoding fibrocystin protein [4]. Mutations of the PC1/2 complex in the renal cells lead to a reduction in intracellular calcium, subsequently increasing the intracellular cAMP level, which stimulates cell proliferation and cyst expansion [5, 6]. An increase in intracellular cAMP stimulates PKA which further stimulates cell proliferation by a sequential activated form of Ras, B-Raf, MEK, and ERK [7]. Cysts are filled with fluid driven by cAMP-stimulated transepithelial $Cl^-$ transport [8, 9]. In PKD cyst epithelia, $Cl^-$ enters the cell through $Na^+$-$K^+$-2 $Cl^-$ cotransporters at the basolateral side, using the $Na^+$ gradient established by the function of $Na^+$-$K^+$ ATPase [10, 11]. $Cl^-$ transports into the luminal side via the cystic fibrosis transmembrane conductance regulator (CFTR) $Cl^-$ channel [12], which plays a crucial role in the accumulation of fluid in the PKD cyst, resulting in cyst enlargement [5]. In addition to CFTR, calcium-activated chloride channels (CaCCs) have been reported as the key channels responsible for fluid accumulation in renal cysts [13]. Among the CaCCs, TMEM16A (anoctamin-1; ANO1) plays a role in the accumulation of fluid in renal cysts. The inhibition of TMEM16A by its inhibitors attenuates renal cyst progression [14–16].

Lansoprazole is a drug used for the treatment of acid-related diseases [17, 18]. It acts as a potent

inhibitor of the gastric acid pump and has been used for short- and long-term treatments without major side effects [19]. Lansoprazole has been reported as a liver x receptor (LXR) agonist [20]. It demonstrates an inhibitory effect on $Cl^-$ secretion and cyst progression [21, 22]. After absorption, lansoprazole is metabolized in the liver through the cytochrome P450 system, specifically through the CYP3A4 and CYP2C19 isozymes [23, 24]. Three metabolites, 5-hydroxy lansoprazole, lansoprazole sulfide, and lansoprazole sulfone, have been identified in plasma following the administration of lansoprazole [25]. Lansoprazole demonstrates a potential therapeutic effect on cyst progression in PKD rats [22]. It would be interesting to establish whether lansoprazole metabolites also contribute to the inhibitory effect of lansoprazole on $Cl^-$ secretion and cyst progression. The present study investigates the in vitro pharmacological effects of 2 major lansoprazole metabolites found in plasma, namely lansoprazole sulfide and lansoprazole sulfone, on *in vitro* CFTR- and CaCC-mediated $Cl^-$ secretion and the progression of renal cell-derived cysts.

## MATERIALS AND METHODS

### Chemicals

Lansoprazole, amiloride, forskolin (FSK), 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), ATP, fetal bovine serum (FBS), insulin, selenium, and transferrin were purchased from Sigma-Aldrich (MO, USA). [Arg8]-vasopressin (AVP) was obtained from the Ferring International Center (Prex, Switzerland). Collagen type I was from Advanced BioMatrix (CA, USA). Antibodies against SREBP-1c were purchased from Merck Millipore Inc. (Bangkok, Thailand). DMEM/F12 media, trypsin-EDTA, penicillin, and streptomycin were from Gibco (NY, USA). The iScript cDNA synthesis kit was obtained from Bio-Rad Laboratories (Bangkok, Thailand). The LUNA® SYBR Green universal master mix was from New England BioLabs (Frankfurt, Germany). All the other chemicals used in this study were of analytical grade and obtained from commercial sources.

### Cell culture

Type I MDCK cells were cultured in DMEM/F12 medium supplemented with 10% FBS, penicillin/streptomycin, and insulin-transferrin-selenium at 37 °C in a humidified incubator with 95% $O_2$ and 5% $CO_2$.

### Electrophysiological analysis

Cells were grown on Snapwell inserts (Corning Life Sciences, MA, USA) at a density of $5 \times 10^5$ cells/well. Media were changed every 2 days with transepithelial resistance measured using an epithelial volt-ohm meter. On day 10, only MDCK polarized cell monolayers with resistance higher than 1000 ohm $\cdot$ cm$^2$ were used to test the effect of lansoprazole and its metabolites on $Cl^-$ secretion. MDCK cell monolayer was mounted on an Ussing Chamber. The chambers were filled with a physiological transport buffer solution containing 117 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM $KH_2PO_4$, 25 mM $NaHCO_3$, and 11 mM D-glucose at 37 °C. The buffer was bubbled with a gas mixture of 95% $O_2$-5% $CO_2$ to maintain the pH of the buffer at 7.4. Positive short-circuit current (Isc) reflects the absorption of $Na^+$ from the apical to the basolateral side of the monolayer or secretion of $Cl^-$ from the basolateral to the apical side [21]. To measure the $Cl^-$ secretion, the $Na^+$ current was inhibited by the addition of 100 μM amiloride. CFTR-mediated $Cl^-$ secretion was measured by adding 20 nM arginine-vasopressin (AVP) to the basolateral side with CaCC-mediated $Cl^-$ secretion determined following the addition of 100 μM ATP to the apical side.

### Cell viability assay

The viability of the cells was determined by the MTT assay. Confluent MDCK cells in a 96-well plate were exposed to lansoprazole and its metabolites at high concentrations (100 μM) for 24 h. After that, the adherent cells were treated with 0.5 mg/ml MTT in serum-free media for 4 h in the humidified incubator under 95% $O_2$ and 5% $CO_2$ at 37 °C. At the end of incubation, the media were removed, then followed by an addition of 100 μl of DMSO, and absorbance was measured at 540 nm. Cell viability was analyzed and compared with the control group.

### Cell proliferation assay

Cell proliferation was performed by counting the viable cells. MDCK cells (50% confluency) in a 24-well plate were exposed to DMEM/F12 medium containing vehicle, lansoprazole, and its metabolites at the same concentration of 50 μM for 24, 48, and 72 h under a humidified atmosphere at 37 °C. The cells were removed by trypsinization and counted on a hematocytometer under a light microscope for comparison between the vehicle and treatment groups.

### MDCK model of cyst formation and cyst growth

MDCK cells with a density of 400–600 cells/well were grown in collagen gel in the presence of forskolin to stimulate cyst formation following the method used in previous studies [21, 26, 27]. The collagen was composed of 10% 10X minimum essential medium (MEM), 27 mM $NaHCO_3$, 10 mM HEPES, and penicillin/streptomycin (adjusted to pH 7.4 with NaOH). For the cyst formation experiment, 1.5 ml of MDCK cell suspension containing 10 μM forskolin with or without test compounds was added to a 24-well plate containing collagen gel. The cells were then cultured in a humidified incubator under 95% $O_2$ and 5% $CO_2$. The medium was changed every 2 days. On day 6, after

seeding, the number of cysts (with diameters > 50 μm) and non-cyst cell colonies were counted by a phase-contrast light inverted microscope. For cyst growth, cysts with a diameter of more than 50 μm were selected to monitor the growth by photographing for 6 days. The cyst size was compared between the control and treatment groups.

### qPCR analysis

The total RNA of the MDCK cells was extracted by RiboEx™ reagent (GeneAll Biotechnology Co., Ltd., Seoul, South Korea), according to the manufacturer's instructions. The amount and quality of extracted RNA were determined using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The cDNA was synthesized by an iScript cDNA synthesis kit (Bio-Rad), and the mRNA expression level was quantified using a LUNA® SYBR Green universal master mix in the ABI Prism 7500 Sequence Detection system (Applied Biosystems, Foster City, CA, USA). Relative quantification was determined using the $2^{-\Delta\Delta C_T}$ method (where $C_T$ is the threshold cycle) [28]. The primers used for amplification of specific mRNA targets were designed based on sequences in the National Center for Biotechnology Information (NCBI) database (Table S1).

### Western blot analysis

The MDCK cells were lyzed with lysis buffer and a protease inhibitor cocktail for 20 min at 4 °C. After that, the sample was centrifuged at 12,000 rpm for 20 min, and the supernatants were then collected at −80 °C until used. Proteins were separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes. GAPDH was used as a loading control. The membranes were incubated in 10% non-fat milk for 2 h, followed by incubation with antibodies against SREBP-1c (1:1,000 dilution) or GAPDH (1:1,000 dilution) overnight at 4 °C. The membranes were washed 3 times and incubated with a horseradish peroxidase-conjugated secondary antibody. The protein expressions were detected using the Electro-Chemi-Luminescence (ECL) system and visualized by the Azure 600 Gel Imaging System (Azure Biosystem, Inc., California, USA). The immunoreactive bound density was reported relative to that of GAPDH.

### Analysis of lansoprazole metabolites

Fourier Transform Infrared (FTIR) spectroscopy was performed on a Bruker ALPHA FTIR model (Bruker, Billerica, MA, USA), whereas the major bands ($\lambda_{max}$) were recorded in wave numbers ($cm^{-1}$). The high-resolution nuclear magnetic resonance spectra were mainly recorded on Bruker Ascend™ 400 and Bruker ADVANCE-500 spectrometers at the Department of Chemistry, Faculty of Science, Mahidol University, in $CDCl_3$ using TMS as the internal standard. EIMS were recorded at 60 eV (probe) on a DART, AccuTOF LC-plus 4G, JEOL mass spectrometer. The solvents for extraction, chromatography, and recrystallization were distilled prior to use at their boiling point ranges. Pre-coated TLC aluminum sheets of silica gel 60 $PF_{254}$ (20 × 20 cm, layer thickness 0.2 mm) were used for analytical purposes, and the bands were visualized by ultraviolet light (at $\lambda_{max}$ 254 and 366 nm).

### Statistical analysis

Data are shown as means ± SD. Statistical differences were determined using a one-way ANOVA followed by Tukey's test. A $p$-value less than 0.05 was considered statistically significant.

## RESULTS

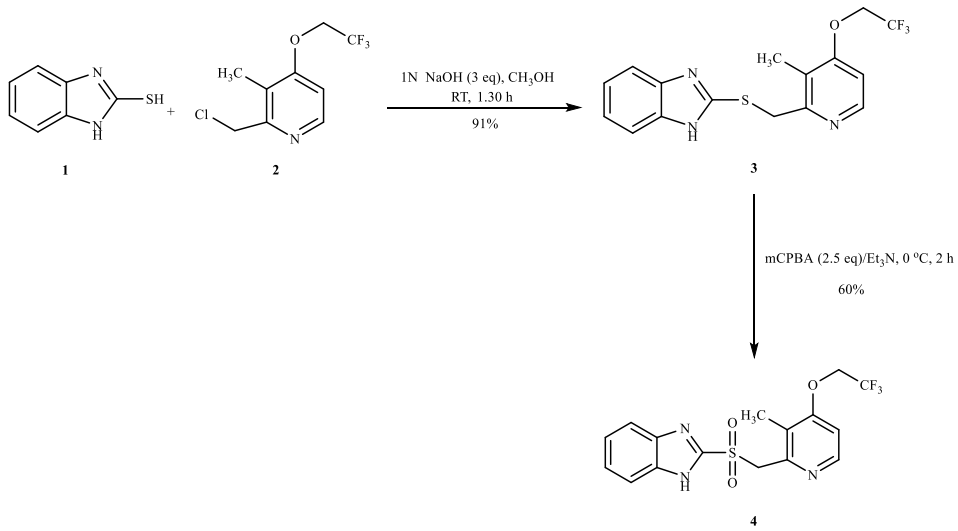
### Synthesis of lansoprazole metabolites

Lansoprazole sulfone and lansoprazole sulfide were detected in plasma after administration of lansoprazole. To investigate the pharmacological effects of these metabolites on CFTR-mediated $Cl^-$ secretion, we performed the total synthesis of these metabolites. The structures of lansoprazole, lansoprazole sulfide, and lansoprazole sulfone are shown in Fig. 1A. Scheme 1 shows the synthesis process of the metabolites.

#### *Preparation of lansoprazole sulfide*

To a stirred solution of 2-mercaptobenzimidazole (1) (107.10 mg, 0.70 mmol, 1 equiv.) and 2-(chloromethyl)-4-methoxy-3, 5-dimethylpyridine hydrochloride (2) (198.9 mg, 0.70 mmol, 1 equiv.) in $CH_3OH$ (3 ml), NaOH (83.9 mg, 2.10 ml, 2.1 mmol, 3 equiv.) was sequentially added. The reaction mixture was continuously stirred at room temperature for 1.3 h (Scheme 1). The reaction was monitored by TLC with EtOAc-hexane (1:1) as an eluent. After evaporation to dryness and extraction with $CH_2Cl_2$ (3 × 20 ml), the combined organic layers were washed respectively with sat. $NH_4Cl$ (20 ml) and sat. NaCl (20 ml), dried over anhydrous $Na_2SO_4$, filtered, and evaporated to dryness to yield a white solid (260.6 mg). Further recrystallization with EtOAc-hexane (1:1) afforded lansoprazole sulfide (192.0 mg, 91%).

Lansoprazole sulfide: FTIR (KBr) $cm^{-1}$: 3416, 3054, 2898, 1578, 1445, 1256, 1175, 1111, 977, 858, 746, 665. $^1$H NMR (500 MHz, $CDCl_3$), $\delta$: 8.41 (1H, $d$, $J$ = 5.7 Hz, H-6′), 7.56–7.53 (2H, $m$, H-4, H-7), 7.21–7.18 (2H, $m$, H-5, H-6), 6.73 (1H, $d$, $J$ = 5.8 Hz, H-5′), 4.43 (2H, $s$, $SCH_2$), 4.43 (2H, $q$, $J$ = 8.0 Hz, $CH_2CF_3$), 2.32 (3H, $s$, $CH_3$). $^{13}$C NMR (500 MHz, $CDCl_3$): 162.5 (C-4), 157.6 (C-2′), 151.2 (C-2), 147.4 (C-6′), 123.9 (C-8, C-9), 122.0 (C-5, C-6), 121.8 (C-3′), 121.7 ($CF_3$), 114.2 (C-4, C-7), 106.0 (C-5′), 65.5 ($CH_2CF_3$), 34.9 ($CH_2S$), 10.7 ($CH_3$). ESI-MS (DART)




**Scheme 1** Synthesis of lansoprazole metabolites. Lansoprazole sulfide and lansoprazole sulfone are depicted as 3 and 4, respectively.

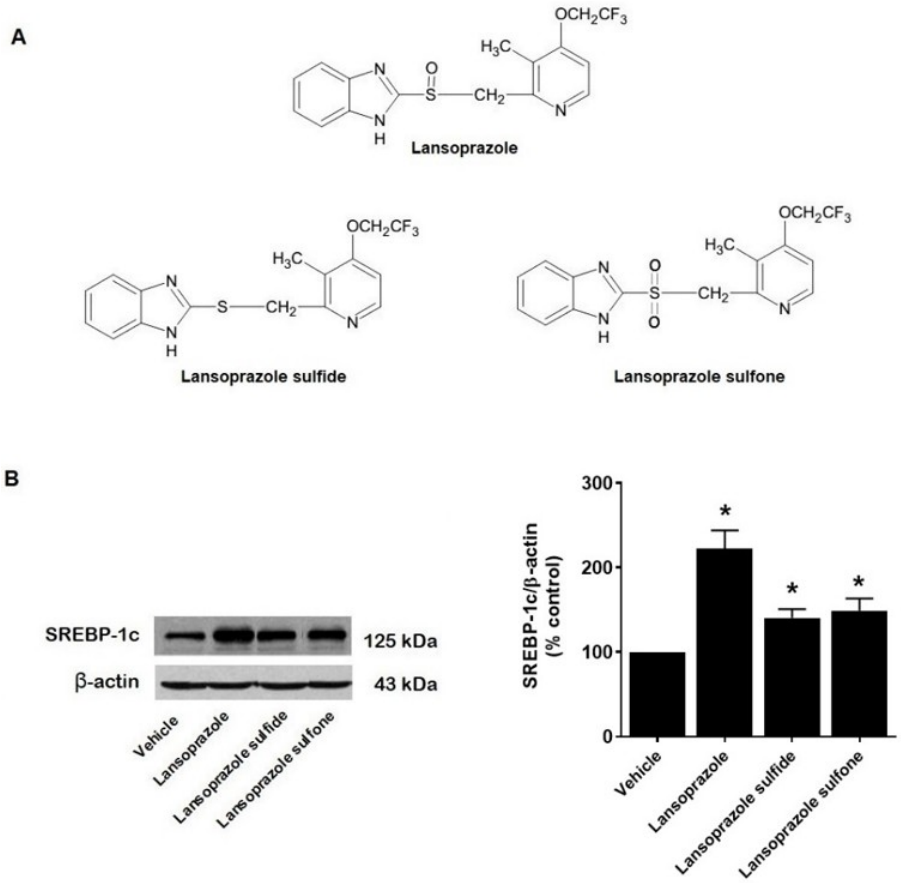



**Fig. 1** (A) Chemical structure of lansoprazole, lansoprazole sulfide, and lansoprazole sulfone; (B) Effect of lansoprazole and its metabolites on SREBP-1c protein expression in MDCK cells. Representative western blot and density analyses are shown for the expression of SREBP-1c in cells treated with vehicle, lansoprazole, lansoprazole sulfide, or lansoprazole sulfone at a concentration of 50 μM for 24 h. Data are presented as mean ± SD from independent experiments, $n = 3$; * $p < 0.05$, compared with vehicle-treated cells.

$m/z$: 354.0880 $[M+H]^+$ (calcd. for $C_{16}H_{15}F_3N_3OS$, 354.0888).

### *Preparation of lansoprazole sulfone*

*Meta*-Chloroperoxybenzoic acid (mCPBA) (77.5 mg, 0.45 mmol, 2.1 equiv.) was added to a stirred solution of lansoprazole sulfide (75.6 mg, 0.21 mmol, 1 equiv.) in dry $CH_2Cl_2$ (6 ml) at 0 °C. Dry $Et_3N$ (0.13 ml) was then added to the reaction mixture and stirred at 0 °C for 2 h (Scheme 1). The reaction was monitored by TLC, using EtOAc-hexane (1:1) as an eluent. After extraction with $CH_2Cl_2$ (3 × 20 ml), the combined organic layers were washed with sat. NaCl (20 ml), dried with anhydrous $Na_2SO_4$, filtered, and evaporated to dryness to yield a white solid (112.9 mg). Further recrystallization with EtOAc-hexane (1:1) afforded lansoprazole sulfone (12.1 mg, 61%).

Lansoprazole sulfone: FTIR (KBr) $cm^{-1}$: 3418, 3091, 2964, 1586, 1480, 1329, 1268, 1165, 1110, 976, 804, 744, 678. $^1$H NMR (400 MHz, $CDCl_3$), $\delta$: 8.26 (1H, *d*, $J$ = 5.6 Hz, H-6′), 7.52 (2H, br *s*, H-4, H-7), 7.39 (2H, *m*, H-5, H-6), 6.69 (1H, *d*, $J$ = 5.6 Hz, H-5′), 5.05 (2H, *s*, $SO_2CH_2$), 4.39 (2H, *q*, $J$ = 7.8 Hz, $CH_2CF_3$), 2.37 (3H, *s*, $CH_3$). $^{13}$C NMR (400 MHz, $CDCl_3$): 162.33 (C-4′, C-2′), 148.2 (C-6′), 147.9 (C-2), 147.5 (C-8, C-9), 126.30 (C-5, C-6), 125.1 (C-3′), 124.1 ($CF_3$), 112.4 (C-4, C-7), 106.5 (C-5′), 65.3 ($CH_2CF_3$), 60.5 ($CH_2SO_2$), 11.4 ($CH_3$). ESI-MS (DART) $m/z$: 386.0778 $[M+H]^+$ (calcd. for $C_{16}H_{15}F_3N_3O_3S$, 386.0786).

Lansoprazole has been reported as an LXR activator and has been shown to inhibit $Cl^-$ secretion and cyst progression [22]. The present study sought to determine whether lansoprazole metabolites, including lansoprazole sulfide and lansoprazole sulfone, can activate LXR in MDCK cells. The protein expression of SREBP-1c, a LXR target protein, was detected and quantified using western blot analysis. The MDCK cells were treated with the vehicle, lansoprazole, lansoprazole sulfide, or lansoprazole sulfone at a concentration of 50 µM for 24 h, followed by the measurement of SREBP-1c expression. The results showed that lansoprazole and its metabolites significantly enhanced the expression of SREBP-1c protein compared with vehicle-treated cells (Fig. 1B). According to the data, lansoprazole sulfide and lansoprazole sulfone induce LXR activation in MDCK cells.

### Effect of lansoprazole metabolites on $Cl^-$ secretion in MDCK cell monolayers

We determined whether the metabolites of lansoprazole, lansoprazole sulfide, and lansoprazole sulfone affected CFTR-mediated $Cl^-$ secretion. The cell monolayers grown on Snapwell inserts were incubated with the vehicle, lansoprazole, lansoprazole sulfide, or lansoprazole sulfone at a concentration of 50 µM for 24 h. As shown in Fig. 2A, incubation with lansoprazole, lansoprazole sulfide, or lansoprazole sulfone significantly decreased AVP-induced $Cl^-$ secretion representing CFTR function, compared with vehicle-treated cell monolayers. In addition to CFTR, the effect of lansoprazole and its metabolites on CaCC-mediated $Cl^-$ secretion was determined. As shown in Fig. 2B, incubation of the cell monolayers for 24 h with lansoprazole and its metabolites did not significantly affect ATP-induced $Cl^-$ secretion representing the CaCC function, compared with vehicle-treated cells. Treatment with lansoprazole, lansoprazole sulfide, or lansoprazole sulfone for 24 h did not reduce the viability of MDCK cells (Fig. 2C).

### Effect of lansoprazole metabolites on mRNA expression of channels and transporters involving $Cl^-$ secretion

Next, we tested whether the inhibitory effects of lansoprazole sulfide and lansoprazole sulfone on AVP-induced $Cl^-$ secretion were mediated by a decrease in CFTR expression. The treatment of MDCK cells for 24 h with lansoprazole and lansoprazole sulfone did not affect CFTR mRNA expression compared with vehicle-treated cells. In contrast, lansoprazole sulfide treatment reduced CFTR mRNA expression (Fig. 3A). In addition, we tested whether lansoprazole and its metabolites affected mRNA expression of TMEM16A, a major CaCC contributor to $Cl^-$ secretion in PKD cells [29, 30]. The results showed that lansoprazole and its metabolites did not significantly affect mRNA expression of TMEM16A (Fig. 3B). Since $Na^+$-$K^+$ ATPase, $Na^+$-$K^+$-2 $Cl^-$ cotransporter, and the $K^+$ channel play a role in basolateral $Cl^-$ influx, we also tested whether lansoprazole and its metabolites affect mRNA expression of these proteins. As shown in Fig. 3C–E, lansoprazole and its metabolites had no significant effect on mRNA expression of $Na^+$-$K^+$ ATPase and $Na^+$-$K^+$-2 $Cl^-$ cotransporter. However, only lansoprazole sulfide significantly reduced the mRNA expression of the $K^+$ channel.

### Effect of lansoprazole metabolites on MDCK-derived cyst growth

In PKD, renal cyst growth is mediated by $Cl^-$ secretion, leading to fluid accumulation. After finding that lansoprazole sulfide and lansoprazole sulfone inhibited CFTR-mediated $Cl^-$ secretion, we next investigated whether the metabolites affected renal cyst growth. The MDCK cell-derived cysts were treated with the vehicle, lansoprazole, lansoprazole sulfide, or lansoprazole sulfone, and cyst growth was monitored by measuring the cyst diameters. As shown in Fig. 4A, as expected, incubation with 50 µM lansoprazole for 3 and 6 days significantly reduced the cyst size compared with vehicle treatment. Lansoprazole sulfide and lansoprazole sulfone at 50 µM showed an inhibitory effect on renal cyst growth. Interestingly, the effect of lanso-

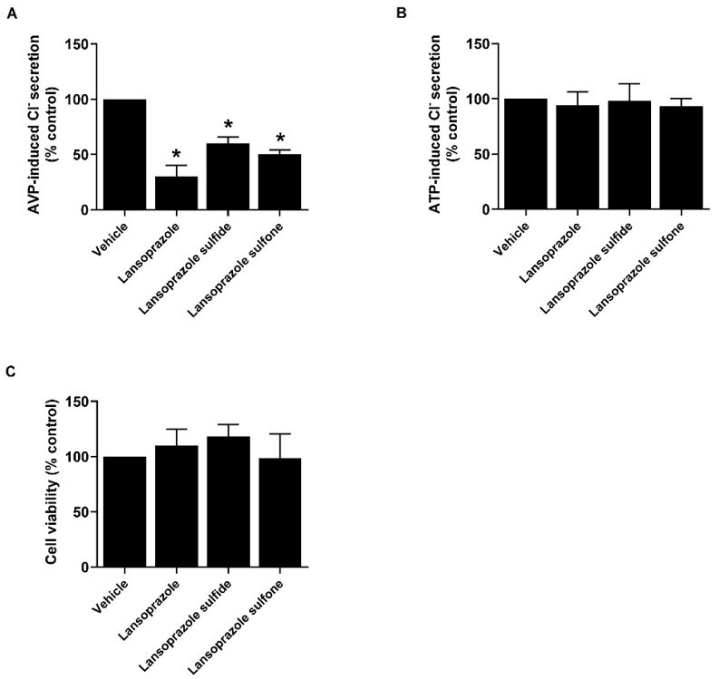



**Fig. 2** Effect of lansoprazole metabolites on $Cl^-$ secretion. MDCK cell monolayers treated with vehicle, lansoprazole, lansoprazole sulfide, or lansoprazole sulfone at a concentration of 50 μM for 24 h, followed by measurement of Isc. (A) AVP-induced $Cl^-$ secretion, (B) ATP-induced $Cl^-$ secretion, and (C) viability of MDCK cells following each treatment. Data are expressed as means ± SD of percentages of control from 3 independent experiments. * $p < 0.05$ compared with the vehicle-treated cells.

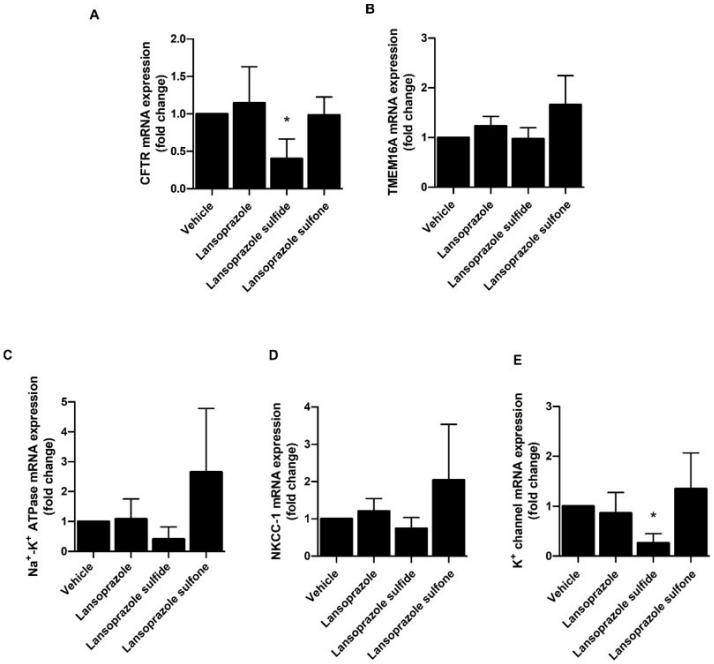



**Fig. 3** Effect of lansoprazole metabolites on mRNA of channels and transporters involved in $Cl^-$ secretion in MDCK cells. MDCK cells were treated with vehicle, lansoprazole, lansoprazole sulfide, or lansoprazole sulfone at a concentration of 50 μM for 24 h, followed by measurement of mRNA expressions of (A) CFTR, (B) TMEM16A, (C) $Na^+$-$K^+$ ATPase, (D) $Na^+$-$K^+$-2 $Cl^-$ cotransporter, and (E) $K^+$ channel. Data are expressed as means ± SD of control from 3 independent experiments. * $p < 0.05$ compared with the vehicle-treated cells.

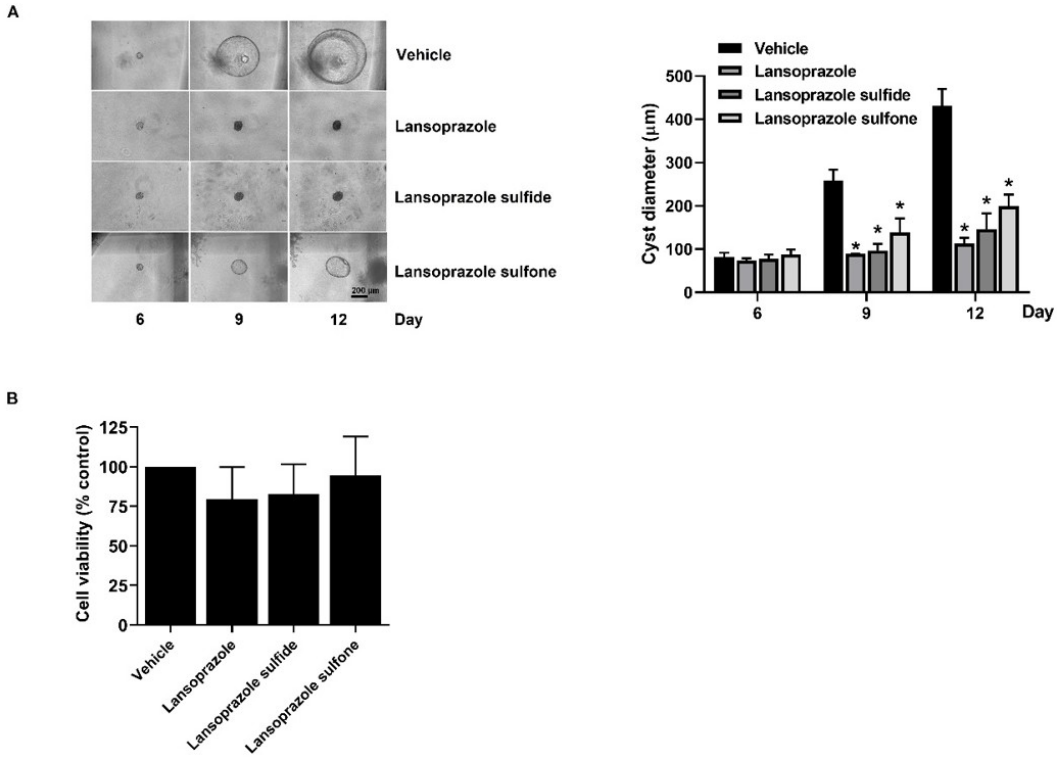



**Fig. 4** Effect of lansoprazole metabolites on MDCK-derived cyst growth. MDCK-derived cysts were incubated with serum-free media containing the vehicle, lansoprazole, lansoprazole sulfide, or lansoprazole sulfone at a concentration of 50 μM from day 6 onward. (A) Cyst growth observed by a phase-contrast light microscope and photographed on days 6, 9, and 12 at 10× magnification. (B) Viability of MDCK cells following treatment with vehicle or drugs for 6 days. Data are expressed as means ± SD from 3 independent experiments. * $p < 0.05$ compared with the vehicle-treated control.

prazole sulfone was less potent than with lansoprazole. Treating the cells with lansoprazole and its metabolites for 6 days did not significantly reduce cell viability (Fig. 4B).

### Effect of lansoprazole metabolites on cyst formation

In addition to cyst growth, the effect of lansoprazole sulfide and lansoprazole sulfone on cyst formation was determined. The MDCK cells grown in collagen gel were treated with the vehicle, lansoprazole, lansoprazole sulfide, or lansoprazole sulfone for 6 days followed by the measurement of cyst numbers. As shown in Fig. 5A, treating cells with 50 μM lansoprazole or lansoprazole sulfide significantly reduced the number of cysts compared with the vehicle, but this was not the case for lansoprazole sulfone. Next, we examined whether the inhibitory effect of lansoprazole sulfide on cyst formation was related to reduced cell proliferation. The MDCK cells were treated with the vehicle, lansoprazole, lansoprazole sulfone, or lansoprazole sulfide for 24–72 h. As expected, lansoprazole and lansoprazole sulfide significantly reduced cell proliferation compared with vehicle-treated cells. Although lansoprazole sulfone showed a decrease in cell proliferation at 24 h of treatment, the decreased cell proliferation was not significant at longer treatment periods (Fig. 5B).

## DISCUSSION

PKD is a genetic disorder causing end-stage renal failure. According to our previous data, lansoprazole, an LXR activator, inhibits CFTR-mediated $Cl^-$ secretion and subsequently suppresses the progression of cyst growth in MDCK cell-derived cysts and PKD rats [22]. The inhibitory effect of lansoprazole on renal cyst progression correlated well with reduced CFTR-mediated $Cl^-$ secretion. Lansoprazole is extensively metabolized in the liver to produce lansoprazole sulfide and lansoprazole sulfone, which can be identified in plasma [25]. We investigated whether the lansoprazole metabolites contribute to the inhibitory effect of lansoprazole on renal $Cl^-$ secretion and cyst growth.

This study reveals the contribution of lansoprazole metabolites to the inhibition of $Cl^-$ secretion and renal cyst progression. Electrophysiological data showed that lansoprazole sulfide and lansoprazole sulfone inhibited AVP-stimulated Isc, representing CFTR-

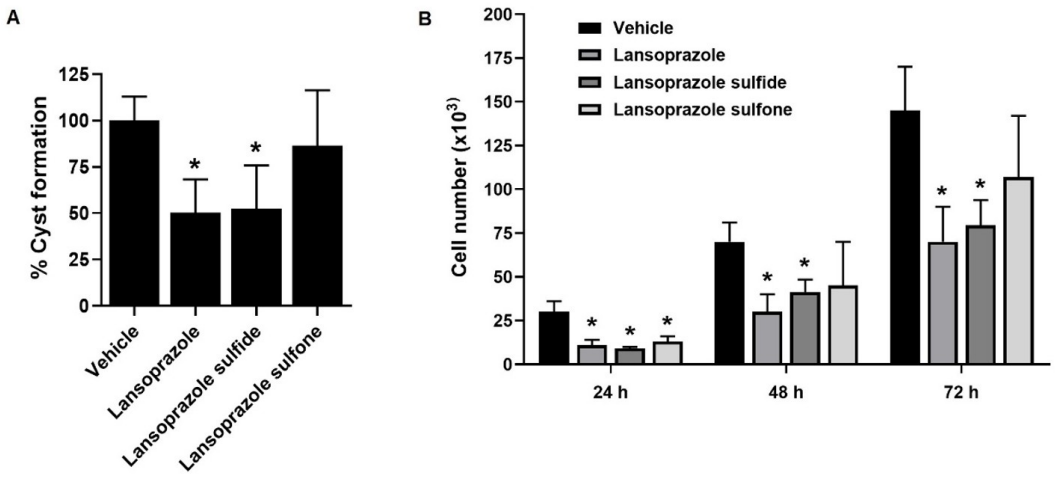



**Fig. 5** Effect of lansoprazole metabolites on MDCK-derived cyst formation. (A) Cells suspended in collagen gel incubated with vehicle, lansoprazole, lansoprazole sulfide, or lansoprazole sulfone at a concentration of 50 μM for 6 days. Cysts with diameters > 50 μm and non-cysts were counted and expressed as a percentage of cyst formation. (B) Cell proliferation determined by measurement of cell number after 24, 48, and 72 h of incubation. Data are presented as means ± SD of 3 independent experiments. * $p < 0.05$ compared with the vehicle-treated control.

mediated $Cl^-$ secretion in MDCK cell monolayers [21, 27]. Since lansoprazole and its metabolites did not affect ATP-induced $Cl^-$ secretion, this supports that the effect of lansoprazole and its metabolites on $Cl^-$ secretion has a selective effect on CFTR but not CaCCs (Fig. 2). The inhibitory effect of the metabolites on CFTR-mediated $Cl^-$ secretion was not the result of metabolite-induced cytotoxicity. The inhibitory effect on CFTR-mediated $Cl^-$ secretion of the lansoprazole metabolites might be at the transcriptional level. Our data reveal that lansoprazole sulfide reduced the mRNA expression of CFTR, whereas lansoprazole and lansoprazole sulfone did not affect the mRNA expression of CFTR. The data correlate well with our previous study, showing that lansoprazole did not affect mRNA expression of CFTR [22]. These results may imply that the lansoprazole and its sulfone metabolite inhibit the CFTR function involved in the post-transcriptional modification of CFTR, whereas its sulfide metabolite affects the transcriptional process of CFTR synthesis (Fig. 3A).

Since $Cl^-$ secretion plays a crucial role in cyst growth [27, 31–35], we hypothesized that the inhibition of $Cl^-$ secretion by lansoprazole metabolites would retard cyst growth. Indeed, our results show that both lansoprazole sulfide and lansoprazole sulfone retarded cyst growth relative to vehicle treatment. These results could be mediated, at least in part, by the decrease in $Cl^-$ secretion, leading to reduced fluid accumulation. Transepithelial $Cl^-$ secretion is involved in the transport of $Cl^-$ from the extracellular fluid into renal collecting duct cells. This is followed by efflux across the apical membrane via $Cl^-$ channels [32, 36]. An increase in intracellular $Cl^-$ concentration is established through the functions of the $Na^+$-$K^+$-2 $Cl^-$ cotransporter, $K^+$ channel, and $Na^+$-$K^+$ ATPase [36]. Therefore, the metabolites of lansoprazole might have additional effects on the basolateral transport of $Cl^-$ in renal cells. Our data reveal that lansoprazole sulfide significantly reduced mRNA expression of the $K^+$ channel but had no significant effect on other channels and transporters. In addition, lansoprazole and lansoprazole sulfone did not significantly alter the mRNA expression of the channels and transporters involved in $Cl^-$ secretion. It could be possible that the inhibitory effect of lansoprazole sulfide on $Cl^-$ secretion might be mediated, in part, by an alteration in the $Cl^-$ influx processes (Fig. 3E). However, the interpretation is based on mRNA data, and the effect of the lansoprazole metabolites on the protein expression of these transporters and channels requires further study.

In addition to cyst growth, our data demonstrate that lansoprazole sulfide significantly decreased MDCK cell-derived cyst formation while lansoprazole sulfone did not. Treating the cells with lansoprazole sulfide for 6 days did not significantly decrease cell viability, suggesting that the inhibitory effect of lansoprazole sulfide on cyst formation was not the result of cytotoxicity (Fig. 4). Cyst formation is known to result from increased cell proliferation [37–39]. As lansoprazole sulfide showed an inhibitory effect of cyst formation, therefore, we determined whether the inhibitory effect of lansoprazole sulfide on cyst formation was mediated by the suppression of cell proliferation. Lansoprazole sulfide was found to have a significant effect on cell proliferation indicating that its effect on cyst formation

might be mediated by suppression of cell proliferation (Fig. 5). Lansoprazole has been shown to reduce cell proliferation via a suppression of mTOR/S6K activation, a key factor in cell proliferation regulation [22]. We speculate that lansoprazole sulfide might inhibit cell proliferation via mTOR/S6K activation, resulting in reduced cyst formation. Although lansoprazole sulfone showed an inhibitory effect on $Cl^-$ secretion, it produced less effect on cyst growth, compared with lansoprazole and lansoprazole sulfide. Cyst growth is contributed by processes of cell proliferation and $Cl^-$ secretion-induced fluid accumulation. This notion might support that lansoprazole sulfone shows less effectiveness on cyst growth compared with lansoprazole and its sulfide metabolite. Taken together, our data reveal that lansoprazole sulfide is a major active metabolite of lansoprazole for suppression of renal cyst progression.

## CONCLUSION

This study demonstrates that lansoprazole and its sulfide metabolite inhibit CFTR-mediated $Cl^-$ secretion but not CaCC-induced $Cl^-$ secretion. These effects cause a decrease in cyst growth derived from MDCK cells. This study demonstrates that lansoprazole sulfide found in plasma might contribute to the inhibitory effect of lansoprazole on the progression of PKD.

### Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.044.

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## Appendix A. Supplementary data

**Table S1** Primers for qPCR.

| Target | Forward Primer 5′–3′ | Reverse Primer 3′–5′ | Amplicon size (bp) |
|---|---|---|---|
| TMEM16A | 5′-AGGTTCCCCTCGTACTCCTC-3′ | 5′-TCACCCCAGTTGCTGAACTC-3′ | 305 |
| CFTR | 5′-TGCCATGATGCTCTTCGAGT-3′ | 5′-GTGGTTGGTTCCCCTTCCAG -3′ | 274 |
| $Na^+$-$K^+$ ATPase | 5′-GTGAGTGAGGGCCCAAACAG-3′ | 5′-CTGCACCACCACGATACTGA-3′ | 186 |
| KCh | 5′-AGGATGGCAAGACTCCAACTC-3′ | 5′-AACACACAGGTGTCTCGACC-3′ | 178 |
| NKCC | 5′-AGGATGGCAAGACTCCAACTC-3′ | 5′-AAAGTAGCCATCGCTCTCCG-3′ | 286 |
| GAPDH | 5′-CCATGTTTGTGATGGGCGTG -3′ | 5′-CATGGACGGTGGTCATGAGG -3′ | 153 |