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CHENODEOXYCHOLIC ACID INHIBITS CFTR-MEDIATED CHLORIDE SECRETION IN RENAL TUBULAR CELLS

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ABSTRACT

Chenodeoxycholic acid (CDCA), a primary bile acid, has been reported to activate cystic fibrosis transmembrane conductance regulator (CFTR)-mediated Cl^- secretion in intestinal cells. However, there have not been any reports on the role of bile acid in the regulation of CFTR-mediated Cl^- secretion in renal cells. In this study, we revealed that CDCA reduced CFTR-mediated Cl^- secretion in Madin-Darby canine kidney (MDCK) cells, renal tubular cells. Incubation MDCK cell monolayers with 20 μM CDCA for 48 h led to a decrease in [Arg^8]-vasopressin (AVP)-induced Cl^-current (I_{Cl^-}). The inhibition on I_{Cl^-} was not a result of CDCA-induced toxicity as shown by the treatment of the cells with CDCA for 72 h did not alter cell viability. Interestingly, acute incubation of MDCK cell monolayers with CDCA did not reduce I_{Cl^-}. Furthermore, basolateral membrane permeabilization of MDCK cell monolayers still decreased apical I_{Cl^-}, indicating that the target of CDCA might be located at the apical membrane. Western blot analysis revealed that inhibition of Cl^- secretion occurred via a decrease in CFTR protein expression. These results reveal that CDCA inhibits Cl^- secretion in renal tubular cells via a decrease in CFTR protein expression.

Keywords: bile acid, MDCK, chloride channel, chloride secretion, kidney

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Introduction

Cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel is a member of the ATP-binding cassette (ABC) superfamily.[1] CFTR protein is mainly expressed in the apical membrane of renal cells of proximal tubule, distal tubule, and collecting duct.[2,3] Although CFTR expressed along renal tubular epithelial cells, its physiological role in renal tubular cells is not fully understood. Dysfunction of CFTR does not present major renal impairments; however, renal capacity to concentrate and dilute urine are altered.[4] Previous studies have revealed the roles of CFTR in regulation of membrane proteins such as potassium channel (ROMK) and epithelial sodium channel (ENaC).[5-8] Importantly, the role of CFTR as the main contribution in mediating Cl^- and fluid secretion in a renal cyst of polycystic kidney disease (PKD) is reported.[9,10] Over activation of CFTR in PKD is a key factor for PKD progression. Previous studies report that arginine vasopressin (AVP) binds to V_2 receptor leads to activation of protein kinase A (PKA), resulting in activation of the CFTR.[11] Therefore, inhibition of CFTR using V_2 receptor antagonist has been developed as a drug for treatment of PKD.[12] Generally, CFTR is upregulated by PKA phosphorylation of the R domain, resulting in transepithelial Cl^- transport.[1] Cl^-enters cells across the basolateral membrane via the Na^+-K^+-$2Cl^-$ cotransporter and exits across the apical membrane into the lumen through the CFTR chloride channel. Moreover, an active Cl^- secretion also requires function of Na^+-K^+ ATPase and K^+ channel.[13]

Bile acids play a crucial role in the digestion and absorption of lipid.[14] Additionally, their regulatory roles in cellular signaling have been recognized.[15] Bile acids exert direct genomic effects via the bile acid-specific nuclear farnesoid X receptor (FXR).[16,17] They can also act via the bile acid-specific membrane G protein-coupled receptor such as Takeda G protein-coupled receptor 5 (TGR5).[18] There is evidence that chenodeoxycholic acid (CDCA), a primary bile acid, has prosecretory actions in intestinal cells. CDCA stimulates CFTR-mediated Cl^- secretion in colonic cells.[19] CDCA-mediated activation of CFTR-dependent Cl^- secretion requires the epidermal growth factor receptor (EGFR) and Ca^{2+} in colonic cells.[20] However, there is no reports concerning the role of bile acids in the regulation of CFTR-mediated Cl^- secretion in renal cells. Here, we investigated the effect of CDCA on CFTR-mediated Cl^- transport in renal collecting duct cells using the Madin–Darby canine kidney (MDCK) cell line as a model to study.

Materials and methods

Chemical reagent and antibodies

Bile acid chenodeoxycholic acid (CDCA), amiloride, amphotericin B, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (MO, USA). [Arg^8]-vasopressin (AVP) was purchased from Ferring Pharmaceuticals (Bangkok, Thailand). A protease inhibitor cocktail was purchased from Roche Diagnostics (Mannheim, Germany). Rabbit anti-CFTR antibody, monoclonal rabbit anti-β-actin, and goat horseradish peroxidase (HRP)-conjugated anti-rabbit IgG antibodies were obtained from Cell Signaling Technology (MA, USA). All other chemicals used were of analytical grade from commercial sources.

Cell culture

Madin–Darby canine kidney (MDCK) cells were obtained from Dr. David N. Sheppard (University of Bristol, Bristol, UK). The cells were grown in DMEM/F-12 medium supplemented with 5% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin and Insulin-Transferrin-Selenium (ITS) at 37°C in a humidified incubator under an atmosphere of 5% CO_2. For the electrophysiological study, MDCK cells were cultured on Snapwell inserts (Corning Life Sciences) until the transepithelial resistance (TER) of the confluent monolayer was higher than 1,000 $\Omega \cdot cm^2$.

Measurement of CFTR-mediated Cl^- transport in intact cell monolayers

CFTR-mediated Cl^- transport was measurement by short-circuit current (I_{SC}) using VC600 voltage clamp (Physiologic Instruments, CA, USA) as previously described.[21] Briefly, MDCK cell monolayers were mounted into hemi-chamber in an Ussing chamber and bathed with physiological buffer containing (in mM) 117 NaCl, 25 $NaHCO_3$, 11 D-glucose, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, and 1.2 KH_2PO_4 which was maintained at 37°C and pH at 7.4 by bubbled the buffer with a 95% O_2–5% CO_2 gas mixture. The positive short-circuit current represents both Na^+ absorption and Cl^- secretion. Then, amiloride, an ENaC blocker, (100 µM) was added into the apical bathing solution to inhibit ENaC-mediated Na^+ current. In order to measure the CFTR-mediated Cl^- currents (I_{Cl}), AVP (20 nM) was added to the basolateral side of the chamber.

Measurement of apical Cl^- transport

MDCK cell monolayers were bathed with asymmetric buffer. The basolateral hemichamber was filled with buffer containing (in mM) 130 NaCl, 2.7 KCl, 1.5 KH_2PO_4), 1 $CaCl_2$, 0.5 $MgCl_2$, 10 Na-HEPES, and 10 glucose, whereas the apical hemichamber was filled with a buffer in which 65 mM NaCl was replaced by sodium 65 mM gluconate to generate a Cl^- gradient. Amphotericin B (250 µg/ml) was added to permeabilize the basolateral membrane of the cell monolayer for 30 min followed by inhibition of ENaC-mediated Na^+ flux. After I_{SC} was stable, apical I_{Cl} was measured following the addition of AVP (20 nM) to the basolateral chamber.

Western blot analysis

MDCK cells grown on 6-well plates were lysed with Triton X-100 lysis buffer containing 50 mM Tris·HCl, 150 mM NaCl, 1 mM NaF, 1 mM EDTA, 1 mM sodium orthovanadate, 100 µM PMSF, protease inhibitor cocktail, and 1% Triton X-100 for 20 min at 4°C. After that, the harvested protein was centrifugated at 17,000 xg for 20 min at 4°C. Supernatant cell lysate proteins were separated by 8% SDS-PAGE and transferred to a Hybond-ECL nitrocellulose membrane (Amersham Biosciences). The membrane was incubated in 10% non-fat milk for 2 h followed by incubation with rabbit polyclonal antibodies against CFTR (Catalog No. 2269; 1:1,000 dilution) or β-actin (Catalog No. 4970; 1:1,000 dilution) overnight at 4°C. The membrane was incubated with secondary HRP-conjugated goat anti-rabbit IgG (Catalog No. AP132P; 1:10,000 dilution) for 1 h at room temperature. Rabbit anti-CFTR antibody and monoclonal rabbit anti-β-actin antibodies were obtained from Cell Signaling Technology (MA, USA). Goat horseradish peroxidase (HRP)-conjugated anti-rabbit IgG antibody was purchased from Sigma-Aldrich (Darmstadt, Germany). Immunoreactive bands were detected by chemiluminescence, recorded on Amersham® hyperfilm (GE Healthcare) using a Western lightning plus-ECL detection kit (Millipore), and quantified using ImageJ analysis software. Band intensity of immunoblot of the protein of interest was normalized with that of β-actin.

Cell viability assay

Cell viability was determined using an MTT assay. In brief, MDCK cells grown on 96-well plates, and the confluent cells were treated with 20 µM CDCA for 24-72 h. Then, the treated cells were incubated with 100 µl medium containing MTT (0.5 mg/ml) for 2.5 h in a humidified 95% incubator with 5% CO_2 gas at 37°C. After the removal of the medium, a 100 µl aliquot of dimethyl sulfoxide (DMSO) was added to dissolve the formazan. Then, the absorbance at 570 nm was measured by a microplate reader (Spark™ 10M spectrophotometer, TECAN).

Statistical analysis

Results are presented as means ± S.D. Statistical differences between control and treatment were determined using a Student t-test and one-way ANOVA followed by Tukey's test. A level of p-value < 0.05 was significant.

Results

Effect of CDCA on Cl^- transport in intact MDCK cell monolayers

CFTR-mediated Cl^- secretion in renal collecting duct cells was measured by Ussing chamber technique as previously reported.[22,23] Firstly, we investigated the effect of bile acid, CDCA, on CFTR-mediated Cl^- secretion in MDCK cell monolayers by measurement of AVP-induced I_{Cl^-}. MDCK cell monolayers treated with 20 μM CDCA for 48 h at the basolateral side significantly decrease AVP-induced I_{Cl^-}. Incubation of the cells with 10 μM CDCA-treated for 48 h at the basolateral side did not significantly change in AVP-induced I_{Cl^-} when compared with vehicle control (Fig. 1A). Since acute of CDCA on biological activity has been reported[18], we tested whether the inhibitory effect of CDCA on I_{Cl^-} was revealed in short time incubation. MDCK cell monolayer was incubated with vehicle or 20 μM CDCA for 30 min at the basolateral side followed by measurement of the I_{Cl^-} induced by AVP. Our results showed that CDCA did not affect I_{Cl^-} (Fig. 1B).

Next, we determined whether apical Cl^- transport was the target of CDCA's effect, MDCK cell monolayers were treated with vehicle (DMSO) or 10 and 20 μM CDCA for 48 h at the basolateral side. Then, the apical I_{Cl^-} measurement was performed following basolateral membrane permeabilization. As shown in Fig. 2, the inhibitory effect of CDCA on I_{Cl^-} was remained indicating the apical Cl^- transport is the target of CDCA.

Effect of CDCA on CFTR expression

MDCK cells were treated with vehicle and CDCA (10 and 20 μM) for 48 h followed by the measurement of CFTR protein expression. As shown in Fig. 3, CDCA at 20 μM significantly decreased CFTR protein expression compared with vehicle-treated cells. The data suggest that the inhibitory effect of CDCA on apical Cl^- transport was mediated by a decrease in CFTR protein expression.

Effect of CDCA on MDCK cell viability

To investigate whether the inhibitory effect of CDCA on I_{Cl^-} was caused by CDCA-induced cytotoxicity, cell viability was measured by using MTT assay. The results showed that incubation MDCK cells with 20 μM CDCA for 24, 48, and 72 h did not alter cell viability compared with vehicle control (Fig. 4). Therefore, the inhibitory effect of CDCA on Cl^- secretion did not drive from the cytotoxicity on MDCK cells.

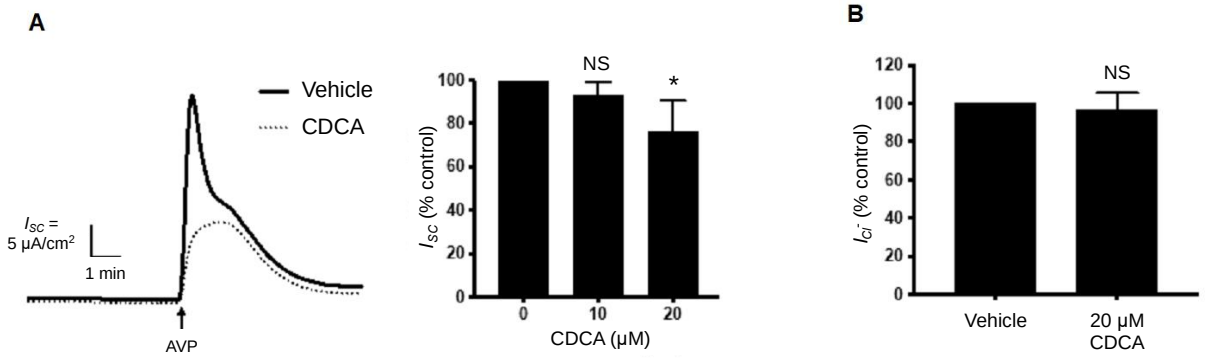

Figure 1 (A) Effect of CDCA on AVP-induced Cl^- secretion in MDCK cell monolayers. MDCK cell monolayers were incubated with CDCA (0-20 μM) for 48 h. Data are shown as mean ± S.D. of 7 independent experiments (3-6 monolayers/experiment). (B) MDCK cell monolayers were incubated with vehicle or 20 μM CDCA for 30 min. Data are shown as means ± S.D. of 3 independent experiments (3 monolayers/experiment). NS; not significant and *p-value < 0.05 compared with the vehicle-treated cells.

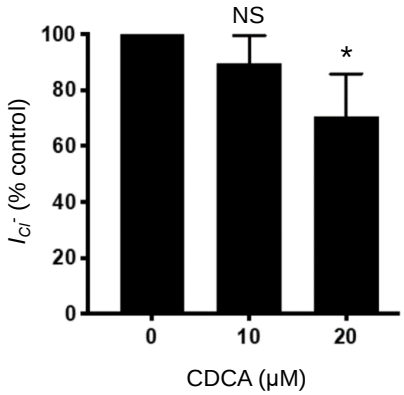

Figure 2 Effect of CDCA on apical I_{Cl^-} in MDCK cell monolayers. Cell monolayers incubated with vehicle, 10 and 20 μM CDCA for 48 h. Data are presented as mean ± S.D. of 3 independent experiments (3-6 monolayers/experiment) NS; not significant and *p-value < 0.05 compared with the vehicle-treated cells.

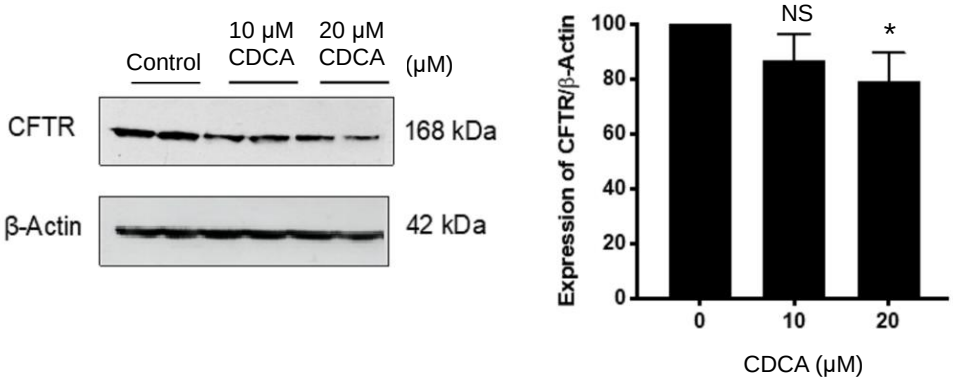

Figure 3 Effect of CDCA on CFTR protein expression in MDCK cells. MDCK cells were incubated with CDCA (10 and 20 μM) as indicated concentration for 48 h. Representative immunoblots of CFTR and β-actin expressions are shown. Data are shown as mean ± S.D. of densitometry obtained from 5 independent experiments. NS; not significant and *p-value < 0.05 compared with the vehicle-treated cells.

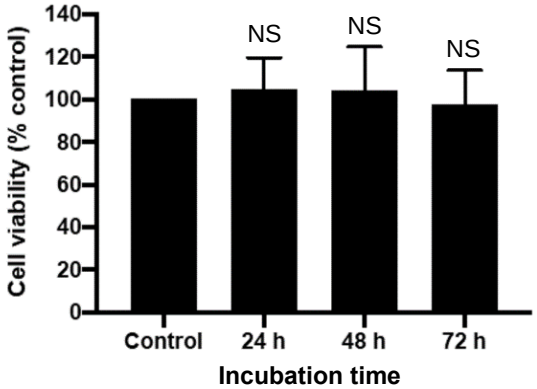

Figure 4 Effect of CDCA on MDCK cell viability. MDCK cells were treated with 20 μM CDCA for 24-72 h followed by measurement of cell viability. Data are expressed as mean of percentages of control ± S.D. from 3 independent experiments. NS; not significant compared with the vehicle-treated control cells.

Discussion

Bile acid is a potential molecule regulating intestinal cystic fibrosis transmembrane conductance regulator (CFTR)-mediated Cl^- transport.[19] Lack of information is concerning their effect in renal CFTR-mediated Cl^- transport, the effect of CDCA on CFTR was investigated in renal cells. We reveal the role of CDCA, a primary bile acid, in the regulation of the CFTR-mediated Cl^- secretion in renal collecting duct cells.

The effect of CFTR-mediated Cl^- transport in renal tubular cells was determined in MDCK cells that show characteristics of collecting duct cells and express CFTR.[2,3] Additionally, MDCK cells can form a monolayer and develop efficient transepithelial resistance for a suitable model for transepithelial transport study.[23,24] CFTR is stimulated by AVP-induced PKA activation[1], we used AVP as a ligand to stimulate CFTR-induced Cl^- transport in collecting duct cells. The inhibitory effect of CDCA on Cl^- transport was not a result of cytotoxicity as CDCA did not reduce the viability of MDCK cells. The inhibitory effect of CDCA on Cl^- secretion in intact MDCK cell monolayer was similar as found in basolateral membrane in permeabilized MDCK monolayers. These data imply that the apical CFTR-mediated Cl^- secretion might be a target of CDCA. The plasma concentration of CDCA under pathological condition such as hepatitis is about 100-400 μM[25]; therefore, these results might imply that CDCA under pathological condition such as hepatitis could interfere with CFTR function.

CDCA has been reported to stimulate CFTR function by the activation of TGR5, a bile acid-specific membrane G protein-coupled receptor. The effect of TGR5 activation on CFTR function was found within 10 min.[26] Our data showed that the inhibitory effect of CDCA on Cl^- transport was not found following 30 min incubation. These results imply that TGR5 might not be required for the inhibitory effect of CDCA on Cl^- secretion in the renal tubular cell. To rule out the involvement of TGR5 in the inhibitory effect of CDCA, the effect of CDCA on CFTR under inhibition of TGR5 needs to be revealed. Our results showed that the inhibitory effect of CDCA on Cl^- secretion was found following 48 h of incubation. These data indicate that the effect of CDCA is the chronic effect and requires time for the action. CDCA is a potent endogenous agonist of FXR which is a transcriptional factor regulating several proteins.[27] It is possible that activation of FXR may be involved in the chronic effect of CDCA.

The Cl^- transport function of CFTR is depended on its membrane expression and the open probability.[28] We examined whether the decrease in CFTR-mediated Cl^- transport was via reduced CFTR expression. Our data demonstrated that CDCA decreased the total protein expression of CFTR, indicating CDCA treatment downregulates CFTR expression. It is unclear whether CDCA affects the transcriptional or translational step of CFTR synthesis. Besides the expression level of CFTR contributing to CFTR transport function, we cannot rule out the regulatory role of CDCA on the open probability of CFTR. Taken together, the present study demonstrates the role of bile acid CDCA in the regulation of CFTR-mediated Cl^- secretion in renal cells. CDCA inhibits Cl^-secretion by inhibiting protein expression of CFTR. Since over activation of CFTR involves in the progression of renal cyst expansion in polycystic kidney disease (PKD)[29], CDCA might be a potential agent for inhibition of renal cyst expansion in PKD. To support the therapeutic application of CDCA in PKD treatment, additional studies concerning the effect of CDCA on cyst development are required.

Conclusion

The present study delineates the novel role of CDCA in regulating chloride transport in renal collecting duct cells. The underlying mechanism of CDCA in inhibiting renal Cl^- transport involves a down-regulation of CFTR protein expression and subsequent decrease in CFTR-mediated Cl^-secretion.

Acknowledgments

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Conflict of Interest

None to declare.

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