Short Communication

Colistin is substrate of the carnitine/organic cation transporter 2 (OCTN2, SLC22A5)

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ABSTRACT

Colistin is a polycation antibiotic used for the treatment of multidrug-resistance (MDR) gram-negative infections; nevertheless, its use is often limited by the high incidence of renal damage. The mechanism underlying colistin-induced nephrotoxicity is not known, but perhaps related to its accumulation in the renal cortex upon extensive reabsorption from the nascent urine. Because little is known about the membrane transport of colistin, the purpose of the present study was to characterize better the transport system involved in colistin renal handling by using HEK293 cells stably transfected with the main organic cation transporters expressed at the apical membrane of the proximal tubule. [^{14}C]Colistin was transported by

the carnitine/organic cation transporter 2 (OCTN2, SLC22A5) but not by the organic cation transporter 1 (OCT1) and N1 (OCTN1). Non-labeled colistin inhibited the OCTN2-mediated transport of [^3H]L-carnitine in a non-competitive manner and that of [^{14}C]tetraethylammonium bromide ([^{14}C]TEA) in a competitive manner. Unlike that of [^3H]L-carnitine, the [^{14}C]colistin OCTN2-mediated uptake was Na^+ -independent. When endogenous OCTN2-mediated colistin transport was inhibited by co-incubation with L-carnitine, primary mouse proximal tubular cells were fully protected from colistin toxicity, suggesting that colistin toxicity occurred upon intracellular accumulation.

Introduction

Colistin, a member of the polymyxin family, represents the mainstay for the treatment of multidrug-resistance (MDR) gram-negative bacterial infections (Falagas and Kasiakou, 2006). The main limitation of colistin treatment is nephrotoxicity (Deryke et al., 2010; Garonzik et al., 2011; Dalfino et al., 2012). The mechanism underlying colistin nephrotoxicity is not known. It has been suggested that colistin acts in a detergent-mode, increasing membrane permeability and, in turn, inducing cell swelling and lysis (Falagas and Kasiakou, 2006). More recently, Yun and colleagues proposed, by using fluorescent-probed polymyxins, that the renal damage induced by polymyxins is exerted upon entering the cells (Yun et al., 2015).

Colistin enters proximal tubular cells from the apical side upon extensive reabsorption from the nascent urine (Li et al., 2003). Due to its polycationic structure colistin poorly diffuses across the plasma membrane so that it requires facilitative transport systems to enter the cells. Studies in isolated perfused rat kidney revealed that tetraethylammonium (TEA) and the dipeptide glycine-glycine (Gly-Gly) could increase colistin clearance, indicating that renal reabsorption of colistin may involve organic cation transporters and peptide transporters (Ma et al., 2009). In line with this, the oligopeptide transporter 2 (PEPT2, SLC15A2) was recently shown to transport [^3H]polymyxin B1 and the uptake was substantially abolished by co-incubation with non-labeled colistin (Lu et al., 2016).

The present work identified the carnitine/organic cation transporter 2 (OCTN2, SLC22A5) as a further transport system of colistin at the

apical side of proximal tubule cells and demonstrated that colistin toxic activity occurred at the intracellular level.

Materials and Methods

Reagents. Radiolabeled colistin [methyl- ^{14}C]methylated ([^{14}C]colistin) was purchased from American Radiolabeled Chemicals (St. Louis, MO). L-[methyl- ^3H]carnitine hydrochloride ([^3H]L-carnitine) was synthesized by Amersham Life Sciences (Piscataway, NJ). [^{14}C]Tetraethylammonium Bromide ([^{14}C]TEA) was provided by Perkin Elmer (Boston, MA). Non-labeled colistin, L-carnitine and TEA were provided by Sigma-Aldrich (St. Louis, MO). All cell culture reagents were purchased from Gibco (Parsippany, NJ).

Cell Lines. Wild-type HEK293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 mg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2 . OCT1-HEK293 cells, previously characterized, were supplemented with Geneticin G-418 (600 $\mu\text{g}/\text{ml}$) (Thévenod et al., 2013). OCTN1- and OCTN2-HEK293 cells were maintained in 800 $\mu\text{g}/\text{ml}$ Geneticin G-418 (Tamai et al., 1997, 2001).

Animals. Animal experiments and protocols conformed to the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health) and the Swiss animal protection laws and were approved by the Cantonal Veterinary Office in Zurich, Switzerland (study number 2012058).

Isolation of Primary Proximal Tubular Cells from Mouse Kidney. Primary proximal tubular cells were isolated from kidneys of female C57/BJ mice as described previously (Gai et al., 2010, 2016). Briefly, kidney cortices were dissected, sliced, minced, and digested in 0.25% collagenase solution (Life Technologies BRL, Grand Island, NY). Collagenase was neutralized with Bovine Serum Albumin (BSA). The suspension was filtered, centrifuged (72.4g for 5 minutes) to pellet the tubules, washed with 10 ml of medium, centrifuged, and washed twice more. The final pellet, containing of renal tubules, was resuspended in culture medium (REBM bullet kit; Clonetics, Basel, Switzerland), plated onto culture dishes and incubated at 37°C in a humidified atmosphere of 5% CO_2 .

Transport Studies in Intact Cells. Uptake of radiolabeled compounds was measured using a protocol designed for uptake determination in cells (Schroeder

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ABBREVIATIONS: OCT1, organic cation transporter; OCTN2, carnitine/organic cation transporter 2; SLC, solute carrier; TEA, tetraethylammonium bromide.

et al., 1998; Visentin et al., 2015). Cells were seeded in 35-mm dishes coated with 0.1 mg/ml poly-D-lysine (Corning, Bedford, MA). Cells were washed and equilibrated in transport buffer (116.4 mM NaCl, 5.3 mM KCl, 1 mM NaH_2PO_4 , 0.8 mM MgSO_4 , 5.5 mM D-glucose and 20 mM Hepes/Tris, pH 7.4) at 37°C then the buffer was aspirated and transport buffer containing the radiolabeled substrate was added. Uptake was stopped by quick aspiration followed by extensive washing with ice-cold transport buffer. Transport in Na^+ -free buffer was measured as described above, in a transport buffer in which NaCl was replaced with choline chloride (116.4 mM choline chloride, 5.3 mM KCl, 1 mM KH_2PO_4 , 0.8 mM MgSO_4 , 5.5 mM D-glucose and 20 mM Hepes/Tris, pH 7.4).

Cells were solubilized and intracellular radioactivity was assessed by liquid scintillation counting. Protein content was determined by the bicinchoninic acid protein assay (Interchim, Montluçon Cedex, France). For Dixon's analysis, OCTN2-independent influx was determined in WT-HEK293 cells and subtracted from total uptake to quantify the OCTN2-mediated influx. Uptake is expressed as picomoles of substrate per milligram of protein.

To evaluate colistin accumulation as a function of the extracellular concentration of L-carnitine, proximal tubular cells were seeded in 96-well plates at a density of 0.5×10^4 cells/well in a medium containing [^{14}C]colistin at the extracellular concentration of 100 μM . After 48 hour exposure cells were extensively washed with ice-cold transport buffer and then processed, as described previously, for radioactivity and protein content.

Viability Assay. Primary proximal tubular cells were seeded in 96-well plates at a density of 0.5×10^4 cells/well in a medium containing a fixed concentration of colistin (100 μM), and varying the concentration of L-carnitine (0.075, 0.3, 1.2, 5 mM). Viability rates were quantified after 48 hour of exposure by alamarBlue assay (Thermo Fisher Scientific, Carlsbad, CA). Fluorescence ($\lambda_{\text{ex}} = 520$ nm, $\lambda_{\text{em}} = 580$ nm) was measured on the GloMax Multi Detection System (Promega, Madison, WI).

Statistical Analysis. Statistical comparisons were performed from three independent measurements, at least, using GraphPad Prism (version 5.0 for Windows; GraphPad Software).

Results

Impact of OCT1, OCTN1 and OCTN2 Expression on the [^{14}C]colistin Net Uptake in HEK293 Cells. To understand the role of the organic cation transporters expressed at the luminal side of the proximal tubular cells in colistin uptake, HEK293 cells stably transfected with OCT1 (a), OCTN1 (b) or OCTN2 (c) were incubated at the indicated times with [^{14}C]colistin at an extracellular concentration of 1 μM . As illustrated in Fig. 1, [^{14}C]colistin was transported only by OCTN2 (Fig. 1c). After 10 minute incubation the intracellular level of [^{14}C]colistin in OCTN2-HEK293 was significantly higher than that in WT-HEK293 cells (9.6 ± 1.2 vs. 7.2 ± 0.7 , $P = 0.04$).

Effect of Colistin on OCTN2-Mediated Transport. The main physiologic role of OCTN2 in the kidney is the tubular reabsorption of carnitine (Nezu et al., 1999). OCTN2 can also transport cations such as tetraethylammonium bromide (TEA). Initial uptake of [^3H]L-carnitine (Fig. 2a) and [^{14}C]TEA (Fig. 2c) was measured in OCTN2- and WT-HEK293 cells. It can be seen the uptake slopes of [^3H]L-carnitine and [^{14}C]TEA was linear over the first 20 seconds and 3 minutes, respectively, reflecting the unidirectional flux (influx) of the radiolabeled compounds into these cells. To study whether colistin could inhibit the OCTN2-mediated transport of [^3H]L-carnitine and [^{14}C]TEA, the inhibitory effect of non-labeled colistin on the influx of [^3H]L-carnitine and [^{14}C]TEA was measured. To further study the nature of the inhibition, the influx of [^3H]L-carnitine or [^{14}C]TEA at three different concentrations of substrate as a function of extracellular non-labeled colistin at the indicated concentrations was measured (Dixon analysis). In Fig. 2b it can be seen that the lines intersect on the x -axis ($-K_i$), indicating that colistin could inhibit the OCTN2-mediated [^3H]L-carnitine transport in a non- or un-competitive manner ($K_i = 22.7 \pm 0.9$ mM), suggesting that colistin and [^3H]L-carnitine did not bind to a common binding site. Figure 2d indicates that the nature of the inhibition

Fig. 1. Time course of the net uptake of [^{14}C]colistin in transfected cells. Uptake of [^{14}C]colistin at the extracellular concentration of 1 μM in OCT1- (A), OCTN1- (B), OCTN2-HEK293 cells (C) was measured as the function of the time and compared with that in wild-type cells. Data represent the mean $\pm$ S.E.M. from three independent experiments.

of [^{14}C]TEA by colistin was competitive and the K_i value could be estimated to be ~ 2.5 mM.

Effect of Na^+ on the Colistin Uptake Mediated by OCTN2. OCTN2 mediates the uptake of the zwitterionic substrate L-carnitine in a Na^+ -dependent manner, whereas the transport of TEA is Na^+ -independent (Tamai, 2013). To investigate whether colistin OCTN2-mediated transport was driven by Na^+ , the uptake of [^{14}C]colistin at an extracellular concentration of 1 μM was assessed in the presence or absence of Na^+ . Figure 3 shows that [^{14}C]colistin uptake mediated by

Fig. 2. Effect of non-labeled colistin on OCTN2-mediated transport. Initial uptake of $[^3\text{H}]\text{L-carnitine}$ (A) and $[^{14}\text{C}]\text{TEA}$ (C) at the extracellular concentration of 0.1 and 50 μM , respectively, in OCTN2- and WT-HEK293 cells. $[^3\text{H}]\text{L-carnitine}$ (15 seconds) (B) and $[^{14}\text{C}]\text{TEA}$ (2 minutes) (D) influx at the indicated extracellular concentrations as a function of increasing extracellular concentrations of non-labeled colistin was measured in OCTN2 transfected cells. Uptake in WT-HEK293 cells was subtracted from that in transfected cells to define the OCTN2-specific transport. The line was derived by plotting $1/v$ against the concentration of colistin (inhibitor) (Dixon analysis).

OCTN2 was similar in the presence and absence of Na^+ in the transport buffer.

Contribution of OCTN2 to Colistin Accumulation and Toxicity in Primary Proximal Tubular Cells. To understand the contribution of endogenous OCTN2 to colistin cellular uptake and toxicity, the accumulation and toxic effect of colistin in mouse renal primary proximal tubular cells was assessed by co-incubation with different extracellular concentrations of L-carnitine (0.075, 0.3, 1.2, and 5 mM). Figure 4 shows that, at an extracellular concentration of 100 μM , colistin alone reduced cell viability by $\sim 25\%$. The intracellular accumulation (H_0 , slope = 0, $P = 0.005$) and the toxic effect (H_0 , slope = 0, $P = 0.01$) of colistin significantly decreased as the extracellular concentration of L-carnitine increased. Cells exposed to the highest extracellular concentration of L-carnitine (5 mM) showed 100% viability and coincided with $\sim 30\%$ less intracellular $[^{14}\text{C}]\text{colistin}$, in line with the K_i value of colistin for the OCTN2-mediated L-carnitine transport.

Discussion

Among the organic cation transporters expressed at the luminal side of the proximal tubule, the carnitine/organic cation transporter 2 (OCTN2, SLC22A5) could transport colistin at a pharmacologically relevant concentration (1 μM), suggesting that this carrier might contribute to the renal handling of colistin in the clinical setting (C_{max} , 1–10 μM) (Couet et al., 2011; Karvanen et al., 2013). The model for OCTN2-mediated

transport envisages two distinct but overlapping binding sites for carnitine and cationic compounds (Ohashi et al., 2002). OCTN2 transports zwitterionic carnitine in a Na^+ -dependent high-affinity manner, whereas organic cation transport (e.g., Tetraethylammonium bromide (TEA)) is a low-affinity, Na^+ -independent process (Tamai, 2013). Colistin is likely to interact with the cationic binding site of the pocket, as suggested by the competitive inhibition of non-labeled colistin with $[^{14}\text{C}]\text{TEA}$ at the OCTN2 level. TEA co-administration could reduce colistin reabsorption in isolated perfused rat kidney. The authors speculated that OCTN1 mediates, at least in part, colistin renal reabsorption (Ma et al., 2009). Our data indicate that OCTN2 and not OCTN1 can transport colistin and that such transport is Na^+ -independent.

Previously colistin was shown to inhibit the transport mediated by PEPT2 at clinically relevant concentrations. The same investigators, in the process of screening different carriers for colistin transport, could not observe any significant inhibition of OCTN2-mediated L-carnitine transport when co-incubated with colistin. This was probably due to the relatively low concentration of colistin (10 μM) used for the cis-inhibition assay, as indicated by the relatively high K_i of colistin for $[^3\text{H}]\text{L-carnitine}$ transport reported in the present study (Lu et al., 2016). Cis-inhibition data from experiments with primary cultured mouse proximal tubular cells suggests that OCTN2 gives a relevant contribution to colistin reabsorption, in line with the work from Ma and colleagues in isolated perfused rat kidney (Ma et al., 2009).

Fig. 3. OCTN2-mediated transport in Na⁺-free buffer. OCTN2-mediated uptake of [³H]L-carnitine and [¹⁴C]colistin at the extracellular concentration of 1 μM. Uptake in WT-HEK293 cells was subtracted from that in transfected cells to define the OCTN2-specific transport. Results are expressed as percentage of the uptake values in transport buffer containing Na⁺. Data represent the mean ± S.E.M. from three independent experiments.

The physiologic role of OCTN2, at the kidney level, is to mediate the reabsorption of carnitine from the glomerular filtrate (Tamai et al., 1998, 2001). Drugs that alter the expression and/or activity of OCTN2 have been associated with carnitine deficiency (Heuberger et al., 1998; Ganapathy et al., 2000; Diao et al., 2010; Lancaster et al., 2010). When the ratio C_{max}/K_i is considered to forecast the possibility of a clinical drug-drug interaction (>0.1), based upon the relatively low C_{max} achievable with the current recommended dosage (1–10 μM) and the high K_i for carnitine transport calculated (~22 mM), colistin-induced carnitine deficiency is unlikely (Greenblatt, 2009; Couet et al., 2011; Karvanen et al., 2013). Nevertheless, rats treated with a single intraperitoneal administration of colistin showed no sign of renal damage, but a 2–fourfold increase in the levels of carnitine in urine (Jeong et al., 2016). The drug-drug interaction observed in the animal study might be due to the much higher C_{max} reached (50–100 μM) as result of the high dose administered (Hengzhuang et al., 2012; Jeong et al., 2016).

The molecular mechanism underlying colistin-induced nephrotoxicity is unknown. The detergent property of the d-aminobutyric acid and fatty acid components may damage the plasma membrane of the cells. This hypothesis, however, does not account for the selective toxicity of colistin toward proximal tubular cells (Falagas and Kasiakou, 2006; Ordoe Javan et al., 2015). Alternatively, nephrotoxicity may be secondary to the intracellular accumulation of colistin (Yun et al., 2015). By co-incubation of primary cultured proximal tubular cells with [¹⁴C]colistin and L-carnitine we provide direct evidence that higher intracellular accumulation of colistin resulted in a more potent toxic effect. While L-carnitine is a high affinity substrate for OCTN2, its inhibitory effect on [¹⁴C]colistin uptake is relatively weak, as the result of the noncompetitive/uncompetitive nature of the inhibition.

Although the colistin detergent-mode activity cannot be completely ruled out, especially at suprapharmacological concentrations of the drug, the results support the hypothesis that colistin toxicity occurs upon intracellular accumulation. Since OCTN2 is abundantly expressed at the luminal side of the kidney proximal tubule, the current findings provide one possible explanation for the particular sensitivity of the kidney parenchyma to colistin exposure (Tamai et al., 1998, 2001).

Fig. 4. Colistin accumulation and cell survival as a function of L-carnitine extracellular concentration in mouse primary proximal tubular cells. Cells were co-exposed to [¹⁴C]colistin at the extracellular concentration of 100 μM and non-labeled L-carnitine at the indicated concentrations. After 48 hour exposure, survival rate and intracellular radioactivity were measured. Left and right y-axes indicate respectively the intracellular [¹⁴C]colistin and the cell survival, as the function of the extracellular concentration of L-carnitine. Data are expressed as percentage of the respective controls and represents the mean ± S.E.M. from four independent experiments.

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Disclosures

The authors have no conflict of interest to declare.

References

- Couet W, Grégoire N, Gobin P, Saulnier PJ, Frasca D, Marchand S, and Mimoz O (2011) Pharmacokinetics of colistin and colistimethate sodium after a single 80-mg intravenous dose of CMS in young healthy volunteers. *Clin Pharmacol Ther* **89**:875–879.
- Dalfino L, Puntillo F, Mosca A, Monno R, Spada ML, Coppolecchia S, Miragliotta G, Bruno F, and Brienza N (2012) High-dose, extended-interval colistin administration in critically ill patients: is this the right dosing strategy? A preliminary study. *Clin Infect Dis* **54**:1720–1726.
- Deryke CA, Crawford AJ, Uddin N, and Wallace MR (2010) Colistin dosing and nephrotoxicity in a large community teaching hospital. *Antimicrob Agents Chemother* **54**:4503–4505.
- Diao L, Ekins S, and Polli JE (2010) Quantitative structure activity relationship for inhibition of human organic cation/carnitine transporter. *Mol Pharm* **7**:2120–2131.
- Falagas ME and Kasiakou SK (2006) Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. *Crit Care* **10**:R27.
- Gai Z, Visentin M, Hiller C, Krajnc E, Li T, Zhen J, and Kullak-Ublick GA (2016) Organic Cation Transporter 2 (OCT2-SLC22A2) overexpression may confer an increased risk of gentamicin-induced nephrotoxicity. *Antimicrob Agents Chemother* **60**:5573–5580.
- Gai Z, Zhou G, Gui T, Itoh S, Oikawa K, Uetani K, and Muragaki Y (2010) Trps1 haploinsufficiency promotes renal fibrosis by increasing Arkadia expression. *J Am Soc Nephrol* **21**:1468–1476.
- Ganapathy ME, Huang W, Rajan DP, Carter AL, Sugawara M, Iseki K, Leibach FH, and Ganapathy V (2000) beta-lactam antibiotics as substrates for OCTN2, an organic cation/carnitine transporter. *J Biol Chem* **275**:1699–1707.
- Garonzik SM, Li J, Thamlikitkul V, Paterson DL, Shoham S, Jacob J, Silveira FP, Forrest A, and Nation RL (2011) Population pharmacokinetics of colistin methanesulfonate and formed

- colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. *Antimicrob Agents Chemother* **55**:3284–3294.
- Greenblatt DJ (2009) Drug-drug noninteractions. *Cardiovasc Ther* **27**:226–229.
- Hengzhuang W, Wu H, Ciofu O, Song Z, and Høiby N (2012) In vivo pharmacokinetic-/pharmacodynamics of colistin and imipenem in *Pseudomonas aeruginosa* biofilm infection. *Antimicrob Agents Chemother* **56**:2683–2690.
- Heuberger W, Berardi S, Jacky E, Pey P, and Krähenbühl S (1998) Increased urinary excretion of carnitine in patients treated with cisplatin. *Eur J Clin Pharmacol* **54**:503–508.
- Jeong ES, Kim G, Moon KS, Kim YB, Oh JH, Kim HS, Jeong J, Shin JG, and Kim DH (2016) Characterization of urinary metabolites as biomarkers of colistin-induced nephrotoxicity in rats by a liquid chromatography/mass spectrometry-based metabolomics approach. *Toxicol Lett* **248**:52–60.
- Karvanen M, Plachouras D, Friberg LE, Paramythiotou E, Papadomichelakis E, Karaiskos I, Tsangaris I, Armaganidis A, Cars O, and Giamarellou H (2013) Colistin methanesulfonate and colistin pharmacokinetics in critically ill patients receiving continuous venovenous hemodiafiltration. *Antimicrob Agents Chemother* **57**:668–671.
- Lancaster CS, Hu C, Franke RM, Filipinski KK, Orwick SJ, Chen Z, Zuo Z, Loos WJ, and Sparreboom A (2010) Cisplatin-induced downregulation of OCTN2 affects carnitine wasting. *Clin Cancer Res* **16**:4789–4799.
- Li J, Milne RW, Nation RL, Turnidge JD, Smeaton TC, and Coulthard K (2003) Use of high-performance liquid chromatography to study the pharmacokinetics of colistin sulfate in rats following intravenous administration. *Antimicrob Agents Chemother* **47**:1766–1770.
- Lu X, Chan T, Xu C, Zhu L, Zhou QT, Roberts KD, Chan HK, Li J, and Zhou F (2016) Human oligopeptide transporter 2 (PEPT2) mediates cellular uptake of polymyxins. *J Antimicrob Chemother* **71**:403–412.
- Ma Z, Wang J, Nation RL, Li J, Turnidge JD, Coulthard K, and Milne RW (2009) Renal disposition of colistin in the isolated perfused rat kidney. *Antimicrob Agents Chemother* **53**:2857–2864.
- Nezu J, Tamai I, Oku A, Ohashi R, Yabuuchi H, Hashimoto N, Nikaido H, Sai Y, Koizumi A, Shoji Y, et al. (1999) Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. *Nat Genet* **21**:91–94.
- Ohashi R, Tamai I, Inano A, Katsura M, Sai Y, Nezu J, and Tsuji A (2002) Studies on functional sites of organic cation/carnitine transporter OCTN2 (SLC22A5) using a Ser467Cys mutant protein. *J Pharmacol Exp Ther* **302**:1286–1294.
- Ordooei Javan A, Shokouhi S, and Sahraei Z (2015) A review on colistin nephrotoxicity. *Eur J Clin Pharmacol* **71**:801–810.
- Schroeder A, Eckhardt U, Stieger B, Tynes R, Schteingart CD, Hofmann AF, Meier PJ, and Hagenbuch B (1998) Substrate specificity of the rat liver Na(+)-bile salt cotransporter in *Xenopus laevis* oocytes and in CHO cells. *Am J Physiol* **274**:G370–G375.
- Tamai I (2013) Pharmacological and pathophysiological roles of carnitine/organic cation transporters (OCTNs: SLC22A4, SLC22A5 and SLC22A21). *Biopharm Drug Dispos* **34**:29–44.
- Tamai I, China K, Sai Y, Kobayashi D, Nezu J, Kawahara E, and Tsuji A (2001) Na(+)-coupled transport of L-carnitine via high-affinity carnitine transporter OCTN2 and its subcellular localization in kidney. *Biochim Biophys Acta* **1512**:273–284.
- Tamai I, Ohashi R, Nezu J, Yabuuchi H, Oku A, Shimane M, Sai Y, and Tsuji A (1998) Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. *J Biol Chem* **273**:20378–20382.
- Tamai I, Yabuuchi H, Nezu J, Sai Y, Oku A, Shimane M, and Tsuji A (1997) Cloning and characterization of a novel human pH-dependent organic cation transporter, OCTN1. *FEBS Lett* **419**:107–111.
- Thévenod F, Ciarimboli G, Leistner M, Wolff NA, Lee WK, Schatz I, Keller T, Al-Monajjed R, Gorboulev V, and Koepsell H (2013) Substrate- and cell contact-dependent inhibitor affinity of human organic cation transporter 2: studies with two classical organic cation substrates and the novel substrate cd2+. *Mol Pharm* **10**:3045–3056.
- Visentin M, Stieger B, Merz M, and Kullak-Ublick GA (2015) Octreotide inhibits the bilirubin carriers organic anion transporting polypeptides 1B1 and 1B3 and the multidrug resistance-associated protein 2. *J Pharmacol Exp Ther* **355**:145–151.
- Yun B, Azad MA, Wang J, Nation RL, Thompson PE, Roberts KD, Velkov T, and Li J (2015) Imaging the distribution of polymyxins in the kidney. *J Antimicrob Chemother* **70**:827–829.

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