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Gene Ablation of Carnitine/Organic Cation Transporter 1 Reduces Gastrointestinal Absorption of 5-Aminosalicylate in Mice

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5-Aminosalicylic acid (5-ASA) is an orally administered therapeutic agent for inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease. We hypothesized that the absorption of 5-ASA is mediated by the polyspecific carnitine/organic cation transporter (OCTN1/SLC22A4), based on the similarity of chemical structure between 5-ASA and other OCTN1 substrates. Therefore, we examined the involvement of this transporter in the disposition of 5-ASA *in vivo* by using *octn1* gene knockout (*octn1*$^{-/-}$) mice. After oral administration of 5-ASA, the plasma concentrations of 5-ASA and its primary metabolite, *N*-acetyl-5-aminosalicylate (Ac-5-ASA), in *octn1*$^{-/-}$ mice were much lower than those in wild-type mice. The time required to reach maximum plasma concentration was also delayed in *octn1*$^{-/-}$ mice. On the other hand, the plasma concentration profiles of both 5-ASA and Ac-5-ASA after intravenous administration of 5-ASA (bolus or infusion) were similar in the two strains. Uptake of 5-ASA from the apical to the basal side of isolated small-intestinal tissues of *octn1*$^{-/-}$ mice, determined in an Ussing-type chamber, was lower than that in wild-type mice. Further, uptake of 5-ASA in HEK293 cells stably transfected with the *OCTN1* gene, assessed as the sum of cell-associated 5-ASA and Ac-5-ASA, was higher than that in HEK293 cells transfected with the vector alone. Overall, these results indicate that OCTN1 is involved, at least in part, in the gastrointestinal absorption of 5-ASA.

Key words organic cation transporter; 5-aminosalicylate; gastrointestinal absorption

5-Aminosalicylic acid (5-ASA) is orally administered for treatment of inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease, and is the standard first-line therapy for mild to moderate ulcerative colitis according to current treatment guidelines.[1,2] Ulcerative colitis and Crohn's disease increase the risk of colorectal cancer,[3] and many *in vitro* and *in vivo* studies have suggested that 5-ASA inhibits proliferation of colorectal cancer cells and induces their apoptosis.[4–6] Therefore, 5-ASA not only ameliorates bowel disease due to its anti-inflammatory action, but may also reduce the risk of cancer.[7,8]

Since 5-ASA is well absorbed from the gastrointestinal tract, a controlled-release formulation (mesalazine) and a prodrug (sulfasalazine) of 5-ASA have been developed to deliver 5-ASA to the lower part of the gastrointestinal tract, which is a primary therapeutic target in ulcerative colitis. 5-ASA is extensively metabolized in the liver, mainly to *N*-acetyl-5-aminosalicylic acid (Ac-5-ASA). Controversial reports have been obtained regarding pharmacological activity of Ac-5-ASA: Ac-5-ASA had no effect on erythrocyte lipid peroxidation[9] whereas inhibition of prostaglandin E_2 synthesis by Ac-5-ASA in human rectal mucosa was reported.[10]

5-ASA is an ionic compound at physiological pH (its pK_a values are 2.6, 5.8 and 12.0), and therefore, membrane permeation of 5-ASA *via* simple diffusion is expected to be minimal according to pH-partition theory. Nevertheless, oral absorption of 5-ASA is quite rapid and almost complete (time to maximum plasma concentration *ca.* 1 h, bioavailability >75%) in humans.[11] Thus, a transport mechanism(s) appears to be involved in gastrointestinal uptake of 5-ASA. *In vitro* studies using cultured cell lines heterologously transfected with transporter genes showed that 5-ASA is a substrate of organic anion transporting polypeptides (OATPs) such as OATP1B1, 1B3 and 2B1.[12] However, it is not yet clear whether these transporters are involved in absorption and/or disposition of 5-ASA in the body.

Carnitine/organic cation transporter 1 (OCTN1/SLC22A4) is expressed ubiquitously in various organs, including kidney, liver, small intestine, brain, skeletal muscle and heart.[13] OCTN1 recognizes both cationic and zwitterionic compounds such as tetraethylammonium, verapamil, quinidine, pyrilamine, ipratropium, oxaliplatin, methimazole, metformin, ergothioneine (ERGO), stachydrine and acetylcholine as substrates.[14–19] In humans, renal tubular secretion of gabapentin is reduced in subjects with polymorphism of *OCTN1* gene (L503F),[20] indicating possible involvement of this transporter in renal disposition. *Octn1* gene knockout mice (*octn1*$^{-/-}$) have been constructed and exhibit a marked reduction in small-intestinal absorption, tissue distribution in various organs, and renal reabsorption of ergothioneine, a typical OCTN1 substrate[21,22]; these results suggest functional expression of OCTN1 in these organs of wild-type animals *in vivo*. Pharmacokinetic study using *octn1*$^{-/-}$ mice suggested the possible involvement of OCTN1 as an efflux system for metformin in murine small intestinal membranes.[23] However, information on the involvement of OCTN1 in the pharmacokinetics of other therapeutic agents is still limited.

5-ASA contains carboxylic and amide groups, like many OCTN1 substrates, and so we hypothesized that 5-ASA is recognized by OCTN1. Interestingly, it is reported that *OCTN1* gene expression is up-regulated in inflamed ileal mucosal segments of Crohn's disease patients[24] and in dextran sodium

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sulfate (DSS)-induced colitis model mice.[25] Thus, OCTN1-mediated 5-ASA uptake could be pharmacologically important. Therefore, the purpose of the present study is to examine the involvement of OCTN1 in the pharmacokinetics of 5-ASA *in vivo*.

MATERIALS AND METHODS

Materials 5-ASA was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ac-5-ASA was prepared from 5-ASA and acetic anhydride according to the reported procedure.[26] All other chemicals were commercial products of analytical grade.

Animals Male mice were used for all experiments at 6–8 weeks of age. The *octn1*$^{-/-}$ mice were generated as reported.[21] The *octn1*$^{-/-}$ and littermates were of a mixed genetic background (C57BL/6J and 129Sv/Ev). They were maintained with free access to food and water. The present study was carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* in Kanazawa University.

Pharmacokinetic Studies Mice were fasted overnight with free access to water. 5-ASA was first dissolved in 0.2 N NaOH at 20 mg/mL, then diluted 10 times with saline and orally administered at 10 mg/kg body weight, intravenously injected as a bolus at 2 mg/kg body weight, or intravenously infused at 25 μg/min/kg body weight. At various intervals after administration, aliquots of blood were collected through the tail vein. All blood samples were immediately centrifuged to obtain plasma. Urine was collected by washing the bladder with saline through polyethylene tubing (SP31, Natsume, Tokyo, Japan). At the end of intravenous infusion of 5-ASA, mice were sacrificed, and tissues were obtained. All samples were stored at −30°C until HPLC determination.

Total body clearance (CL_{tot}) and renal clearance (CL_{renal}) were calculated as follows:

$$CL_{tot} = R_{inf} / C_{ss,plasma} \quad (1)$$

$$CL_{renal} = V_{ss,urine} / C_{ss,plasma} \quad (2)$$

where R_{inf}, $C_{ss,plasma}$ and $V_{ss,urine}$ represent the infusion rate (μg/min/kg), steady-state plasma concentration (μg/mL) and steady-state urinary excretion rate (μg/min/kg), respectively. $C_{ss,plasma}$ was estimated as the mean value of plasma concentration at 60, 90 and 120 min. $V_{ss,urine}$ was estimated as the mean value of excretion rate from 60 to 90 min and that from 90 to 120 min.

Intestinal Transport Study in Ussing-Type Chamber The permeation of 5-ASA in upper region of small-intestinal tissues was assessed in an Ussing-type chamber as described previously.[27] The transport buffer was composed of 128 mM NaCl, 5.1 mM KCl, 1.4 mM $CaCl_2$, 1.3 mM $MgSO_4$, 21 mM $NaHCO_3$, 1.3 mM KH_2PO_4, 10 mM NaH_2PO_4 and 5 mM D-glucose [adjusted to pH 6.0 or 7.4 for the apical or basal side, respectively], and gassed with O_2 before and during the transport experiment. 5-ASA was first dissolved in dimethyl sulfoxide (DMSO) and then diluted with transport buffer to give final concentrations of 5-ASA and DMSO in the donor side of 300 μM and 0.3%, respectively. At the designated times, a 200 μL aliquot was sampled from the acceptor side and replaced with an equal volume of prewarmed fresh buffer.

Transport Studies in HEK293/mOCTN1 Cells HEK293 cells stably transfected with mouse OCTN1 (HEK293/mOCTN1) were previously prepared.[23] For uptake experiments, these cells were cultured in poly-L-lysine coated 12-well plates. At 24 h before uptake study, sodium butyrate (final 50 μM) was added to induce expression of OCTN1. The cells were washed with transport buffer containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM H_2PO_4, 1.2 mM $MgSO_4$, and 25 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES) (adjusted to pH 7.4 with 1 N NaOH) twice. Cells were preincubated at 37°C for 10 min, followed by aspiration of the medium and addition of fresh medium containing 150 μM 5-ASA or 1 mM Ac-5-ASA. The concentration of each compound was set to be the lowest value for the observed uptake to be above detection limit in HPLC. At the designated times, the medium was aspirated, and the cells were washed with ice-cold transport buffer 3 times, and then solubilized with 0.2 N NaOH at room temperature overnight and subjected to HPLC determination.

Measurement of 5-ASA and Ac-5-ASA by HPLC Pre-column derivatization using propionic anhydride was performed to determine 5-ASA and Ac-5-ASA as described previously[28] with some modifications. Briefly, to 10 or 20 μL of plasma and diluted urine samples, 10 μL of water, 20 or 10 μL of internal standard, respectively, and 2 μL of propionic anhydride were added. The samples were then gently mixed for 20 min at room temperature, and 50 μL of 6% perchloric acid was added to precipitate protein. Tissue samples were mixed with the same volume of internal standard and three volumes of phosphate buffered saline (PBS), and homogenized, followed by derivatization with 2 μL of propionic anhydride and protein precipitation with perchloric acid. After centrifugation at 15000 rpm and 4°C for 5 min, the supernatant was subjected to HPLC. The HPLC system consisted of a constant-flow pump (LC-10Avp, Shimadzu, Kyoto, Japan), a fluorimetric detector (RF-10A$_{XL}$, Shimadzu), an automatic sample injector (SIL-10A, Shimadzu), and an integrator (CLASS-VP, Shimadzu). The reversed-phase column (Cosmosil 5C18-AR-II, 4.6×150 mm; Nacalai Tesque) was maintained at 40°C in a column oven (CTO-10Avp, Shimadzu). The mobile phase was a mixture of 20 mM sodium phosphate buffer (pH 2.5) and methanol (77 : 23), and the fluorimetric detector was set at 355 nm (excitation) and 515 nm (emission). The flow rate was 1.0 mL/min. 4-Aminosalicylic acid (4-ASA) was used as an internal standard.

Data Analysis The statistical significance of differences was determined by means of Student's *t*-test or one-way ANOVA with the Bonferroni test, and $p<0.05$ was regarded as denoting a significant difference.

RESULTS

Effect of *octn1* Gene Deletion on Plasma Concentration of 5-ASA after Oral Administration To investigate possible involvement of OCTN1 in intestinal absorption of 5-ASA, 5-ASA was orally administered to wild-type and *octn1*$^{-/-}$ mice (Fig. 1). The plasma concentration of 5-ASA after oral administration in *octn1*$^{-/-}$ mice was significantly lower than that in wild-type mice, the difference being greater at the early phase after administration (Fig. 1A). In *octn1*$^{-/-}$ mice, the peak of plasma concentration was also lower, and

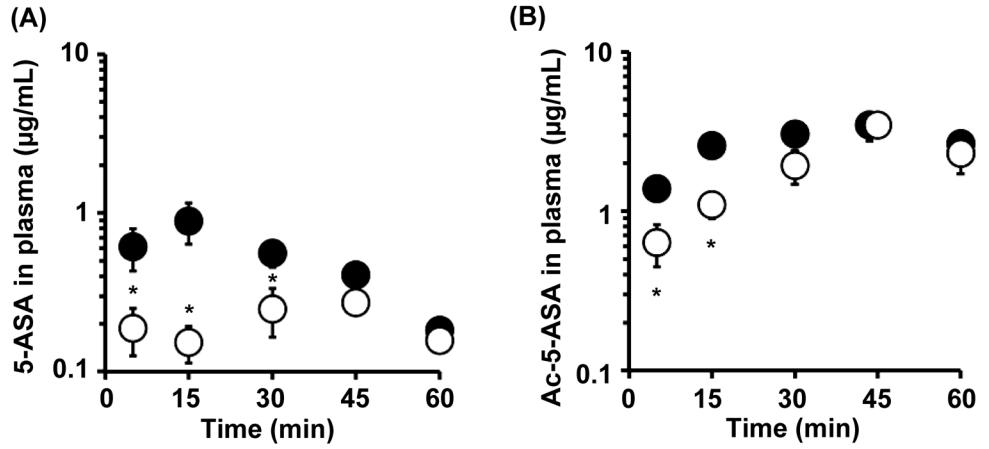

Fig. 1. Effect of *octn1* Gene Depletion on Plasma Concentration–Time Profiles of 5-ASA and Its Metabolite Ac-5-ASA after Oral Administration of 5-ASA

Plasma concentrations of 5-ASA (A) and Ac-5-ASA (B) were determined in wild-type (●) and *octn1*$^{-/-}$ mice (○) after oral administration of 5-ASA (10 mg/kg). Each value represents the mean±S.E.M. (n=9). When error bars are not shown, they were smaller than the symbols. *Significantly different from wild-type mice (p<0.05).

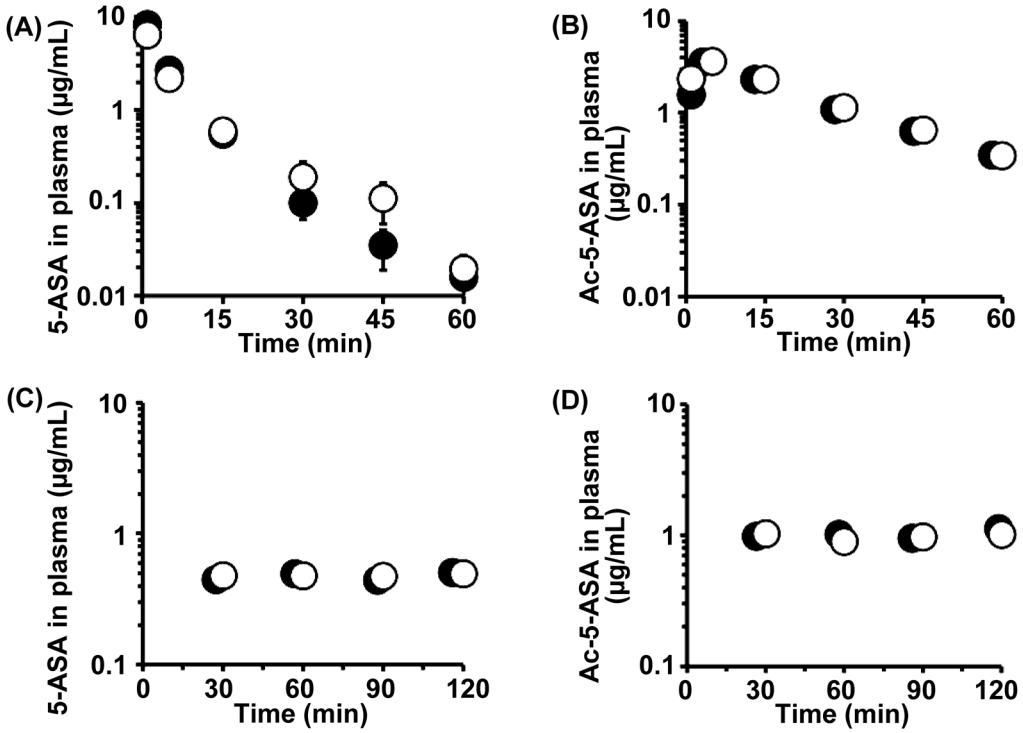

Fig. 2. Effect of *octn1* Gene Depletion on Plasma Concentration–Time Profiles of 5-ASA and Ac-5-ASA after Intravenous Administration of 5-ASA

Plasma concentrations of 5-ASA (A) and Ac-5-ASA (B) were determined in wild-type (●) and *octn1*$^{-/-}$ mice (○) after an intravenous bolus (2 mg/kg) (A, B) and during intravenous infusion (25 μg/min/kg) following an intravenous loading dose of 5-ASA (0.6 mg/kg) (C, D). Each value represents the mean±S.E.M. (n=5–6). When error bars are not shown, they were smaller than the symbols.

the time of peak plasma concentration tended to be delayed compared with that in wild-type mice (Fig. 1A). Since 5-ASA is metabolized to Ac-5-ASA by *N*-acetyltransferase (NAT),[29] we also measured the plasma concentration of Ac-5-ASA (Fig. 1B). The plasma concentration of Ac-5-ASA in *octn1*$^{-/-}$ mice was also lower than that in wild-type mice (Fig. 1B).

Minimal Effect of *octn1* Gene Deletion on Systemic Elimination of 5-ASA Next, to examine the possible role of OCTN1 in systemic elimination of 5-ASA, 5-ASA was intravenously administered to wild-type and *octn1*$^{-/-}$ mice (Fig. 2). In contrast to the results after oral administration (Fig. 1), the plasma concentrations of both 5-ASA and Ac-5-ASA after an intravenous bolus or during constant intravenous infusion were similar in the two strains (Fig. 2), showing comparable systemic elimination (represented as $CL_{tot,plasma}$; Table 1). Tissue distribution and urinary excretion were further examined (Fig. 3). Concentrations of 5-ASA in liver and kidney at the end of the infusion, and urinary excretion of 5-ASA during the intravenous infusion were below the detection limit in both strains, probably because of rapid metabolism of 5-ASA (see Discussion). On the other hand, the tissue-to-plasma concentration ratio (Kp) of Ac-5-ASA in both liver and kidney at the end of 5-ASA infusion showed no significant difference between wild-type and *octn1*$^{-/-}$ mice (Figs. 3A, B).

The $V_{ss,urine}$ values of Ac-5-ASA were also similar in the two strains (60–80% of the infusion rate of 5-ASA; Fig. 3C), indicating substantial metabolism of 5-ASA and recovery as Ac-5-ASA in the urine. CL_{tot} and CL_{renal} for Ac-5-ASA showed little difference between the two strains (Table 1).

Intestinal Membrane Permeation of 5-ASA Was Reduced in *octn1*$^{-/-}$ Mice To directly investigate the membrane permeability of 5-ASA in small intestine, permeation of 5-ASA was next examined in an Ussing-type chamber. We used upper region of small intestinal tissues because regional differentiation was not clearly observed in functional expression of OCTN1 in mouse small intestine assessed as the uptake of [^{3}H]ERGO from the apical side.[22] The permeation of 5-ASA in the absorptive direction (from apical to basal side) in intestine of *octn1*$^{-/-}$ mice was significantly lower than that in wild-type mice (Fig. 4A), whereas that in the secretory direction (from basal to apical side) was similar in wild-type and *octn1*$^{-/-}$ mice (Fig. 4B). Intestinal permeation of 5-ASA in the two strains was much higher in the absorptive direction than in the secretory direction (Fig. 4).

Table 1. Pharmacokinetic Parameters of 5-ASA and Ac-5-ASA Estimated during Intravenous Infusion (25 μg/min/kg) of 5-ASA in Wild-Type and *octn1*$^{-/-}$ Mice[a]

	Wild-type	*octn1*$^{-/-}$
$CL_{tot,plasma}$ (mL/min/kg)[b]	54.9±7.1	50.0±4.9
$CL_{renal,5\text{-}ASA}$ (mL/min/kg)[c]	<2.0	<2.0
$CL_{renal,Ac\text{-}5\text{-}ASA}$ (mL/min/kg)[d]	15.9±1.3	19.6±1.4

a) Data were expressed as the mean±S.E.M. (n=5–6). *b*) Total clearance of 5-ASA. *c*) Renal clearance of 5-ASA. *d*) Renal clearance of Ac-5-ASA.

Uptake Study of 5-ASA and Ac-5-ASA in OCTN1-Transfected Cell Lines We next investigated whether 5-ASA and/or Ac-5-ASA are substrates of OCTN1. During incubation of 5-ASA with HEK293/mOCTN1 cells, Ac-5-ASA was detected in both HEK293/mOCTN1 cells and medium, indicating substantial metabolism of 5-ASA in HEK293 cells. Therefore, uptake of 5-ASA was measured as the sum of 5-ASA and Ac-5-ASA in the cells, and Ac-5-ASA in the medium. On this basis, time-dependent uptake of 5-ASA was observed in both HEK293/mOCTN1 and HEK293/mock cells, the 5-ASA uptake in the former case being slightly higher than that in

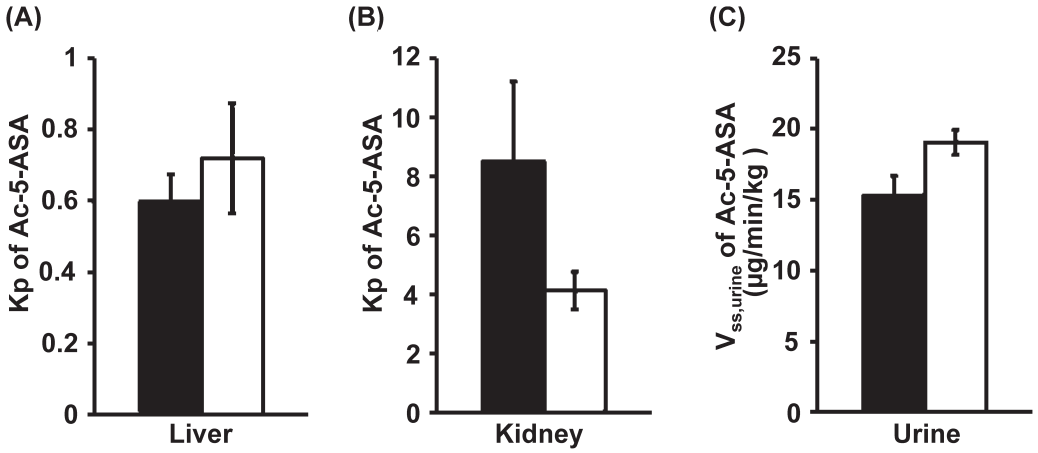

Fig. 3. Tissue-to-Plasma Concentration Ratio and Urinary Excretion Rate of Ac-5-ASA during Intravenous Infusion of 5-ASA

Tissue-to-plasma concentration ratio (Kp) (A, B) and steady-state urinary excretion rate ($V_{ss,urine}$) (C) of Ac-5-ASA in wild-type (closed column) and *octn1*$^{-/-}$ mice (open column) were determined at the end of intravenous infusion of 5-ASA (25 μg/min/kg) for 120 min. Each value represents the mean±S.E.M. (n=5–6).

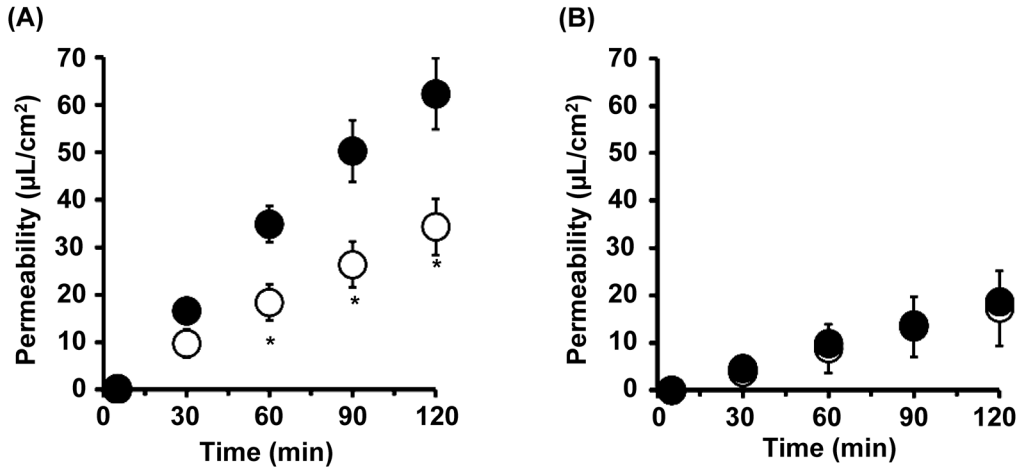

Fig. 4. Membrane Permeation of 5-ASA in Small Intestine of Wild-Type and *octn1*$^{-/-}$ Mice

The permeation of 5-ASA (300 μM) from the apical to basal side (absorptive direction; A) and that from basal to apical side (secretory direction; B) were measured in an Ussing-type chamber using small intestinal tissues obtained from wild-type (●) and *octn1*$^{-/-}$ mice (○). Each value represents the mean±S.E.M. (n=4–5). When error bars are not shown, they were smaller than the symbols. *Significantly different from wild-type mice (p<0.05).

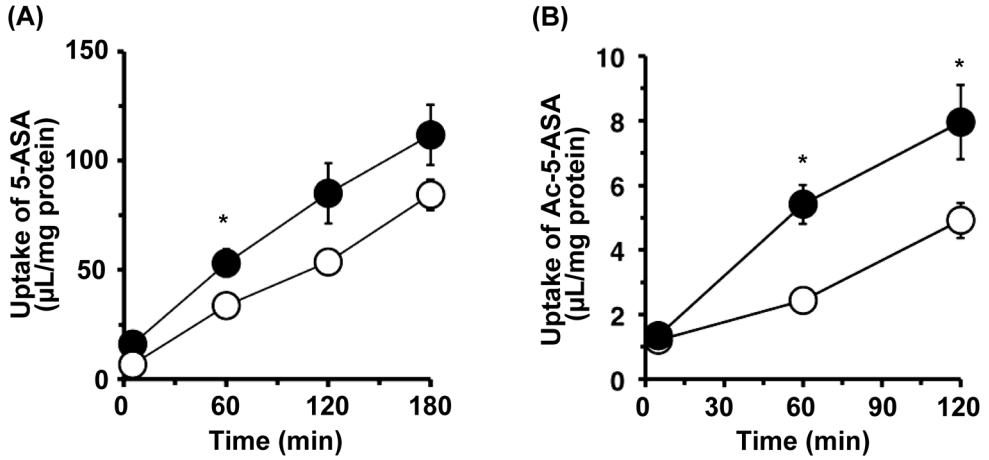

Fig. 5. Uptake of 5-ASA and Ac-5-ASA in HEK293/mOCTN1 Cells

Uptake of 5-ASA (150 μM; A) and Ac-5-ASA (1 mM; B) was measured in HEK293/mOCTN1 (●) and HEK293/mock (○) cells. Each value represents the mean±S.E.M. (n=3). When error bars are not shown, they were smaller than the symbols. *Significantly different from HEK293/mock cells (p<0.05).

the latter (Fig. 5A). Uptake of Ac-5-ASA was also measured during incubation with Ac-5-ASA, and the uptake in HEK293/mOCTN1 was higher than that in HEK293/mock cells.

DISCUSSION

Although 5-ASA is orally administered for treatment of ulcerative colitis and Crohn's disease, the mechanism of its intestinal uptake has not been established. Here, we found that the plasma concentration of 5-ASA after oral administration in *octn1*$^{-/-}$ mice was much lower than that in wild-type mice, and there was a similar difference in the plasma concentration of its metabolite, Ac-5-ASA, between the two strains. On the other hand, there was no marked difference in plasma concentration of 5-ASA or Ac-5-ASA after intravenous administration, suggesting that systemic elimination of 5-ASA is similar in the two strains. Since renal excretion of 5-ASA was negligible, systemic elimination may occur predominantly *via* the liver. Furthermore, CL_{tot} of 5-ASA in both strains (Table 1) was close to the hepatic plasma flow rate (*ca.* 50 mL/min/kg body wt),[30] which may therefore be the rate-limiting step in systemic elimination of 5-ASA.

To confirm the role of OCTN1 in intestinal uptake of 5-ASA, we used an Ussing-type chamber system to examine transport of 5-ASA across small-intestinal tissues. We found that the membrane permeability of 5-ASA in the absorptive direction in *octn1*$^{-/-}$ mice was lower than that in wild-type mice, in accordance with the lower plasma concentration of 5-ASA in *octn1*$^{-/-}$ mice after oral administration. Since OCTN1 is expressed on apical membrane of small intestinal epithelial cells in humans and mice,[22] it seems likely that the reduction in plasma 5-ASA profile in *octn1*$^{-/-}$ mice is due to loss of OCTN1 function at the intestinal plasma membrane. This is the first evidence that *octn1* gene product mediates gastrointestinal absorption of 5-ASA in mouse small intestine.

However, it should be noted that 5-ASA is orally absorbed to some extent even in *octn1*$^{-/-}$ mice, indicating that other mechanisms besides OCTN1 are involved in absorption of 5-ASA. OATPs are expressed in human intestinal epithelial cells and may have a role in drug absorption.[31,32] Indeed, OATP2B1 recognizes 5-ASA as a substrate.[12] Therefore, plural transporters are likely to contribute to intestinal membrane permeability of 5-ASA. On the other hand, 5-ASA (mesalamine; mesalazine) is classified as a class 2 compound in the Biopharmaceutics Drug Disposition Classification System (BDDCS),[33] and the BDDCS predicts that the effect of efflux transporter(s) could be predominant in the intestinal absorption of class 2 drugs.[34] This is inconsistent with the present finding that uptake transporter(s) mediate absorption of 5-ASA. However, the BDDCS does not reliably predict the effect of transporters.[33] Also, there may be species differences in the mechanisms of intestinal membrane permeation of 5-ASA.

To directly demonstrate recognition of 5-ASA by OCTN1, we performed uptake studies in HEK293/mOCTN1 cells. However, 5-ASA was markedly metabolized in HEK293 cells. Addition of NAT inhibitors such as fisetin (100 μM) and quercetin (50 μM) reduced the formation of Ac-5-ASA (data not shown), suggesting functional expression of NAT in HEK293 cells. Nevertheless, these inhibitors also reduced uptake of a typical OCTN1 substrate, [^{3}H]ergothioneine. Therefore, since it was difficult to estimate OCTN1 function, the uptake of 5-ASA was assessed as the sum of 5-ASA and Ac-5-ASA in the cells, and Ac-5-ASA in the medium in the present study (Fig. 5A). On this basis, the uptake of 5-ASA in HEK293/mOCTN1 cells was slightly higher than that in HEK293/mock cells (Fig. 5A), suggesting a direct interaction of OCTN1 with 5-ASA. Rapid metabolism in cell lines is generally problematic in studies to estimate transport function. König *et al.* addressed this issue by measuring OATP-mediated 5-ASA transport based the uptake of total radioactivity derived from [^{3}H]5-ASA in HEK293 cells stably transfected with OATP genes.[12] Further studies may be required to estimate the contributions of individual transporters to membrane permeation of 5-ASA in the body.

5-ASA has an anti-inflammatory effect at pathological lesions in inflammatory bowel diseases. Interestingly, gene expression of *OCTN1* and accumulation of its typical substrate ergothioneine are up-regulated in inflamed intestinal tissues of both Crohn's disease patients and gastrointestinal inflammation model mice, compared with those in non-inflamed tissues.[24,25] Therefore, the present finding regarding possible association of OCTN1 with 5-ASA disposition may imply that OCTN1 contributes to the delivery of 5-ASA to inflamed

intestinal tissues. Further studies are required to examine possible association of this transporter with pharmacological activity of 5-ASA.

Uptake of Ac-5-ASA in HEK293/mOCTN1 was slightly higher than that in HEK293/mock cells (Fig. 5B). In addition, Kp of Ac-5-ASA in kidney tended to be lower in *octn1*$^{-/-}$ mice, whereas $V_{ss,urine}$ of Ac-5-ASA tended to be higher in *octn1*$^{-/-}$ mice (Fig. 3). These results can be explained by partial involvement of OCTN1 in renal reabsorption of Ac-5-ASA although further studies are required to examine this hypothesis.

In conclusion, deletion of the *octn1* gene reduces gastrointestinal absorption of 5-ASA in mice. Therefore, OCTN1 could be at least partially involved in small-intestinal uptake of 5-ASA. Further investigation is needed to establish the molecular mechanism of the interaction between 5-ASA and OCTN1.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) The National Institute for Health and Care Excellence (NICE) clinical guideline 166. "Ulcerative colitis: Management in adults, children and young people.": ‹https://www.nice.org.uk/guidance/cg166›, published in June 2013.
2) Kornbluth A, Sachar DB, Practice Parameters Committee of the American College of Gastroenterology. Ulcerative colitis practice guidelines in adults: American College of Gastroenterology, Practice Parameters Committee. *Am. J. Gastroenterol.*, **105**, 501–523, quiz, 524 (2010).
3) Jess T, Rungoe C, Peyrin-Biroulet L. Risk of colorectal cancer in patients with ulcerative colitis: a meta-analysis of population-based cohort studies. *Clin. Gastroenterol. Hepatol.*, **10**, 639–645 (2012).
4) Baan B, Dihal AA, Hoff E, Bos CL, Voorneveld PW, Koelink PJ, Wildenberg ME, Muncan V, Heijmans J, Verspaget HW, Richel DJ, Hardwick JC, Hommes DW, Peppelenbosch MP, van den Brink GR. 5-Aminosalicylic acid inhibits cell cycle progression in a phospholipase D dependent manner in colorectal cancer. *Gut*, **61**, 1708–1715 (2012).
5) Munding J, Ziebarth W, Pox CP, Ladigan S, Reiser M, Hüppe D, Brand L, Schmiegel W, Tannapfel A, Reinacher-Schick AC. The influence of 5-aminosalicylic acid on the progression of colorectal adenomas *via* the *β*-catenin signaling pathway. *Carcinogenesis*, **33**, 637–643 (2012).
6) Rousseaux C, El-Jamal N, Fumery M, Dubuquoy C, Romano O, Chatelain D, Langlois A, Bertin B, Buob D, Colombel JF, Cortot A, Desreumaux P, Dubuquoy L. The 5-aminosalicylic acid antineoplastic effect in the intestine is mediated by PPAR*γ*. *Carcinogenesis*, **34**, 2580–2586 (2013).
7) Reinacher-Schick A, Schoeneck A, Graeven U, Schwarte-Waldhoff I, Schmiegel W. Mesalazine causes a mitotic arrest and induces caspase-dependent apoptosis in colon carcinoma cells. *Carcinogenesis*, **24**, 443–451 (2003).
8) Zhao LN, Li JY, Yu T, Chen GC, Yuan YH, Chen QK. 5-Aminosalicylates reduce the risk of colorectal neoplasia in patients with ulcerative colitis: an updated meta-analysis. *PLoS ONE*, **9**, e94208 (2014).
9) Greenfield SM, Boswell DJ, Punchard NA, Thompson RP. The effects of 5-aminosalicylic acid and acetyl-5-aminosalicylic acid on lipid peroxidation in erythrocytes and prostaglandin production by mononuclear cells. *Aliment. Pharmacol. Ther.*, **6**, 671–683 (1992).
10) Hawkey CJ, Lo Casto M. Inhibition of prostaglandin synthetase in human rectal mucosa. *Gut*, **24**, 213–217 (1983).
11) Yu DK, Elvin AT, Morrill B, Eichmeier LS, Lanman RC, Lanman MB, Giesing DH. Effect of food coadministration on 5-aminosalicylic acid oral suspension bioavailability. *Clin. Pharmacol. Ther.*, **48**, 26–33 (1990).
12) König J, Glaeser H, Keiser M, Mandery K, Klotz U, Fromm MF. Role of organic anion-transporting polypeptides for cellular mesalazine (5-aminosalicylic acid) uptake. *Drug Metab. Dispos.*, **39**, 1097–1102 (2011).
13) Tamai I, Yabuuchi H, Nezu J, Sai Y, Oku A, Shimane M, Tsuji A. Cloning and characterization of a novel human pH-dependent organic cation transporter, OCTN1. *FEBS Lett.*, **419**, 107–111 (1997).
14) Yabuuchi H, Tamai I, Nezu J, Sakamoto K, Oku A, Shimane M, Sai Y, Tsuji A. Novel membrane transporter OCTN1 mediates multispecific, bidirectional, and pH-dependent transport of organic cations. *J. Pharmacol. Exp. Ther.*, **289**, 768–773 (1999).
15) Kawasaki Y, Kato Y, Sai Y, Tsuji A. Functional characterization of human organic cation transporter OCTN1 single nucleotide polymorphisms in the Japanese population. *J. Pharm. Sci.*, **93**, 2920–2926 (2004).
16) Gründemann D, Harlfinger S, Golz S, Geerts A, Lazar A, Berkels R, Jung N, Rubbert A, Schömig E. Discovery of the ergothioneine transporter. *Proc. Natl. Acad. Sci. U.S.A.*, **102**, 5256–5261 (2005).
17) Nakamura T, Nakanishi T, Haruta T, Shirasaka Y, Keogh JP, Tamai I. Transport of ipratropium, an anti-chronic obstructive pulmonary disease drug, is mediated by organic cation/carnitine transporters in human bronchial epithelial cells: implications for carrier-mediated pulmonary absorption. *Mol. Pharm.*, **7**, 187–195 (2010).
18) Jong NN, Nakanishi T, Liu JJ, Tamai I, McKeage MJ. Oxaliplatin transport mediated by organic cation/carnitine transporters OCTN1 and OCTN2 in overexpressing human embryonic kidney 293 cells and rat dorsal root ganglion neurons. *J. Pharmacol. Exp. Ther.*, **338**, 537–547 (2011).
19) Pochini L, Scalise M, Galluccio M, Indiveri C. Regulation by physiological cations of acetylcholine transport mediated by human OCTN1 (SLC22A4). Implications in the non-neuronal cholinergic system. *Life Sci.*, **91**, 1013–1016 (2012).
20) Urban TJ, Brown C, Castro RA, Shah N, Mercer R, Huang Y, Brett CM, Burchard EG, Giacomini KM. Effects of genetic variation in the novel organic cation transporter, OCTN1, on the renal clearance of gabapentin. *Clin. Pharmacol. Ther.*, **83**, 416–421 (2008).
21) Kato Y, Kubo Y, Iwata D, Kato S, Sudo T, Sugiura T, Kagaya T, Wakayama T, Hirayama A, Sugimoto M, Sugihara K, Kaneko S, Soga T, Asano M, Tomita M, Matsui T, Wada M, Tsuji A. Gene knockout and metabolome analysis of carnitine/organic cation transporter OCTN1. *Pharm. Res.*, **27**, 832–840 (2010).
22) Sugiura T, Kato S, Shimizu T, Wakayama T, Nakamichi N, Kubo Y, Iwata D, Suzuki K, Soga T, Asano M, Iseki S, Tamai I, Tsuji A, Kato Y. Functional expression of carnitine/organic cation transporter OCTN1/SLC22A4 in mouse small intestine and liver. *Drug Metab. Dispos.*, **38**, 1665–1672 (2010).
23) Nakamichi N, Shima H, Asano S, Ishimoto T, Sugiura T, Matsubara K, Kusuhara H, Sugiyama Y, Sai Y, Miyamoto K, Tsuji A, Kato Y. Involvement of carnitine/organic cation transporter OCTN1/SLC22A4 in gastrointestinal absorption of metformin. *J. Pharm.*

Sci., **102**, 3407–3417 (2013).
24) Taubert D, Jung N, Goeser T, Schomig E. Increased ergothioneine tissue concentrations in carriers of the Crohn's disease risk-associated 503F variant of the organic cation transporter OCTN1. *Gut*, **58**, 312–314 (2009).
25) Shimizu T, Masuo Y, Takahashi S, Nakamichi N, Kato Y. Organic cation transporter Octn1-mediated uptake of food-derived antioxidant ergothioneine into infiltrating macrophages during intestinal inflammation in mice. *Drug Metab. Pharmacokinet.*, in press.
26) Caliendo G, Santagada V, Perissutti E, Severino B, Fiorino F, Warner TD, Wallace JL, Ifa DR, Antunes E, Cirino G, de Nucci G. Synthesis of substituted benzamides as anti-inflammatory agents that inhibit preferentially cyclooxygenase 1 but do not cause gastric damage. *Eur. J. Med. Chem.*, **36**, 517–530 (2001).
27) Nishimura T, Amano N, Kubo Y, Ono M, Kato Y, Fujita H, Kimura Y, Tsuji A. Asymmetric intestinal first-pass metabolism causes minimal oral bioavailability of midazolam in cynomolgus monkey. *Drug Metab. Dispos.*, **35**, 1275–1284 (2007).
28) Hussain FN, Ajjan RA, Moustafa M, Anderson JC, Riley SA. Simple method for the determination of 5-aminosalicylic and *N*-acetyl-5-aminosalicylic acid in rectal tissue biopsies. *J. Chromatogr. B: Biomed. Sci. Appl.*, **716**, 257–266 (1998).
29) Zhou SY, Fleisher D, Pao LH, Li C, Winward B, Zimmermann EM. Intestinal metabolism and transport of 5-aminosalicylate. *Drug Metab. Dispos.*, **27**, 479–485 (1999).
30) Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm. Res.*, **10**, 1093–1095 (1993).
31) Imanaga J, Kotegawa T, Imai H, Tsutsumi K, Yoshizato T, Ohyama T, Shirasaka Y, Tamai I, Tateishi T, Ohashi K. The effects of the SLCO2B1 c.1457C>T polymorphism and apple juice on the pharmacokinetics of fexofenadine and midazolam in humans. *Pharmacogenet. Genomics*, **21**, 84–93 (2011).
32) Ieiri I, Doi Y, Maeda K, Sasaki T, Kimura M, Hirota T, Chiyoda T, Miyagawa M, Irie S, Iwasaki K, Sugiyama Y. Microdosing clinical study: pharmacokinetic, pharmacogenomic (SLCO2B1), and interaction (grapefruit juice) profiles of celiprolol following the oral microdose and therapeutic dose. *J. Clin. Pharmacol.*, **52**, 1078–1089 (2012).
33) Benet LZ, Broccatelli F, Oprea TI. BDDCS applied to over 900 drugs. *AAPS J.*, **13**, 519–547 (2011).
34) Shugarts S, Benet LZ. The role of transporters in the pharmacokinetics of orally administered drugs. *Pharm. Res.*, **26**, 2039–2054 (2009).