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Lansoprazole identified as a prophylactic agent for oxaliplatin-induced peripheral neuropathy using integrated *in silico*, *in vitro*, and *in vivo* analyses

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ABSTRACT

Oxaliplatin (L-OHP) is a platinum-based anticancer drug used to treat various malignant tumors. However, L-OHP-induced peripheral neuropathy (OIPN) is a major clinical problem that often limits treatment. As OIPN occurs when L-OHP accumulates in the dorsal root ganglion (DRG) *via* organic cation transporter 2 (OCT2/*SLC22A2*), drugs with OCT2 inhibitory properties may serve as prophylactic agents for OIPN. This study aimed to explore the potential prophylactic agents for OIPN using integrated model with the quantitative structure–activity relationship screening for human organic cation transporter 2 (hOCT2) inhibitors and two real-world data analyses of the Food and Drug Administration Adverse Event Reporting System and Japanese Adverse Drug Event Report, and subsequently evaluate the protective effects of the identified drugs against OIPN. Our integrated model identified lansoprazole, a proton pump inhibitor, as a potential prophylactic agent. *In vitro* uptake study using hOCT2-expressing HEK293 cells demonstrated that lansoprazole significantly inhibited the hOCT2-mediated transport of L-OHP. In the OIPN mouse model, concomitant lansoprazole drastically suppressed mechanical allodynia and cold hypersensitivity after repeated L-OHP administration. Moreover, the concentration of L-OHP in the DRG was significantly decreased by the concomitant administration of lansoprazole. In primary cultured mouse DRG neurons, cotreatment with lansoprazole significantly inhibited L-OHP uptake and restored the L-OHP-induced decrease in neurite length. These findings suggest that the concomitant administration of lansoprazole ameliorates OIPN by inhibiting OCT2-mediated L-OHP uptake in the mouse DRG. Our findings provide important insights for the establishment of novel protective approaches against OIPN.

1. Introduction

Oxaliplatin (L-OHP) is a platinum-based anticancer drug widely used to treat various cancers, including colorectal cancer and other malignancies [1]. However, L-OHP-induced peripheral neuropathy (OIPN) is a major clinical problem associated with L-OHP owing to its high incidence [2]. OIPN manifests as acute and chronic symptoms, including paresthesia, dysesthesia, and sensory ataxia, substantially affecting patients' quality of life [3–5]. The development of OIPN frequently necessitates dose reduction or discontinuation, thereby compromising therapeutic outcomes [6]. Despite its prevalence and considerable impact, effective preventive strategies against OIPN have not been established. Therefore, effective and safe preventive strategies for OIPN are urgently required.

Organic cation transporter 2 (OCT2/*SLC22A2*), a membrane protein expressed in dorsal root ganglion (DRG) neurons, plays a key role in OIPN development [7]. Sprowl et al. [7] demonstrated that OCT2 was the primary mediator of L-OHP uptake into DRG neurons and established a direct correlation between transporter activity and neurotoxicity. OCT2-mediated L-OHP uptake results in its preferential accumulation in DRG neurons, leading to concentration-dependent neurotoxicity [8,9]. To date, genetic deletion or inhibition of OCT2 reduces L-OHP-induced neurotoxicity in mice [7,10]. In addition, cimetidine, a typical OCT2 inhibitor, ameliorates mechanical allodynia and cold hypersensitivity after L-OHP administration at concentrations significantly higher than the clinical concentrations in mice [7]. These

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findings suggest that OCT2 is an important factor in OIPN development. Therefore, drugs with OCT2 inhibitory properties may serve as prophylactic therapeutic agents for OIPN in clinical settings.

Drug repositioning, the process of finding novel indications for previously approved drugs, is of growing interest to academia and industry because it reduces the time and costs associated with drug development [11]. Real-world clinical databases, including the Food and Drug Administration Adverse Event Reporting System (FAERS) and Japanese Adverse Drug Event Report (JADER), have been widely analyzed to identify potential novel prophylactic therapeutic agents [12]. Moreover, a quantitative structure–activity relationship (QSAR) model for identifying the relationship between chemical structure and biological activity has been applied to drug repositioning [13]. Recently, we established a machine learning-based QSAR model for human organic cation transporter 2 (hOCT2) inhibitors based on the ChEMBL database [14]. Thus, we hypothesized that an integrated QSAR model screening and real-world data analysis could be used to identify novel therapeutic agents for OIPN.

In the present study, we aimed to explore the therapeutic agents for OIPN by a novel drug repositioning method using QSAR model screening for hOCT2 inhibitors and two real-world data analyses, FAERS and JADER, and evaluate the protective effects against OIPN through *in vitro* and *in vivo* experiments.

2. Materials and methods

2.1. Materials

L-OHP was obtained from LC Laboratories (Woburn, MA, USA). Lansoprazole and cimetidine were purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan). 4-(4-(dimethylamino)styryl)-*N*-methylpyridinium (ASP^+) was purchased from Oakwood Chemical (Columbia, SA, USA). All other chemicals utilized were of the highest available purity.

2.2. Screening of hOCT2 inhibitors from the DrugBank database using machine learning-based QSAR model

Based on previous methods [14], we predicted the hOCT2 inhibitory activities of 11,281 compounds registered in the DrugBank database [15] using our constructed QSAR model for hOCT2 inhibitor. Subsequently, the compounds with a predicted value of hOCT2 inhibitor ≥ 0.90 were extracted.

2.3. FAERS and JADER analyses for OIPN

Data on patient demographic information, drug information, adverse events, and primary diseases were obtained from the FAERS database (http://www.fda.gov/) from April 2004 to December 2023 and the JADER database (https://www.pmda.go.jp/) from April 2004 to September 2024. The preferred term of "Neuropathy peripheral" was used for searching OIPN. According to a previous report [16], the reporting odds ratio (ROR) and 95 % confidence interval (CI) for each drug was calculated. Negative signals were defined as the upper limit of the 95 % CI for ROR < 1.

2.4. Cell culture

The hOCT2-expressing human embryonic kidney cell line, HEK293 (HEK-hOCT2), and mock-transfectants obtained by transfecting CMV vector with HEK293 (HEK-vector) were generously provided by Dr. Atsushi Yonezawa (Department of Pharmacy, Kyoto University Hospital, Japan). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) containing G418 (0.5 mg/mL). The HEK-hOCT2 and HEK-vector cells were used between passage numbers 84 and 89, respectively. All cell lines were maintained at 37°C under 5 % CO_2 in a humidified atmosphere.

2.5. Uptake study of L-OHP using HEK-hOCT2 and HEK-vector cells

The cells (1×10^6 cells/dish) were seeded in 35-mm dishes with culture medium in the absence of G418. After 48 h of culture, the cell monolayers were used for the uptake study of L-OHP. The incubation medium was 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose, and 5 mM HEPES (pH 7.4). The cells were incubated with 10 μM L-OHP for specified durations (15, 30, 60, and 120 min) at 37°C. In inhibition experiments, the cells were incubated for 15 min with 10 μM L-OHP in the absence or presence of 10 μM lansoprazole or 1 mM cimetidine (a typical inhibitor of hOCT2) at 37°C. To evaluate the concentration of L-OHP in the cells, they were suspended in 1 mL of ultrapure water. Platinum concentration in the cells was determined using inductively coupled plasma mass spectrometry (ICP-MS). The protein content of cells solubilized in 1 N NaOH was measured using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA).

2.6. Animals

Four-week-old male C57BL/6 J mice were obtained from the SLC Japan Co. (Shizuoka, Japan). The mice were kept in cage at 23.0 ± 1.5°C with 45 ± 15 % humidity under a regular 12-h dark/light cycle with ad libitum access to pellet food and water. All animal procedures were approved by the Animal Experiments Committee of The University of Osaka (No. 05–019–000) and conducted in accordance with the Japanese Law for the Protection and Management of Animals, Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain, and other relevant regulations and guidelines for animal experimentation.

2.7. In vivo experimental design using mice

We developed an OIPN mouse model by modifying a previously established protocol [17]. Briefly, L-OHP (10 mg/kg) or a 5 % glucose solution (vehicle) was injected intraperitoneally once a week for 6 weeks in the absence or presence of lansoprazole (2.0 mg/kg) or cimetidine (30 mg/kg) in mice. When we preliminarily examined the plasma concentration of lansoprazole after intraperitoneally administration of lansoprazole (2 mg/kg) in mice, the maximum plasma concentration of lansoprazole was comparable to clinical concentration. Therefore, the dose of lansoprazole was set at 2 mg/kg. Neuropathy was assessed at baseline and weekly intervals from the initiation of treatment through a 6-week period. At 6 weeks, the mice were anesthetized with an intraperitoneal injection of a mixture of medetomidine, midazolam, and butorphanol at doses of 0.75, 4.0, and 5.0 mg/kg, respectively. Blood samples and DRG (L4–L6) were collected, and the platinum concentrations in the plasma and DRG were determined using ICP-MS.

2.8. Assessment of mechanical allodynia and cold hypersensitivity in mice

Mechanical allodynia was assessed by measuring the 50 % paw withdrawal threshold against stimulation with von Frey filaments (0.04–4.0 g; Aesthesio, DanMic Global LLC, San Jose, CA) according to the up-down method described by Chaplan et al. [18]. Cold sensitivity was assessed by quantifying the cold-escape behavior for 60 s after the application of acetone (10 μL) to the plantar surface of the hind paw [17].

2.9. Preparation of primary cultured mouse DRG neurons

Primary cultured mouse DRG neurons were prepared according to established protocols with minor modifications [19]. Briefly, the DRGs (L1–L6) were isolated from 5-week-old male mice anesthetized with a mixture of medetomidine, midazolam, and butorphanol. Isolated DRGs were digested using 2 mg/mL collagenase type III (Worthington Biochemical Corp., Lakewood NJ) at 37°C for 90 min. The digested

DRGs were incubated with Hank's Balanced Salt Solution containing trypsin (2.5 mg/mL) at 37°C. After 15 min, trypsin activity was neutralized by adding trypsin inhibitor (50 μg/mL). Subsequently, DRG neurons were purified by density gradient centrifugation using Percoll® (Cytiva, Uppsala, Sweden) at 200 × *g* for 5 min. The neuron-enriched fraction was collected and was resuspended in DMEM/Ham's F-12 supplemented 10 % FBS, 2 % B-27 supplement (Thermo Fisher Scientific), 100 U/mL penicillin, and 100 μg/mL streptomycin. DRG neurons were maintained at 37°C under 5 % CO_2 in a humidified atmosphere.

2.10. Uptake study of ASP^+ and L-OHP using primary cultured mouse DRG neurons

Primary cultured mouse DRG neurons (1×10^6 cells/dish) were seeded in 35-mm dishes with the culture medium. After 48 h of culturing, an uptake study of ASP^+ (a well-established substrate of OCT2) and L-OHP was performed using primary cultured mouse DRG neurons. Primary cultured mouse DRG neurons were incubated with incubation medium containing 5 μM ASP^+ or 10 μM L-OHP for specified durations (15, 30, 60, and 120 min) at 4°C or 37°C. In inhibition experiments, mouse DRG neurons were incubated for 15 min with incubation medium containing 10 μM L-OHP in the absence or presence of 1 mM cimetidine or 10 μM lansoprazole at 37°C. To evaluate the concentration of ASP^+ in the primary cultured mouse DRG neurons, primary cultured mouse DRG neurons were lysed with 10 % sodium dodecyl sulfate solution, and fluorescence was detected using a fluorescence spectrophotometer (SH-9000lab, CORONA, Ibaraki, Japan) at 485 nm excitation/607 nm emission. To evaluate the concentration of L-OHP in the primary cultured mouse DRG neurons, mouse DRG neurons were suspended in 1 mL of ultrapure water. The concentration of platinum in the mouse DRG neurons was determined using ICP-MS. The protein content of solubilized cells was measured using a BCA protein assay kit (Thermo Fisher Scientific).

2.11. Assessment of neurite length in the primary cultured mouse DRG neurons

Primary cultured mouse DRG neurons were seeded on 12 mm poly-L-lysine-coated round coverslips in a 24-well plate (5×10^3 cells/well). After 24 h of culture, primary cultured mouse DRG neurons were treated with culture medium containing 10 μM L-OHP in the absence or presence of 10 μM lansoprazole for 72 h. Subsequently, primary cultured mouse DRG neurons were fixed with 4 % paraformaldehyde and blocked with phosphate buffered saline containing 5 % normal goat serum and 0.3 % Triton X-100. Immunostaining was conducted using anti-β-III tubulin antibody (rabbit, 1:1000, ab18207, Abcam, Cambridge, MA), which is a neuronal marker, followed by Alexa Fluor 568-conjugated secondary antibody (goat, 1:1000, A11011, Thermo Fisher Scientific). Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (1 μg/mL; Southern Biotech, Birmingham, AL). Image acquisition was performed using an Olympus FV3000 confocal microscope (1024 × 1024 pixels, 0.5 μm z-steps, Olympus, Tokyo, Japan). Five random fields per coverslip were captured, and the experiments were conducted in triplicate. Neurite length was quantified using the IMARIS software (v9.5.1; Bitplane AG, Zurich, Switzerland) with manual verification.

2.12. Determination of platinum in cells, plasma, and DRG

The cell suspensions, plasma, and DRG were mineralized with 70 % HNO_3 and then completely dried at 100°C. The platinum concentrations were determined using an Agilent 7700x ICP-MS (Agilent Technologies, Santa Clara, CA, USA). The instrument settings were optimized to obtain the maximum sensitivity of platinum. Dry platinum-containing material was dissolved in 1 mL of 5 % HNO_3 with 0.1 ng/mL thallium, which was used as an internal standard. The most abundant platinum and thallium isotopes were observed at *m/z* 195 and 205, respectively.

2.13. Statistical analyses

The *in vitro* and *in vivo* experimental data are expressed as means ± standard error (S.E.) and mean ± standard deviation (S.D.), respectively. Statistical comparisons between the two groups were performed using an unpaired Student's *t*-test. For multiple group comparisons, one- or two-way analysis of variance followed by Tukey's post-hoc test was used. Statistical analyses were performed using GraphPad Prism version 10.3.1 (GraphPad Software Inc., San Diego, CA, USA). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Integrated QSAR model screening for hOCT2 inhibitors and real-world data analysis using the FAERS and JADER analyses

To identify potential prophylactic therapeutic agents for OIPN, we analyzed the DrugBank database using the QSAR model for hOCT2 inhibitors and the FAERS and JADER databases. The compounds with a predicted value ≥ 0.90 by QSAR analysis and those with upper limit of 95 % CI for the ROR of < 1 by FAERS and JADER database analyses are listed in Supplementary Table 1. Venn diagrams of the candidate compounds of hOCT2 inhibitors by QSAR model screening and potential prophylactic agents for OIPN by FAERS and JADER analyses are shown in Fig. 1. As shown in Fig. 1 and Supplementary Table 1, two compounds (lansoprazole and granisetron) were identified as potential prophylactic agents against OIPN. Although little information is available regarding the preventive effects of granisetron on the development of OIPN in patients with cancer receiving L-OHP and/or in OIPN animal models, our recent retrospective study revealed a significantly lower incidence of OIPN in patients who concomitantly received proton pump inhibitors (PPIs), especially lansoprazole [20]. Therefore, we focused on "lansoprazole" as a potential prophylactic agent against OIPN.

3.2. hOCT2-mediated L-OHP uptake and inhibition by lansoprazole and cimetidine

To elucidate the role of hOCT2 in L-OHP uptake, we initially assessed the time-dependent accumulation of L-OHP in HEK-hOCT2 cells compared with that in HEK-vector cells. As shown in Fig. 2A, the uptake of L-OHP in HEK-hOCT2 cells increased in a time-dependent manner and

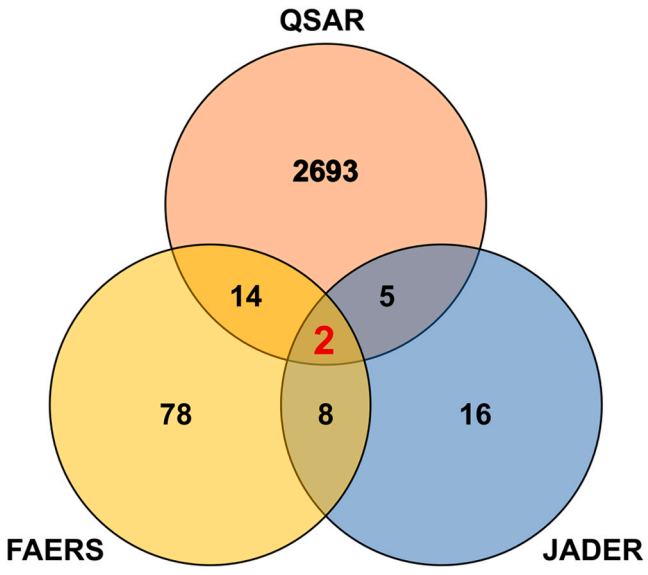


Fig. 1. Venn diagrams of candidate compounds of hOCT2 inhibitors by QSAR model screening and potential prophylactic agents for OIPN by analyzing FAERS and JADER.

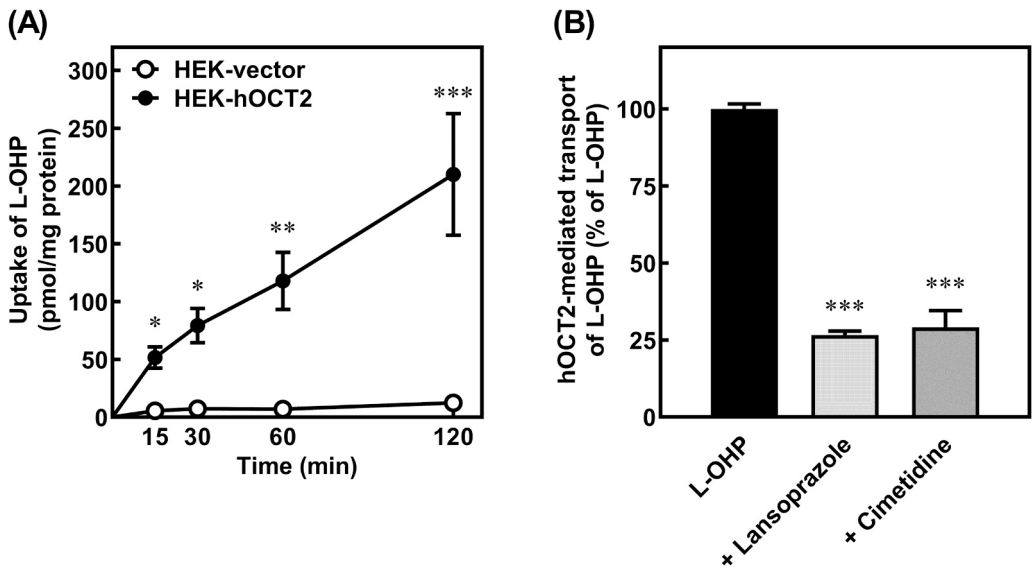


Fig. 2. Inhibition of lansoprazole and cimetidine against hOCT2-mediated transport of L-OHP. (A) HEK-hOCT2 or HEK-vector cells were incubated with L-OHP (10 μM, pH 7.4) for specified durations (15, 30, 60, and 120 min) at 37°C. (B) HEK-hOCT2 or HEK-vector cells were incubated for 15 min at 37°C in the absence or presence of lansoprazole (10 μM) or cimetidine (a typical OCT2 inhibitor, 1 mM). The hOCT2-meditated transport of L-OHP was determined by subtracting the uptake in HEK-vector cells from that in HEK-hOCT2 cells. The hOCT2-mediated transport of L-OHP after treatment of L-OHP was set at 100 %. Each data represents the mean ± S.E. of three separate experiments using three monolayers. When the standard errors of the means were small, they were contained within the symbols. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with HEK-vector cells or L-OHP.

was significantly higher than that in HEK-vector cells, the corresponding controls, at all time points (15, 30, 60, and 120 min). Subsequently, we evaluated the inhibitory effects of lansoprazole and cimetidine (a typical inhibitor of hOCT2) on hOCT2-mediated L-OHP transport for 15 min (Fig. 2B). Lansoprazole (10 μM) and cimetidine (1 mM) potently inhibited hOCT2-mediated L-OHP uptake ($p < 0.001$).

3.3. *Effect of lansoprazole and cimetidine on body weight, mechanical allodynia, and cold hypersensitivity after repeated administration of L-OHP in mice*

We evaluated the preventive effects of lansoprazole and cimetidine on OIPN using an OIPN mouse model. As shown in Fig. 3A, the body weights of L-OHP-treated mice were significantly lower than those of control mice at 3, 4, 5, and 6 weeks after repeated administration of L-OHP. However, the concomitant administration of lansoprazole and cimetidine did not decrease body weight following repeated administration of L-OHP (Fig. 3A). In terms of OIPN, a significantly decreased 50 % withdrawal threshold for mechanical allodynia and an increased frequency of licking and shaking behaviors for acetone application were observed after 1 week of L-OHP administration (Fig. 3B and C). Mechanical allodynia and cold hypersensitivity were observed during 6 weeks of repeated L-OHP administration. Notably, the concomitant administration of lansoprazole or cimetidine drastically suppressed mechanical allodynia and cold hypersensitivity.

3.4. *Effect of lansoprazole and cimetidine on L-OHP concentration in plasma and DRG*

The concentrations of L-OHP in the plasma or DRG at 6 weeks after L-OHP administration are shown in Table 1. The concomitant administration of lansoprazole and cimetidine did not significantly affect the plasma concentration of L-OHP. In contrast, L-OHP concentrations in the DRG of mice treated with concomitant lansoprazole or cimetidine were significantly lower than those in mice treated with L-OHP alone.

3.5. *Inhibition of ASP^+ or L-OHP uptake in primary cultured mouse DRG neurons by lansoprazole and cimetidine*

To confirm OCT2 activity in the primary cultured mouse DRG neurons, we conducted the uptake study of 5 μM ASP^+ at 4°C or 37°C (Fig. 4A). Uptake of ASP^+ in the primary cultured mouse DRG neurons increased in a time-dependent manner at 37°C. The uptake of ASP^+ at 37°C was significantly higher than that at 4°C at all time points. These results confirmed the activity of OCT2 in DRG neurons. To validate the uptake of L-OHP in the primary cultured mouse DRG neurons, we assessed the time-dependent accumulation of L-OHP in the primary cultured mouse DRG neurons. As shown in Fig. 4B, L-OHP accumulation in primary cultured mouse DRG neurons increased in a time-dependent manner. To assess whether lansoprazole and cimetidine inhibit L-OHP uptake in the primary cultured mouse DRG neurons, we determined the uptake of L-OHP (10 μM) for 15 min in the absence or presence of 10 μM lansoprazole and 1 mM cimetidine (Fig. 4C). Lansoprazole and cimetidine significantly inhibited L-OHP uptake in primary cultured mouse DRG neurons. In particular, lansoprazole exhibited a more potent inhibitory effect than cimetidine.

3.6. *Effect of lansoprazole on decreased neurite length induced by L-OHP in primary cultured mouse DRG neurons*

In this study, we investigated the effects of lansoprazole on L-OHP-induced neurite degeneration in primary cultured mouse DRG neurons. Fig. 5A shows representative photomicrographs of DRG neurons immunostained with an anti-β-III tubulin antibody and DAPI. Quantification of neurite outgrowth was performed by measuring the total length of β-III tubulin-positive neurites (Fig. 5B). As shown in Fig. 5B, L-OHP treatment significantly reduced neurite length compared with that in the control (vehicle). Cotreatment with lansoprazole significantly restored the L-OHP-induced decrease in neurite length.

4. Discussion

To date, both basic and clinical studies have investigated prophylaxis and/or therapy for OIPN; however, effective prevention strategies for

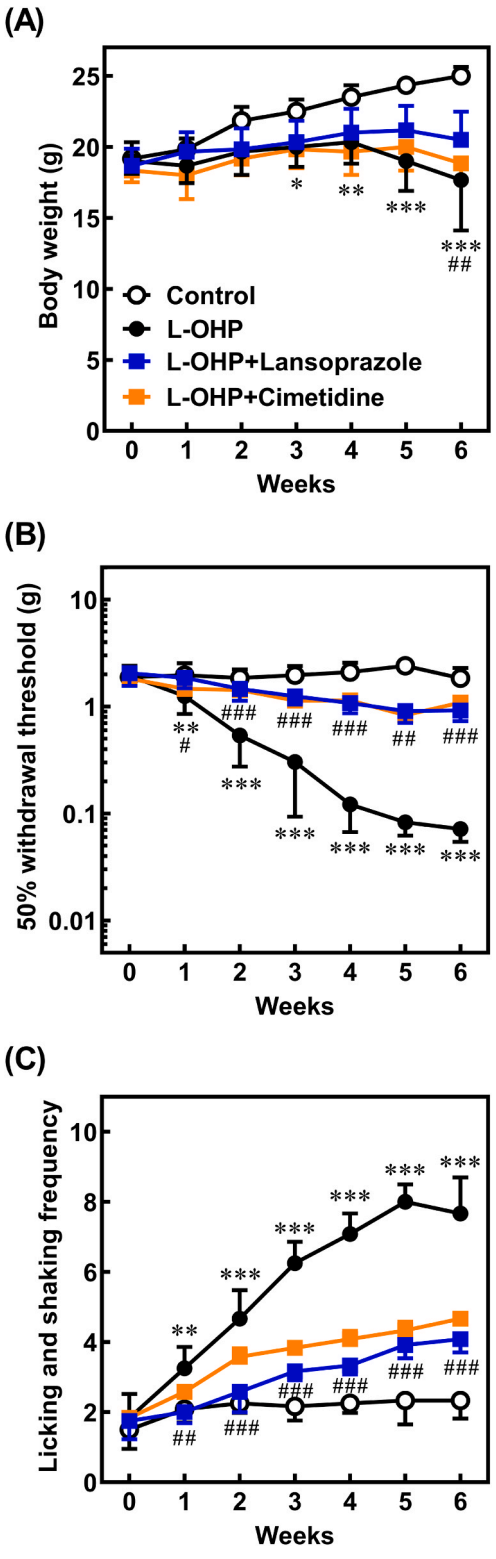


Fig. 3. Effect of lansoprazole and cimetidine on body weight (A), mechanical allodynia (B), and cold hypersensitivity (C) after repeated administration of L-OHP in mice. L-OHP (10 mg/kg) or vehicle (control) was injected intraperitoneally once a week for 6 weeks in the absence or presence of lansoprazole (2.0 mg/kg) or cimetidine (30 mg/kg) in mice. Mechanical allodynia and cold sensitivities were assessed by von Frey filament and acetone tests, respectively. Each point represents the means ± S.D. (n = 6). When the standard deviations of the means were small, they were contained within the symbols. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control mice; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. mice treated with L-OHP alone.

Table 1
Effect of lansoprazole and cimetidine on the concentration of L-OHP in plasma or DRG at 6 weeks after repeated administration of L-OHP in mice.

Samples	L-OHP concentration		
	L-OHP	L-OHP + lansoprazole	L-OHP + cimetidine
Plasma (nmol/mL)	0.090 ± 0.050	0.085 ± 0.028	0.091 ± 0.037
DRG (nmol/g tissue)	0.390 ± 0.154	$0.111 \pm 0.035^{***}$	$0.194 \pm 0.046^{**}$

Each value represents the mean ± S.D. (n = 6). $^{**}p < 0.01$, $^{***}p < 0.001$ vs. L-OHP-treated mice. DRG, dorsal root ganglion; L-OHP, oxaliplatin.

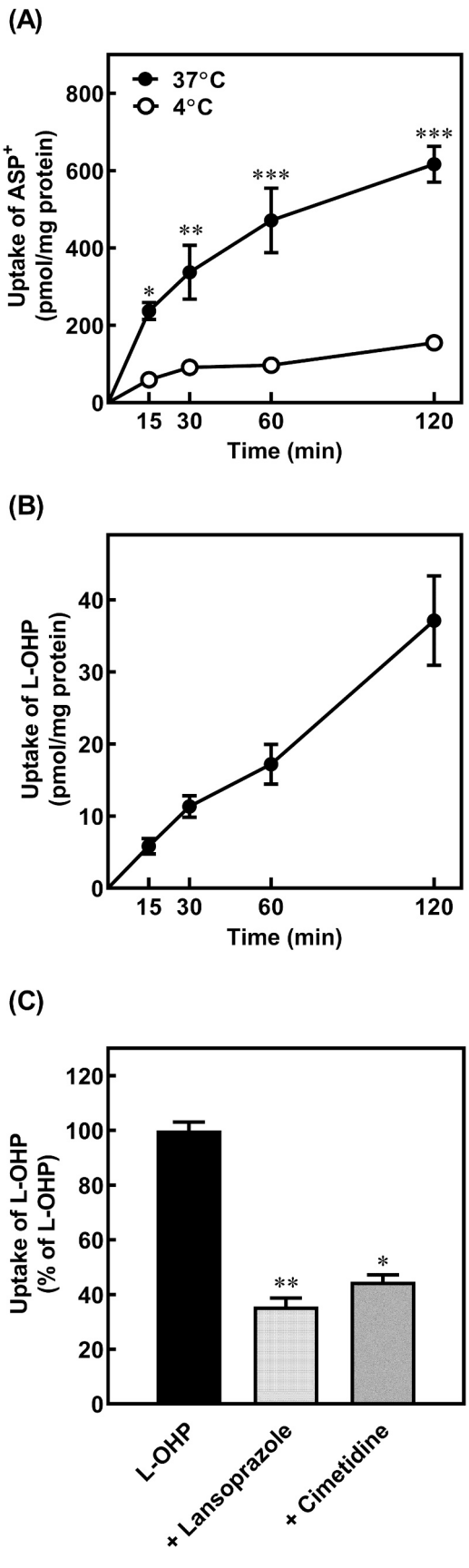
OIPN are yet to be established. In the present study, we identified lansoprazole as a potential prophylactic agent for OIPN using an integrated model with QSAR screening of hOCT2 inhibitors and real-world data analyses using FAERS and JADER. In addition, our study demonstrated for the first time that the concomitant use of lansoprazole reduced the accumulation of L-OHP in the mouse DRG by inhibiting its uptake *via* OCT2, which should alleviate OIPN by suppressing L-OHP-induced neurite degeneration.

As shown in Fig. 1 and Supplementary Table 1, we identified two compounds (lansoprazole and granisetron) as potential prophylactic agents for OIPN using an integrated model with QSAR screening of hOCT2 inhibitors and real-world data analysis using FAERS and JADER. Both compounds exert off-target effects *via* OCT2 inhibition [21,22]. Our recent retrospective study using electronic medical records demonstrated that the incidences of OIPN (grade ≥ 2) and discontinuation of L-OHP were significantly lower in patients with PPIs than in those without PPIs [20]. In the present uptake study of L-OHP using HEK-hOCT2 cells, we confirmed the inhibitory effect of lansoprazole for hOCT2-mediated transport of L-OHP (Fig. 2B). Based on these findings, we focused on lansoprazole as a potential prophylactic agent against OIPN.

Because we hypothesized that co-administration of lansoprazole may ameliorate OIPN by inhibiting the DRG accumulation of L-OHP *via* OCT2, we confirmed whether lansoprazole ameliorates OIPN in mouse models. As shown in Fig. 3B and C, we demonstrated for the first time that concomitant lansoprazole drastically suppressed OIPN in mice after repeated L-OHP administration. Moreover, a significantly decreased concentration of L-OHP was observed in the mouse DRG, but not in the plasma, after concomitant lansoprazole (Table 1). As the accumulation of L-OHP in the DRG represents the primary mechanism underlying the development of OIPN [23], these findings suggest that concomitant lansoprazole administration ameliorates OIPN by decreasing the accumulation of L-OHP in the mouse DRG.

Various transporters are expressed in the DRG [9]. In addition to OCT2, L-OHP is transported by OCT3 (*SLC22A3*) and multidrug and toxin extrusion 1 (MATE1/*SLC47A1*) but not OCT1 (*SLC22A1*) in both mice and humans [9]. OCT1/2 knockout mice exhibit significant protection against both acute and chronic OIPN, whereas OIPN development of OIPN is not reduced in OCT3 and MATE1 knockout mice [9]. Although the organic cation/carnitine transporter 1 (OCTN1/*SLC22A4*) has also been implicated in the uptake of L-OHP into the rat DRG [24,25], L-OHP is not a substrate for mouse or human OCTN1 [9]. An L-OHP uptake study showed that OCT2 was the most efficient L-OHP transporter in both mice and humans [9]. As shown in Fig. 4, L-OHP uptake was observed in primary cultured mouse DRG neurons and was potently inhibited by cimetidine (a typical OCT2 inhibitor). Thus, these findings strongly suggest that OCT2 plays a critical role in L-OHP uptake by DRG neurons.

To validate the contribution of OCT2 in OIPN development, we evaluated the preventive effects of cimetidine (a typical OCT2 inhibitor) against OIPN in mice (Fig. 3B and C). Concomitant cimetidine drastically suppressed OIPN, similar to previous results [7]. In addition,



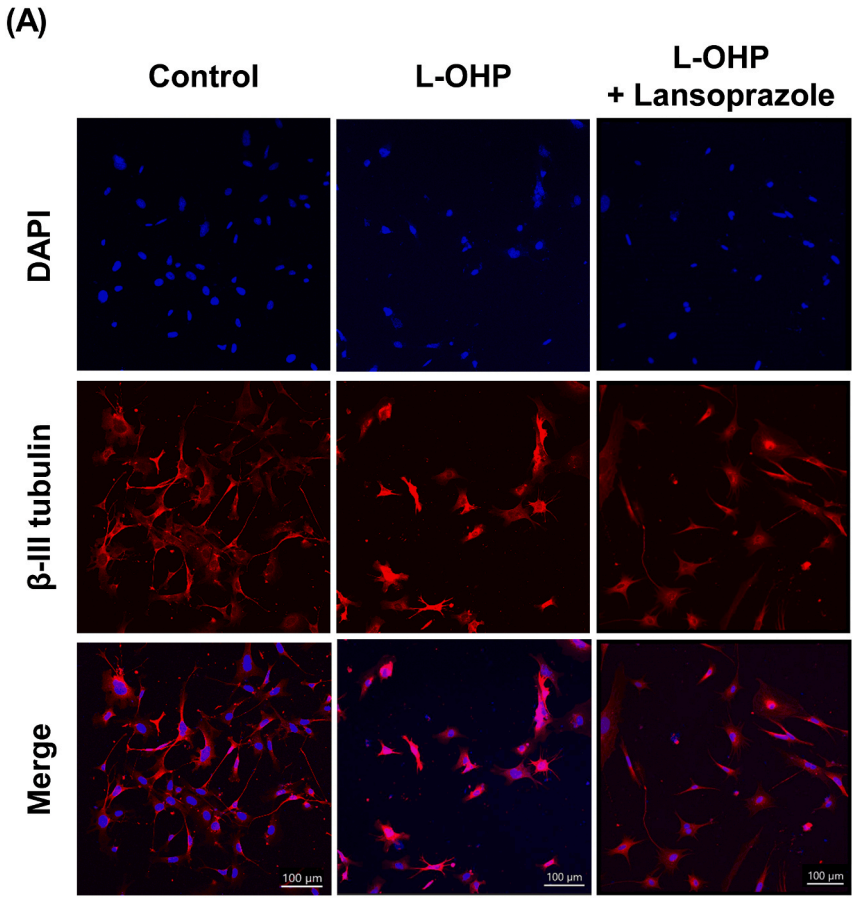
(caption on next column)

Fig. 4. Effect of lansoprazole and cimetidine on the uptake of ASP^+ or L-OHP in primary cultured mouse DRG neurons. (A) Primary cultured mouse DRG neurons were incubated with ASP^+ (5 μM, pH 7.4) for specified durations (15, 30, 60, and 120 min) at 4°C or 37°C. (B) Primary cultured mouse DRG neurons were incubated with L-OHP (10 μM, pH 7.4) for specified durations (15, 30, 60, and 120 min) at 37°C. (C) Primary cultured mouse DRG neurons were incubated at 37°C for 15 min with L-OHP (10 μM) in the absence or presence of lansoprazole (10 μM) or cimetidine (1 mM). Each data represents the mean ± S. E. of three independent experiments using three monolayers. The value of L-OHP uptake (10.5 ± 2.1 pmol/mg protein) after single treatment of L-OHP was set at 100 %. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with 4°C or L-OHP.

L-OHP concentration in the DRG was significantly decreased by cimetidine administration (Table 1). Similar with the results for cimetidine, concomitant lansoprazole drastically suppressed OIPN and decreased L-OHP accumulation in the DRG (Fig. 3B, C and Table 1). Lansoprazole significantly inhibited L-OHP uptake by primary cultured mouse DRG neurons (Fig. 4C). Therefore, lansoprazole should ameliorate OIPN by decreasing the accumulation of L-OHP in the mouse DRG through the inhibition of OCT2-mediated uptake of L-OHP. Although renal Oct2 mRNA expression in male mice is approximately 2-fold higher than that in female mice [26], no information is available regarding sex-related differences of OCT2 expression in mice DRG. In addition, previous studies have demonstrated no significant sex-related differences in OIPN, nor in the pharmacokinetics and tissue distribution of L-OHP between male and female mice [7,9]. As these findings suggested that the role of OCT2 in the pathogenesis of OIPN could be comparable between male and female mice, it is presumed that the neuroprotective of lansoprazole is not sex-specific.

L-OHP is mainly eliminated from the kidney, and OCT2 expressed in the renal proximal tubules is responsible for the urinary secretion of L-OHP [27]. Because lansoprazole has OCT2 inhibitory effects as an off-target effect, it is possible that lansoprazole influences the pharmacokinetics and toxicity of L-OHP. However, increased plasma L-OHP concentrations were not observed in mice receiving lansoprazole (Table 1). In addition, no significant differences in plasma L-OHP concentrations were observed between OCT2 knockout and wild-type mice [10]. Although the antitumor effects of lansoprazole on L-OHP were not examined in the present study, a previous report demonstrated that omeprazole, another PPI, had no effect on L-OHP-induced tumor growth suppression in tumor-bearing mice [28]. In our retrospective study, no significant differences were noted in the incidence of hematological toxicity, an adverse effect influenced by blood concentration, between patients treated with and without PPIs [20]. Moreover, no significant differences were observed in therapeutic outcomes (time to treatment failure) following combination therapy with L-OHP and capecitabine between patients treated with and without PPIs [20,29]. Thus, concomitant lansoprazole administration should decrease L-OHP accumulation in the DRG without affecting systemic pharmacokinetics.

To evaluate the neurotoxicity of L-OHP in mouse DRG neurons, we examined the effect of lansoprazole on the L-OHP-induced decrease in neurite length in primary cultured mouse DRG neurons. As shown in Fig. 5, co-incubation with lansoprazole significantly reversed the L-OHP-induced decrease in neurite length. In addition to its inhibitory effects on hOCT2, lansoprazole has various off-target effects, including antioxidant and anti-inflammatory effects [30]. Previous studies have suggested that the pathogenesis of OIPN is related to inflammation and oxidative stress [31–33]. Therefore, the antioxidant and anti-inflammatory effects of lansoprazole may contribute to OIPN prevention. Moreover, our recent clinical study demonstrated that PPIs could ameliorate capecitabine-induced hand-foot syndrome associated with inflammation [29]. Thus, lansoprazole may attenuate L-OHP-induced neurotoxicity not only by inhibiting hOCT2-mediated accumulation of L-OHP in the DRG but also by its anti-inflammatory and antioxidant properties.



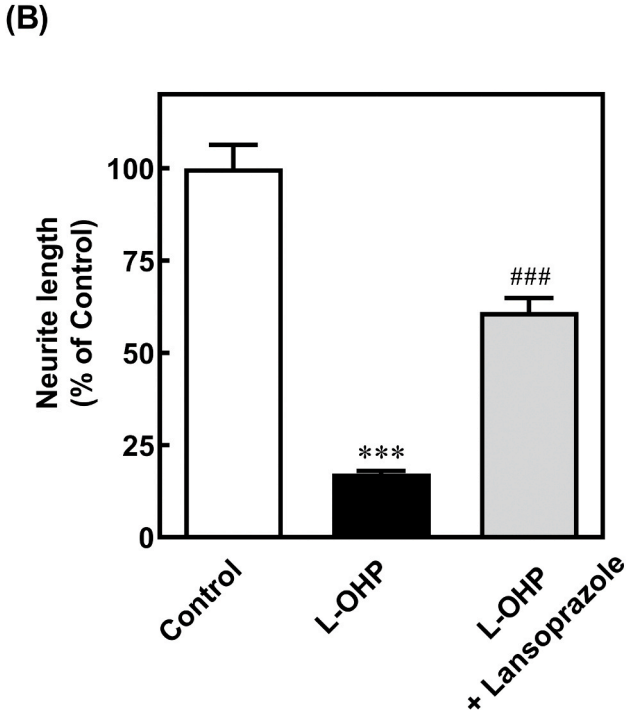


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Fig. 5. Effect of lansoprazole on L-OHP-induced neurite length in primary cultured mouse DRG neurons. Primary cultured mouse DRG neurons were incubated with L-OHP (10 μM) for 72 h in the presence or absence of lansoprazole (10 μM). (A) Representative photomicrographs of DRG neurons immunostained with an anti-β-III tubulin antibody (1:1000) and DAPI. Images were captured using a confocal microscope equipped with a 20 × objective lens. Scale bar = 100 μm. (B) Quantification of neurite outgrowth was performed by measuring the total length of β-III tubulin-positive neurites. Each column represents the mean ± S.E. of three independent experiments using three monolayers. The neurite length after treatment of vehicle (control) was set at 100 %. $^{***}p < 0.001$ compared with control, $^{\#\#\#}p < 0.001$ compared with L-OHP.

5. Conclusion

We identified lansoprazole as a potential prophylactic agent for OIPN in an integrated model using QSAR screening for hOCT2 inhibitors and real-world data analysis using FAERS and JADER. To the best of our knowledge, our study demonstrates for the first time that concomitant lansoprazole ameliorates OIPN through the inhibition of OCT2-mediated accumulation of L-OHP in the mouse DRG. These findings provide important information for establishing novel protective approaches to minimize OIPN.

CRediT authorship contribution statement

Akihide Kobayashi: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Kenji Ikemura:** Writing – review & editing, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Manami Ueno:** Investigation. **Eri Wakai:** Writing – review & editing, Methodology, Investigation. **Fumihiro Yamane:** Investigation. **Masahiro Okuda:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2025.118272.

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