Concomitant lansoprazole ameliorates cisplatin-induced nephrotoxicity by inhibiting renal organic cation transporter 2 in rats

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Short running title

LPZ ameliorates nephrotoxicity by CDDP

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Conflict of interest

The authors declare that there are no conflicts of interest to disclose.

Abstract

Cisplatin is widely used for the treatment of multiple solid tumors. Cisplatin-induced nephrotoxicity is caused by renal accumulation of cisplatin via human organic cation transporter 2 (hOCT2). As lansoprazole (LPZ), a proton pump inhibitor (PPI), is known to inhibit hOCT2 activity, LPZ might ameliorate cisplatin-induced nephrotoxicity. Previous study showed that concomitant LPZ administration ameliorated nephrotoxicity in patients receiving cisplatin. However, the detailed mechanism remains to be clarified. In the present study, the drug-drug interaction between LPZ and cisplatin was examined using hOCT2-expressing cultured cells and rat renal slices. Moreover, we investigated the effect of LPZ on cisplatin-induced nephrotoxicity and pharmacokinetics of cisplatin in rats. In the uptake study, LPZ potently inhibited uptake of cisplatin in hOCT2-expressing cultured cells and rat renal slices. *In vivo* rat study showed that concomitant LPZ significantly ameliorated cisplatin-induced nephrotoxicity and reduced renal accumulation of platinum (Pt) up to approximately 60% of cisplatin alone at 72 h after cisplatin intraperitoneal administration. Furthermore, renal uptake of Pt at 3 min after intravenous cisplatin administration in rats with cisplatin and LPZ decreased to 78% of rats with cisplatin alone. In addition, there was no significant difference in plasma Pt concentration between rats treated with and without LPZ at 3 min after cisplatin intravenous administration. These findings suggested that concomitant LPZ ameliorated cisplatin-induced nephrotoxicity by inhibiting rOCT2-mediated cisplatin uptake in rats, thus decreasing cisplatin accumulation in the kidney. The present findings provided important information for the establishment of novel protective approaches to minimize cisplatin-induced nephrotoxicity.

Keywords: cisplatin, lansoprazole, nephrotoxicity, organic cation transporter 2

Introduction

Cisplatin, a platinum anticancer agent, is widely used for the treatment of multiple solid tumors (Go & Adjei, 1999). The major side effects of cisplatin include nephrotoxicity, ototoxicity, myelosuppression, and peripheral neuropathy (Arany & Safirstein, 2003). An increase in serum creatinine has been reported in 41% of patients treated with a high dose of cisplatin, although hydration was carried out to prevent these side effects (de Jongh et al., 2003). Because cisplatin dosage is restricted by its nephrotoxicity (Pabla & Dong, 2008), the development of renal protective strategies during chemotherapy with cisplatin is crucial for the success of chemotherapy.

Cisplatin accumulation is specific to the kidney, rather than other organs or plasma (Litterst, Gram, Dedrick, Leroy, & Guarino, 1976). Cisplatin-induced nephrotoxicity is known to be localized mainly to the proximal tubules (Dobyan, Levi, Jacobs, Kosek, & Weiner, 1980). Cisplatin accumulation in the kidney is known to be mainly mediated by organic cation transporter 2 (OCT2/*SLC22A2*), which is highly expressed at the renal basolateral membrane (Yonezawa & Inui, 2011). It is reported that a nonsynonymous single-nucleotide polymorphism at 808G>T in human OCT2 (hOCT2) was inversely correlated with the development of cisplatin-induced nephrotoxicity (Iwata et al., 2012). In addition, a previous report showed that administration of cimetidine, a typical OCT2 inhibitor, reduces cisplatin-induced nephrotoxicity in mice (Ciarimboli et al., 2010). Therefore, cisplatin-induced nephrotoxicity should be ameliorated through reduction or inhibition of hOCT2 activity in the kidney.

Proton pump inhibitors (PPIs) are frequently used for the treatment of gastroesophageal hyperacidity (Targownik, Metge, Roos, & Leung, 2007). The usage rate of PPI in cancer patients for alleviating symptoms of gastroesophageal reflux was estimated to be 20% (Smelick et al., 2013). Recent studies reported that PPIs inhibit hOCT2-mediated transport of

metformin (a typical substrate of hOCT2) (Hacker, Maas, Kornhuber, Fromm, & Zolk, 2015; Nies et al., 2011). Considering these findings, we hypothesized that co-administration of a PPI may ameliorate cisplatin-induced nephrotoxicity by inhibiting renal accumulation of cisplatin via hOCT2. Recent clinical retrospective study revealed that co-administration of PPIs, especially lansoprazole (LPZ), ameliorates nephrotoxicity without exacerbating hematological toxicity in patients receiving cisplatin therapy (Ikemura et al., 2017). However, it remains unclear whether LPZ directly inhibits hOCT2-mediated uptake of cisplatin in the kidney.

In the present study, we investigated the drug interaction between cisplatin and LPZ using hOCT2-expressing cultured cells and rat renal slices. In addition, the effect of concomitant LPZ administration on the development of cisplatin-induced nephrotoxicity and pharmacokinetics of cisplatin was examined in rats.

Materials and Methods

Materials

Cisplatin and LPZ were purchased from WAKO Pure Chemical (Osaka, Japan). [ethyl-1-^{14}C] Tetraethylammonium bromide ([^{14}C]TEA, 55 mCi/mmol) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Unlabeled TEA and cimetidine were purchased from Nacalai Tesque (Kyoto, Japan). All other chemicals used were of the highest purity available.

Cell culture

Human OCT2-expressing HEK293 cells (HEK-hOCT2) and mock-transfectants obtained by transfecting vector-CMV vector into HEK293 cells (HEK-vector) were cultured in complete medium consisting of Dulbecco's modified Eagle's medium (WAKO Pure Chemical) supplemented with 10% fetal bovine serum containing G418 (0.5 mg/mL; Sigma-Aldrich St. Louis, MO). HEK-hOCT2 and HEK-vector cells were used between passage numbers 80 and 96, respectively. These cells were maintained at 37°C in a humidified 5% CO_2 atmosphere.

Uptake experiments using HEK-hOCT2 and HEK-vector cells

For the uptake study, cells (1.0×10^6 cells/dish) were seeded in 3.5-cm dishes with culture medium containing G418. Cell monolayers that formed after 48 h of culture were used for the uptake study. Cellular uptake of [^{14}C]TEA, which is a well-established substrate of OCT2, and cisplatin was determined using monolayer cultures of HEK-hOCT2 and HEK-vector cells at 37°C. The composition of the incubation medium was as follows: 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). HEK-hOCT2 and HEK-vector cells were incubated for 2 min with 5 μM [^{14}C]TEA and for 2, 5, 15,

30, and 60 min with 10 μM cisplatin to obtain detectable uptake of platinum (Pt) in HEK-vector cells, respectively. For inhibition experiments, the cells were incubated for 5 min with 1 μM cisplatin, which is comparable with the unbound concentration of cisplatin in human (Urien & Lokiec, 2004).

Determination of [^{14}C]TEA radioactivity in HEK293 cells

To determine the radioactivity of [^{14}C]TEA in cells, HEK293 cells were lysed in 600 μL of 1 N NaOH. The radioactivity in the cell lysate (400 μL) was counted in 4 mL of Clearsol II (Nacalai Tesque, Kyoto, Japan) containing 400 μL of 1 N HCl by a liquid scintillation counter LSC-5100 (Aloka, Tokyo, Japan). The protein content of the solubilized cells (200 μL) was measured using the Bradford method (Bradford, 1976). Finally, the radioactivity and protein content in cell lysate (600 μL) were calculated.

Determination of platinum in HEK293 cells

A cellular pellet in the uptake and inhibition experiments of cisplatin was suspended in 1 mL of ultrapure water to obtain homogeneous cell suspension. Aliquots were solubilized in 0.5 N NaOH. The remaining cell suspension was mineralized with 70% HNO_3 and then completely dried at 100°C. Platinum content was determined by inductively coupled plasma mass spectrometry with an Agilent 7500cx (Agilent Technologies, Santa Clara, CA). Instrument settings were optimized to obtain the maximum sensitivity of Pt. Dry Pt-containing material was dissolved in 2 mL of 5% HNO_3 with thallium (Tl), which was used as an internal standard. The most abundant isotopes of Pt and Tl were measured at m/z 195 and 205, respectively.

Uptake experiments using rat renal slices

Nine-week-old male Wistar rats (SLC Japan Co., Shizuoka, Japan) were allowed free access to water and pellet food. The experiments were approved by Mie University Review Board for Animal Investigation and conducted according to the guidelines for animal experiments of the National Institute of Health. Slices (0.3 mm thickness (Arakawa et al., 2019; Berndt, 1976)) were prepared from cortical area of whole kidney in rats, and were put in ice-cold oxygenated incubation buffer (pH 7.5) consisting of 120 mM NaCl, 16.2 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 10 mM NaH_2PO_4/Na_2HPO_4 as described previously (Urakami et al., 1999). Slices, each weighting 21-94 mg, were randomly selected and then incubated with 10 mL of oxygenated incubation buffer for 5, 10, and 20 min with 50 μM cisplatin to detect uptake of Pt in rat renal slices. The uptake of cisplatin was carried out at 25ºC and 4ºC under bubbling of 100% oxygen. After incubation for an appropriate time, each slice was rapidly washed twice in 10 mL of ice-cold incubation medium, blotted on filter paper and weighed. For inhibition experiments, the renal slices were incubated for 5 min.

Effect of concomitant LPZ administration on the nephrotoxicity and renal accumulation of cisplatin in rats

Rat model with cisplatin-induced nephrotoxicity was usually evaluated after 72 h of intraperitoneal administration of cisplatin at a dose of 7.5 mg/kg (Abd El-Kader & Taha, 2020; Alibakhshi, Khodayar, Khorsandi, Rashno, & Zeidooni, 2018). The rats received cisplatin (7.5 mg/kg, i.p.) with or without LPZ (1-2 mg/kg, i.p.). To collect urine samples, the rats were maintained in metabolic cages from 48 to 72 h after the administration of cisplatin. After 72 h of cisplatin administration, the rats were sacrificed under anesthesia by injection of pentobarbital (50 mg/kg, i.p.). Plasma and urinary creatinine, as well as blood urea nitrogen (BUN) were determined using diagnostic kits obtained from WAKO Pure Chemical. Urinary liver-type fatty acid binding protein (L-FABP; a biomarker of renal proximal tubular

function) was measured with a sandwich ELISA kit following the manufacturer's protocol (R&D Systems, Minneapolis, MN). Creatinine clearance (CLcr) was calculated using the following equation: CLcr (mL/min/kg) = Urine volume (mL/min/kg) × (Urinary creatinine (mg/dL) / Plasma creatinine (mg/dL)). Renal tissues were gently washed, weighed, and homogenized. Then the homogenized suspension was mineralized with 70% HNO_3 and then completely dried at 100°C. The amounts of Pt were measured by atomic absorption spectrophotometer, as described below.

Effect of concomitant LPZ administration on the pharmacokinetics and renal accumulation of cisplatin in rats

Rats were fasted for 12 h, but allowed free access to water before the experiments. After anesthetization by injection of pentobarbital (50 mg/kg, i.p.), the rats received intravenous (i.v.) injection of cisplatin (1 mg/kg), which is comparable with the clinical dose (Homma et al., 2011), through the right femoral vein with and without LPZ (2 mg/kg, i.v.) or cimetidine (20 mg/kg, i.v.). Blood samples were obtained from the right femoral artery at 0.5, 1, 1.5, 2, 2.5, and 3 min after cisplatin administration. Renal tissues were immediately collected after sacrifice. Pt concentrations in plasma and the whole kidney were determined by atomic absorption spectrophotometer, as mentioned below.

Determination of Pt in rat plasma, kidney, and kidney slices

The amounts of Pt in plasma and the kidney were determined by atomic absorption spectrophotometer (AA-6400G; Shimadzu, Kyoto, Japan). The detection wavelength was 265.9 nm. Standards were prepared in plasma or distilled water for determination of Pt concentration in plasma and kidney samples, respectively, by using a platinum standard solution (WAKO Pure Chemical).

Statistical analysis

The *in vitro* and *in vivo* experimental data are expressed as the mean ± standard error (S.E.) and the mean ± standard deviation (S.D.), respectively. Statistical comparisons between two groups were performed using unpaired *t*-test and differences among multiple groups were determined by one-way analysis of variance followed by Dunnett's test or Tukey's multiple comparison test with GraphPad Prism version 5.01 (GraphPad Software Inc., San Diego, CA). Differences were considered significant at $p < 0.05$.

Results

Uptake of [^{14}C]TEA in HEK-vector and HEK-hOCT2 cells

The uptake study with [^{14}C]TEA (a well-established substrate of hOCT2) was conducted to confirm the functionality of hOCT2 in HEK-hOCT2 cells. Uptake of [^{14}C]TEA (5 μM) was evaluated in HEK-vector and HEK-hOCT2 cells for 2 min (Figure 1). [^{14}C]TEA uptake in HEK-hOCT2 cells was approximately 42-fold higher than that in HEK-vector cells, the corresponding control.

Time course of cisplatin uptake in HEK-vector and HEK-hOCT2 cells

The time-dependent uptake of cisplatin (10 μM) was evaluated in HEK-vector and HEK-hOCT2 cells (Figure 2). Cisplatin uptake in HEK-hOCT2 cells increased in a time-dependent manner. Cisplatin uptake in HEK-hOCT2 cells was significantly higher than that in HEK-vector cells at all time-points, and the amount of cisplatin in HEK-hOCT2 cells was 14-fold higher than that in HEK-vector cells at the 5 min time point.

Inhibition of hOCT2-mediated uptake of cisplatin in HEK-hOCT2 cells

To assess whether LPZ inhibits hOCT2-mediated uptake of cisplatin, the uptake of cisplatin (1 μM) was measured for 5 min in the absence or presence of LPZ (100 μM) or cimetidine (a typical inhibitor of hOCT2, 100 μM) in HEK-hOCT2 cells (Figure 3). LPZ and cimetidine significantly inhibited hOCT2-mediated uptake of cisplatin to 11% and 18% of the control (vehicle).

Time course of cisplatin uptake in rat renal slices

The time-dependent uptake of cisplatin (50 μM) was evaluated in rat renal slices (Figure 4). Pt uptake in rat renal slices increased in a time-dependent manner. In rat renal slices, the

uptake of Pt at 25°C was significantly higher compared with that at 4°C at all time-points. The amount of Pt in rat renal slices at 25°C was approximately 8-fold higher than that at 4°C at the 5 min time point.

Inhibition of the uptake of cisplatin in rat renal slices

The uptake of cisplatin (50 μM) was measured for 5 min in absence or presence of LPZ (100 μM) or cimetidine (100 μM) in rat renal slices (Figure 5). LPZ and cimetidine significantly inhibited the uptake of Pt to 41% and 39% of control (vehicle).

Effect of LPZ on renal functions at 72 h after intraperitoneal administration of cisplatin in rats

Cisplatin-induced nephrotoxicity was assessed by determining plasma creatinine, BUN, and urinary L-FABP levels at 72 h after administration of cisplatin (7.5 mg/kg, i.p.). Renal functions are summarized in Table 1. Plasma creatinine, BUN, and urinary L-FABP levels in rats treated with cisplatin significantly increased compared with those in sham rats. These renal markers were significantly attenuated by concomitant LPZ administration in a dose dependent manner. In particular, the elevated urinary L-FABP level by cisplatin administration was restored to the basal level in the rats treated with Cisplatin + LPZ at an LPZ dose of above 1 mg/kg. CLcr significantly decreased in cisplatin-treated rats compared with that in sham rats, and the deterioration of CLcr was significantly suppressed by co-administration of LPZ.

Renal Pt accumulation at 72 h after intraperitoneal administration of cisplatin in rats

Renal Pt accumulation at 72 h after cisplatin administration (7.5 mg/kg, i.p.) is shown in Figure 6. Renal accumulation of Pt in the Cisplatin + LPZ-treated rats significantly decreased

compared with that in cisplatin-treated rats. Renal accumulation of Pt was reduced to 68% and 52% of cisplatin alone by concomitant administration of LPZ at 1 and 2 mg/kg, respectively.

Pharmacokinetics of cisplatin within 3 min after intravenous administration of cisplatin in rats

The plasma concentration-time profiles of Pt within 3 min after cisplatin (1 mg/kg, i.v.) administration are shown in Figure 7A. Plasma Pt concentrations in the Cisplatin + Cimetidine-treated rats were significantly higher than those in cisplatin-treated rats at all-time points. Moreover, there were no significant differences in plasma Pt concentrations between Cisplatin + LPZ-treated and cisplatin-treated rats. Renal Pt accumulations at 3 min after cisplatin intravenous administration are shown in Figure 7B. Concomitant LPZ administration significantly decreased renal Pt accumulation in cisplatin-treated rats to 78% of control, whereas concomitant cimetidine administration significantly increased renal Pt accumulation in cisplatin-treated rats to 134% of control.

Discussion

Little is known about the detailed mechanism of the ameliorative effect of LPZ on cisplatin-induced nephrotoxicity. To the best of our knowledge, this is the first study to report the protective effect of LPZ on cisplatin-induced nephrotoxicity through inhibition of renal rOCT2-mediated uptake of cisplatin in rats.

Although Hacker et al. (2015) and Nies et al. (2011) reported that LPZ inhibits the transport of metformin (a typical hOCT2 substrate) in HEK-hOCT2 cells, the hOCT2-mediated drug interaction between LPZ and cisplatin remains to be clarified. As shown in Figure 3, our study clearly showed that LPZ significantly inhibited hOCT2-mediated transport of cisplatin.

In addition, LPZ significantly inhibited the uptake of Pt in rat renal slices (Figure 5). Although rat OCT1 (rOCT1/*SLC22A1*) is known to be expressed in the kidney and liver (Grundemann, Gorboulev, Gambaryan, Veyhl, & Koepsell, 1994; Martel et al., 1996), cisplatin is known to be not a substrate of rOCT1 (Yonezawa et al., 2005). In human, hOCT1 is expressed in the liver, not in the kidney (Gorboulev et al., 1997), and cisplatin is weakly transported by hOCT1 (Yonezawa, Masuda, Yokoo, Katsura, & Inui, 2006). Thus, rOCT2 and hOCT2 should be a major transporter for renal uptake of cisplatin in both of rats and human (Yonezawa & Inui, 2011). The inhibitory effects of LPZ for the transport of cisplatin were comparable to those of cimetidine in HEK-hOCT2 cells and rat renal slices as shown in Figure 3 and 5. Therefore, there findings suggested that LPZ reduced the accumulation of cisplatin in rat renal slices mainly by inhibiting rOCT2-mediate transport of cisplatin.

In the present study, concomitant LPZ administration ameliorated cisplatin-induced nephrotoxicity (Table1) as well as decreased renal Pt accumulation at 72 h after cisplatin administration (Figure 6) and initial renal uptake of Pt at 3 min (Figure 7B) in rats. It is reported that concomitant administration of OCT2 inhibitors (imatinib or carvedilol) prevents

cisplatin-induced nephrotoxicity in mice or rats through reduction of renal accumulation of cisplatin via mouse and rat OCT2 (Guo et al., 2018; Tanihara, Masuda, Katsura, & Inui, 2009). These findings suggested that renal accumulation of Pt could be attributed to the degree of cisplatin-induced nephrotoxicity. Therefore, concomitant LPZ administration should ameliorate cisplatin-induced nephrotoxicity by decreased renal accumulation of cisplatin from an early period through inhibition of rOCT2-mediated uptake of cisplatin in rats.

When 30 mg of LPZ was administered in human, the maximum plasma concentration of LPZ was approximately 2.5-4.9 μM (Ieiri et al., 2001). In the pharmacokinetic study using rats, when we examined the plasma concentration of LPZ after intravenous administration of LPZ (2 mg/kg) in rats, the maximum plasma concentration of LPZ was approximately 5.9 μM (Data not shown). Moreover, we used 1-2 mg/kg LPZ in rats in accordance with clinical dose (0.3-1.2 mg/kg). Thus, dose and plasma concentration of LPZ in rats were almost comparable to those in human. Although there were no reports showing the inhibitory effect of PPI on the OCT2-mediated transport of cisplatin, Hacker et al. (2015) demonstrated that PPIs (lansoprazole and pantoprazole) inhibit hOCT2-mediated transport of metformin (a typical substrate of OCT2) at clinical concentrations. In addition, these PPIs were tested for clinical drug interaction with metformin in patients and healthy subjects (Ding et al., 2014; Kim et al., 2014). Therefore, we speculate that the activity of hOCT2 may be inhibited by PPI at clinical concentration.

Inhibition of rOCT2-mediated transport of cisplatin by LPZ could be expected to increase plasma Pt concentration. However, increased plasma Pt concentration was not observed in rats receiving LPZ, whereas co-administration of cimetidine, which inhibited rOCT2-mediated uptake (Figure 5), increased the plasma and renal concentrations of Pt (Figure 7). It is known that several OCT2 inhibitors simultaneously inhibit multidrug and

toxin extrusion 1 (MATE1/*SLC47A1*) (Tsuda et al., 2009). hMATE1 transports cisplatin from renal proximal tubular cells to the tubular lumen via brush-border membranes (Yonezawa et al., 2006). Cimetidine is known to be a more potent inhibitor of hOCT2 than hMATE1 (Nakamura, Yonezawa, Hashimoto, Katsura, & Inui, 2010; Tsuda et al., 2009). In addition, Nakamura et al. (2010) revealed that the plasma concentration and renal accumulation of cisplatin in MATE1 knock-out mice are significantly higher than those in wild-type mice. Thus, these findings suggested that inhibition or deletion of MATE1 could elevate plasma and renal concentrations of Pt. Although LPZ exerts inhibitory effect against hMATE1 (Guo et al., 2018), this effect is weaker than that of cimetidine (Guo et al., 2018; Ito et al., 2012). In addition, LPZ is not transported into cells by hOCT2, in contrast to cimetidine (Nies et al., 2011; Tahara et al., 2005). In fact, renal concentration of LPZ at 3 min after intravenous administration of LPZ was approximately 5.7 μM, which is comparable to clinical blood concentration. Moreover, this concentration is much lower than the IC_{50} of hMATE1 for metformin (25.7 μM) (Guo et al., 2018). Furthermore, the glomerular filtration rate of cisplatin is approximately 2.5-fold higher than its tubular secretion rate in rats (Hanada, Ninomiya, & Ogata, 2000), suggesting that cisplatin is excreted mainly through glomerular filtration and partly through tubular secretion. Therefore, concomitant LPZ administration deceased renal Pt accumulation but did not increase plasma Pt concentration through inhibition of rOCT2.

A previous report showed that pretreatment with omeprazole (a PPI) for 5 days alleviates cisplatin-induced nephrotoxicity and reduces renal Pt accumulation through downregulation of rOCT2 expression in rats (Gao et al., 2019). Although a single dose of LPZ was concomitantly administered with cisplatin in our present study, the repeated administration of LPZ might have altered the expression levels of rOCT2, as shown in the omeprazole study. Further studies are needed to elucidate the effect of LPZ on rOCT2 expression.

In the present study, we examined the effect of LPZ on cisplatin-induced nephrotoxicity and hOCT2-medaited transport of cisplatin. Interestingly, other PPIs also have inhibitory effect on hOCT2 activity (Hacker et al., 2015; Nies et al., 2011). In previous retrospective clinical study, other PPIs (including esomeprazole, rabeprazole, and omeprazole) also ameliorate nephrotoxicity in patients treated with cisplatin and fluorouracil (Ikemura et al., 2017). Thus, other PPIs might ameliorate cisplatin-induced nephrotoxicity through inhibition of hOCT2-mediated transport of cisplatin. However, further studies are required to clarify the effect of other PPIs on cisplatin-induced nephrotoxicity.

Conclusion

Our study is the first to show that co-administration of LPZ ameliorated nephrotoxicity in cisplatin-treated rats through inhibition of rOCT2-mediated renal uptake of cisplatin. The present findings provided important information for the establishment of novel protective approaches to minimize the nephrotoxicity of cisplatin.

References

Abd El-Kader, M., & Taha, R. I. (2020). Comparative nephroprotective effects of curcumin and etoricoxib against cisplatin-induced acute kidney injury in rats. *Acta Histochemica, 122*(4), 151534. doi:10.1016/j.acthis.2020.151534

Alibakhshi, T., Khodayar, M. J., Khorsandi, L., Rashno, M., & Zeidooni, L. (2018). Protective effects of zingerone on oxidative stress and inflammation in cisplatin-induced rat nephrotoxicity. *Biomedicine and Pharmacotherapy, 105*, 225-232. doi:10.1016/j.biopha.2018.05.085

Arakawa, H., Kubo, H., Washio, I., Staub, A. Y., Nedachi, S., Ishiguro, N., . . . Tamai, I. (2019). Rat Kidney Slices for Evaluation of Apical Membrane Transporters in Proximal Tubular Cells. *Journal of Pharmaceutical Sciences, 108*(8), 2798-2804. doi:10.1016/j.xphs.2019.03.031

Arany, I., & Safirstein, R. L. (2003). Cisplatin nephrotoxicity. *Seminars in Nephrology, 23*(5), 460-464.

Berndt, W. O. (1976). Use of the tissue slice technique for evaluation of renal transport processes. *Environmental Health Perspectives, 15*, 73-88. doi:10.1289/ehp.761573

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248-254. doi:10.1006/abio.1976.9999

Ciarimboli, G., Deuster, D., Knief, A., Sperling, M., Holtkamp, M., Edemir, B., . . . Schlatter, E. (2010). Organic cation transporter 2 mediates cisplatin-induced oto- and nephrotoxicity and is a target for protective interventions. *American Journal of Pathology, 176*(3), 1169-1180. doi:10.2353/ajpath.2010.090610

de Jongh, F. E., van Veen, R. N., Veltman, S. J., de Wit, R., van der Burg, M. E., van den Bent, M. J., . . . Verweij, J. (2003). Weekly high-dose cisplatin is a feasible treatment

option: analysis on prognostic factors for toxicity in 400 patients. *British Journal of Cancer, 88*(8), 1199-1206. doi:10.1038/sj.bjc.6600884

Ding, Y., Jia, Y., Song, Y., Lu, C., Li, Y., Chen, M., . . . Wen, A. (2014). The effect of lansoprazole, an OCT inhibitor, on metformin pharmacokinetics in healthy subjects. *European Journal of Clinical Pharmacology, 70*(2), 141-146. doi:10.1007/s00228-013-1604-7

Dobyan, D. C., Levi, J., Jacobs, C., Kosek, J., & Weiner, M. W. (1980). Mechanism of cis-platinum nephrotoxicity: II. Morphologic observations. *Journal of Pharmacology and Experimental Therapeutics, 213*(3), 551-556.

Gao, H., Zhang, S., Hu, T., Qu, X., Zhai, J., Zhang, Y., . . . Song, Y. (2019). Omeprazole protects against cisplatin-induced nephrotoxicity by alleviating oxidative stress, inflammation, and transporter-mediated cisplatin accumulation in rats and HK-2cells. *Chemico-Biological Interactions, 297*, 130-140. doi:10.1016/j.cbi.2018.11.008

Go, R. S., & Adjei, A. A. (1999). Review of the comparative pharmacology and clinical activity of cisplatin and carboplatin. *Journal of Clinical Oncology, 17*(1), 409-422. doi:10.1200/JCO.1999.17.1.409

Gorboulev, V., Ulzheimer, J. C., Akhoundova, A., Ulzheimer-Teuber, I., Karbach, U., Quester, S., . . . Koepsell, H. (1997). Cloning and characterization of two human polyspecific organic cation transporters. *DNA and Cell Biology, 16*(7), 871-881. doi:10.1089/dna.1997.16.871

Grundemann, D., Gorboulev, V., Gambaryan, S., Veyhl, M., & Koepsell, H. (1994). Drug excretion mediated by a new prototype of polyspecific transporter. *Nature, 372*(6506), 549-552. doi:10.1038/372549a0

Guo, D., Yang, H., Li, Q., Bae, H. J., Obianom, O., Zeng, S., . . . Shu, Y. (2018). Selective Inhibition on Organic Cation Transporters by Carvedilol Protects Mice from

Cisplatin-Induced Nephrotoxicity. *Pharmaceutical Research, 35*(11), 204. doi:10.1007/s11095-018-2486-2

Hacker, K., Maas, R., Kornhuber, J., Fromm, M. F., & Zolk, O. (2015). Substrate-Dependent Inhibition of the Human Organic Cation Transporter OCT2: A Comparison of Metformin with Experimental Substrates. *PloS One, 10*(9), e0136451. doi:10.1371/journal.pone.0136451

Hanada, K., Ninomiya, K., & Ogata, H. (2000). Pharmacokinetics and toxicodynamics of cisplatin and its metabolites in rats: relationship between renal handling and nephrotoxicity of cisplatin. *Journal of Pharmacy and Pharmacology, 52*(11), 1345-1353. doi:10.1211/0022357001777496

Homma, A., Inamura, N., Oridate, N., Suzuki, S., Hatakeyama, H., Mizumachi, T., . . . Fukuda, S. (2011). Concomitant weekly cisplatin and radiotherapy for head and neck cancer. *Japanese Journal of Clinical Oncology, 41*(8), 980-986. doi:10.1093/jjco/hyr086

Ieiri, I., Kishimoto, Y., Okochi, H., Momiyama, K., Morita, T., Kitano, M., . . . Ishizaki, T. (2001). Comparison of the kinetic disposition of and serum gastrin change by lansoprazole versus rabeprazole during an 8-day dosing scheme in relation to CYP2C19 polymorphism. *European Journal of Clinical Pharmacology, 57*(6-7), 485-492. doi:10.1007/s002280100342

Ikemura, K., Oshima, K., Enokiya, T., Okamoto, A., Oda, H., Mizuno, T., . . . Okuda, M. (2017). Co-administration of proton pump inhibitors ameliorates nephrotoxicity in patients receiving chemotherapy with cisplatin and fluorouracil: a retrospective cohort study. *Cancer Chemotherapy and Pharmacology, 79*(5), 943-949. doi:10.1007/s00280-017-3296-7

Ito, S., Kusuhara, H., Yokochi, M., Toyoshima, J., Inoue, K., Yuasa, H., & Sugiyama, Y.

(2012). Competitive inhibition of the luminal efflux by multidrug and toxin extrusions, but not basolateral uptake by organic cation transporter 2, is the likely mechanism underlying the pharmacokinetic drug-drug interactions caused by cimetidine in the kidney. *Journal of Pharmacology and Experimental Therapeutics, 340*(2), 393-403. doi:10.1124/jpet.111.184986

Iwata, K., Aizawa, K., Kamitsu, S., Jingami, S., Fukunaga, E., Yoshida, M., . . . Saito, H. (2012). Effects of genetic variants in SLC22A2 organic cation transporter 2 and SLC47A1 multidrug and toxin extrusion 1 transporter on cisplatin-induced adverse events. *Clinical and Experimental Nephrology, 16*(6), 843-851. doi:10.1007/s10157-012-0638-y

Kim, A., Chung, I., Yoon, S. H., Yu, K. S., Lim, K. S., Cho, J. Y., . . . Chung, J. Y. (2014). Effects of proton pump inhibitors on metformin pharmacokinetics and pharmacodynamics. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 42*(7), 1174-1179. doi:10.1124/dmd.113.055616

Litterst, C. L., Gram, T. E., Dedrick, R. L., Leroy, A. F., & Guarino, A. M. (1976). Distribution and disposition of platinum following intravenous administration of cis-diamminedichloroplatinum(II) (NSC 119875) to dogs. *Cancer Research, 36*(7 PT 1), 2340-2344.

Martel, F., Vetter, T., Russ, H., Grundemann, D., Azevedo, I., Koepsell, H., & Schomig, E. (1996). Transport of small organic cations in the rat liver. The role of the organic cation transporter OCT1. *Naunyn-Schmiedebergs Archives of Pharmacology, 354*(3), 320-326. doi:10.1007/bf00171063

Nakamura, T., Yonezawa, A., Hashimoto, S., Katsura, T., & Inui, K. (2010). Disruption of multidrug and toxin extrusion MATE1 potentiates cisplatin-induced nephrotoxicity. *Biochemical Pharmacology, 80*(11), 1762-1767. doi:10.1016/j.bcp.2010.08.019

Nies, A. T., Hofmann, U., Resch, C., Schaeffeler, E., Rius, M., & Schwab, M. (2011). Proton pump inhibitors inhibit metformin uptake by organic cation transporters (OCTs). *PloS One, 6*(7), e22163. doi:10.1371/journal.pone.0022163

Pabla, N., & Dong, Z. (2008). Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. *Kidney International, 73*(9), 994-1007. doi:10.1038/sj.ki.5002786

Smelick, G. S., Heffron, T. P., Chu, L., Dean, B., West, D. A., Duvall, S. L., . . . Ware, J. A. (2013). Prevalence of acid-reducing agents (ARA) in cancer populations and ARA drug-drug interaction potential for molecular targeted agents in clinical development. *Molecular Pharmaceutics, 10*(11), 4055-4062. doi:10.1021/mp400403s

Tahara, H., Kusuhara, H., Endou, H., Koepsell, H., Imaoka, T., Fuse, E., & Sugiyama, Y. (2005). A species difference in the transport activities of H2 receptor antagonists by rat and human renal organic anion and cation transporters. *Journal of Pharmacology and Experimental Therapeutics, 315*(1), 337-345. doi:10.1124/jpet.105.088104

Tanihara, Y., Masuda, S., Katsura, T., & Inui, K. (2009). Protective effect of concomitant administration of imatinib on cisplatin-induced nephrotoxicity focusing on renal organic cation transporter OCT2. *Biochemical Pharmacology, 78*(9), 1263-1271. doi:10.1016/j.bcp.2009.06.014

Targownik, L. E., Metge, C., Roos, L., & Leung, S. (2007). The prevalence of and the clinical and demographic characteristics associated with high-intensity proton pump inhibitor use. *American Journal of Gastroenterology, 102*(5), 942-950. doi:10.1111/j.1572-0241.2007.01106.x

Tsuda, M., Terada, T., Ueba, M., Sato, T., Masuda, S., Katsura, T., & Inui, K. (2009). Involvement of human multidrug and toxin extrusion 1 in the drug interaction between cimetidine and metformin in renal epithelial cells. *Journal of Pharmacology and Experimental Therapeutics, 329*(1), 185-191. doi:10.1124/jpet.108.147918

Urakami, Y., Nakamura, N., Takahashi, K., Okuda, M., Saito, H., Hashimoto, Y., & Inui, K. (1999). Gender differences in expression of organic cation transporter OCT2 in rat kidney. *FEBS Letters, 461*(3), 339-342. doi:10.1016/s0014-5793(99)01491-x

Urien, S., & Lokiec, F. (2004). Population pharmacokinetics of total and unbound plasma cisplatin in adult patients. *British Journal of Clinical Pharmacology, 57*(6), 756-763. doi:10.1111/j.1365-2125.2004.02082.x

Yonezawa, A., & Inui, K. (2011). Organic cation transporter OCT/SLC22A and H(+)/organic cation antiporter MATE/SLC47A are key molecules for nephrotoxicity of platinum agents. *Biochemical Pharmacology, 81*(5), 563-568. doi:10.1016/j.bcp.2010.11.016

Yonezawa, A., Masuda, S., Nishihara, K., Yano, I., Katsura, T., & Inui, K. (2005). Association between tubular toxicity of cisplatin and expression of organic cation transporter rOCT2 (Slc22a2) in the rat. *Biochemical Pharmacology, 70*(12), 1823-1831. doi:10.1016/j.bcp.2005.09.020

Yonezawa, A., Masuda, S., Yokoo, S., Katsura, T., & Inui, K. (2006). Cisplatin and oxaliplatin, but not carboplatin and nedaplatin, are substrates for human organic cation transporters (SLC22A1-3 and multidrug and toxin extrusion family). *Journal of Pharmacology and Experimental Therapeutics, 319*(2), 879-886. doi:10.1124/jpet.106.110346

Zolk, O., Solbach, T. F., Konig, J., & Fromm, M. F. (2009). Functional characterization of the human organic cation transporter 2 variant p.270Ala>Ser. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 37*(6), 1312-1318. doi:10.1124/dmd.108.023762

Figure legends

Figure 1. Uptake of [^{14}C]TEA in HEK-hOCT2 and HEK-vector cells. HEK-hOCT2 (closed column) or HEK-vector (open column) cells were incubated with [^{14}C]TEA (5 μM, pH 7.4) for 2 min at 37°C. Each point represents the mean ± S.E. of three separate experiments using three monolayers. ***: $p < 0.001$ compared with HEK-vector cells. When the standard errors of the means are small, they are contained within the columns.

Figure 2. Time course of cisplatin uptake in HEK-hOCT2 cells. HEK-hOCT2 (closed circles) or HEK-vector (open circles) cells were incubated with cisplatin (10 μM, pH 7.4) for the specified duration (2, 15, 30, and 60 min) at 37°C. Each point represents the mean ± S.E. of three separate experiments using three monolayers. **: $p < 0.01$, ***: $p < 0.001$ compared with HEK-vector cells. When the standard errors of the means were small, error bars are hidden behind the symbols.

Figure 3. Inhibition of LPZ and cimetidine on hOCT2-mediated transport of cisplatin. HEK-hOCT2 cells were incubated at 37°C for 5 min with cisplatin (1 μM) in the absence or presence of LPZ (100 μM) or cimetidine (100 μM). Each point represents the mean ± S.E. of three separate experiments using three monolayers. ***: $p < 0.001$ compared with Control (vehicle).

Figure 4. Time course of Pt uptake in rat renal slices. Renal slices were incubated with cisplatin (50 μM, pH 7.5) at 25ºC (closed circles) and 4ºC (open circles) for the specified duration (5, 10, and 20 min). Each point represents the mean ± S.E. of three separate experiments using three slices. **: $p < 0.01$, ***: $p < 0.001$ compared with 4ºC. When the standard deviation of the means were small, error bars are hidden behind the symbols.

Figure 5. Inhibition of LPZ and cimetidine on the uptake of Pt in rat renal slices. Renal slices were incubated with cisplatin (50 μM, pH 7.5) at 25ºC for 5 min in the absence or presence of LPZ (100 μM) or cimetidine (100 μM). Each point represents the mean ± S.E. of three separate experiments using three slices. **: $p < 0.01$ compared with Control.

Figure 6. Effect of concomitant LPZ administration on renal Pt accumulation at 72 h after cisplatin (7.5 mg/kg, i.p.) administration in rats. Each column represents the mean ± S.D. of five rats. **: $p < 0.01$, ***: $p < 0.001$ compared with cisplatin rats.

Figure 7. Effect of concomitant LPZ (2 mg/kg) and cimetidine (20 mg/kg) administration on the pharmacokinetics of Pt within 3 min after cisplatin (1 mg/kg) intravenous administration in rats. (A) Plasma concentration-time profiles of Pt. (B) Renal Pt accumulation. Each point and column represent the mean ± S.D. of five rats. *: $p < 0.05$, **: $p < 0.01$ compared with Control (cisplatin only) rats. Control rats (open circles), Cisplatin + LPZ rats (closed circles), and Cisplatin + Cimetidine rats (closed squares)

Table

Table 1. Renal functions in rats at 72 h after intraperitoneal administration cisplatin

	Sham	Cisplatin	Cisplatin + LPZ (1 mg/kg)	Cisplatin + LPZ (2 mg/kg)
Pcr (mg/dL)	0.4 ± 0.0	1.3 ± 0.1***	1.1 ± 0.2 $^{***, \#}$	0.8 ± 0.1 $^{***, \#\#\#, \dagger\dagger}$
CLcr (mL/min/kg)	4.0 ± 0.4	1.3 ± 0.2 ***	2.2 ± 0.3 $^{***, \#\#}$	2.8 ± 0.3 $^{***, \#\#\#, \dagger}$
BUN (mg/dL)	11 ± 1	97 ± 7 ***	51 ± 17 $^{***, \#\#\#}$	37 ± 9 $^{**, \#\#\#}$
Urinary L-FABP (μg/g creatinine)	2.3 ± 1.3	76.4 ± 31.2 ***	2.9 ± 1.8 $^{\#\#\#}$	2.6 ± 2.4 $^{\#\#\#}$

Results are mean ± S.D. of five rats. **: $p < 0.01$, ***: $p < 0.001$ compared with Sham rats, $^{\#}$: $p < 0.05$, $^{\#\#}$: $p < 0.01$, $^{\#\#\#}$: $p < 0.001$ compared with Cisplatin rats, †: $p < 0.05$, ††: $p < 0.01$ compared with Cisplatin + LPZ (1 mg/kg) rats.

BUN: blood urea nitrogen, CLcr: creatinine clearance, L-FABP: liver-type fatty acid binding protein, LPZ: lansoprazole, Pcr: plasma creatinine

Figure 1

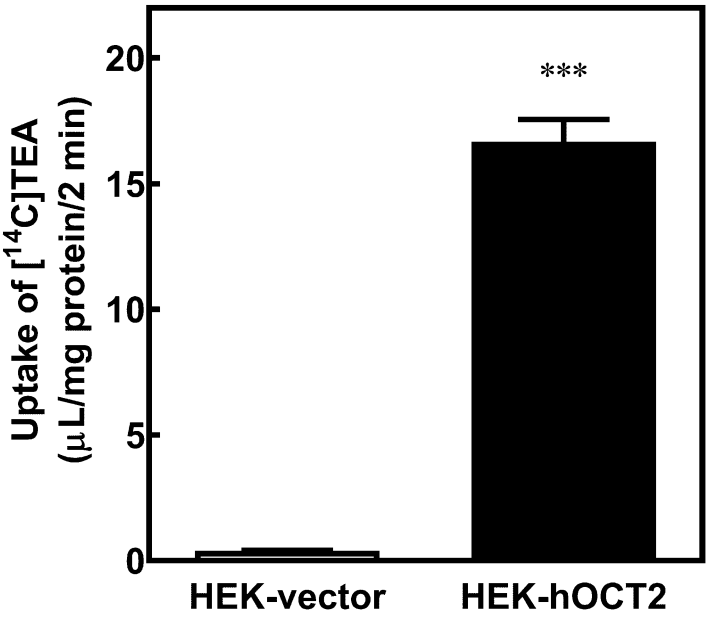

Uptake of [14C]TEA
(μL/mg protein/2 min)
20
15
10
5
0
***
HEK-vector
HEK-hOCT2

Figure 2

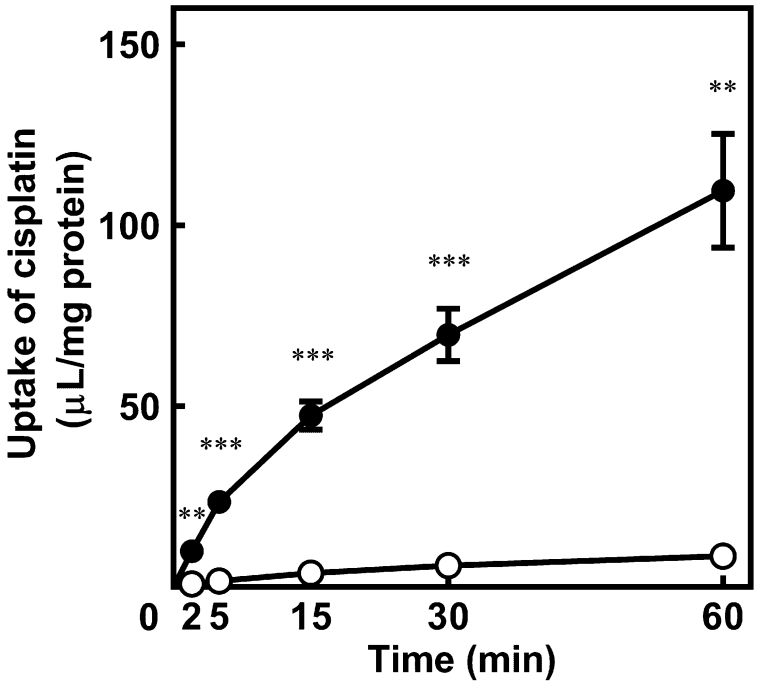

150
100
50
0
Uptake of cisplatin
(μL/mg protein)
**
***
***
***
**
2
5
15
30
60
Time (min)

Figure 3

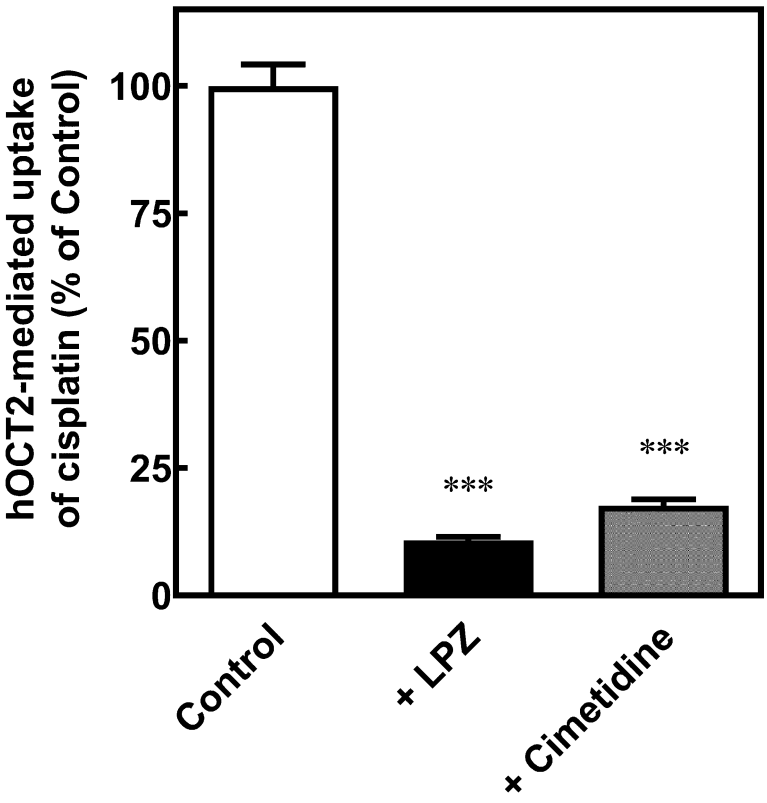

hOCT2-mediated uptake
of cisplatin (% of Control)
100
75
50
25
0
***
***
Control
+ LPZ
+ Cimetidine

Figure 4

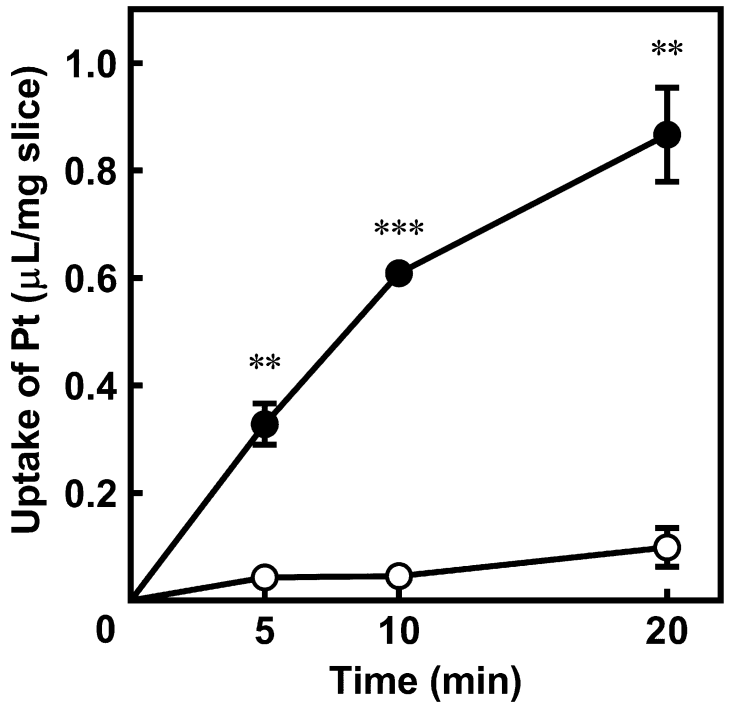


Figure 5

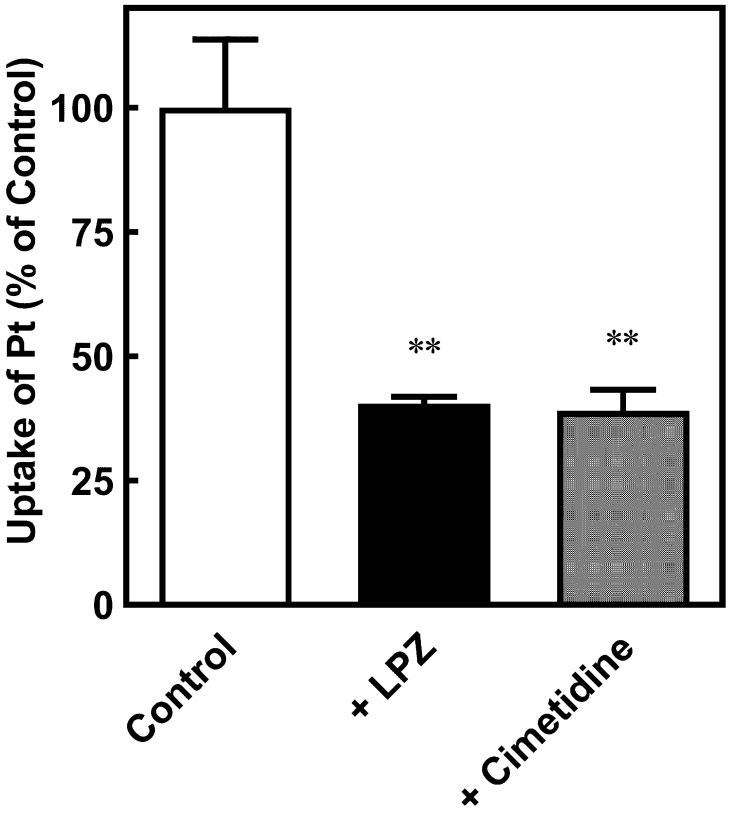


Figure 6

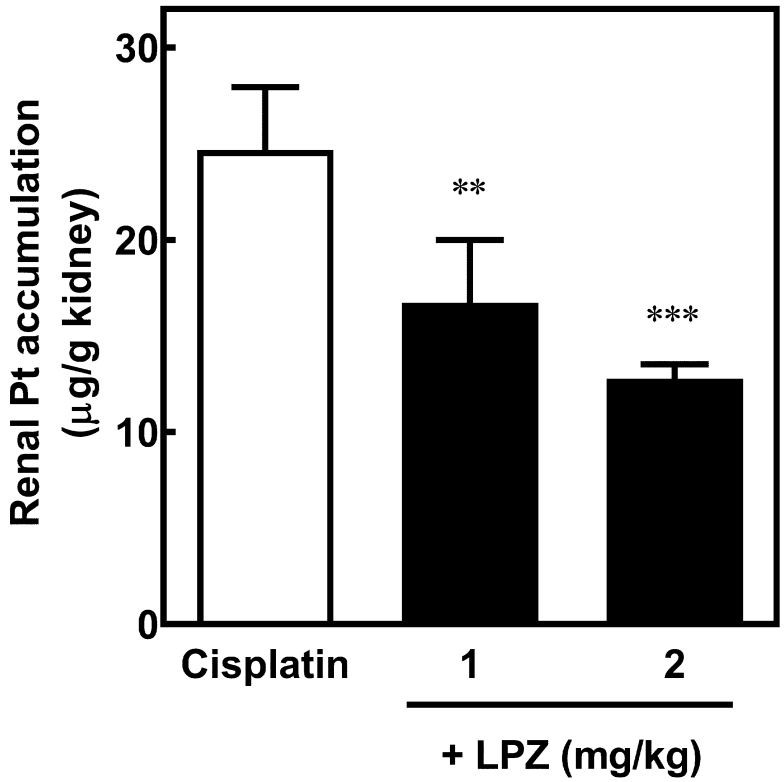
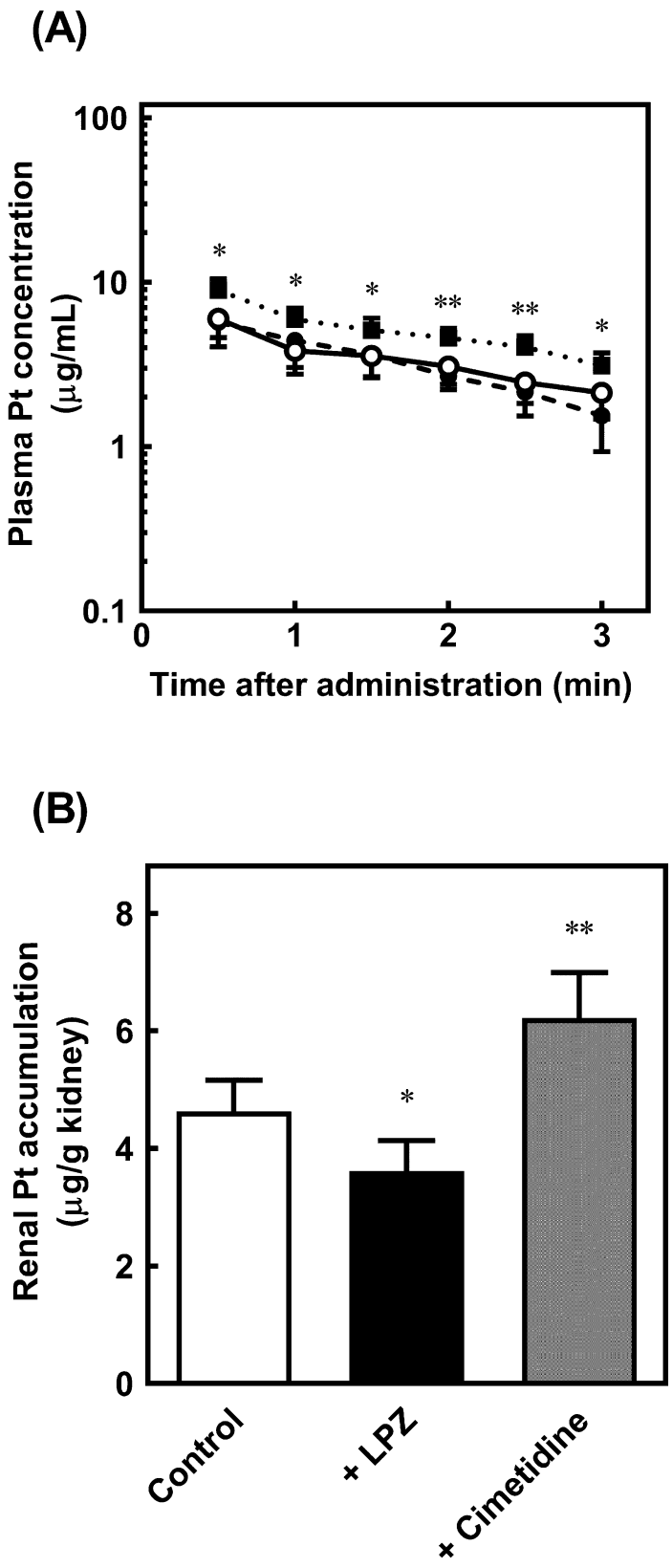

Renal Pt accumulation
(μg/g kidney)
30
20
10
0
**
***
Cisplatin
1
2
+ LPZ (mg/kg)

Figure 7


(A)
Plasma Pt concentration (μg/mL)
100
10
1
0.1
0
1
2
3
Time after administration (min)
*
*
*
**
**
*
(B)
Renal Pt accumulation (μg/g kidney)
8
6
4
2
0
*
**
Control
+ LPZ
+ Cimetidine