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Conjunctive therapy of cisplatin with the OCT2 inhibitor cimetidine: influence on antitumor efficacy and systemic clearance

JA Sprowl[1], **L van Doorn**[2], **S Hu**[1], **L van Gerven**[2], **P de Bruijn**[2], **L Li**[1], **AA Gibson**[1], **RH Mathijssen**[2], and **A Sparreboom**[1,2]

[1]Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, Tennessee, USA [2]Department of Medical Oncology, Erasmus MC, Rotterdam, the Netherlands

Abstract

The organic cation transporter 2 (OCT2) regulates uptake of cisplatin in proximal tubules and inhibition of OCT2 protects against severe cisplatin-induced nephrotoxicity. However, it remains uncertain whether potent OCT2 inhibitors such as cimetidine can influence the antitumor properties and/or disposition of cisplatin. Using an array of preclinical assays, we found that cimetidine had no effect on the uptake and cytotoxicity of cisplatin in ovarian cancer cells with high OCT2 mRNA levels (IGROV-1). Moreover, the antitumor efficacy of cisplatin in mice bearing luciferase-tagged IGROV-1 xenografts was unaffected by cimetidine ($P = 0.39$). Data obtained in 18 patients receiving cisplatin (100 mg/m^2) in a randomized crossover fashion with or without cimetidine (800 mg×2) revealed that cimetidine did not alter exposure to unbound cisplatin, a marker of antitumor efficacy (4.37 vs 4.38 μg×h/mL; $P = 0.86$). These results support the future clinical exploration of OCT2 inhibitors as specific modifiers of cisplatin-induced nephrotoxicity.

Keywords

SLC22A2 (OCT2); Cisplatin; Nephrotoxicity; cimetidine

Cis-diamminedichloroplatinum (cisplatin) is a commonly used anticancer drug that is effective for the treatment of various malignant solid tumors.[1] It is currently understood that

Correspondence: Alex Sparreboom, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, CCC, Room I5308. Phone: (901) 595-5346; Fax: (901) 595-3125; alex.sparreboom@stjude.org.

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AUTHOR CONTRIBUTIONS

JAS and AS wrote the manuscript; JAS, LL, RHM, and AS designed research; JAS, LvD, SH, LvG, PdB, LL, AAG, and RHM performed research; PdB and LL contributed new reagents/analytical tools; JAS, LL, RHM, and AS analyzed data.

CONFLICT OF INTEREST

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cisplatin covalently binds with DNA to form adducts that activate signal-transduction pathways involved in DNA-damage recognition and repair, cell-cycle arrest, and programmed cell death/apoptosis.[2] The clinical use of cisplatin is associated with a dose-limiting renal tubular dysfunction typically occurring in up to 40% of patients despite the introduction of preventative measures such as intravenous hydration therapy and prolonging the infusion duration.[3–7]

Cisplatin is known to abundantly accumulate in the human renal cortex against a concentration gradient, and it can competitively inhibit active uptake of the cation tetraethylammonium (TEA) in mouse kidney slices and basolateral membrane vesicles of the renal cortex of rats.[8–10] Furthermore, cisplatin has been shown to inhibit the renal clearance of organic ions from the basolateral site in isolated-perfused rat kidney.[11] These observations led to the recognition that an organic cation transporter (OCT) was likely regulating the cellular uptake of cisplatin into renal proximal tubular cells. In recent years, this transporter has been identified as OCT2, encoded by the gene *SLC22A2*, which is expressed at high levels in the basolateral membrane of renal tubular epithelial cells.[12,13] Indeed, mice with a deficiency of the murine ortholog transporters Oct1 and Oct2 are protected from experiencing severe tubular damage following exposure to cisplatin,[14,15] and similarly, patients carrying a variant in the OCT2 gene associated with reduced function are protected from cisplatin-induced nephrotoxicity.[14,16] Moreover, concurrent administration of cisplatin with the OCT2 inhibitor cimetidine[17–19] was demonstrated to result in at least partial protection against renal toxicity.[15,20]

While OCT2 has been linked to cisplatin-induced nephrotoxicity, it remains unclear if this transporter has a contribution to the antitumor properties of cisplatin. In the current study, we evaluated the extent to which the transport of cisplatin by OCT2 is an important source of interindividual variability in efficacy and systemic disposition of cisplatin-based chemotherapy which is a marker of anti-tumor efficacy in patients with head and neck cancer.[21,22] We used *in vitro* and *in vivo* experimental approaches involving cell lines with variable expression levels of OCT2, a murine tumor model, and a cohort of patients with head and neck cancer receiving cisplatin with or without cimetidine in a randomized crossover fashion.

RESULTS

Effect of cimetidine on cisplatin uptake and cytotoxicity *in vitro* and *in vivo*

Previous studies reported that, among the NCI60 panel of cell lines, expression of the OCT2 gene *SLC22A2* was the highest in the ovarian cancer cell lines SKOV-3[19] and IGROV-1.[23] A direct comparison indicates that the expression of *SLC22A2* in IGROV-1 cells is equivalent to that observed in SKOV-3 cells (Figure 1a). Since SKOV-3 cells, unlike IGROV-1 cells, are p53-deficient and thereby experience increased intrinsic resistance to cisplatin,[24,25] we focused on IGROV-1 cells to assess the influence of OCT2 inhibition on cisplatin-induced cytotoxicity. Despite the detectable expression levels of *SLC22A2*, co-incubation with cimetidine did not result in altered cisplatin uptake in IGROV-1 cells compared to cells exposed to cisplatin alone ($P = 0.86$) (Figure 1b). Moreover, co-

incubation of cimetidine with cisplatin had no effect on cell growth inhibition when compared to IGROV-1 cells exposed to cisplatin alone (Figure 1c).

We next sought to assess whether co-administration of cimetidine with cisplatin would alter treatment efficacy *in vivo* using immunodeficient *nu/nu* mice. Phenotypic characterization of these mice indicated that the cumulative urinary excretion of cisplatin was only about 25% of the dose, which is considerably lower than that observed in FVB mice used previously in toxicity studies (see Supplementary Figure 1a online). In line with this observation, we found that the *nu/nu* mice are relatively resistant to cisplatin-induced nephrotoxicity compared to FVB mice, as determined by both histological examination of the kidney and by the toxicity markers BUN and serum creatinine (see Supplementary Figure 1b–d online). These findings suggests that the effects of cimetidine on the anti-tumor efficacy of cisplatin can be properly assessed in *nu/nu* mice without considering injurious effects on the kidney that may impact morbidity and mortality.

Administration of either cimetidine alone, cisplatin alone, or a combination of cisplatin and cimetidine to female *nu/nu* mice xenografted with luciferase-positive IGROV-1 cells had no effect on overall body weight, whereas mice that received saline alone experienced a significant increase in weight ($P = 0.04$), presumably due to a progressively increasing tumor size (Figure 2a). The IGROV-1 tumor volume, measured based on luciferase intensity, was dramatically decreased in mice that received cisplatin or the combination of cisplatin and cimetidine, compared to controls and did not significantly differ from each other ($P = 0.39$) (Figure 2b/c). Mice that had received cimetidine alone had no changes in tumor volume compared to control mice ($P = 0.09$).

In order to further understand why cimetidine does not alter the uptake of cisplatin or cisplatin-induced cytotoxicity in cells expressing *SLC22A2*, we assessed localization of the OCT2 protein in these cells by immunofluorescence. We found that OCT2 was not detected at the plasma membrane of IGROV-1 or SKOV-3 cells (Figure 3a/b), while HEK293 cells engineered to overexpress OCT2, used as a positive control, had an abundance of OCT2 localized to this region (Figure 3c). OCT2 was also absent from the plasma membrane of HEK293 cells transfected with a vector control (Figure 3d).

Effects of cimetidine co-administration on cisplatin pharmacokinetics

A total of 19 patients diagnosed with head and neck cancer were recruited to the study, but samples from one patient had undetectable levels of cimetidine, and data from this patient were excluded from subsequent analyses. Baseline demographic data from the remaining 18 patients are summarized in Table 1. Of these patients, 10 received co-administration of cimetidine with cisplatin during cycle 1 and cisplatin alone during cycle 2 (Arm A), while 8 patients received the reverse sequence (Arm B). Two of 18 patients (both in Arm A) were found to be heterozygous for the *SLC22A2* 808G>T variant (rs316019). The pharmacokinetic parameter estimates were not significantly altered when data from these 2 patients were excluded, and therefore data from all patients were included irrespective of *SLC22A2* genotype. Two patients had received proton-pump inhibitors, which have been previously identified as OCT2 inhibitors,[26] however pharmacokinetic parameter estimates were not significantly altered when data from these 2 patients were excluded

(Supplementary Figure 2), and therefore data reported in this study included all patients. The area under the curve (AUC) of cimetidine in patients enrolled in Arm A and Arm B was 29.0 ± 2.40 μg×h/mL and 24.5 ± 2.14 μg×h/mL, respectively, which is in line with previous estimates.[27] The concentrations of unbound cimetidine were consistently above the observed levels of unbound cisplatin at all time-points in both Arm A and Arm B (Figure 4a–b), with an average AUC ratio of unbound cimetidine to unbound cisplatin of 5.55 ± 0.50. Since a cimetidine to cisplatin concentration ratio of 2:1 was previously found to reduce OCT2-mediated uptake of cisplatin already by 4-fold,[19] these clinical findings support the selection of our cimetidine dosing regimen.

In Arm A, the AUC of unbound cisplatin was unaffected by concurrent use of cimetidine (4.47 ± 0.21 *vs* 4.47 ± 0.20 μg×h/mL; $P = 0.80$), and similar results were obtained in Arm B (4.26 ± 0.24 *vs* 4.27 ± 0.40 μg×h/mL; $P = 0.99$) (Figure 5a–b; Table 2). Likewise, the clearance of unbound cisplatin was unaffected by cimetidine, independently of the treatment sequence (Figure 5c–d; Table 2). Combining results from the two treatment arms generated similar results (Table 2). Additionally, urinary excretion of platinum was unaffected by cimetidine (Table 2), which is consistent with previous findings that showed patients with the reduced functional *SLC22A2* 808G>T variant do not have altered urinary excretion.[28]

DISCUSSION

The current study addressed the concern as to whether co-administration of cisplatin with the OCT2 inhibitor cimetidine would alter the efficacy and pharmacokinetic properties of cisplatin. Using *in vitro* models, we showed that cimetidine had no effect on the cytotoxicity of cisplatin or tumoral uptake. Moreover, co-administration of cimetidine with cisplatin in mice bearing IGROV-1 xenografts demonstrated no significant change in efficacy compared to mice receiving cisplatin alone. Finally, we report that patients with head and neck cancer who received a combination of cisplatin and cimetidine showed no alterations in cisplatin pharmacokinetics as compared to when the same patients received cisplatin alone.

The incentive for our study was based on a growing body of literature which suggests that concurrent use of cimetidine can be protective against cisplatin-induced nephrotoxicity. The mechanism by which cimetidine affects cisplatin-induced nephrotoxicity is believed to be due to competitive inhibition of the renal tubular transporter OCT2, thereby restricting the accumulation of cisplatin in the kidney and subsequent downstream events resulting in apoptosis. This supposition is consistent with both *ex vivo* preclinical findings indicating that cimetidine can alter cisplatin uptake in mouse kidney slices,[29] as well as *in vivo* observations in rodents and patients with cancer.[20,30] Although the above studies reveal that cimetidine can be of great benefit in ameliorating cisplatin-induced nephrotoxicity, evidence to show whether its use would alter treatment efficacy is limited.

Currently available expression data demonstrate that OCT2 is either absent or detectable only at low levels in tumors, suggesting that it would be highly unlikely that this transporter regulates uptake of cisplatin into tumor cells.[14,23] Our current study is in agreement with this notion, where even in cells that are among the highest expressers of OCT2, cimetidine does not appear to alter tumor cell uptake or efficacy *in vitro* or *in vivo*. These observations

are consistent with other recently reported studies involving cell lines with high expression of OCT2 either in culture or in xenograft models.[19,31] Moreover, we provide data to show that although tumor cells may express high levels of OCT2, localization and function of this transporter is not necessarily a factor in the uptake of cisplatin into tumor cells. This process may instead rely on other transporters, such as the organic anion transporting polypeptide OATP1B3[32] or the copper transporter 1 (CTR1),[33] although involvement of CTR1 in cisplatin transport has recently been questioned.[34] In addition to the preclinical observations, patient data obtained throughout our study indicate that inhibition of OCT2 function with cimetidine has no impact on plasma levels or systemic clearance of cisplatin, which is consistent with results found in mice with a deficiency of both Oct1 and Oct2.[14]

Although our study supports further clinical exploration of cimetidine as a modulator of cisplatin toxicity, it should be pointed out that the introduction of cimetidine to cisplatin-containing chemotherapy regimens in routine practice could remain problematic. Indeed, while loss of OCT2 function *in vivo* substantially diminishes cisplatin-induced nephrotoxicity, the degree of protection that is offered remains only partial,[14] suggesting that other pathways unaffected by cimetidine may contribute to the overall side effect profile. Furthermore, it should be kept in mind that the action of cimetidine on OCT2 is via a competitive inhibitory mechanism and that patients with a genetic predisposition to poor cimetidine absorption and/or rapid elimination may experience inadequate renoprotection, or that cimetidine can inhibit the metabolism of other chemotherapeutics that are sometimes given in combination with cisplatin, such as paclitaxel.[35] Adding further to the complexity, the Ki of cimetidine for MATE1 and MATE2-K, two solute carriers expressed on the luminal membrane of renal tubular cells linked with the terminal elimination of platinum chemotherapeutic drugs,[36] are 1.1 and 7.3 μM, respectively, which is comparable to the Ki for OCT2.[37–40] Therefore cimetidine is likely to inhibit function of MATE1 and MATE2-K at relatively low doses and may lead to non-optimal efflux of cisplatin out of tubular cells.

In conclusion, our results indicate that use of the OCT2 inhibitor cimetidine does not sacrifice the antitumor effects of cisplatin, irrespective of tumoral OCT2 expression, and that cimetidine has no influence of the disposition of cisplatin. These results support the future exploration of treatment strategies aimed at ameliorating cisplatin-induced nephrotoxicity using OCT2 inhibitors that can offer improved protection and selectivity compared to cimetidine.

METHODS

In vitro and *in vivo* antitumor efficacy

Expression of *SLC22A2* in IGROV-1 and SKOV-3 cells were assessed as previously described and normalized to expression of *GAPDH*.[19] Cellular cisplatin uptake was measured by determining total platinum levels following incubation with cisplatin (500 μM) for 30 minutes in the presence or absence of cimetidine (1 mM), as previously described.[19] The cell growth inhibitory potential of cisplatin in IGROV-1 cells in the presence or absence of 100 μM cimetidine was evaluated using an MTT assay following continuous exposure for 72 hours.

Efficacy studies were done in female, immunodeficient CD-1 *nu/nu* mice (Charles River, Wilmington, MA) injected with of 3×10^6 Luc^+-YFP^+-tagged IGROV-1 cells which were transduced with a lenti-vector (obtained from Vector Core Facility at St. Jude Children's Research Hospital) containing the firefly luciferase and yellow fluorescent protein genes (CL20SF2-Luc2aYFP). Yellow fluorescent protein-positive cells were sorted by flow cytometry and expanded in culture before injection. Following 21 days, cisplatin (10 mg/kg, i.p.) was administered in the presence or absence of cimetidine (7.5 mg/kg, i.v.) once a week for 3 weeks. Efficacy was assessed by measuring total body weight and tumor volume, as measured by luciferase imaging of mice and as a percentage of pretreatment values. Housing, handling, sample collection and analysis were performed as previously described.[14] The experiments were approved according to guidelines of the IACUC of St Jude Children's Research Hospital (Memphis, TN).

Cellular localization of OCT2

Localization of OCT2 was assessed in IGROV-1 and SKOV-3 cells, as well as in HEK293 cells that were transfected with human OCT2 or a control vector.[41] A total of 250,000 cells were plated in 6-well plates with sterile glass coverslips and incubated under standard conditions overnight. Cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton-X and incubated in phosphate buffer-saline with 4% fetal bovine serum after which a rat anti-human OCT2 antibody (Sigma-Aldrich, St Louis, MO) at a 1:40 dilution was added and left overnight at 4°C. Specificity of this antibody has been demonstrated by the Human Protein Atlas (http://www.proteinatlas.org/ENSG00000112499/subcellular). Samples were then incubated with a goat anti-rabbit Alexa Fluor 555 secondary antibody (1:500 dilution; Invitrogen, Carlsbad, CA) and co-stained with Alexa Fluor 488 phalloidin (1:100 dilution; Invitrogen, Carlsbad, CA). Coverslips were mounted on glass microscope slides with Prolong Gold antifade reagent with DAPI (Invitrogen, Carlsbad, CA) for observation by confocal microscopy (LSM 510 Meta; Carl Zeiss) with a 63× objective lens.

Patient selection

All patients had a histologically or cytologically confirmed diagnosis of irresectable and/or metastatic head and neck cancer, which at the time was not treated with cisplatin. Eligibility criteria included: (i) availability to receive treatment with high dose cisplatin (3-weekly 100 mg/m^2), without any other systemic anti-cancer treatment; (ii) at least 18 years of age; (iii) WHO performance status ≤ 1; (iv) adequate hematological functions (absolute neutrophil count > 1.5×10^9/L; platelets > 100×10^{12}/L); (v) adequate renal [serum creatinine < 1.25×upper limit of institutional normal (ULN)] and hepatic functions (bilirubin < 1.25×ULN, ALAT and ASAT < 2.5×ULN; in case of liver metastasis < 5×ULN; alkaline phosphatase < 5×ULN). All patients provided written informed consent and the study protocol was approved by the Erasmus MC institutional review board. Specifically excluded were patients if they were: (i) pregnant or lactating; (ii) experiencing serious illness or were in a medically unstable condition requiring treatment; (iii) having symptomatic CNS-metastases or a history of psychiatric disorder that prohibited the understanding and providing of informed consent; (iv) currently undergoing cisplatin therapy; (v) previously diagnosed with chronic kidney disease; (vi) undergoing major surgery within 4 weeks of the start of treatment; (vii) chronic users of CYP3A and/or ABCB1/ABCG2 inhibiting and/or

inducing medication, dietary supplements, or other inhibiting compounds of putative relevance to cimetidine (http://medicine.iupui.edu/flockhart/table.htm); (viii) unwilling to change medication; and (ix) using cimetidine within 4 weeks prior to study entry.

Pretherapy evaluations

Urine samples were collected within 24 hours of the start of treatment to establish baseline kidney function and blood samples were collected to allow subsequent genotyping of patients. A complete blood count was performed to assess WBC differentiation, and serum biochemistry, including sodium, potassium, calcium, phosphorus, creatinine, creatine kinase, transaminases, γ-glutamyltranspeptidase, alkaline phosphatase, total and conjugated bilirubin, total protein, α1-acid glycoprotein, albumin, glucose, blood urea nitrogen, urea within two weeks prior to therapy. All patients were genotyped for the *SLC22A2* 808G>T variant, as previously described.[14] Baseline creatinine clearance was determined by the Cockcroft-Gault formula and Estimated Glomerular Filtration (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula which considers serum creatinine, blood urea nitrogen, and albumin levels.

Study design and treatment

Patients were deemed evaluable when treated once every 3 weeks with cisplatin at an i.v. dose of 100 mg/m^2 given as a 3-hour infusion for at least 2 cycles of treatment, and when a complete pharmacokinetic sampling and toxicity assessment had been performed. Enrollment of patients continued until at least 18 patients had evaluable pharmacokinetic data for both cimetidine and cisplatin. Under these conditions, the probability is 91% that the study will detect a difference in the pharmacokinetics of unbound cisplatin between treatment groups at a two-sided 5.0% significance level, if the true difference between the treatments is 0.300 units. This is based on the assumption that the within-patient standard deviation of the response variable is 0.200.[42] No reductions or alterations of cisplatin dose were permitted in this study and therefore if patients experienced severe nephrotoxicity following the first cycle they were immediately removed from the study. As a result, the randomized crossover study design of this trial did not allow for accurate assessment as to the renoprotective effects of cimetidine which would instead require a randomized, parallel, two arm, placebo-controlled, and adequately powered study to definitively address its protective effects.

Participants were randomized such that approximately half of the patients received concomitant administration of cimetidine with cisplatin on the initial day of treatment and no cimetidine on the second day of treatment (arm A), while the remaining patients received cisplatin alone on the initial day of treatment and concomitant administration of cimetidine with cisplatin on the second day of treatment (arm B). Cimetidine was given at a dose of 800 mg orally 30 minutes prior to the initiation of cisplatin infusion and again 800 mg orally 1.5 hours after the start of infusion in order to achieve an unbound concentration of at least 3-times that of cisplatin which would be necessary to achieve renoprotection.[16]

Patients also received aprepitant (125 mg), granisetron (1 mg), and dexamethasone (10 mg) on the day of cisplatin infusion and continued aprepitant (80 mg a day) on days 2 and 3 after

infusion and dexamethasone (6 mg/day) on days 2 to 4. Some patients also received a proton pump inhibitors and most received pain-killing medication (acetaminophen, and morphine). These agents were found not to interfere with the function of OCT2 in preclinical studies at clinically achievable concentrations (Supplementary Figure 3).[19] No other anticancer chemotherapy or use of biological response modifiers, hormone therapy or immunotherapy was permitted during the study period.

Clinical pharmacokinetic studies

The pharmacokinetics of unbound and total cisplatin was assessed on the first day of hospitalization up to the following day in each treatment cycle. Blood samples were collected from the peripheral vein immediately prior to cisplatin administration, and then at 1, 2, 3, 3.5, 4, 5, 6, and 24 hours following the start of infusion. Samples were processed to obtain plasma, which was stored frozen until analysis. Cisplatin concentrations were measured by a validated atomic absorption method, as previously described.[14] The same samples were also used for the determination of cimetidine concentrations using liquid chromatography-tandem mass spectrometry (MS/MS). In brief, quantitation of cimetidine was carried out with a Waters ACQUITY separation system (Milford, MA) and TQD triple-quadrupole system (Beverly, MA). Separation was achieved on a Waters ACQUITY $BEHC_{18}$ column (1.7 μm, 100 × 2.1 mm) using a column heater operating at 40°C with a Waters ACQUITY in-line filter. The autosampler temperature was maintained at 15 ± 5°C, and a gradient mobile phase was composed of acetonitrile and 10mM ammonium bicarbonate in water. The flow rate was 0.6 ml/min and the separation was completed within 3.5 min. The instrument was equipped with an electrospray interface, and was controlled by Masslynx 4.1 software (Micromass, UK). The analysis was performed in MRM mode: *m/z* 253.08>158.95 for cimetidine, and cimetidine_d3 (*m/z* 256.02>162.02) was used as the internal standard. The MS/MS conditions were as follows: capillary voltage: 0.7 kV; source temperature: 150°C; desolvation temperature: 450 °C; cone gas flow: 10 l/h; desolvation gas flow: 900 l/h. Calibration curves of cimetidine were created by plotting the peak area ratios of analyte to the internal standard against the analyte concentrations in the spiked plasma. The within- and between-run precisions for cimetidine were always less than 5.0%, and the mean measured concentrations (accuracy) were always within ±5.3% of the nominal value. All pharmacokinetic parameters were calculated using WinNonlin 6.2 (Pharsight, St. Louis, MO).

Statistical considerations

All data are presented as mean values with standard error. Group differences as a function of cell type or treatment arm were evaluated using a t-test. Two-tailed *P* values of less than 0.05 were considered as statistically significant. All statistical calculations were performed using NCSS version 2004 (Number Cruncher Statistical System, Kaysville, UT).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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STUDY HIGHLIGHTS

What is the current knowledge on the topic?

Inhibitors of the organic cation transporter 2 (OCT2) can ameliorate cisplatin-induced nephrotoxicity.

What question this study addressed?

Do renoprotective strategies involving the OCT2 inhibitor cimetidine influence the antitumor properties and systemic disposition of cisplatin?

What this study adds to our knowledge?

Use of cimetidine does not influence the pharmacokinetics or antitumor effects of cisplatin, irrespective of tumoral OCT2 expression.

How this might change clinical pharmacology and therapeutics?

This study provides support to future exploration of treatment strategies aimed at ameliorating cisplatin-induced nephrotoxicity using OCT2 inhibitors.

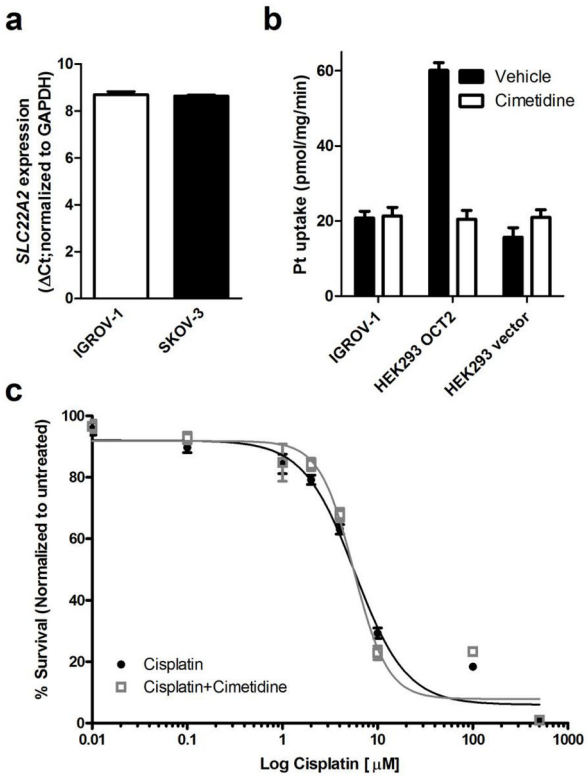

Figure 1.

Expression of *SLC22A2* (OCT2) normalized to expression of *GAPDH*, in IGROV-1 and SKOV-3 tumor cells (**a**). Cellular accumulation of platinum in IGROV-1 cells as well as HEK293 cells transfected with OCT2 or a vector control following a 30 minute incubation with cisplatin (500μM) in the absence or presence of cimetidine (1 mM) (**b**). Survival of IGROV-1 cells exposed to various concentrations of cisplatin and normalized to untreated controls in the presence or absence of cimetidine (100 μM) (**c**). Error bars represent standard error of the mean (n=6).

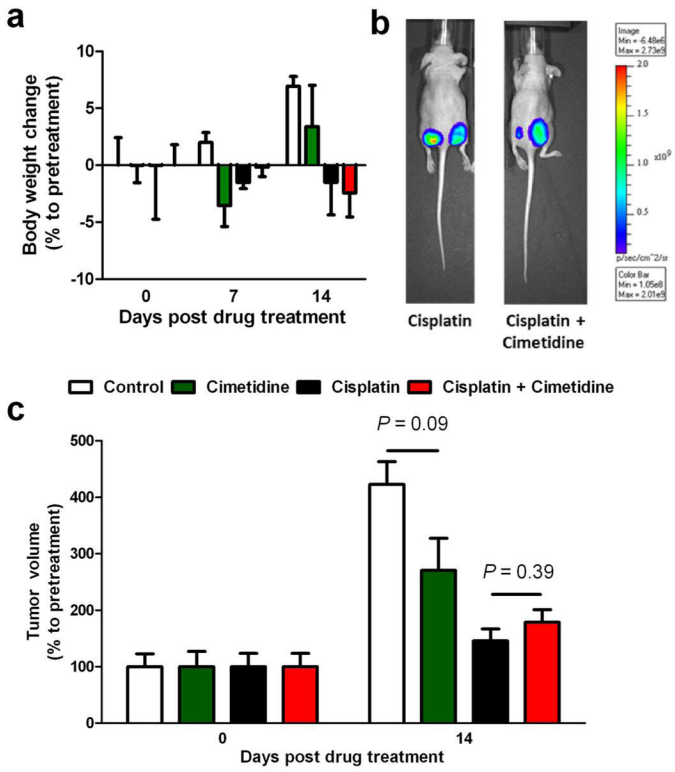

Figure 2.

Change in body weight from baseline of female immunodeficient CD-1 *nu/nu* mice injected with IGROV-1 cells following 7 and 14 days of receiving cisplatin (10 mg/kg i.p.), cimetidine (7.5 mg/kg i.v.) or a combination of cisplatin and cimetidine (**a**). Representative luminescence images of female immunodeficient CD-1 *nu/nu* mice bearing IGROV-1 cells following 14 days of receiving cisplatin (10 mg/kg i.p.) or a combination of cisplatin and cimetidine (7.5 mg/kg i.v.) (**b**). Signal intensity is measured by quantitative biophotonic imaging analysis (photons/sec/cm^2/sr). Tumor growth and volume as measured by luciferase activity in female immunodeficient CD-1 *nu/nu* mice injected with IGROV-1 cells following 7 and 14 days of receiving cisplatin (10 mg/kg i.p.), cimetidine (7.5 mg/kg i.v.) or a combination of cisplatin and cimetidine (**c**). Error bars represent standard error of the mean (n=6). P-values above the bars denote statistical comparison between treatments.

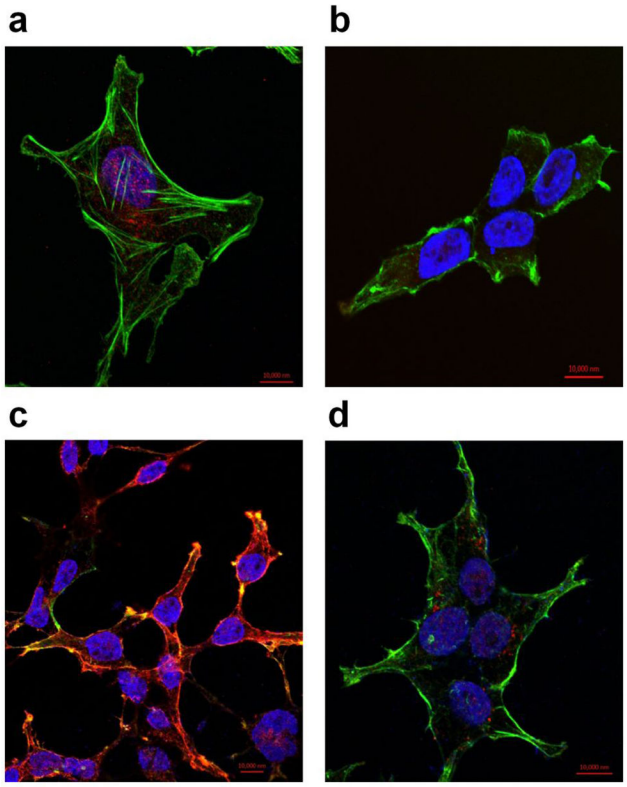

Figure 3.

Cellular localization of OCT2 (red) in IGROV-1 (**a**) and SKOV-3 cells (**b**), or HEK293 cells transfected with OCT2 (**c**), or a vector control (**d**). Cells were also co-stained with phalloidin (green) and DAPI (blue) to visualize actin and DNA, respectively.

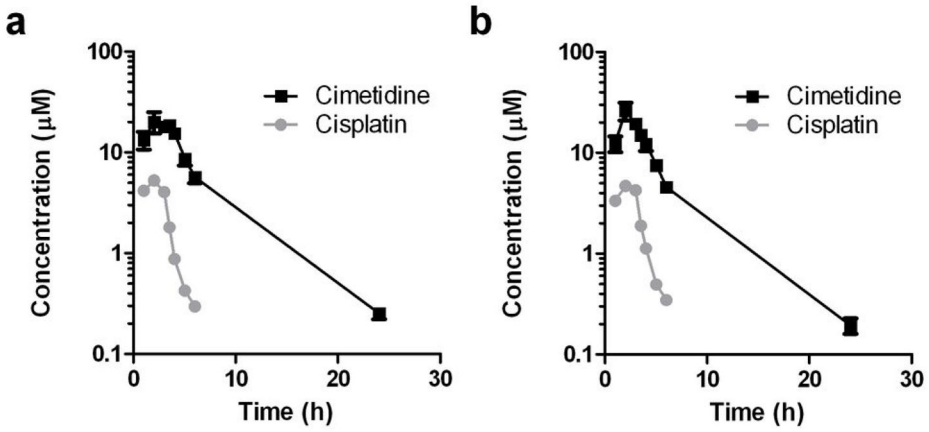

Figure 4.

Systemic levels of unbound cimetidine and unbound cisplatin in patients enrolled in Arm A (**a**) or Arm B (**b**) over time. Data is represented by mean values and error bars represent standard error of the mean (Arm A, n = 10; Arm B, n = 8).

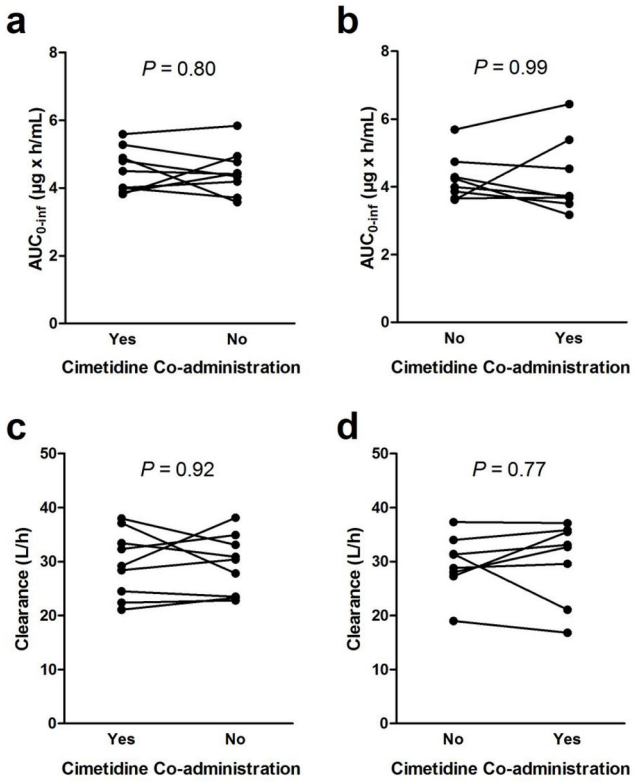

Figure 5.

The area under the curve (AUC) of unbound cisplatin in patients who received co-treatment with cimetidine in the first cycle only (Arm A) (**a**) or second cycle only (Arm B) (**b**). The overall mean clearance of unbound cisplatin in patients who received co-treatment with cimetidine in the first cycle only (Arm A) (**c**) or second cycle only (Arm B) (**d**). The difference in pharmacokinetics of cisplatin between the period *without* and the period *with* concomitant use of cimetidine, was evaluated using a paired Student's *t*–test for comparison of the mean absolute difference between both periods.

Table 1

Patient demographics.

Patient Characteristics	Total	Arm A	Arm B
Patients	18	10	8
Sex			
Male	15 (83.3%)	8 (80%)	7 (87.5%)
Female	3 (16.7%)	2 (20%)	1 (12.5%)
Age (years)			
Mean (Range)	57.5 (43–71)	59.1 (43–71)	55.5 (46–70)
Race			
Caucasian	16 (88.9%)	10 (100%)	6 (75.0%)
Asian	2 (11.1%)	0 (0%)	2 (25.0%)
SLC22A2 808G>T Genotype (GT)	2 (11.1%)	2 (20%)	0 (0%)
Tumor type			
Head and Neck	18 (100%)	10 (100%)	8 (100%)
BSA			
Mean (Range)	1.95 (1.55–2.26)	1.98 (1.55–2.26)	1.90 (1.66–2.10)
Accumulative Cisplatin Dose (mg)			
Mean (Range)	387.5 (310–452)	395.9 (310–452)	377.0 (332–420)
Serum Creatinine (μmol/L)			
Mean (Range)	72.7 (45–101)	72.2 (47–99)	73.4 (45–101)
Blood Urea Nitrogen (mmol/L)			
Mean (Range)	5.0 (2.3–7.2)	5.2 (2.3–7.2)	4.8 (2.3–6.0)
Estimated GFR (mL/min/1.73m^2)			
Mean (Range)	105.7 (71.3–141.6)	110.1 (87.9–141.6)	100.7 (71.3–124.0)
Hemoglobin (mmol/L)			
Mean (Range)	8.49 (6.0–9.7)	8.66 (7.9–9.7)	8.3 (6.0–9.7)
Thrombocytes (×10^9/L)			
Mean (Range)	260.5 (171–405)	236.8 (176–405)	290.1 (171–378)
Leukocytes (×10^9/L)			
Mean (Range)	8.26 (4.6–12.4)	8.4 (6.0–12.2)	8.1 (4.6–12.4)
Bilirubin (umol/L)			
Mean (Range)	6.3 (3.0–13.0)	6.2 (3.0–13.0)	6.5 (4.0–12.0)
Alkaline Phosphatase (U/L)			

Patient Characteristics	Total	Arm A	Arm B
Mean (Range)	75.5 (40–105)	75.9 (40.0–97.0)	75 (63–105)
Gamma-glutamyl transpeptidase (U/L)			
Mean (Range)	39.8 (9–110)	39.2 (9.0–110.0)	40.6 (20–75)
Alanine Aminotransferase (U/L)			
Mean (Range)	25.7 (11–43)	26.4 (14–28)	24.9 (11–43)
Glutamate-Pyruvate-Transaminase (U/L)			
Mean (Range)	28.3 (7.0–88.0)	32.7 (9.0–88.0)	22.7 (7.0–46.0)

Table 2

Pharmacokinetic data of patients treated with cisplatin and cimetidine.

	ARM A			ARM B			ARM A + ARM B		
Parameters	**Cisplatin**	**Cisplatin + Cimetidine**	***P***	**Cisplatin**	**Cisplatin + Cimetidine**	***P***	**Cisplatin**	**Cisplatin + Cimetidine**	***P***
Cisplatin dose (mg)	196.4 (155–226)	199.5 (155–226)		189.7 (166–210)	187.2 (155–210)		193.4 (155–226)	194.1 (155–226)	
AUC unbound $_{0\text{-inf}}$ (μg*h/mL)	4.47 (3.71–5.84)	4.47 (3.82–5.59)	0.800	4.26 (3.62–5.69)	4.27 (3.12–6.44)	0.991	4.37 (3.62–5.84)	4.38 (3.12–6.44)	0.859
Fractional AUC Unbound	0.073 (0.056–0.088)	0.082 (0.069–0.112)	0.082	0.072 (0.062–0.079)	0.072 (0.054–0.106)	0.956	0.072 (0.056–0.088)	0.078 (0.054–0.112)	0.213
Clearance unbound (L/h)	29.4 (22.8–38.1)	29.6 (21.1–38.0)	0.920	29.6 (19.0–37.3)	30.2 (16.8–37.1)	0.772	29.5 (19.0–38.1)	29.9 (16.8–38.0)	0.772
Urinary excretion (% of dose)	34.7 (30.2–38.8)	37.3 (32.0–40.2)	0.008	38.3 (32.6–44.7)	33.7 (25.9–38.9)	0.024	36.3 (30.2–44.7)	35.7 (25.9–40.2)	0.609