

Pantoprazole, an Inhibitor of the Organic Cation Transporter 2, Does Not Ameliorate Cisplatin-Related Ototoxicity or Nephrotoxicity in Children and Adolescents with Newly Diagnosed Osteosarcoma Treated with Methotrexate, Doxorubicin, and Cisplatin

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TRIAL INFORMATION

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- **Principal Investigator** Frank Balis
- **IRB Approved** Yes

LESSONS LEARNED

- Using a randomized crossover design and continuous variables such as change in hearing threshold and biomarkers of acute renal injury as short-term endpoints, it was determined that pantoprazole, an organic cation transporter 2 inhibitor, did not ameliorate cisplatin-associated nephrotoxicity or ototoxicity.
- Cystatin C is a robust method to estimate glomerular filtration rate in patients with cancer. Using a patient-reported outcome survey, all patients identified tinnitus and subjective hearing loss occurring "at least rarely" after cycle 1, prior to objective high-frequency hearing loss measured by audiograms.
- New therapies that improve outcome with less acute and long-term toxicity are needed.

ABSTRACT

Background. Organic cation transporter 2 (OCT2), which is a cisplatin uptake transporter expressed on renal tubules and cochlear hair cells but not on osteosarcoma cells, mediates cisplatin uptake. Pantoprazole inhibits OCT2 and could ameliorate cisplatin ototoxicity and nephrotoxicity. Using a randomized crossover design, we evaluated audiograms, urinary acute kidney injury (AKI) biomarkers, and glomerular filtration rate (GFR) estimated from cystatin C (GFR_{cysC}) in patients receiving cisplatin with and without pantoprazole.

Materials and Methods. Cisplatin ($60 \text{ mg/m}^2 \times 2$ days per cycle) was administered concurrently with pantoprazole (intravenous [IV], 1.6 mg/kg over 4 hours) on cycles 1 and 2 or cycles 3 and 4 in 12 patients with osteosarcoma (OS) with a median (range) age of 12.8 (5.6–19) years. Audiograms, urinary AKI biomarkers, and serum cystatin C were monitored during each cycle.

Results. Pantoprazole had no impact on decrements in hearing threshold at 4–8 kHz, post-treatment elevation of urinary AKI biomarkers, or GFR_{cysC} (Fig. 1, Table 1). Histological response (percent necrosis) after two cycles was similar with or without

pantoprazole. All eight patients with localized OS at diagnosis are alive and in remission; three of four patients with metastases at diagnosis have died.

Conclusion. Pantoprazole did not ameliorate cisplatin ototoxicity or nephrotoxicity. The decrease in GFR_{cysC} and increase in N-acetyl- β -glucosaminidase (NAG) and creatinine demonstrate that these biomarkers can quantify cisplatin glomerular and proximal tubular toxicity. OCT2 inhibition by pantoprazole did not appear to alter antitumor response or survival. *The Oncologist* 2018;23:762–e79

DISCUSSION

Standard methotrexate, doxorubicin, and cisplatin for OS cause substantial acute and long-term toxicity, including permanent hearing loss and kidney injury. Developing selective rescue approaches to alleviate these toxicities without antagonizing the antitumor effect of the chemotherapy is essential.

Cisplatin uptake into cochlear hair cells and renal tubular cells is mediated by membrane transporters, such as OCT2. In

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Table 1. Biomarkers of renal injury and glomerular filtration rate before and after cisplatin administered without and with intravenous pantoprazole infused over 4 hours

Cycle day	Urinary acute kidney injury markers, median (range)						Glomerular filtration rate, median (range)					
	NAG, U/g Cr		KIM-1, µg/g Cr		NGAL, µg/g Cr		FEMg, %, median (range)		GFR _{cr} , mL/min per 1.73 m ²		GFR _{cysC} , mL/min per 1.73 m ²	
	–P	+P	–P	+P	–P	+P	–P	+P	–P	+P	–P	+P
1	22 (6–64)	23 (9–97)	1.7 (0.3–15)	1.7 (0.5–5.9)	10 (2.9–165)	18 (2–900)	3.1 (0.9–7.8)	3.1 (1.7–5.4)	132 (95–252)	131 (96–252)	120 (87–180)	126 (89–169)
2	58 (11–134)	66 (18–316)	3.3 (0.2–8.1)	2.1 (0.2–182)	18 (1.1–209)	27 (2.6–542)						
8	43 (7–91)	32 (18–260)	4.6 (0.4–11)	4.1 (0.5–11)	12 (1.6–841)	12 (2.2–1193)	4.1 (0.7–45)	3.1 (1.4–8.7)	124 (81–252)	120 (81–252)	109 (72–186)	105 (78–193)
22	26 (7–72)	25 (11–218)	2.8 (0.5–7.9)	2.6 (0.4–23)	14 (1.4–114)	18 (1–1927)			141 (64–252)	134 (96–252)	125 (77–176)	122 (94–160)

The day 1 sample was pretreatment, and the day 2 sample was after two daily doses of 60 mg/m² per dose cisplatin infused over 4 hours. Urinary acute kidney injury biomarkers were normalized to the urine creatinine concentration to correct for dilutional effects from intravenous fluid hydration. Abbreviations: +P, with intravenous pantoprazole; –P, without intravenous pantoprazole; Cr, creatinine; FEMg, fractional excretion of magnesium; GFR_{cr}, estimated glomerular filtration rate using serum creatinine and the Schwartz formula; GFR_{cysC}, estimated glomerular filtration rate using serum creatinine, blood urea nitrogen, and cystatin C (CysC); KIM-1, urine kidney injury molecule-1 normalized to urine creatinine; NAG, urine N-acetyl-β-glucosaminidase normalized to urine creatinine; NGAL, urine neutrophil gelatinase-associated lipocalin normalized to urine creatinine.

OS cells, which do not express OCT2, cisplatin uptake is mediated by copper transporters. We hypothesized that blocking OCT2 could selectively rescue normal cells from cisplatin-associated toxicity [1].

Pantoprazole was infused concurrently with cisplatin at a dose estimated by pharmacokinetic simulations to achieve >80% inhibition of OCT2 [2]. Mean hearing threshold at 4–8 kHz provided an objective, continuous measure of ototoxicity. Participants also reported tinnitus and subjective hearing loss in patient-reported outcome questionnaires. OCT2 inhibition by pantoprazole did not prevent hearing loss, suggesting that more complete inhibition of OCT2 is required or that other transporters are involved in cisplatin uptake.

GFR was estimated from serum creatinine (GFR_{cr}) and cystatin C. Urine biomarkers of AKI included kidney injury molecule-1 (KIM-1), measuring proximal tubular damage; neutrophil gelatinase-associated lipocalin (NGAL), discriminating prerenal from intrinsic AKI; and NAG, indicating proximal tubular damage [3]. Urine biomarkers were normalized to urine

creatinine to account for dilution from hydration during cisplatin. Pretreatment GFR was normal, with good agreement between GFR_{cr} and GFR_{cysC}. After cisplatin, GFR_{cysC} decreased, but GFR_{cr} increased, possibly related to loss of muscle mass. GFR_{cysC} appears to be more a robust method because cystatin C is not secreted by tubules and is not dependent on muscle mass. Change in AKI biomarkers during cisplatin indicates that acute intrinsic AKI and proximal tubular damage were not altered by pantoprazole. Fractional excretion of magnesium (FEMg) was not a useful measure because magnesium was unmeasurable in urine after hydration.

Estimated GFR_{cysC}, a simple, robust measure of GFR, may supplant creatinine clearance [4]. The role of urinary AKI biomarkers in assessing chemotherapy-related nephrotoxicity deserves further study. Although pantoprazole did not ameliorate cisplatin-related ototoxicity or nephrotoxicity, this randomized, crossover study design was an efficient way to rapidly conduct this pilot study and may be useful for future evaluation of agents to ameliorate cisplatin-related toxicity.

TRIAL INFORMATION

Disease	Pediatric cancer – Osteosarcoma
Stage of Disease/Treatment	Primary
Prior Therapy	None
Type of Study – 1	Phase I/II
Type of Study – 2	Randomized cross over design
Primary Endpoint	Correlative endpoint
Secondary Endpoint	Toxicity

Additional Details of Endpoints or Study Design

The sample size in this pilot study was too small to derive a statistically valid stopping rule with sufficient sensitivity. After the first 12 patients completed treatment, we evaluated outcome measures including tolerability, tumor necrosis, and outcome measures of nephrotoxicity and ototoxicity.

The effect of IV pantoprazole on cisplatin nephrotoxicity was assessed by comparing urinary biomarkers of AKI, FEMg, and serum cystatin C and creatinine. The effect of IV pantoprazole on cisplatin ototoxicity was assessed by comparing audiograms performed prior to each cisplatin infusion and at the end of therapy. All subjects received cisplatin with and without pantoprazole in this crossover design, and subjects therefore served as their own controls. For the analyses of nephrotoxicity using biomarker endpoints of AKI (urinary KIM-1, NAG, and NGAL), data were analyzed by course or by dose. Each patient had two courses of cisplatin with pantoprazole and two courses without pantoprazole.

A power calculation was conducted under the framework of a multivariate general linear hypothesis for general linear models, using Wilks' lambda test with a significance level of 0.05. Because the comparison was within subject, a larger within-subject correlation provided greater power for the test. For AKI biomarkers, we had 80% power to detect a 0.60-standard deviation (SD) difference for with versus without pantoprazole. Assuming a moderate correlation of 0.5, we had 80% power to detect a smaller difference of 0.42 SD. Variability (SD) for these biomarkers in children with AKI was not available, but in a healthy adult population, the mean value of KIM-1 was 0.228 with a SD of 0.094, so, assuming a similar mean and SD for pediatric patients, we had 80% power to detect a 0.056 difference (about 25% decrease) in the mean KIM-1 value with pantoprazole, assuming independence among repeated measures. We had 80% power to detect a difference of 0.039 (about 17% decrease) if the correlation were moderate (e.g., 0.5).

For the analyses of ototoxicity data (hearing level thresholds from 4 to 8 kHz in dB), treatment cycle was the analysis unit. Each patient had two cycles with pantoprazole and two cycles without pantoprazole. We had 80% power to detect a 0.60-SD difference, assuming a within-subject correlation of 0, and to detect a 0.42-SD difference, assuming a correlation of 0.5.

Investigator's Analysis	Level of activity did not meet planned endpoint
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DRUG INFORMATION

Generic/Working Name	Pantoprazole
Trade Name	Protonix IV
Company Name	Generic commercial drug supply
Drug Type	Small molecule
Drug Class	3
Dose	1.3 micrograms (mcg) per kilogram (kg)
Route	Continuous intravenous infusion (CIV)

PATIENT CHARACTERISTICS

Number of Patients, Male	4
Number of Patients, Female	8
Stage	Localized or metastatic osteosarcoma
Age	Median (range): 12.8 (5.6–19) years
Number of Prior Systemic Therapies	0
Performance Status: ECOG	0 — 1 — 2 — 3 — Unknown — 12

PRIMARY ASSESSMENT METHOD

Title	Total Patient Population
Number of Patients Enrolled	12
Number of Patients Evaluable for Toxicity	12
Number of Patients Evaluated for Efficacy	12
Evaluation Method	RECIST version 1.0
Response Assessment CR	$n = 8$
Response Assessment PD	$n = 3$
Response Assessment OTHER	$n = 1$

ADVERSE EVENTS

No adverse events attributed to pantoprazole

ASSESSMENT, ANALYSIS, AND DISCUSSION

Completion	Study completed
Investigator's Assessment	Level of activity did not meet planned endpoint

The cellular transport of cisplatin is mediated by membrane transporters, including copper transporter-1 and -2, copper-transporting ATPases (ATP7A, ATP7B), and organic cation transporter-2 (OCT2). In murine models, expression of OCT mediates cisplatin-related ototoxicity and nephrotoxicity. In human tumors, epigenetic modifications appear to downregulate expression of OCT2, providing the potential tissue specificity necessary to ameliorate ototoxicity and nephrotoxicity without abrogating anticancer effects [5]. Pantoprazole inhibits OCT2 with a half maximal inhibitory concentration of 2.8 μM ; the peak pantoprazole concentration in children receiving 1.6 mg/kg is 27 μM , which would inhibit OCT2 by >80% [6, 7].

The reported prevalence of hearing loss in children from platinum analogs ranges from 2% to 90%, and risk factors are unclear, in part because of the heterogeneity of patient populations, variation in anticancer treatment regimens included in clinical trial reports, and variation in scales to quantify hearing, as well as definitions of hearing loss. Tinnitus from cisplatin has not been well documented [8]. With these limitations in mind, we designed a randomized crossover study in which patients served as their own controls, and we included only patients with newly diagnosed osteosarcoma treated with the same chemotherapy regimen. In addition, we evaluated high-frequency hearing loss as a continuous variable, defined as the average hearing level threshold in decibels rather than using a categorical toxicity scale [9]. Although our primary endpoint was confined to the initial four cycles of chemotherapy, we performed a retrospective review of hearing loss in patients with osteosarcoma at our institution between 2000 and 2016 and determined that after the completion of six cycles of therapy, there was no difference in high-frequency hearing threshold in patients receiving pantoprazole versus historical controls ($p = .18$) [10]. The inclusion of patient-reported outcome to evaluate tinnitus is a unique feature of our study and should be included in future studies of cisplatin ototoxicity.

Cisplatin affects glomerular filtration rate (GFR) and renal tubular function. We incorporated new biomarkers of glomerular and tubular function, including serum cystatin C to estimate GFR (GFR_{cysC}) and biomarkers of acute kidney injury (AKI) to quantify cisplatin nephrotoxicity. Our results demonstrate the

limitation of using serum creatinine to estimate GFR (GFR_{cr}) in this patient population. The variability of GFR_{cr} at all time points and estimated GFR_{cr} values up to 250 mL per min per 1.73 m^2 during therapy demonstrate that GFR_{cr} is insensitive to functional changes related to cisplatin nephrotoxicity. GFR_{cysC} was less variable and consistently demonstrated a 10%–15% acute, reversible decrease in GFR on day 8 after cisplatin infusions. Thus, serum cystatin C appears to be more sensitive for detecting small changes in GFR. Urinary biomarkers of AKI (kidney injury molecule-1 [KIM-1], neutrophil gelatinase-associated lipocalin, N-acetyl- β -glucosaminidase [NAG]) were highly variable. This may, in part, reflect the hyper-hydration regimen used to ameliorate cisplatin nephrotoxicity and the need to normalize urine AKI concentration to urine creatinine concentration. Urinary NAG levels were increased on days 2 and 8 after cisplatin and returned to baseline by day 21 of the treatment cycle. Although we were unable to detect differences in the degree of NAG elevations with and without pantoprazole, our data suggest that NAG may be a useful acute biomarker of cisplatin renal tubular toxicity.

The goal of this pilot study was to screen pantoprazole as a rescue agent for cisplatin-related ototoxicity and nephrotoxicity. Using short-term continuous biomarker endpoints in a small sample size over multiple treatment cycles, we did not detect a statistical or clinically meaningful abrogation of cisplatin-related ototoxicity or nephrotoxicity. However, this trial design and acute endpoints may be useful to screen the growing number of antioxidant, anti-inflammatory, and other agents with potential to ameliorate of cisplatin toxicity [11].

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DISCLOSURES

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(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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FIGURES AND TABLES

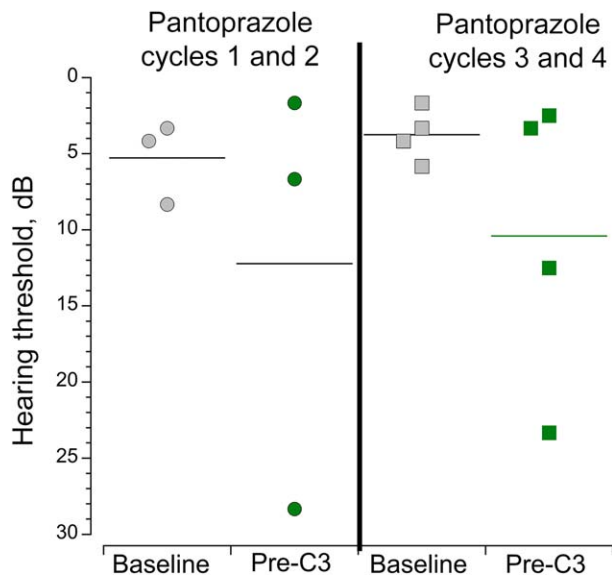


Figure 1. Change in high-frequency hearing threshold (db) from baseline to before cycle 3. Three patients received pantoprazole during cisplatin administration in cycles 1 and 2; four patients received pantoprazole during cisplatin administration in cycles 3 and 4. There was no difference in change in high-frequency hearing threshold in these groups prior to cycle 3.

Abbreviations: C3, cycle 3; dB, decibels.

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