Development of an Acute Renal Safety Model in Sprague Dawley Rats for Relative Evaluation of Platinum-based Therapeutics

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ABSTRACT

ICH guidance S7A[1] outlines considerations for the conduct of safety pharmacology studies, which are typically performed using a range of acute doses of a study compound to characterize potential off-target, adverse pharmacology. When conducting safety studies, ICH S7A suggests the use of positive control treatments to demonstrate the sensitivity of the model. The objectives of this study were to identify the optimal timing and magnitude of effects on serum and urinary endpoints following a single dose of Cisplatin in male Sprague Dawley rats against which alternative platinum-based agents could be evaluated for relative safety. Twelve 11-week old male Sprague Dawley rats were equally divided into two groups receiving intravenous injections of either saline or 6 mg/kg Cisplatin. Paired serum and urine samples were collected for biochemistry evaluations, including, but not limited to, urine volume, fractional excretion of electrolytes in urine, urine proteins, urine pH, urine glucose concentration, urinary markers of tubular injury N-acetyl-beta-D-glucosaminidase (NAG) and Gamma-glutamyltransferase (GGTs), and serum creatinine and urea nitrogen at 2, 4, and 6 days following cisplatin administration. As expected, Cisplatin administration produced signs of functional deficits relating to progressive renal tubule injury characterized by a maximal increase in serum urea nitrogen and creatinine of 255% and 194% at six days following administration, respectively. Urinary GGT increased by 166% at four days following dosing, but began to decrease by Day six. Urinary NAG concentrations progressively increased following dosing and reached 167% of control values at six days following dosing. The greatest effects were observed in urine quantitative glucose values which increased to 6987% of controls at six days following dosing. This study suggests that single-dose renal safety studies using platinum-based chemotherapeutic should be conducted for at least six days to allow detection of the largest toxicological impact. Additionally, this study demonstrated the suitability of the selected serum and urinary markers for detection of Cisplatin-induced renal toxicity in a range suitable to allow characterization of comparator platinum-based therapeutics.

METHODOLOGY

The study was conducted using 11-week old male Sprague Dawley rats weighing between 374 to 414 g. Twelve animals were equally divided into two groups which were administered saline or 6 mg/kg Cisplatin[2]. Cisplatin was formulated in saline at 3 mg/ml and filtered through a 0.22 µM nylon syringe filter before dosing. The vehicle and cisplatin were administered once via intravenous injection at a dose volume of 2 mL/kg. Body weight measurements were conducted before dosing and study termination (Day 6).

Clinical pathology assessments were conducted on study Days 2, 4, and 6 (24, 72, 120 hours after dosing). For assessment, whole blood samples were collected from each animal via the sublingual vein. All whole blood samples were placed into serum separators with no anticoagulant and allowed to clot for at least 30 minutes. For urine collection, all animals were food fasted overnight but had free access to water. The animals were placed into steel metabolism cages and urine was collected for approximately 16 hours. Samples were collected on wet ice. Urine and blood samples were centrifuged at 1500 g for 5–10 minutes.

The following endpoints were measured in each sample:

Urine:
Volume, color and appearance, specific gravity, pH, protein, glucose, bilirubin, ketones, blood, urobilinogen, microscopy of centrifuged sediment, sodium/16 hours, sodium fractional excretion, potassium/16 hours, potassium fractional excretion, chloride/16 hours, chloride fractional excretion, creatinine/16 hours, creatinine clearance, calcium/16 hours, fractional excretion calcium, quantitative glucose/16 hours, quantitative glucose/creatinine ratio, GGT/16 hours, GGT/creatinine ratio, NAG/16 hours, NAG/creatinine ratio, quantitative urine protein (QUP)/16 hours, and QUP/creatinine ratio.

Serum:
Alkaline phosphatase, total bilirubin, aspartate aminotransferase, alanine aminotransferase, urea nitrogen, creatinine, total protein, albumin, globulin and A/G (albumin/globulin) ratio, glucose, total cholesterol, triglycerides, electrolytes (sodium, potassium, chloride), calcium, and phosphorus. Urine and serum chemistry evaluations were conducted using an Olympus AU2700 or AU640. Additional urinalysis was performed using Clinitek Urine Analyzers. Urine osmolality was evaluated using an Advanced Micro-Osmometer Model 3320. Urine sediment was microscopically analyzed for the presence of casts, crystals, epithelial cells, white or red blood cells, and other miscellaneous cell types (sperm, yeast, bacteria, etc.) under 10X magnification. Urine-specific gravity was analyzed with a Reichert® Vet 360 refractometer.

At study termination, all animals were euthanized by carbon dioxide inhalation. Following euthanasia, both kidneys were collected for microscopic evaluation. The kidneys were fixed for at least 48 hours in neutral buffered formalin. Kidneys were processed using a Leica ASP300 automated tissue processor and then embedded in paraffin. The kidneys were sectioned and stained with hematoxylin and eosin using a Tissue-Tek® Prisma™ automated slide stainer. The slides were evaluated by a board-certified veterinary pathologist.

Treatment effects on serum chemistry values were analyzed using a one-way analysis of variance, followed by linear contrasts for group pair-wise comparisons. Treatment effects on urinalysis and urine chemistry values were conducted by rank transformation, followed by one-way analysis of variance with linear contrasts for group pair-wise comparisons.

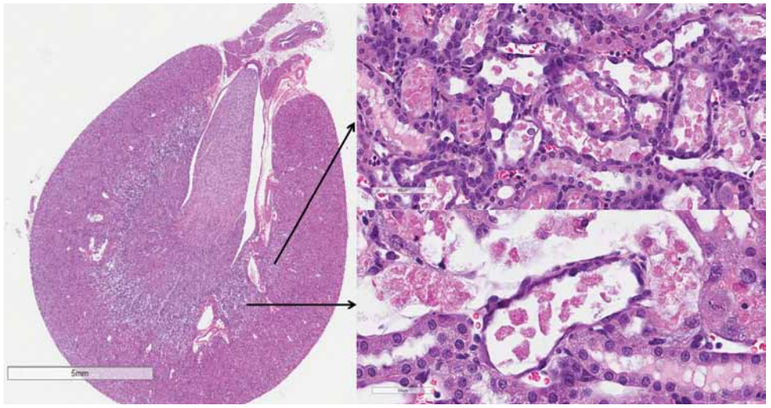
RESULTS

Table 1. Summary of urinalysis and urine chemistry endpoints. Specific gravity and pH values represent the absolute difference from control.

Endpoint	Interval	6 mg/kg Cisplatin % Change from Control	Endpoint	Interval	6 mg/kg Cisplatin % Change from Control
Volume (mL)	Day 2	**+367**[A]	GGT/16 Hr	Day 2	**+101**[B]
	Day 4	+61		Day 4	**+196**[B]
	Day 6	**+230**[B]		Day 6	**+164**[B]
Specific Gravity	Day 2	-0.0215	GGT/Creatinine Ratio	Day 2	+58
	Day 4	-0.0092		Day 4	**+166**[B]
	Day 6	-0.0217		Day 6	**+108**[B]
pH	Day 2	0	NAG/16 Hr	Day 2	+67
	Day 4	-0.16		Day 4	**+120**[B]
	Day 6	**-0.5**[B]		Day 6 +	**167**[B]
Fractional Excretion Sodium	Day 2	**+51**[A]	NAG/Creatinine Ratio	Day 2	+22
	Day 4	+42		Day 4	**+77**[A]
	Day 6	**+134**[B]		Day 6	**+146**[B]
Fractional Excretion Potassium	Day 2	+7	Quantitative Urine Protein (QUP)	Day 2	+58
	Day 4	**+82**[B]		Day 4	+56
	Day 6	**+155**[B]		Day 6	+103
Fractional Excretion Chloride	Day 2	-1	QUP/Creatinine Ratio	Day 2	+16
	Day 4	+25		Day 4	+37
	Day 6	**+192**[B]		Day 6	+73
Fractional Excretion Calcium	Day 2	**+151**[A]	Glucose/16 Hr	Day 2	+103
	Day 4	+54		Day 4	**+1636**[B]
	Day 6	**+784**[B]		Day 6	**+6987**[B]
Creatinine Clearance (mL/min)	Day 2	+39	Glucose/Creatinine Ratio	Day 2	+45
	Day 4	-32		Day 4	**+1660**[B]
	Day 6	**-52**[B]		Day 6	**+5310**[B]

(A) Significantly different from saline control p<0.05, (B) Significantly different from saline control p<0.01

Figure 1. Representative kidney slide images from a Cisplatin-treated animal showing tubular necrosis, epithelial loss, epithelial pavementing, lumen dilation, and cellular/acellular debris.



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RESULTS CONTINUED

Table 2. Summary of serum chemistry endpoints

Endpoint	Interval	6 mg/kg Cisplatin % Change from Control	Endpoint	Interval	6 mg/kg Cisplatin % Change from Control
Sodium (mEq/L)	Day 2	+0.2	Urea Nitrogen (mg/dL)	Day 2	+7
	Day 4	+0.4		Day 4	**+68**[A]
	Day 6	-0.6		Day 6	**+255**[B]
Potassium (mEq/L)	Day 2	-2	Creatinine (mg/dL)	Day 2	-6
	Day 4	-9		Day 4	**+83**[B]
	Day 6	+4		Day 6	**+194**[B]
Chloride (mEq/L)	Day 2	+0.5	Total Protein (g/dL)	Day 2	+2
	Day 4	+2		Day 4	-4
	Day 6	-0.8		Day 6	-4
Calcium (mEq/L)	Day 2	+3	Albumin (g/dL)	Day 2	+3
	Day 4	+0.1		Day 4	-3
	Day 6	+0.5		Day 6	-4
Phosphorus (mg/dL)	Day 2	-8	Globulin (g/dL)	Day 2	0
	Day 4	-4		Day 4	-6
	Day 6	+5		Day 6	-4
Alkaline Phosphatase (U/L)	Day 2	-6	Albumin/Globulin Ratio	Day 2	+5
	Day 4	-33		Day 4	+7
	Day 6	-28		Day 6	-2
Total Bilirubin (mg/dL)	Day 2	0	Triglyceride (mg/dL)	Day 2	+3
	Day 4	-20		Day 4	-15
	Day 6	+50		Day 6	-5
AST (U/L)	Day 2	-20	Cholesterol (mg/dL)	Day 2	+5
	Day 4	-19		Day 4	+7
	Day 6	-12		Day 6	+8
ALT (U/L)	Day 2	+7	Glucose (mg/dL)	Day 2	**+29**[B]
	Day 4	**-27**[A]		Day 4	-5
	Day 6	+14		Day 6	-2

(A) Significantly different from saline control p<0.05, (B) Significantly different from saline control p<0.01

DISCUSSIONS & CONCLUSIONS

Urinalysis and Urine Chemistry: There were moderate increases in urine volume on Days 2 and 6 in Cisplatin-treated animals. These changes were generally associated with decreased urine-specific gravity and urine pH relative to concurrent controls. These findings were typical of decreased renal ability to concentrate urine and indicative of renal tubular injury and/or renal functional alterations, as related to increases in serum urea nitrogen, creatinine, and decreased albumin. On Days 4 and 6, Cisplatin-treated animals had mild to moderate, progressive increases in urine glucose relative to concurrent controls. These changes are indicative of renal functional alterations associated with renal tubular injury. Cisplatin-induced renal tubule effects were also indicated by mild to moderate increases in urinary excretion of sodium, potassium, chloride, and/or calcium, as measured by fractional excretion. There were also mild to moderate increases in quantitative urine protein, urine protein to creatinine ratios, quantitative urine glucose, and/or glucose- to-creatinine ratios, which were indicative of renal functional alterations secondary to renal tubular injury. Mild to moderate decreases in creatinine clearance were also observed, and were indicative of decreased GFR. Cisplatin-treated animals also had mild to moderate increases in gamma-glutamyltransferase (GGT), GGT to creatinine ratios, N-acetyl-B-D-glucosaminidase (NAG), and/or NAG to creatinine ratios indicative of renal tubular injury.

Serum Chemistry: There were marked progressive increases in serum urea nitrogen and creatinine relative to concurrent controls on Days 4 and 6. These changes were indicative of decreased glomerular filtration rate (GFR) and typical of renal functional alterations. On Days 4 and 6, Cisplatin produced mild decreases in albumin and/or globulin relative to concurrent controls. These animals generally also had concomitant minimal to mild decreases in total protein and/or albumin to globulin ratios. These changes were consistent with renal functional alterations resulting in urinary protein loss (see above). Cisplatin also produced mild decreases in alkaline phosphatase and/or phosphorus relative to concurrent controls on Days 2, 4, and 6. These findings are suggestive of decreased food consumption.

Renal Histopathology: Microscopic evaluation of kidney sections from Cisplatin-treated animals confirmed the clinical pathology data suggesting renal tubule injury. Histopathological evaluation found evidence of renal tubular necrosis with epithelial loss, epithelial pavementing, luminal dilation, and cellular/acellular debris.

Conclusions: These data suggest that acute single-dose renal safety studies should be conducted for at least six days to capture the peak effect on renal endpoints related to tubular injury. Furthermore, the serum and urinary biomarkers selected for use on this study have been shown to be suitable for detecting progressive tubular injury.