Deleterious effects of gentamicin and cisplatin on renal function in rats and early detection of drug-induced kidney injury using biomarkers

Eric DELPY, Marine HABAULT, Catherine LE QUEMENT and Christophe DRIEU LA ROCHELLE
Biotrial Pharmacology, Non-clinical Pharmacology Department, Rennes, France

INTRODUCTION

Drug-induced kidney damage constitutes an important cause of acute renal failure and chronic kidney diseases in clinical setting. Moreover, unlike other organs, disturbance of kidney function is apparent only when a renal insult is well advanced. Thus, there is an need during safety pharmacology assessment to detect early injury to the kidney.

We decided to further characterize the known renal toxicity of gentamicin and cisplatin in rats by simultaneous evaluation of renal function and quantification of novel nephrotoxicity biomarkers.

MATERIAL & METHODS

- **Animals:** male Wistar rats, 180-220 g (Centre d'Elevage R. Janvier, France)
- **Drugs:** gentamicin (Panpharma) and cisplatin (Sigma-Aldrich)
- **Experimental protocol:**

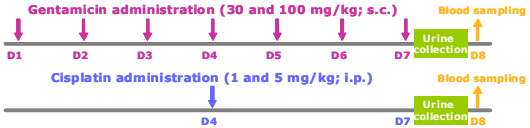
Habituation: The day before the experiment, animals were placed in metabolic cages to acclimate to environmental conditions for at least 1 hour .

Treatment: gentamicin at dose levels of 30 and 100 mg/kg was administered subcutaneously (2.5 mL/kg), once a day, for 7 consecutive days (n=8 animals in each group). Cisplatin at dose levels of 1 and 5 mg/kg was administered once intraperitoneally (5 mL:kg; n=8 animals in each group). NaCl 0.9% was administered as vehicle (n=6 animals).

Urine sampling: just after the last administration of gentamicin or three days after cisplatin injection, rats were placed individually in metabolic cages for urine collection over 24 hours. During this period, animals had free access to water but no access to food.

Blood sampling: at the end of the 24-hour urine collection period, animals were anaesthetized with pentobarbital (60 mg/kg i.p.) and blood was obtained from the vena cava in lithium heparinate tubes and centrifuged to obtain plasma.

Plasma and urine samples were immediately frozen and stored at -20°C pending analysis.



- **Parameters measured:**

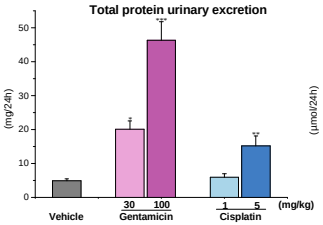
Renal function parameters: urinary and plasma concentrations of Na^+, K^+, creatinine, urea, microalbumin, glucose and total proteins were measured using a medical automated analyser (Olympus AU400). Urinary excretion and clearance were calculated.

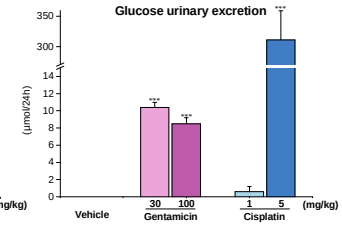
Renal Biomarkers: urinary concentration of KIM-1, clusterin, cystatin C and beta2microglobulin were determined using multiplex technology which combines a sandwich ELISA immobilized on microparticle beads and flow cytometry.

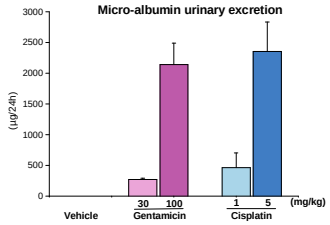
- **Statistical analysis:** comparison of gentamicin or cisplatin versus vehicle was performed using one-way ANOVA followed by Dunnett's test.

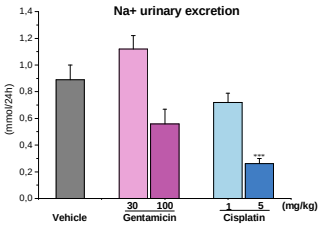
RESULTS

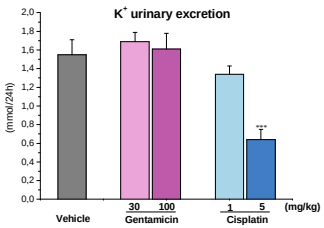
	Vehicle	Gentamicin (mg/kg) 30	Gentamicin (mg/kg) 100	Cisplatin (mg/kg) 1	Cisplatin (mg/kg) 5
Water consumption (ml/24h)	5,2 ± 2,1	7,4 ± 2,6	8,8 ± 3,1	16,3 ± 5,8	11,3 ± 4,0
Urine flow rate (ml/24h)	12,0 ± 4,9	15,6 ± 5,5	15,0 ± 5,3	20,0 ± 7,1	10,4 ± 3,7

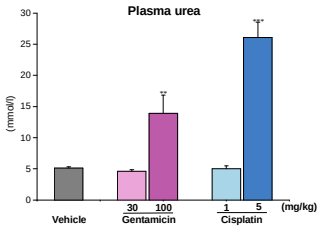


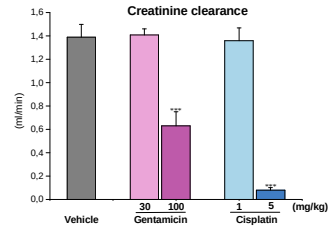


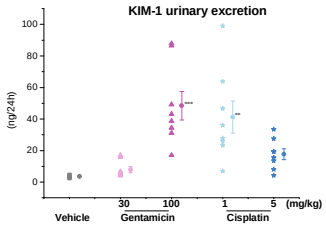


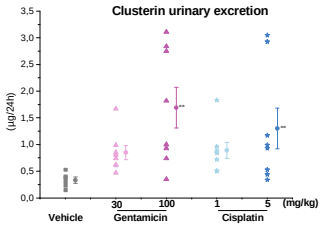


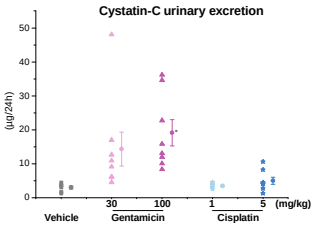


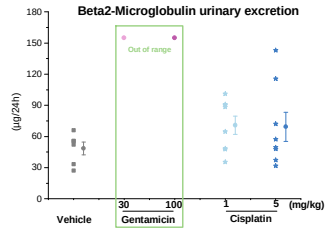












CONCLUSION

Our results showed that gentamicin at 30 mg/kg and cisplatin at 1 mg/kg had almost no effect on renal function parameters. On the contrary, gentamicin at 100 mg/kg and cisplatin at 5 mg/kg induced a marked alteration of renal function. Concerning urinary biomarkers, gentamicin induced a dose-dependent increase in microalbumin, KIM-1, clusterin, cystatin C and β-2-microglobulin, with increases already detectable at 30 mg/kg. Cisplatin induced a dose-dependent increase in microalbumin urinary excretion. At 1 mg/kg, cisplatin increased KIM-1 and at 5 mg/kg increased clusterin urinary excretion.
In conclusion, quantification of these new biomarkers in renal safety pharmacology studies may therefore be useful to detect drug-induced kidney damage whereas renal function remains unaffected. This should enable early identification of potentially nephrotoxic compounds.

ACKNOWLEDGMENTS

We thank warmly Véronique QUILLIEN and his team (Centre Eugène Marquis, Rennes, France) for their collaboration on this work.