For general inquiries:
(514) 457-7400

For marketing and study inquiries:
(514) 457-8264
marketing@itrlab.com

Poster Board number: 286 Poster Board -471

Abstract Title: Acute Kidney Injury Biomarkers Following Low- Dose Gentamicin Treatment of Rats

Presenter: Christian Gauthier (cgauthier@itrlab.com)

Session Title: Safety Assessment: Drug Development I

Presentation Date & Time: March 24, 2014 from 9:30 AM to 12:30 PM

Acute Kidney Injury Biomarkers Following Low Dose Gentamicin Treatment of Rats

G. Marceau[1], L. Kolodzieyski[2], G. Diagne[1], J. Tremblay[1], S. Thébaud[1], M. Toneva[1], K. Scribner Doering[3], C. Kostrup[4], A. Nelson[1]

([1])Immunology, ITR Laboratories Canada Inc., Montreal, Canada; ([2])Pathology, ITR Laboratories Canada Inc., Montreal, Canada; ([3]) ALPCO Diagnostics, Salem NH; ([4])CK Scientific, Belmont CA

Rationale:

The use of biomarkers as part of the preclinical assessment of kidney injury is now a well established goal. Current routine markers of acute kidney injury such as serum creatinine (SCr) and blood urea nitrogen (BUN) are relatively insensitive in identifying early stages of renal dysfunction and structural changes resulting from injury. This insensitivity and lack of specificity of traditional markers of kidney injury has complicated and delayed drug development. Moreover, numerous benefits can be expected from the use of non lethal methods of detecting kidney injury, including lower costs and reduction in use of animals, but mostly the opportunity for longitudinal evaluation of kidney injury progression and recovery.

Published analysis of multiple datasets from the toxicology industry has convincingly identified a panel of urine and serum biomarkers which correlate with drug induced kidney injury.

The objective of this study was to identify biomarkers that correlate with histopathological evidence of kidney damage from low dose daily regimens of Gentamicin sulfate, a known nephrotoxicant. Out of a panel of nine biomarkers, a group of six were found to be increased in animals showing unequivocal signs of histopathological changes.

Experimental Procedures:

Male Sprague-Dawley rats received daily subcutaneous injections of Gentamicin sulfate for up to 15 days.

Animal groups daily injection doses of gentamicin sulfate	
Controls (n=6 to 12)	Saline only
Low Dose (n=3 to 9)	5 mg/kg/day
Medium Dose (n=3 to 9)	15 mg/kg/day
High Dose (n=6 to 12)	50 mg/kg/day
Very High Dose (n=3)	150 mg/kg/day

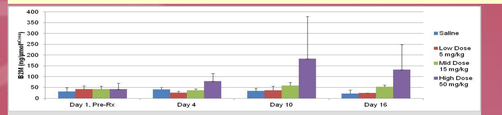
Urine samples were collected over 16-18 hours for biomarker assessment at multiple timepoints (Pre-treatment, Days 4, 10, and 16). Blood was also collected at the end of a fasting period and serum preserved. A panel of biomarkers was tested in urine and serum by enzyme-linked immunosorbent assay (ELISA, kits from ALPCO Diagnostics NH).

Kidney Injury Biomarkers tested in urine by ELISA	
Low molecular weight proteins	Albumin β2 microglobulin (B2M) Retinol binding protein (RBP)
Tubular enzyme	N-acetyl-β-(D)-glucosaminidase (NAG)
Tubular damage response	Clusterin Cystatin C (urine and serum) Kidney Injury Molecule-1 (Kim-1) Neutrophil gelatinase-associated lipocalin (NGAL)
Glomerular filtration dysfunction	Serum Cystatin C
Standard markers	Blood Urea Nitrogen (BUN) (Hitachi 912) Creatinine (Cobas c311)

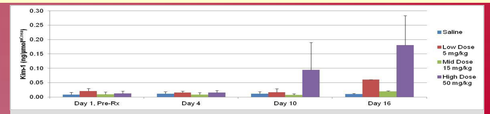
Kidney histology was assessed with hematoxilin & eosin stainings on days 4, 10 and 16.

Results:

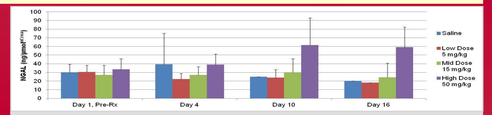
From a panel of nine biomarkers selected, six were elevated (B2M, Cystatin C, Kim-1, NAG, NGAL, and RBP) in urine at doses of 50mg/kg and less in animals with clear histopathological findings of kidney injury. Three biomarkers were not increased (Albumin, Clusterin, and serum Cystatin C) and are not shown here.



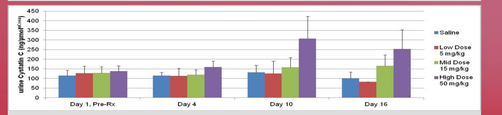
B2M urine levels normalized on urine Creatinine levels in rats treated daily with Gentamicin sulfate



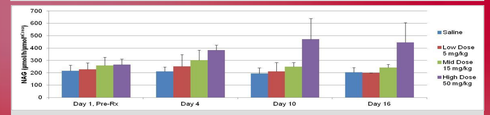
Kim-1 urine levels normalized on urine Creatinine levels in rats treated daily with Gentamicin sulfate



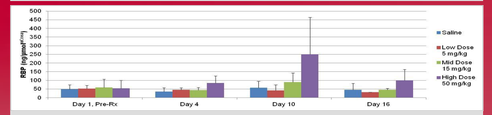
NGAL urine levels normalized on urine Creatinine levels in rats treated daily with Gentamicin sulfate



Cystatin C urine levels normalized on urine Creatinine levels in rats treated daily with Gentamicin sulfate

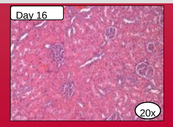


NAG urine levels normalized on urine Creatinine levels in rats treated daily with Gentamicin sulfate



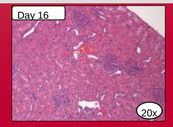
RBP urine levels normalized on urine Creatinine levels in rats treated daily with Gentamicin sulfate

Controls



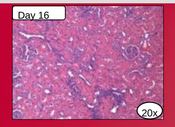
Test	Average (Day 16)
Weight (g)	428.17 ± 25.595
Kidney weight (g) (relative to body)	2.82 ± 0.10 (0.69 ± 0.06)
Creatinine (µmol/L) -Serum -Urine	28.17 ± 4.40 3533.83 ± 1733.51
BUN (mg/dL)	15.33 ± 3.44
Urine Proteins (g/L)	0.18 ± 0.12

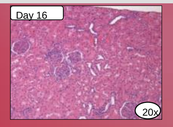
Low Dose

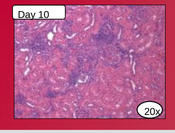


Test	Average (Day 16)
Weight (g)	418.33 ± 18.15
Kidney weight (g) (relative to body)	2.93 ± 0.17 (0.70 ± 0.01)
Creatinine (µmol/L) -Serum -Urine	21.33 ± 1.53 5996.00 ± 2186.47
BUN (mg/dL)	12.00 ± 1.00
Urine Proteins (g/L)	0.44 ± 0.32

Medium Dose

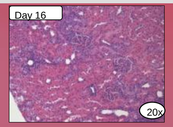


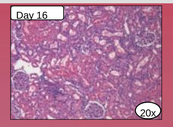


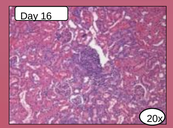


Test	Average (Day 16)
Weight (g)	426.00 ± 3.61
Kidney weight (g) (relative to body)	3.18 ± 0.29 (0.75 ± 0.07 *)
Creatinine (µmol/L) -Serum -Urine	25.33 ± 1.53 2645.50 ± 379.47
BUN (mg/dL)	12.33 ± 1.53
Urine Proteins (g/L)	0.71 ± 1.00

High Dose

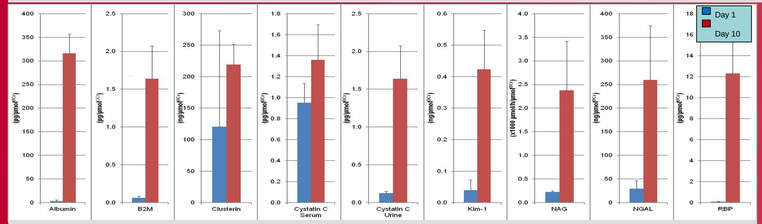




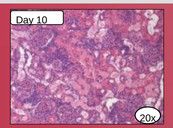


Test	Average (Day 16)
Weight (g)	394.50 ± 31.04
Kidney weight (g) (relative to body)	3.22 ± 0.09 * (0.84 ± 0.03 *)
Creatinine (µmol/L) -Serum -Urine	38.00 ± 10.32 4035.50 ± 2333.84
BUN (mg/dL)	20.83 ± 5.64
Urine Proteins (g/L)	0.43 ± 0.34

Very High Dose



Panel of biomarkers tested in urine and serum of rats (n=3) treated daily for 10 days with very high doses (150 mg/kg) of Gentamicin sulfate (normalized on urine Creatinine)



Test	Average (Day 10)
Weight (g)	255.33 ± 11.06 (***)
Kidney weight (g) (relative to body)	3.62 ± 0.54 (1.01 ± 0.11 *)
Creatinine (µmol/L) -Serum -Urine	148.33 ± 45.94 (*) 5151.67 ± 1570.01
BUN (mg/dL)	59.67 ± 18.58 (*)
Urine Proteins (g/L)	2.62 ± 1.37

Histopathology findings	
Low Dose	There were no findings which could be considered to be test item-related
Mid Dose	Day 10: Absent to mild renal tubular degeneration was observed. Day 16: Minimal renal tubular degeneration and regeneration was observed in all males. This finding was regularly seen in association with mononuclear interstitial cell infiltrate and occasionally with tubular single cell necrosis.
High Dose	Day 10: Absent to minimal renal tubular degeneration was observed. Day 16: Pale discoloration of the kidneys was observed in all animals. Mild to moderate renal tubular degeneration and regeneration was observed in all animals. This finding was regularly seen in association with mononuclear interstitial cell infiltrate and occasionally with tubular single cell necrosis.

Discussion:

•Histopathological changes were present occasionally in Mid and High Dose animal on Day 10 with no significant differences between both groups. Pathological injury progressed on Day 16 in High dose animals;

•Creatinine (serum and urine) and BUN levels were increased on Day 16 in some High Dose animals;

•Relative to body weight, kidney weight was increased on necropsy for High and Mid Dose animals on Day 10 and 16;

•Urine Albumin, Clusterin and serum Cystatin C levels were not increased at any dose levels from Day 1 to Day 16;

•RBP urine levels in High Dose animals increased on Day 10 and decreased on Day 16;

•Cystatin C, Kim-1, and NAG urine levels in High Dose animals increased from Day 10;

•B2M urine levels started increasing in High Dose animals from Day 4, before clear histopathological changes;

•NGAL urine levels in High dose animals and some Mid Dose animals increased from Day 10.

•Serum creatinine, BUN, and all panel biomarkers were elevated in all Very High Dose animals, along with clear and extended histopathological disruption.

Conclusions:

As previously reported, serum creatinine and BUN levels were poorly sensitive to low levels of acute kidney injury. Increased urine excretion of B2M, Cystatin C, Kim-1, NAG, NGAL, and RBP was associated with histopathological findings.

Among the six biomarkers identified in the present study, urine B2M was the only one with some increased levels starting prior to histopathological findings.

From the panels of tested biomarkers, urine NGAL was the only to be elevated in animals treated with the mid dose (15mg/kg/day) on day 10 and 16, concurring with histological evaluation and damage progression.

These six urine biomarkers are candidate diagnostics and potential surrogates to histological evaluation of acute kidney injury. More studies are required to establish strong correlation between histological findings of low levels injury with increased levels of biomarkers in urine. From these results B2M and NGAL appear to be the best candidates for identifying early kidney injury. Attention should also be given to the potential of theses biomarkers to detect kidney damage in female.

Bibliography:

- Bonventre, JV, Vaidya VS, Schmouder R, *et al*. 2010. Next-Generation Biomarkers for Detecting Kidney Toxicity. Nature Biotechnology **28**: 436-440.
- Dieterle, F, Perentes E, Cordier A, *et al*. 2010. Urinary Clusterin, Cystatin C, beta2-Microglobulin and Total Protein as Markers to Detect Drug-Induced Kidney Injury. Nature Biotechnology **28**: 463-469.
- Hoffmann, D, Adler M, Vaidya VS, *et al*. 2010. Performance of Novel Kidney Biomarkers in Preclinical Toxicity Studies. *Toxicological Sciences* **116**: 8-22.
- Hoffmann, D, Fuchs TC, Henzler T, *et al*. 2010. Evaluation of a Urinary Kidney Biomarker Panel in Rat Models of Acute and Subchronic Nephrotoxicity. *Toxicology* **277**: 49-58.
- Nykjaer, A, Schambye H, Pedersen AH, and H Nielsen. 2009. Assessing Low-Dose Gentamicin-Induced Kidney Injury in Rats by Analysis of Urine. *Journal of Pharmacological and Toxicological Methods* 60: 316-320.
- Ozer, JS, Dieterle F, Troth S, *et al*. 2010. A Panel of Urinary Biomarkers to Monitor Reversibility of Renal Injury and a Serum Marker with Improved Potential to Assess Renal Function. *Nature Biotechnology* **28**: 486-494.
- Rouse, RL, Zhang J, Stewart SR, *et al*. 2011. Comparative Profile of Commercially Available Urinary Biomarkers in Preclinical Drug-Induced Kidney Injury and Recovery in Rats. *Kidney International* **79**: 1186-1197.
- Xie, H-G, Wang S-K, Cao C-C, and E Harpur. 2013. Qualified Kidney Biomarkers and Their Potential Significance in Drug Safety Evaluation and Prediction. *Pharmacology & Therapeutics* 137: 100-107.