

Novel Kidney Injury Biomarker Panels To Detect Nephrotoxicity in Mouse Models

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Abstract

The kidney is uniquely susceptible to drug-induced toxicity due to several factors, including high blood flow and concentrating mechanisms. The application of kidney injury biomarkers has greatly improved the detection of nephrotoxicity during drug development and will likely accelerate the introduction of effective and safe drugs to market. Recently, a panel of novel urinary kidney biomarkers has been qualified by the FDA, EMA and PMDA to detect acute nephrotoxicity in preclinical rat studies. Even though mouse models are widely used in the drug development process, the lack of reliable assays to quantitatively assess multiple protein markers in small volumes of mouse urine has limited the application of these markers in mouse models. In the present study, we developed two panels of multiplexed immunoassays for mouse kidney injury urinary biomarkers using Luminex xMAP® technology. Furthermore, we validated the sensitivity and specificity of these assays in detecting kidney injury in both ischemia- and drug-induced mouse kidney injury models. To induce kidney injury, BALB/C mice were subjected to either bilateral ischemic reperfusion (I/R) for 30 minutes or one dose of Cisplatin (20 mg/kg i.p) treatment. Urine specimens were collected and analyzed for urinary protein markers with MILLIPLEx® MAP Mouse Kidney Injury Magnetic Bead Panels. There was a significant increase in many of the markers in both I/R and Cisplatin-treated mouse models, reflecting tubular damage. The simultaneous measurement of these proteins with multiplex technology offered a robust and convenient method to study these biomarkers in small volumes of mouse urine samples.

Methods

Mouse models for kidney injury

Two different mouse kidney injury models were used.

- Drug-induced tubular toxicity:** 4 individual BALB/C male mice were injected with one dose of Cisplatin (20 mg/kg i.p). Urine samples were collected at 24, 48 and 72 hours from both the control and treated groups.
- Ischemia-induced injury:** 4 individual BALB/C male mice were subjected to bilateral renal ischemia at 37 °C for 30 minutes followed by reperfusion for 6-120 hours. Urine samples were collected at 6, 12, 24, 48, 72 and 120 hours from both the control and I/R groups.

Assays

- The urine samples were evaluated for urinary biomarker concentrations using the MILLIPLEx® MAP Mouse Kidney Injury Magnetic Bead Panels 1 and 2, which have been developed based on Luminex xMAP® technology. Mouse Kidney Injury Panel 1 (Cat. No. MK11MAG-94K) detects 6 analytes (β-2-Microglobulin, IP-10, KIM-1, Renin, TIMP-1 and VEGF); Mouse Kidney Injury Panel 2 (Cat. No. MK12MAG-94K) detects 5 analytes (Clusterin, Cystatin C, EGF, Lipocalin-2/NGAL and Osteopontin).

- Urine creatinine levels were also measured. For data analysis, all of the biomarker concentrations were normalized to urine creatinine concentrations.

Results

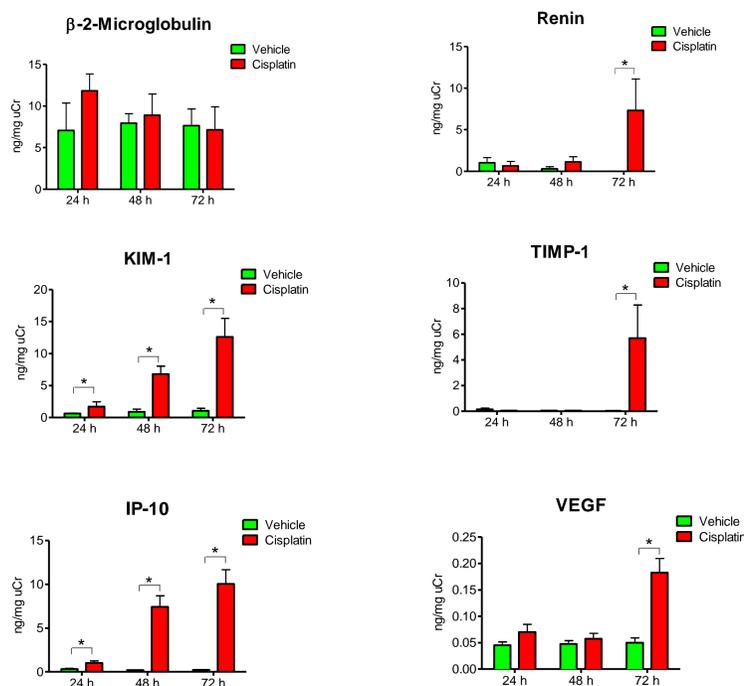


Figure 1. Protein Biomarkers in Cisplatin-Induced Kidney Injury Mouse Model. Urine samples were collected at 24, 48 and 72 hours from both vehicle- and drug-treated groups (n=4 for both groups) and analyzed at a dilution of 1:25 with MILLIPLEx® MAP Mouse Kidney Injury Panel 1 (Cat. No. MK11MAG-94K). The calculated concentrations were normalized to urine creatinine concentrations for data analysis. * indicates significant difference between the groups with p <0.05.

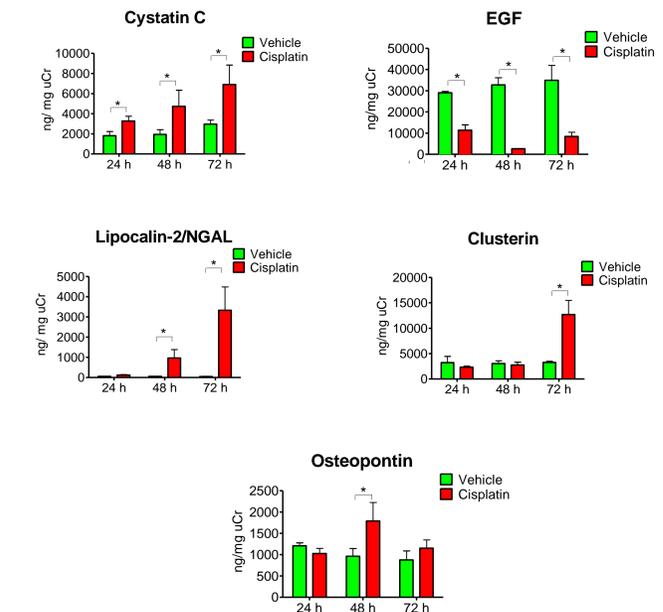


Figure 2. Protein Biomarkers in Cisplatin-Induced Kidney Injury Mouse Model. Urine samples were collected at 24, 48 and 72 hours from both vehicle and treated groups (n=4 for both groups) and analyzed at a dilution of 1:1000 with MILLIPLEx® MAP Mouse Kidney Injury Panel 2 (Cat. No. MK12MAG-94K). The calculated concentrations were normalized to urine creatinine concentrations for data analysis. * indicates significant difference between the groups with p <0.05.

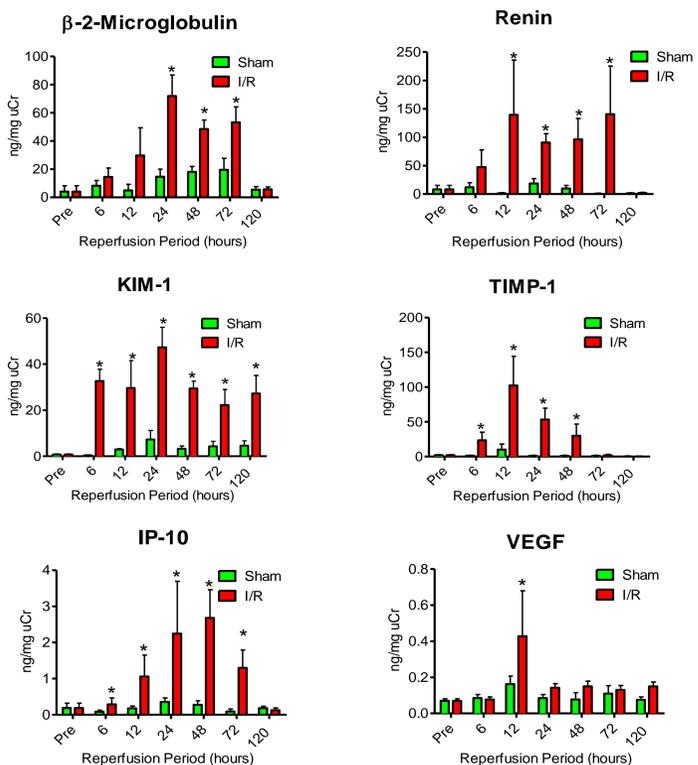


Figure 3. Protein Biomarkers in Ischemia-Reperfusion Kidney Injury Mouse Model. Urine samples were collected before treatment and also at 6, 12, 24, 48, 72 and 120 hours after treatment from both control ("sham") and treated groups (n=4 for both groups) and analyzed at a dilution of 1:25 with MILLIPLEx® MAP Mouse Kidney Injury Panel 1 (Cat. No. MK11MAG-94K). The calculated concentrations were normalized to urine creatinine concentrations for data analysis. * indicates significant difference between the groups with p <0.05.

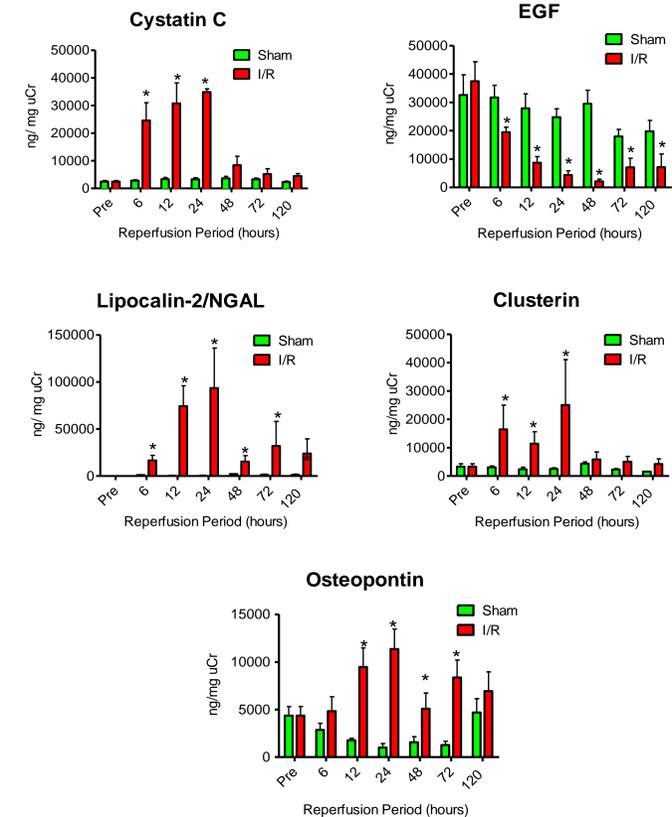


Figure 4. Protein Biomarkers in Ischemia-Reperfusion Kidney Injury Mouse Model. Urine samples were collected before treatment and also at 6, 12, 24, 48, 72 and 120 hours after treatment from both control ("sham") and treated groups (n=4 for both groups) and analyzed at a dilution of 1:1000 with MILLIPLEx® MAP Mouse Kidney Injury Panel 2 (Cat. No. MK12MAG-94K). The calculated concentrations were normalized to urine creatinine concentrations for data analysis. * indicates significant difference between the groups with p <0.05.

Summary

➤ In the present study, we have used two panels of multiplex immunoassays based on Luminex xMAP® technology to quantify eleven candidate biomarkers relevant in nephrotoxicity/kidney injury in mouse.

➤ Two mouse models (bilateral ischemic reperfusion mouse model and a Cisplatin-treated mouse model) were used to evaluate sensitivity and specificity of all of the biomarkers. Our initial findings indicate significant changes in urinary levels of most of the analytes in both models.

➤ In the Cisplatin-induced kidney injury mouse model, Renin, KIM-1, TIMP-1, VEGF, IP-10, Cystatin C, Lipocalin-2/NGAL and Clusterin levels reached their peak levels in 72 hours after Cisplatin administration, while Osteopontin and EGF showed maximum change after 48 hours. No significant change was observed in β-2-Microglobulin levels.

➤ In the ischemic reperfusion mouse model, temporary modulations were observed with biomarkers at different time points. Significant changes in the levels of some biomarkers were observed as early as 6 hours after reperfusion. Renin, TIMP-1 and VEGF showed maximum elevation in 12 hours followed by β-2-Microglobulin, KIM-1, Cystatin C, Lipocalin-2/NGAL, Clusterin and Osteopontin, which peaked at 24 hours. IP-10 and EGF showed maximum changes after 48 hours of reperfusion. Most of the targets started showing recovery by 120 hours after reperfusion.

➤ This study suggests that, with their great sensitivity and specificity, multiplex panels can be a very useful tool for biomarker screening and protein analysis to study nephrotoxicity in mouse in preclinical drug safety assessment.