# Early peritoneal dialysis reduces lung inflammation in mice with ischemic acute kidney injury



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**Although dialysis has been used in the care of patients with acute kidney injury (AKI) for over 50 years, very little is known about the potential benefits of uremic control on systemic complications of AKI. Since the mortality of AKI requiring renal replacement therapy (RRT) is greater than half in the intensive care unit, a better understanding of the potential of RRT to improve outcomes is urgently needed. Therefore, we sought to develop a technically feasible and reproducible model of RRT in a mouse model of AKI. Models of low- and high-dose peritoneal dialysis (PD) were developed and their effect on AKI, systemic inflammation, and lung injury after ischemic AKI was examined. High-dose PD had no effect on AKI, but effectively cleared serum IL-6, and dramatically reduced lung inflammation, while low-dose PD had no effect on any of these three outcomes. Both models of RRT using PD in AKI in mice reliably lowered urea in a dose-dependent fashion. Thus, use of these models of PD in mice with AKI has great potential to unravel the mechanisms by which RRT may improve the systemic complications that have led to increased mortality in AKI. In light of recent data demonstrating reduced serum IL-6 and improved outcomes with prophylactic PD in children, we believe that our results are highly clinically relevant.**

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KEYWORDS: acute kidney injury; cytokines; dialysis; uremia



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The benefits of renal replacement therapy (RRT) in acute kidney injury (AKI) include electrolyte management, volume control, and prevention of uremic complications such as pericarditis and encephalopathy. Whether control of uremia has benefits beyond these traditional effects is unknown. A better understanding of the potential of RRT to improve outcomes is urgently needed, as the mortality rate of AKI requiring dialysis in the intensive care unit is currently 50% to 60%.[1]

The study of the potential benefits of RRT would be greatly aided by an animal model of AKI and RRT. In this regard, mechanistic studies of RRT timing and dose on systemic complications that affect mortality could be performed. Currently, RRT in the form of hemodialysis or continuous RRT can only be studied in large animals. Large animal studies in AKI are costly and logistically difficult to perform; thus, the majority of basic research in AKI is conducted in small animals such as rats and mice.

Currently, the only feasible method for performing RRT in small animals is via peritoneal dialysis (PD). Notably, however, the primary purpose of the current body of PD studies in rodents has been to examine peritoneal membrane properties such as ultrafiltration, inflammation, and fibrosis. Thus, the vast majority of PD studies in murine models have utilized healthy rodents and studies of PD in uremic models are rare.[2,3] In 1 review, only 12 reports of PD in rodents with uremia were identified over the past 20 years, 2 in mice and 10 in rats.[3] In these reports, PD was studied in the uremic models of bilateral nephrectomy or 5/6 nephrectomy. Because the primary endpoint was peritoneal membrane properties, PD as a therapy was not tested, and no particular dose or acute systemic consequence was evaluated.

Therefore, in this study, we sought to establish a technically feasible, reproducible mouse model of PD as a therapy that could be utilized to examine the effect of RRT on the systemic consequences of AKI. We hypothesized that PD in ischemic AKI would be associated with a reduction in systemic inflammation and lung injury based on clinical studies demonstrating reduced serum interleukin (IL)-6 with PD[4] and the known role of IL-6 in mediating lung injury after AKI.[5–7]

## RESULTS

### Narrative description of pilot studies involved in the development of the PD model

Pilot experiments were performed to (i) develop a suitable PD catheter and overcome technical considerations, (ii) develop a dialysis procedure that resulted in a reproducible lowering of blood urea nitrogen (BUN), and (iii) develop an appropriate sham PD control as discussed in the following section. Once the model was established, <5% of mice needed to be removed for technical failures or died. Experiments in the established model are presented in this report (Figures 1–12); in the following section, we describe some of the considerations and pilot experiments performed before these experiments.

### Catheter development and technical considerations

We sought to develop a PD catheter that would allow for multiple fluid exchanges and was flexible and easily accessible. To that end, a custom PD catheter was made from sterile PE50 tubing and a 2-cm loop was created to increase surface volume (Figure 13a); six to eight 25-gauge holes were inserted along the loop to allow ample entry and exit sites for dialysis fluid during dwells. To simplify access for fluid exchanges as well as to prevent destruction of the catheter by the mouse from chewing or other manipulation, the catheter was tunneled through the back of the mouse (Figure 13b and c). To prevent catheter leakage, the peritoneum was closed with suture and sealed with Vetbond, and then the skin was sutured.

### Development of the dialysis prescription

We optimized the PD prescription in mice with bilateral nephrectomy and measured BUN as our marker of PD dose. Because we were interested in the effect of PD on cytokines and lung inflammation, which are increased 4 hours after AKI, all PD experiments were performed within a time frame of 4 hours.

Several combinations of instillation volumes and exchange frequencies were studied; 1-, 1.5-, 2-, and 3-ml PD fluid volumes were studied, and 2 ml gave the best result in terms of urea reduction and tolerance by the mouse. Of note, volumes typically utilized in mouse PD models range from

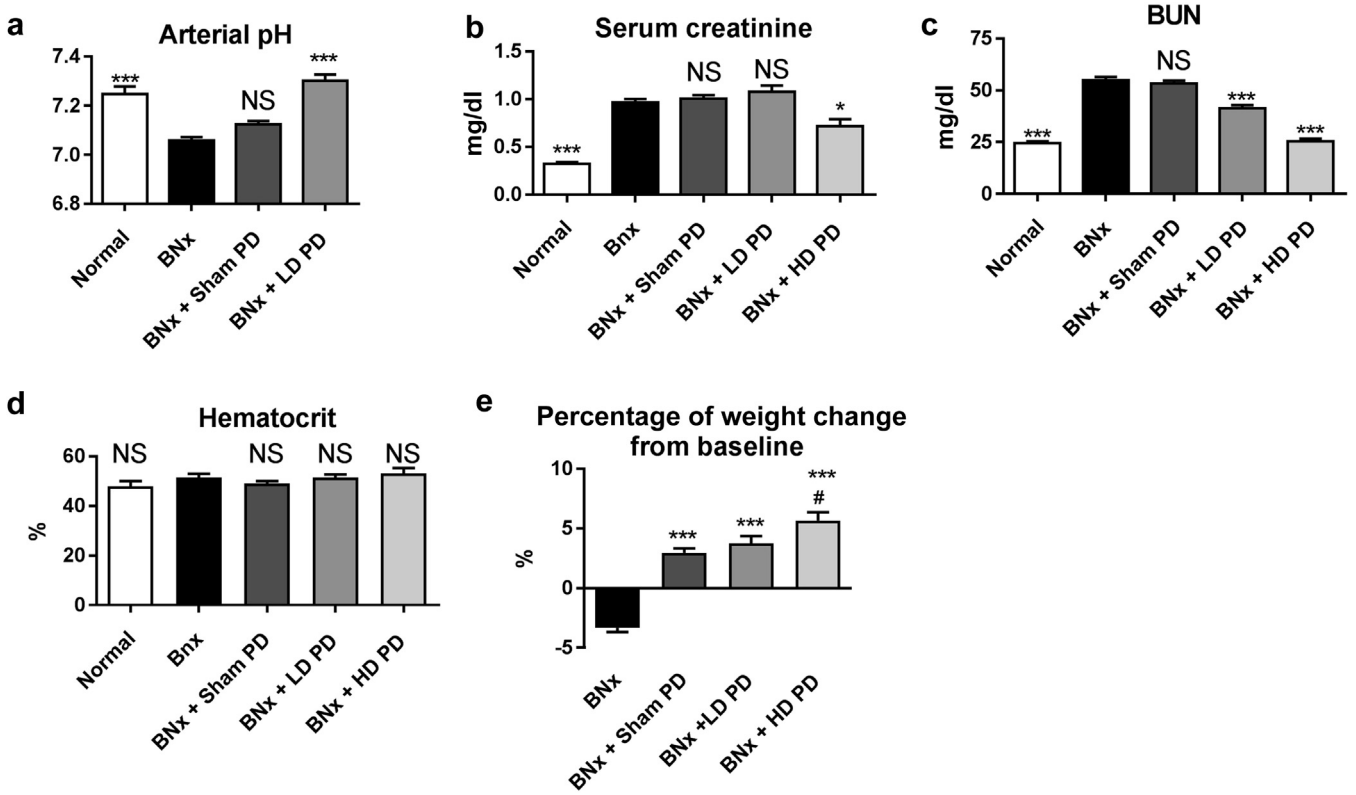


**Figure 1 | Effect of low-dose (LD) and high-dose (HD) peritoneal dialysis (PD) in mice with bilateral nephrectomy 4 hours after initiation of PD.** LD or HD PD was performed 1 hour after bilateral nephrectomy (BNx). LD PD consisted of PD for 4 hours with peritoneal fluid exchanges occurring every hour (4 exchanges in 4 hours); HD PD consisted of 4 hours of PD with peritoneal exchanges occurring every 15 minutes (16 exchanges in 4 hours). Normal mice (no procedure), mice with BNx, and mice with BNx + Sham PD were also studied. pH (**a**), serum creatinine (**b**), blood urea nitrogen (BUN) (**c**), hematocrit (**d**), and weight change from baseline (**e**) were determined. Comparisons were made using analysis of variance with Dunnett's multiple comparison procedure using BNx as the comparison group: *$P < 0.05$ versus BNx, ***$P < 0.0001$ versus BNx NS (not significant) versus BNx; #NS versus BNx + Sham PD or BNx + LD PD. Group numbers for arterial pH are as follows: normal ($N = 4$), BNx ($N = 5$), BNx + Sham PD ($N = 10$), BNx + LD PD ($N = 8$). Group numbers for hematocrit, BUN, serum creatinine, and weight change: normal ($N = 5$), BNx ($N = 6$), BNx + Sham PD ($N = 14$), BNx+ LD PD ($N = 5$), BNx + HD PD ($N = 11$).

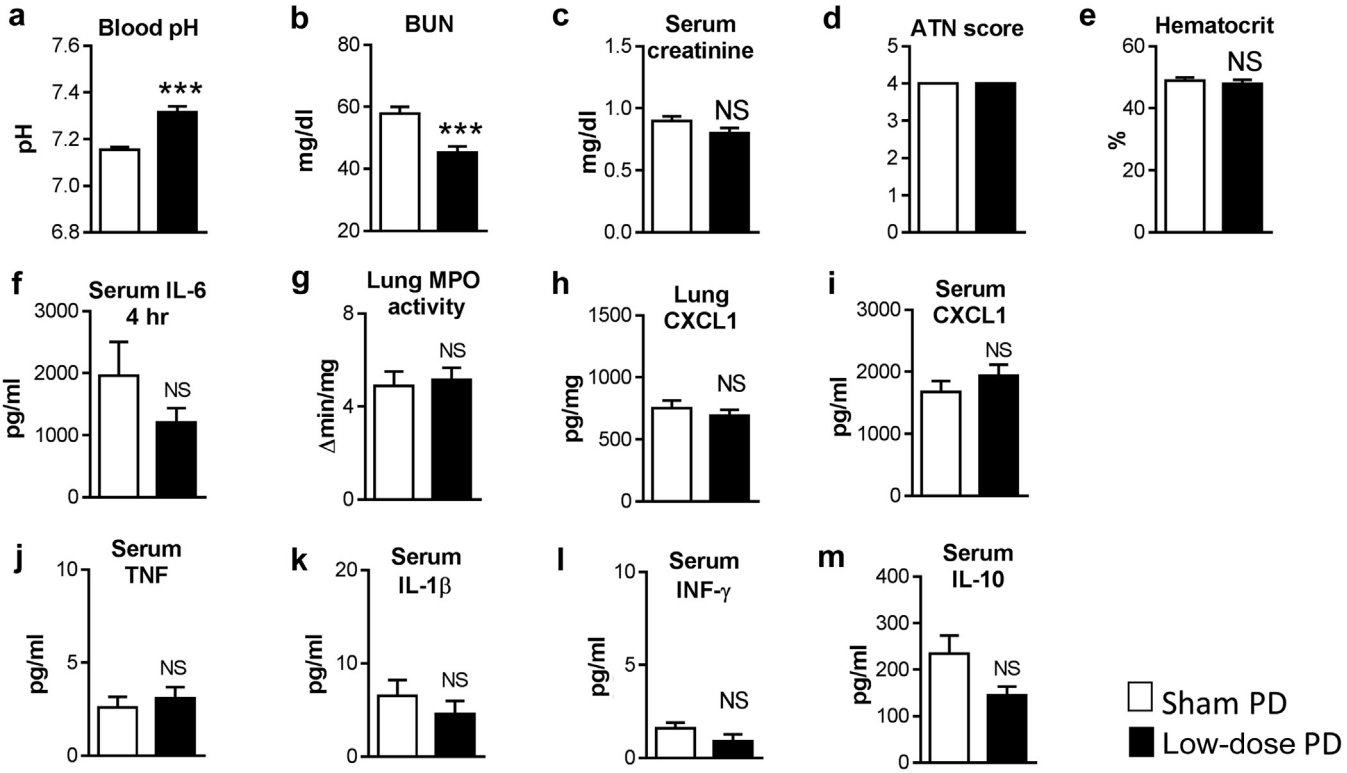


**Figure 2 | Effect of low-dose (LD) peritoneal dialysis (PD) in ischemic acute kidney injury 4 hours after initiation of PD.** LD PD or Sham PD was performed 1 hour after ischemic acute kidney injury for 4 hours with peritoneal dialysate exchanges occurring every hour (i.e., 1 exchange per hour for 4 hours for a total of 4 exchanges). (**a**) pH was increased with LD PD. (**b**) blood urea nitrogen (BUN) was significantly reduced with LD PD. There were no differences between Sham PD and LD PD in any of the other parameters measured, including serum creatinine (**c**), acute tubular necrosis (ATN) score (**d**), hematocrit (**e**), serum interleukin (IL)-6 (**f**), lung myeloperoxidase (MPO) activity (**g**), lung C-X-C motif chemokine ligand 1 (CXCL1) (**h**), serum CXCL1 (**i**), serum tumor necrosis factor (TNF) (**j**), serum IL-1β (**k**), serum IFN (interferon)-γ (**l**), and serum IL-10 (**m**). Analysis with unpaired *t* test, $N = 9–10$, $^{***}P < 0.0001$. NS, not significant.

1.5 to 2.5 ml.[3] We tested the following frequency of exchanges within a 4-hour time frame: (i) every hour (4 exchanges), (ii) every 30 minutes (8 exchanges), (iii) every 20 minutes (12 exchanges), and (iv) every 15 minutes (16 exchanges). We found that the urea rapidly equilibrates between the serum and dialysate (within 15 minutes) and that increasing the frequency of exchanges predictably reduced BUN levels.

Finally, 2 prescriptions of PD were established: (i) low dose and (ii) high dose. Low-dose PD consists of 2 ml of PD fluid instillation, which is then withdrawn and exchanged with

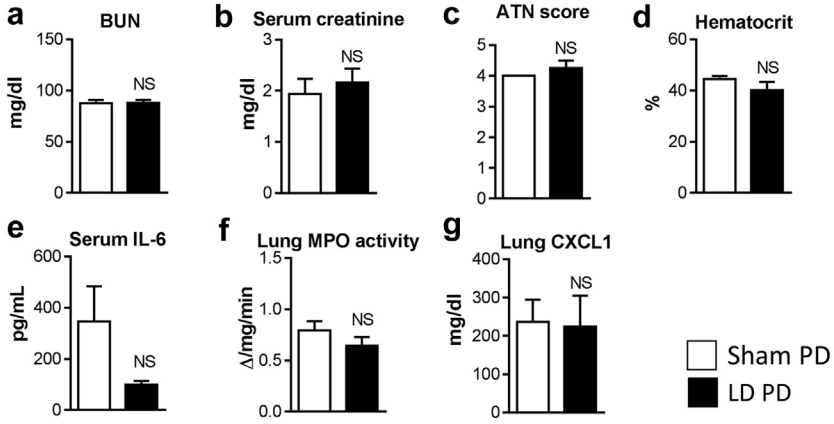


**Figure 3 | Effect of low-dose (LD) peritoneal dialysis (PD) in ischemic acute kidney injury 24 hours after initiation of PD.** Sham PD or LD PD was performed 1 hour after ischemic acute kidney injury for 4 hours with peritoneal fluid exchanges occurring every hour (4 exchanges in 4 hours). (**a**) Blood urea nitrogen (BUN), (**b**) serum creatinine, (**c**) acute tubular necrosis (ATN) score, (**d**) hematocrit, (**e**) serum interleukin (IL)-6, (**f**) lung myeloperoxidase (MPO) activity, and (**g**) lung C-X-C motif chemokine ligand 1 (CXCL1) were determined 24 hours after initiation of Sham PD or LD PD and were not different. Analysis with unpaired *t* test, $N = 3–4$. NS, not significant.

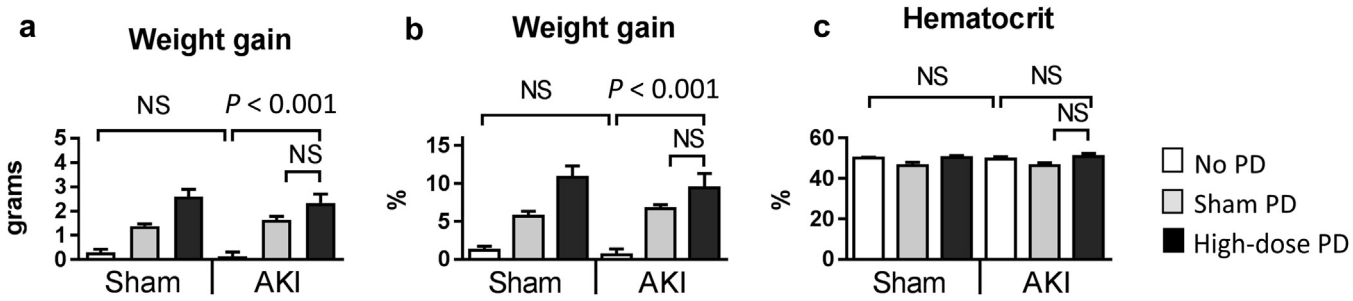


**Figure 4 | Effect of high-dose (HD) peritoneal dialysis (PD) in ischemic acute kidney injury (AKI) 4 hours after initiation of PD on fluid balance.** PD or Sham PD was performed 1 hour after sham operation (Sham) or ischemic AKI for 4 hours with peritoneal dialysate exchanges occurring every 15 minutes (i.e., 4 exchanges per hour for 4 hours for a total of 16 exchanges). Sham with no additional procedure (Sham + no PD) and AKI with no additional procedure (AKI + no PD) were also compared at the same time point. The following were determined 5 hours postprocedure for Sham or AKI: weight gain (absolute value in grams) (**a**), weight gain (relative value percentage) (**b**), and hematocrit (**c**). Comparisons were made using analysis of variance with Tukey's multiple comparison procedure. Group numbers: Sham + no PD ($N = 5$), Sham + Sham PD ($N = 5$), Sham + HD PD ($N = 5$), AKI + no PD ($N = 4$), AKI + Sham PD ($N = 5$), AKI + HD PD ($N = 5$). NS, not significant.

2 ml of fresh PD fluid every hour for 4 hours (for a total of 4 exchanges). High-dose PD consists of 2 ml of PD fluid instillation, which is then withdrawn and exchanged with 2 ml of fresh PD fluid every 15 minutes for 4 hours (for a total of 16 exchanges). PD is initiated 1 hour after surgery for bilateral nephrectomy, ischemic AKI, or sham (surgery alone).

### Development of an appropriate model of sham PD

To develop an appropriate control for PD, we developed a model of sham PD. Our final model of sham PD consisted of placement of a PD catheter and instilling 100 μl of peritoneal dialysis fluid every hour (control for low-dose PD) or every 15 minutes (control for high-dose PD). This method thus accounts for catheter placement and the potential effect of manipulating the PD catheter as well as the administration of PD fluid. Additionally, mice were weighed, and supplemental fluid was given subcutaneously every hour so that weight gains would be comparable to those of the mice receiving PD. Of note, this control does not account for the potential effects of the large volumes of fluid administered with PD; we found that administration of larger volumes PD fluid, even if removed soon after administration, resulted in a decrease in BUN and thus was not an adequate control. Likewise, administration of even small volumes of PD fluid resulted in some clearance of BUN.

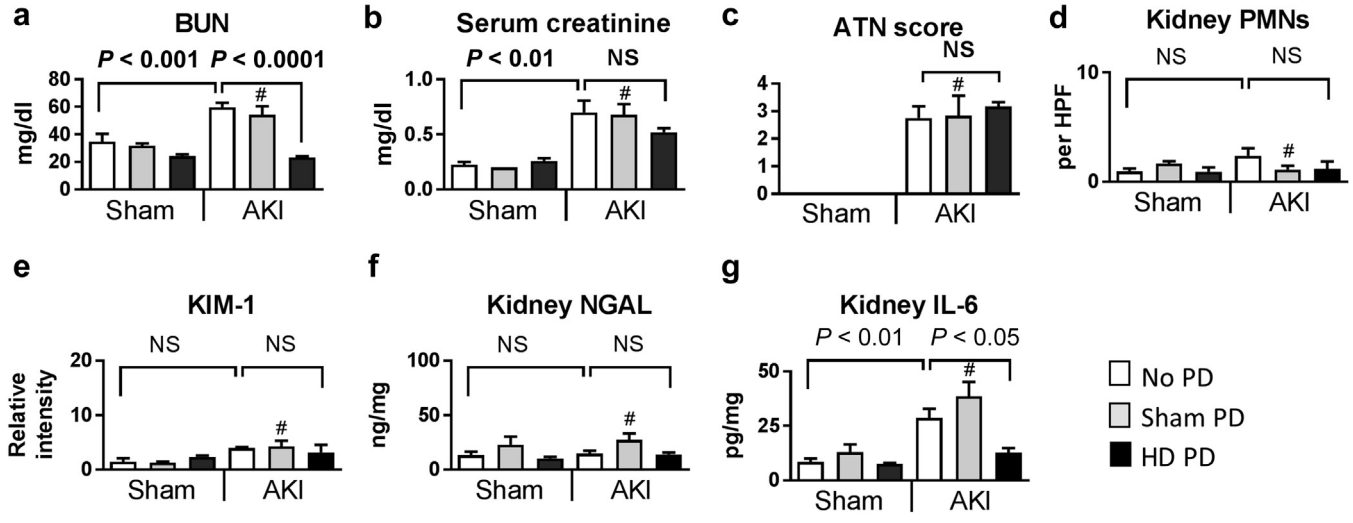


**Figure 5 | Effect of high-dose (HD) peritoneal dialysis (PD) on kidney injury in ischemic acute kidney injury (AKI) 4 hours after initiation of PD.** PD or sham PD was performed 1 hour after sham operation (Sham) or ischemic AKI for 4 hours with peritoneal dialysate exchanges occurring every 15 minutes (i.e., 4 exchanges per hour for 4 hours for a total of 16 exchanges). Sham with no additional procedure (Sham + no PD) and AKI with no additional procedure (AKI + no PD) were also compared at the same time point. The following were determined 5 hours post-Sham or AKI procedure: blood urea nitrogen (BUN) (**a**), serum creatinine (**b**), acute tubular necrosis (ATN) scores (**c**), infiltration of neutrophils (PMNs) into the kidney (**d**), kidney injury molecule 1 (KIM-1) by immunohistochemistry (**e**), kidney neutrophil gelatinase-associated lipocalin (NGAL) (**f**), and kidney interleukin (IL)–6 (**g**). Comparisons were made using analysis of variance with Tukey's multiple comparison procedure. #NS versus AKI + no PD. Group numbers: Sham + no PD ($N = 5$), Sham + Sham PD ($N = 5$), Sham + HD PD ($N = 5$), AKI + no PD ($N = 4$), AKI + Sham PD ($N = 5$), and AKI + HD PD ($N = 5$). HPF, high-power field; NS, not significant.

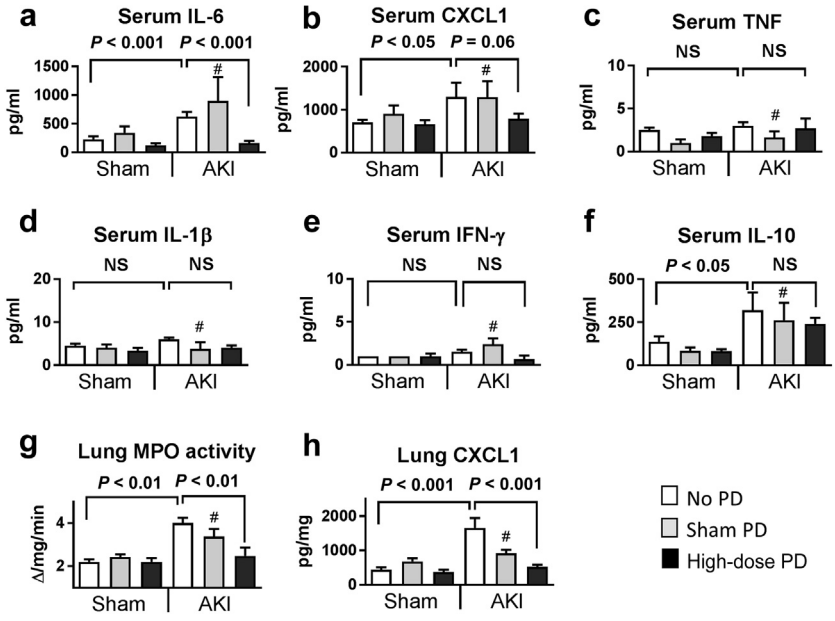


**Figure 6 | Effect of high-dose (HD) peritoneal dialysis (PD) on serum cytokines and lung inflammation in ischemic acute kidney injury (AKI) 4 hours after initiation of PD.** PD or sham PD was performed 1 hour after sham operation (Sham) or ischemic AKI for 4 hours with peritoneal dialysate exchanges occurring every 15 minutes (i.e., 4 exchanges per hour for 4 hours for a total of 16 exchanges). Sham with no additional procedure (Sham + no PD) and AKI with no additional procedure (AKI + no PD) were also compared at the same time point. The following were determined 5 hours post-Sham or AKI procedure: serum interleukin (IL)–6 (**a**), serum CXCL1 (**b**), serum tumor necrosis factor (TNF) (**c**), serum IL-1β (**d**), serum interferon (IFN)-γ (**e**), serum IL-10 (**f**), lung myeloperoxidase (MPO) activity (**g**), and lung C-X-C motif chemokine ligand 1 (CXCL1) (**h**). Comparisons were made using analysis of variance with Tukey's multiple comparison procedure. #NS versus AKI + no PD. Group numbers: Sham + no PD: $N = 5$; Sham + Sham PD: $N = 5$; Sham + HD PD: $N = 5$; AKI + no PD: $N = 4$; AKI + Sham PD: $N = 5$; AKI + HD PD: $N = 5$.

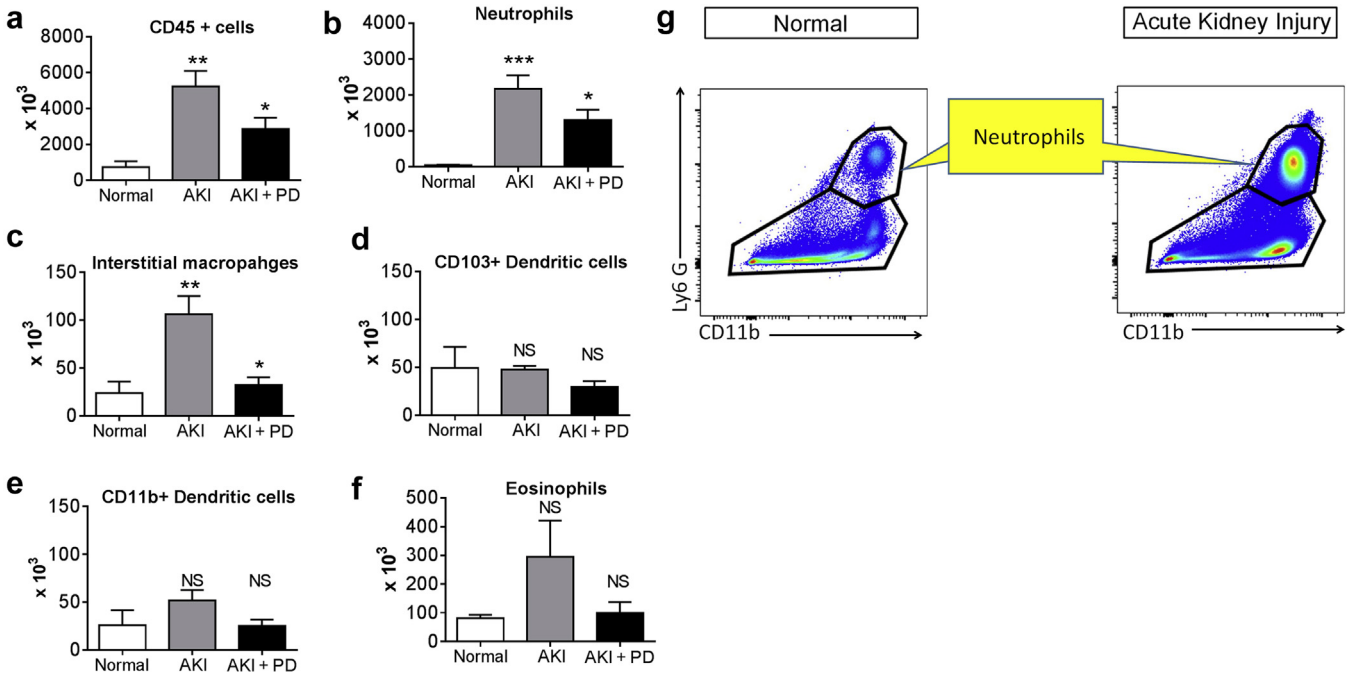


**Figure 7 | Effect of high-dose (HD) peritoneal dialysis (PD) in ischemic acute kidney injury (AKI) on lung leukocyte infiltration as assessed by flow cytometry.** HD PD was performed 1 hour after ischemic AKI for 4 hours with peritoneal dialysate exchanges occurring every 15 minutes (i.e., 4 exchanges per hour for 4 hours for a total of 16 exchanges). Normal mice and mice 5 hours after ischemic AKI were also studied. Flow cytometry was performed on the entire lung and CD45+ cells (**a**), neutrophils (**b**), interstitial macrophages (**c**), CD103 + dendritic cells (**d**), CD11b + dendritic cells (**e**), and eosinophils (**f**). CD45+ cells, neutrophils, and interstitial macrophages were significantly reduced in mice with peritoneal dialysis. (**g**) Representative gating strategy for neutrophil detection. Normal ($N = 4$), AKI ($N = 9$), and AKI + PD ($N = 9$). *$P < 0.05$ versus AKI; **$P < 0.01$ versus normal; ***$P < 0.001$ versus normal; NS, not significant (vs. normal or AKI); 1-way analysis of variance with Dunnett's *post hoc* procedure with AKI as the comparison group. Group numbers: normal ($N = 4$), AKI ($N = 9$), and AKI + PD ($N = 9$).

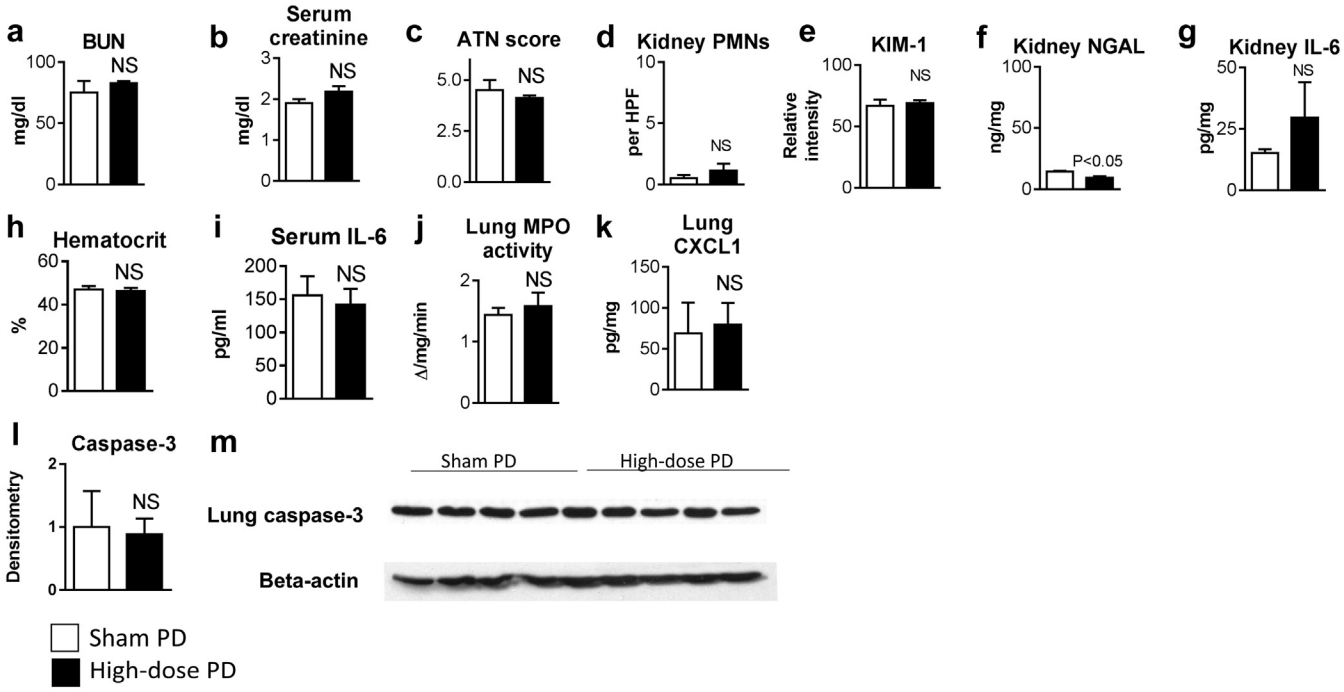


**Figure 8 | Effect of high-dose peritoneal dialysis (PD) in ischemic acute kidney injury (AKI), 24 hours after initiation of PD.** PD or Sham PD was performed 1 hour after ischemic AKI for 4 hours with peritoneal fluid exchanges occurring every 15 minutes (4 exchanges every hour for 4 hours for a total of 16 exchanges). Blood urea nitrogen (BUN) (**a**), serum creatinine (**b**), acute tubular necrosis (ATN) score (**c**), infiltration of neutrophils (PMNs) into the kidney (Kidney PMNs) (**d**), kidney injury molecule-1 (KIM-1) immunohistochemistry (**e**), kidney neutrophil gelatinase-associated lipocalin (NGAL) (**f**), kidney interleukin (IL)-6 (**g**), hematocrit (**h**), serum IL-6 (**i**), lung myeloperoxidase (MPO) activity (**j**), lung C-X-C motif chemokine ligand 1 (CXCL1) (**k**), and lung caspase-3 were determined 24 hours after initiation of PD and were not different (**l,m**). (Analysis with unpaired *t* test, $N = 3–5$). NS, not significant.

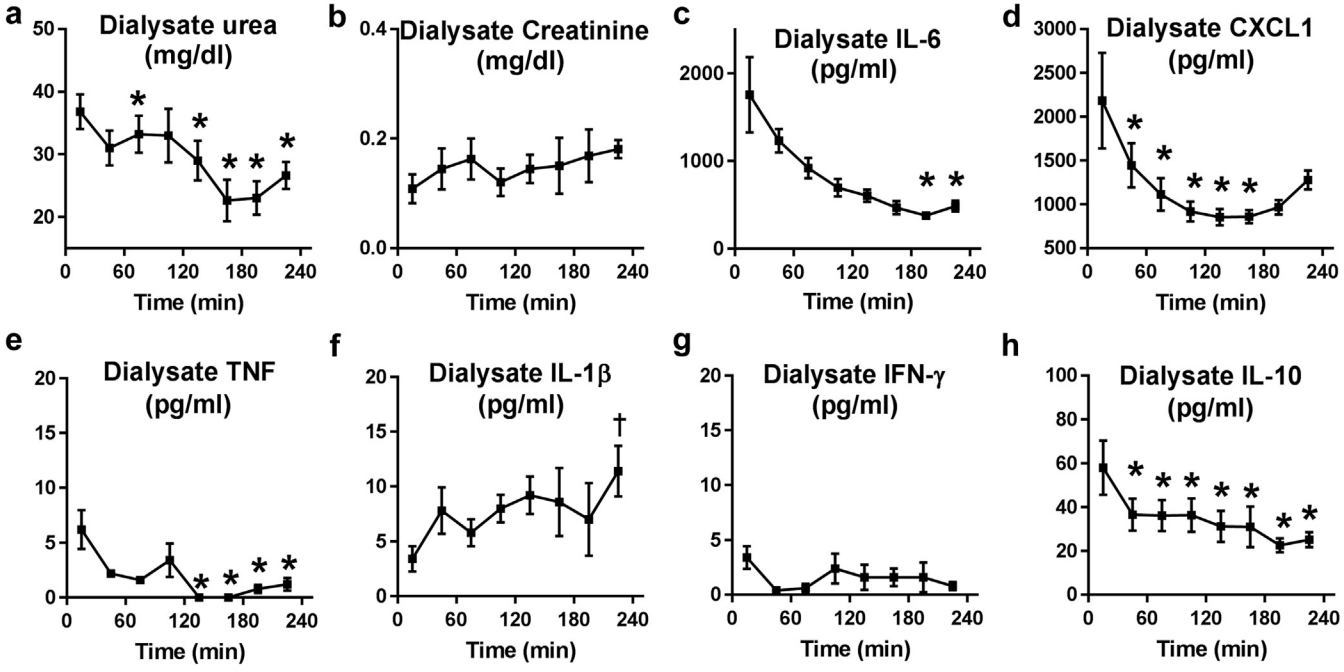


**Figure 9 | Dialysate concentrations of urea, creatinine, and cytokines with high-dose peritoneal dialysis after ischemic acute kidney injury (AKI).** Peritoneal dialysis was initiated 1 hour after ischemic AKI with peritoneal dialysate exchanges occurring every 15 minutes (i.e., 4 exchanges per hour for 4 hours for a total of 16 exchanges). Dialysate concentrations of urea (**a**), creatinine (**b**), interleukin (IL)-6 (**c**), C-X-C motif chemokine ligand 1 (CXCL1) (**d**), tumor necrosis factor (TNF)-α (**e**), IL-1 (**f**), interferon (IFN)-γ (**g**), and IL-10 (**h**) were measured in the dialysate recovered after 15-, 45-, 75-, 105-, 135-, 165-, 205-, and 235-minute dwells. $^*P < 0.05$ (decrease) versus the 15-minute dwell; †$P < 0.05$ (increase) versus the 15-minute dwell; $N = 5$ (**a**–**h**) for each time point. These data show that urea, IL-6, CXCL1, TNF, and IL-10 levels are significantly decreasing in the peritoneal fluid with subsequent PD fluid dwells.

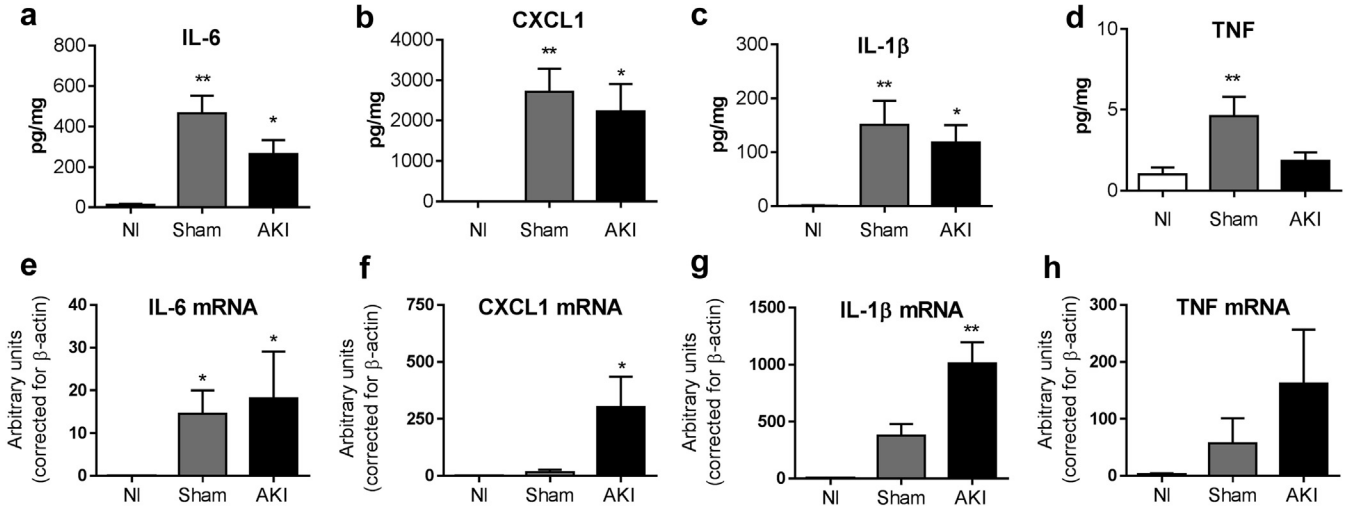


**Figure 10 | Cytokine production after acute kidney injury (AKI) in peritoneal cells.** Four hours after sham operation (Sham) or ischemic AKI, peritoneal cells were removed by lavage and subsequent centrifugation and were analyzed for the cytokines interleukin (IL)-6, C-X-C motif chemokine ligand 1 (CXCL1), IL-1β, and tumor necrosis factor (TNF) by protein (**a–d**) and mRNA (**e–h**). Peritoneal cells from normal mice (no procedure) (NI) were also examined (*$P < 0.05$ vs. NI, **$P < 0.01$ vs. NI). Analysis of variance with Dunnett's multiple comparison procedure with NI as the control group. (**a–d**) $N = 5–6$, (**e–h**) $N = 3–5$.

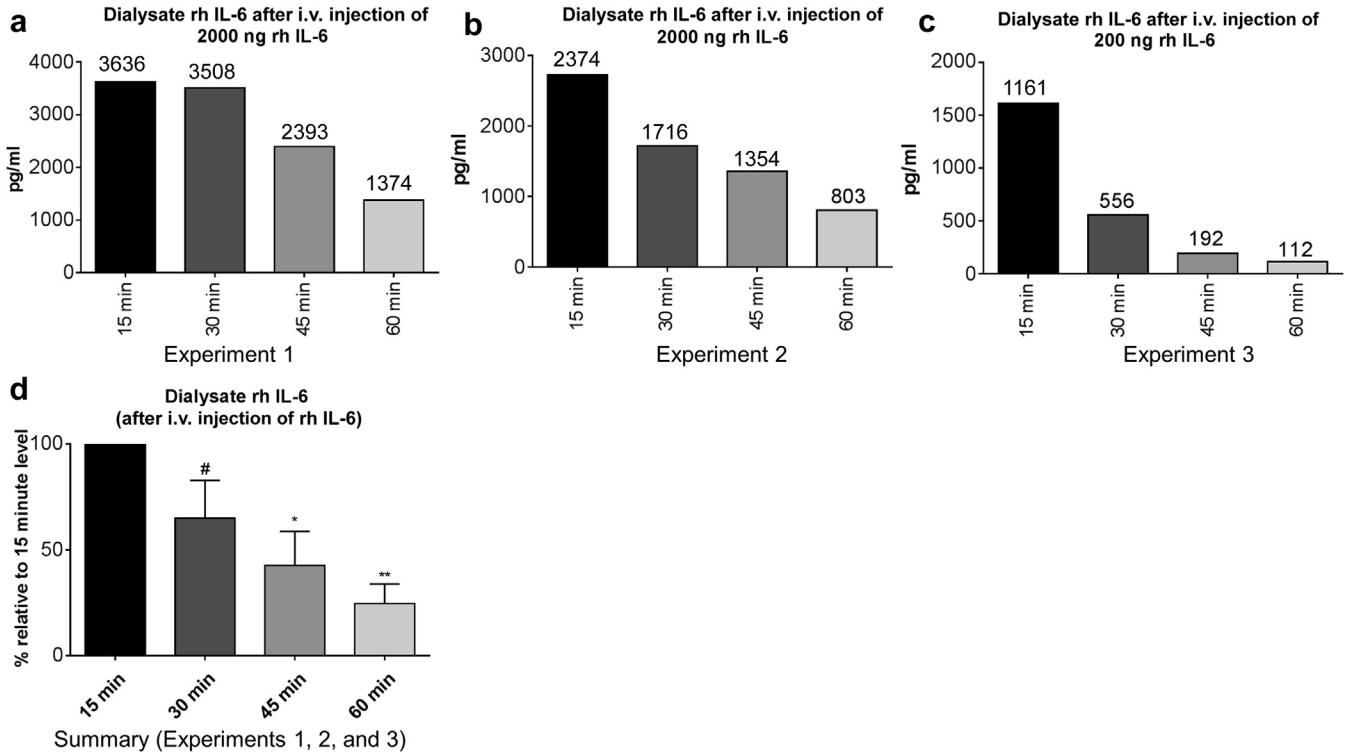


**Figure 11 | Dialysate concentration of recombinant human interleukin-6 (rh IL-6) after i.v. injection of rh IL-6 (a).** Immediately after ischemic acute kidney injury, 2000 ng/ml ($N = 2$; **a** and **b**) or 200 ng/ml ($N = 1$; **c**) of rh IL-6 was administered i.v., and peritoneal dialysis was started. Peritoneal dialysis fluid (dialysate) was collected and exchanged every 15 minutes for 1 hour; thus, dialysate samples were analyzed at 15, 30, 45, and 60 minutes. Absolute values of rh IL-6 at each time point are shown in **a–c**. Data are reported relative to the rh IL-6 level in the dialysate in the 15-minute sample in the same animal as in (**d**). Comparisons were made using analysis of variance with multiple comparisons using uncorrected Fisher's least significant difference and the 15-minute group as the comparator: #$P = 0.089$, *$P = 0.013$, **$P = 0.003$; $N = 3$. Because rh IL-6 is detected in the dialysate after i.v. injection, these data indicate that circulating IL-6 may be removed by peritoneal dialysis.

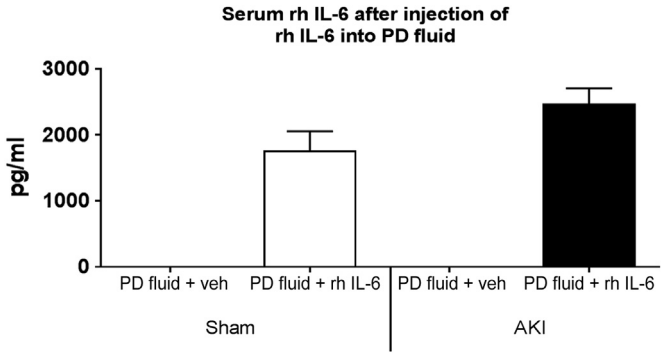


**Figure 12 | Serum concentration of recombinant human interleukin-6 (rh IL-6) after i.p. instillation of peritoneal dialysate fluid containing rh IL-6.** Immediately after sham operation (Sham) or ischemic acute kidney injury (AKI), 2 ml of peritoneal dialysis (PD) fluid containing vehicle (veh) (1% bovine serum albumin) or 200 ng of rh IL-6 was instilled peritoneally; 15 minutes later, dialysate and serum were collected for rh IL-6 measurement. Serum rh IL-6 was increased in Sham and AKI receiving dialysate fluid with rh IL-6 (Sham + PD + IL-6, AKI + PD + IL-6, respectively), and absent in Sham and AKI + veh (Sham + PD + veh, AKI + PD + veh, respectively) (Sham + PD + IL-6 vs. AKI + PD + IL-6, $P$ = NS, $N = 5$). NS, not significant; veh, vehicle.

### Effect of sham and low- and high-dose PD after bilateral nephrectomy

The effect of PD on pH, serum creatinine, BUN, hematocrit, and percentage of weight change was determined in mice with bilateral nephrectomy, as shown in Figure 1. In this experiment, bilateral nephrectomy was performed, and 1 hour later sham PD, low-dose PD, or high-dose PD was performed for 4 hours, and endpoints were determined at that time point. Mice with bilateral nephrectomy and no additional procedure (i.e., no PD) were also studied.

As shown in Figure 1a, pH was significantly reduced 5 hours after bilateral nephrectomy and was restored to normal with low-dose PD. Hematocrit was not affected by sham PD, low-dose PD, or high-dose PD, suggesting that the models of sham, low-dose, and high-dose PD did not cause ultrafiltration (Figure 1d). Hematocrit is an accepted marker of intravascular volume in veterinary medicine, and we previously demonstrated its reliability for volume assessment in mice.[8] Low-dose PD reduced BUN from 55 ± 2 mg/dl to 41 ± 2 mg/dl ($P < 0.001$), whereas high-dose PD reduced BUN to normal (25 ± 1 mg/dl with high-dose PD, 24 ± 1 mg/dl in normal mice, $P$ = not significant). Serum creatinine was also significantly reduced.

Mice with either low- or high-dose PD gained weight that was in proportion to the amount of fluid retained in the abdomen on completion of PD. Specifically, 2.1 ± 0.3 ml were retained in the abdomen after completion of PD, and the net weight gain was 2.0 ± 0.2 g. Because hematocrit was normal, these data suggest that our model of PD is not associated with either ultrafiltration (i.e., fluid removal) or fluid retention and that the net effect on fluid balance is neutral.

### Effect of PD on survival after bilateral nephrectomy

Death after bilateral nephrectomy usually occurs by 36 hours. To assess the effect of PD on survival, low-dose PD was performed daily; 2 of 2 mice survived until planned killing on day 3, and 2 of 5 mice survived until planned killing on day 8 (mice died because of the following: catheter leakage [killed], pulled catheter out [killed], and unexpected death during dialysis on day 5). Activity was dramatically improved with each PD session. Of the mice that survived until day 8, 1 was killed before PD and had a BUN level of 213 mg/dl and a serum creatinine level of 3.9 mg/dl; the other mouse was killed after PD and had a BUN level of 171 mg/dl and a serum creatinine level of 3.9 mg/dl.

### Effect of low-dose or high-dose PD after ischemic AKI

Having established a model of PD in bilateral nephrectomy, we next sought to determine the effect of 4 hours of either

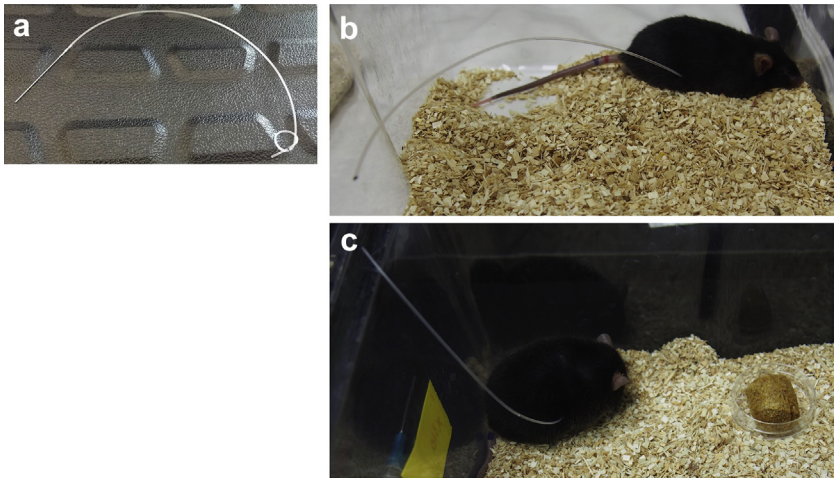


**Figure 13 | Dialysis catheter and placement in mice.** (**a**) Representative image of the custom peritoneal dialysis (PD) catheter is shown. PD catheters consisted of 8 to 10 in. of PE50 sterile tubing with a 2-cm loop and 6 to 8 holes. (**b,c**) Images of catheter placement in mice. Mice were singly housed, and the PD catheter was tunneled through the dorsum of the mouse to allow for easy access for PD fluid exchanges and to be out of reach so that the mouse would be unable to chew or pull out the catheter.

low- or high-dose PD on kidney injury, serum cytokines, and lung inflammation after ischemic AKI. PD was initiated 1 hour after sham operation or ischemic AKI, and endpoints were measured 4 or 24 hours after PD initiation.

### Effect of low-dose PD in ischemic AKI

As shown in Figures 2 and 3, low-dose PD restored pH to normal and significantly reduced BUN, yet there was no effect on kidney injury, serum cytokines, or lung inflammation at either 4 or 24 hours.

### Effect of high-dose PD in ischemic AKI

The effect of high-dose PD in ischemic AKI after 4 hours is shown in Figures 4, 5, and 6.

As with PD in bilateral nephrectomy, mice gained weight with PD in ischemic AKI (Figure 4). The absolute weight gain was 2.5 ± 0.6 g; of note, the amount of fluid retained in the abdomen after the PD procedure was 2.3 ± 0.6 ml. Because weight gain is essentially equivalent to the amount of PD fluid retained in the abdomen after the PD procedure, we suggest that neither ultrafiltration nor systemic fluid resorption is occurring. This conclusion is further supported by the fact that hematocrit is not affected. Thus, our model of PD in ischemic AKI does not affect fluid balance in either direction, and the effect on fluid balance is neutral.

As shown in Figure 5a, high-dose PD effectively cleared BUN and creatinine, and BUN levels were normal. High-dose PD had no effect on kidney injury as judged by histology (acute tubular necrosis scores and neutrophil infiltration), kidney injury molecule-1 immunohistochemistry, and kidney neutrophil gelatinase-associated lipocalin concentration (Figure 5c–f). Interestingly, kidney IL-6 concentrations were reduced, which is likely due to the reduction in serum IL-6 (discussed in the following).

As shown in Figure 6, 4 hours of high-dose PD in ischemic AKI was associated with a significant reduction in serum IL-6 and lung inflammation (as judged by lung myeloperoxidase activity and lung C-X-C motif chemokine ligand 1 [CXCL1]) (Figure 6a, g, h). There was a trend in serum CXCL1 reduction ($P = 0.06$); however, other serum cytokines were not affected (Figure 6b–f).

To better characterize the effect of high-dose PD on lung injury post-AKI, additional experiments were performed to assess leukocyte infiltration and pulmonary edema. In these experiments, mice underwent ischemic AKI with or without 4 hours of high-dose PD. Sham PD was not studied because the results between sham PD and AKI alone were similar (Figures 5 and 6). To increase the severity of kidney injury after AKI, clamp time was increased to 25 minutes; at 5 hours post-procedure, BUN was 59 ± 1 mg/dl in AKI alone and 26 ± 1 mg/dl in AKI with PD ($P < 0.0001$, $N = 19$ per group); serum creatinine was 0.81 ± 0.46 mg/dl in AKI alone and 0.32 ± 0.02 mg/dl in AKI with PD ($P < 0.0001$, $N = 19$ per group). Thus, the level of kidney dysfunction in ischemic AKI and the effect of PD were consistent.

### Leukocyte infiltration by flow cytometry

Flow cytometry for myeloid lineage cells was performed on digests of the entire lung tissue. As shown in Figure 7, neutrophils and interstitial macrophages were significantly reduced in mice that received high-dose PD. In this experiment, serum IL-6 was determined and was 723 ± 103 pg/ml in AKI versus 317 ± 41 pg/ml in AKI + PD ($P = 0.0012$), $N = 9$ for both groups and are thus similar to the results shown in Figure 6.

### Pulmonary edema

In separate experiments, we determined the whole lung wet-to-dry lung weight percentage and bronchoalveolar lavage fluid protein. The wet-to-dry lung weight percentage is an indicator of cardiogenic and noncardiogenic pulmonary edema and was 26 ± 3% in AKI and 24 ± 2% in AKI + PD ($P$ = not significant [0.65], $N = 5$); bronchoalveolar lavage fluid protein is a marker of cardiogenic pulmonary edema and was 83 ± 13 mg/dl in AKI and 76 ± 12 mg/dl in AKI + PD ($P$ = not significant, $N = 5$).

In sum, our data demonstrate that lung inflammation is markedly reduced with high-dose PD, which is independent of an effect on volume status or pulmonary edema.

### Effect of high-dose PD in ischemic AKI after 24 hours

Kidney injury and lung inflammation were assessed at 24 hours after high-dose PD in ischemic AKI. As shown in Figure 8, kidney injury, serum IL-6, and lung inflammation were similar with and without early PD. Because apoptosis is a feature of AKI-mediated lung injury at 24 hours,[9] we also measured caspase-3 and found that it was also similar. Thus, the protection against lung inflammation with high-dose PD was not sustained.

### Clearance of urea, creatinine, and cytokines with high-dose PD in ischemic AKI

To assess solute and cytokine removal by PD, dialysate levels of urea, creatinine, and cytokines were determined in PD fluid collected from high-dose PD in ischemic AKI. As shown in Figure 9, dialysate urea, IL-6, CXCL1, IL-10, and tumor necrosis factor (TNF) were significantly reduced from the initial 15-minute value. Together with serum levels, these data suggest that urea and IL-6 may be cleared from the serum with PD in this model.

### Role of peritoneal cells in cytokine levels in dialysate

To determine the contribution of peritoneal cells to cytokine levels in the PD fluid, peritoneal cell cytokines were determined 4 hours after sham operation and ischemic AKI, as well as in normal mice. Cytokines were increased after both sham and AKI versus normal but were not increased in AKI versus sham (Figure 10). Thus, peritoneal cells produce cytokines after abdominal surgery, which is not further increased by AKI. Thus, PD could be expected to remove peritoneal cytokines with or without AKI.

### Clearance of serum IL-6 with PD

To directly test whether circulating IL-6 is cleared by PD, we administered recombinant human (rh) IL-6 i.v. to wild-type mice with AKI and then performed high-dose PD (instillation and removal of PD fluid every 15 minutes) for 1 hour. Spent PD fluid was then analyzed for rh IL-6. As shown in Figure 11, PD fluid contained high levels of rh IL-6, which steadily decreased at subsequent time points. Because there is no cross-reactivity between endogenous murine IL-6 and rh IL-6 by enzyme-linked immunosorbent assay, these data indicate that circulating IL-6 enters PD fluid and can be effectively cleared because the source of all the rh IL-6 detected in the dialysis fluid would be from what was administered i.v.

To determine whether IL-6 in the peritoneal cavity enters the serum, rh IL-6 was added to peritoneal fluid and serum rh IL-6 was examined 15 minutes after instillation. As shown in Figure 12, serum rh IL-6 levels were increased in mice with either sham operation or ischemic AKI that had rh IL-6 in the peritoneal dialysate.

Together, these data indicate that there is communication between the dialysate and serum regarding IL-6 and that circulating IL-6 accumulates in the PD fluid and can been effectively cleared by PD.

## DISCUSSION

In this study, we successfully developed a therapeutic model of PD that reliably reduces BUN in a dose-dependent fashion in 2 models of AKI: bilateral nephrectomy and ischemic AKI. Our study is the first successful application of PD in a mouse model of acute uremia and lays the ground work for future studies to investigate the systemic effects of RRT in AKI in mice. Because the majority of ongoing clinical trials in AKI are focused on manipulations of RRT regarding dose, timing, and prescription,[10] we believe that establishment of this model of RRT is of substantial clinical relevance that may ultimately inform RRT clinical trial design.

In terms of solute clearance, we suggest that our low-dose model of PD is similar to intermittent hemodialysis and that our high-dose model is similar to both PD and continuous venovenous hemofiltration (CVVH)/continuous venovenous hemodiafiltration (CVVHDF). Clinically, intermittent hemodialysis, PD, and CVVH/CVVHDF are similar, and all remove the same solutes up to a molecular weight of ~10 kDa. However, both PD and CVVH/CVVHDF can theoretically clear solutes and small proteins with a molecular weight ranging from to 20 to 30 kDa (depending on the filters used). Peritoneal transport parameters between murine and human PD are similar with regard to low-weight (<10 kDa) and middle-weight (20–30 kDa) solutes.[11] Thus, the solutes cleared in murine PD are similar to those cleared in human PD and are comparable to CVVH/CVVHDF.

We found that high-dose PD after ischemic AKI significantly reduces serum IL-6. Our data suggest that this is due to removal of both circulating IL-6 and peritoneal IL-6. Clearance of IL-6 by PD is biologically plausible because the molecular weight of IL-6 is ~26 kDa. Indeed, studies in patients indicate that removal of IL-6 occurs with PD.[4]

The reduction in serum IL-6 with high-dose PD was associated with significant protection against lung inflammation that was independent of volume status or pulmonary edema. AKI-mediated lung injury is well described in animal models and is predominantly characterized by lung inflammation, as judged by increased cytokines, chemokines, and neutrophils by 4 hours post-AKI.[12] Noncardiogenic pulmonary edema is variably present.[12] By 24 hours, T-cell infiltration, necroptosis, parthanatos, apoptosis, and increased caspase-3 activity are present.[13,14] Established circulating mediators of lung injury include IL-6,[5] T cells,[14] TNF,[15] and high-mobility group box 1 protein.[16] T cells and TNF may mediate lung injury via neutrophil infiltration and activation of apoptosis via caspase-3. We did not assess T cells or high-mobility group box 1 protein in this report; however, TNF was not affected by PD, and caspase-3 at 24 hours was not affected by early PD. Future studies of PD after AKI could investigate these other factors.

We conclude that the protection against lung inflammation with high-dose PD is due to IL-6 removal. Circulating IL-6 mediates lung inflammation by binding to lung endothelial cells and upregulating production of the neutrophil chemokine CXCL1 via classic signaling, which then facilitates neutrophil accumulation.[5] Our data in this report are consistent with this mechanism because lung CXCL1 and neutrophil accumulation were reduced. Further supporting the role of IL-6 are our data demonstrating that low-dose PD did not lower IL-6 and did not protect against lung inflammation and that other proinflammatory cytokines that mediate lung inflammation (e.g., IL-1, TNF) were not affected by PD.

Among cytokines that have been studied in AKI, IL-6 has particular clinical relevance. Serum IL-6 increases after as early as 2 hours in patients with AKI,[17] and increased levels predict prolonged mechanical ventilation[17] and increased mortality.[18] Of particular relevance to the present report are the results of 2 recent clinical trials investigating the effects of early RRT in AKI demonstrating reduced serum IL-6 that was associated with reduced mechanical ventilation.[19,20] In the more recent study, early RRT versus later RRT was performed with CVVHDF, a convective technique, in critically ill adults in the intensive care unit with AKI; early RRT was associated with a significant reduction in serum IL-6 24 hours after RRT initiation and reduced mechanical ventilation time (125 hours vs. 181 hours).[19] The improvement in mechanical ventilation time could not be attributed to an effect on fluid accumulation as fluid balance was similar in the 2 groups. Although the role of convective therapies in cytokine removal in AKI remains controversial, the result of this trial is consistent with the notion that IL-6 removal in patients with AKI may improve pulmonary outcomes. In the second study, children who received PD immediately after bypass-requiring cardiac surgery had a significant reduction in serum IL-6 that was associated with

improved clinical outcomes including shorter duration of mechanical ventilation (71 hours vs. 125 hours).[21] Because of better clinical outcomes,[4,20,21] prophylactic PD is actually standard practice after cardiac surgery in children at some centers.[20,22] Thus, our model of PD in AKI seems to be particularly clinically relevant to children receiving PD early after cardiac surgery, which is associated with a high risk of AKI.

### Limitations and future directions

Whether cytokines other than IL-6 may be removed by PD is not clear from our experiments. We initiated PD 1 hour after ischemic AKI when serum IL-6 concentrations are high (>1000 pg/ml), but serum levels of IL-1 and TNF are not (<20 pg/ml); thus, a sufficient concentration gradient may not have been present to facilitate removal of other cytokines. It would be interesting, therefore, to test the high-dose PD model in murine sepsis in which TNF and IL-1β levels are high and play an known role in early distant organ injury.[23] In addition, given the interest in the potential of RRT to remove cytokines in established AKI, the study of our model of PD in the later stages of AKI (rather than early, as in our study) would also be of interest. We tested whether early PD had an effect on kidney injury and found that AKI was neither improved nor exacerbated; an important future study would be to examine whether different RRT doses for a longer duration might either improve or delay recovery of kidney injury. Finally, systemic effects were only evaluated after 1 session of PD, and the lung was the only remote organ examined. Because animal models have demonstrated that AKI also adversely affects the heart,[24–26] liver,[25,26] brain,[27] and intestine,[28] future studies with >1 PD session and the effect of PD on other organ injuries would be of interest.

In summary, we developed a reproducible model of PD that reliably corrects uremia in a dose-dependent fashion that will be useful to study the systemic complications of AKI and will be of potential benefit of RRT in mice. Our model of high-dose PD effectively clears IL-6 and reduces lung inflammation, which is clinically relevant to recent studies showing reduced serum IL-6 and better clinical outcomes in children who received prophylactic PD after cardiac surgery. We suggest that clinical trials of early PD after cardiac surgery in children that target normalization of serum IL-6 levels are warranted.

## METHODS

### Surgical procedures

Bilateral nephrectomy, ischemic AKI, and sham operation were performed as previously described.[29] For ischemic AKI, renal pedicles were clamped for 22 or 25 minutes. Blood was collected and processed as previously described[29]; hematocrit was determined as previously described.[8] Dialysates were centrifuged at 3000*g* for 5 minutes, and the supernatant collected. For mice receiving sham PD or PD, a custom PD catheter was placed during surgery (see Supplementary Methods for details).

### Kidney and lung injury measurements

Serum creatinine and BUN were measured using a VetAce autoanalyzer (Alfa Wassermann, West Caldwell, NJ). Lung myeloperoxidase activity was determined on one-quarter lung sections, as previously described.[29] Blood pH was measured using a blood gas analyzer (Siemens RapidLab 248, Washington DC). Acute tubular necrosis scores and other markers of kidney injury were determined as previously described[30] and as detailed in Supplementary Methods. Serum, dialysate, and peritoneal cell cytokines were determined using a mouse proinflammatory 7-plex ultrasensitive kit (MesoScale Discovery, Gaithersburg, MD) per the manufacturer's instructions. Serum IL-6 and CXCL1 were measured by ELISA (R & D), per manufacturer's instructions. Other lung injury assessments and flow cytometry for myeloid derived cells were performed as detailed in the Supplementary Methods, and gating strategy for myeloid-derived cells in the mouse lung is presented in Supplementary Figure S1.

### Cytokine production by peritoneal cells

Peritoneal cells were isolated by injection and withdrawal of 5 ml of ice cold PBS (with 3% FCS) into the peritoneal cavity using a 32g needle. The collected suspension was centrifuged at 1500 RPM for 8 minutes and cells collected. Cytokines were measured on cell lysates by individual enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) and corrected for protein. In separate samples, cytosolic RNA was isolated using the RNeasy kit (Qiagen, Valencia, CA). Before real-time PCR, RNA was converted to cDNA using the iScript reverse transcriptase kit (Bio-Rad, Hercules, CA), as described by the manufacturer. Reverse transcriptase polymerase chain reaction primers were designed as previously reported.[6] Reverse transcriptase polymerase chain reaction was performed as previously described.[6]

## DISCLOSURE

All the authors declared no competing interests.

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## SUPPLEMENTARY MATERIAL

**Supplementary Methods**.

**Figure S1.** Gating strategy for identification of myeloid cell subsets in the mouse lung.

Supplementary material is linked to the online version of the paper at www.kidney-international.org.

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