Anti-Histon IgG attenuates CKD upon AKI related to postischemic tubular necrosis in mice

Lukas Rath, Julian Marschner, Daigo Nakazawa, Alexander Holderied and Hans-Joachim Anders

Renal Division, Medizinische Klinik und Poliklinik IV, LMU University, Munich

Background: AKI-related nephron loss is an underappreciated determinant of subsequent chronic kidney disease. Thus, controlling necroinflammation in the acute injury phase of AKI may preserve nephrons and reduce the risk for CKD upon AKI. We have previously shown that histones released from dying tubular cells as well as from neutrophils forming neutrophil extracellular traps (NETs) are central elements of AKI-related necroinflammation as they are directly cytotoxic and also activate Toll-like receptor 2 and 4 (JASN 2012&2017). Therefore, we hypothesized that neutralizing extracellular histones using the anti-histone antibody BWA3, known to neutralize the cytotoxic and pro-inflammatory effects of extracellular histones, may improve the long term outcome upon postischemic AKI by reducing histone mediated cell death.

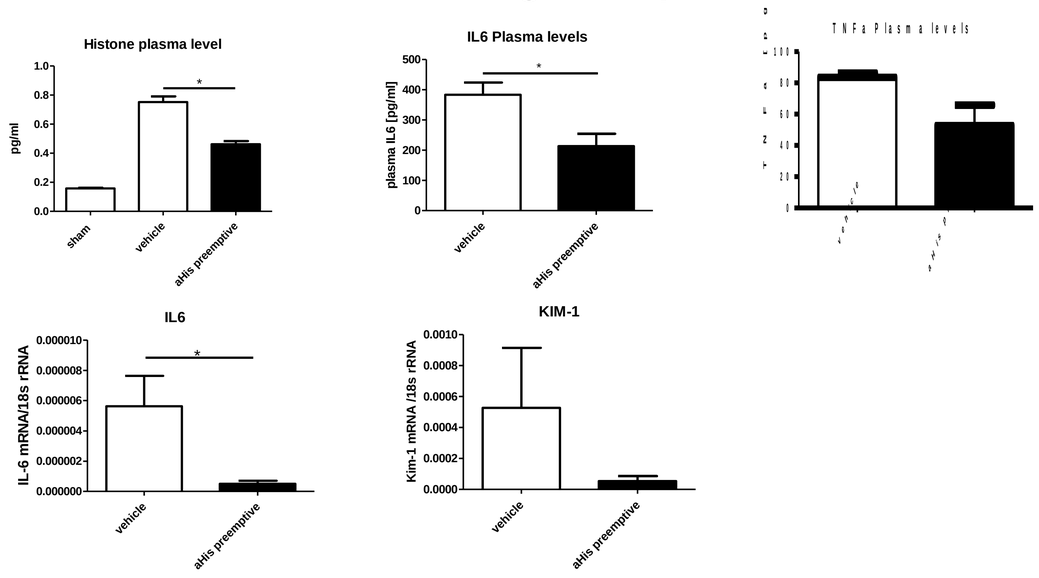
Methods: Bilateral and unilateral ischemia/reperfusion injury (IRI) was induced by renal pedicle clamping for 35 min in male C57BL/6 mice. Anti-histone IgG was administered preemptively, 3h, and 6h post AKI, vehicle groups received control IgG preemptively.

Results: After 35 min of bilateral ischemia and 24h of reperfusion, histone neutralization by preemptive single injection of anti-histone IgG did not affect the reduction in kidney function, but reduced the mRNA expression of KIM-1 and IL-6 as well as the plasma levels of IL-6 and TNF-α. After 35 min of unilateral ischemia and 5 weeks reperfusion the preemptive single application of anti-histone antibody reduced the mean deposition of α-SMA by 42%, induction of collagen by 35% and attenuated the chronic on acute kidney injury by reducing the loss of GFR by an average of 28%.

Conclusion: Together these data suggest, that a single injection of anti-histone IgG can improve long term outcomes of AKI, probably by limiting irreversible nephron loss.

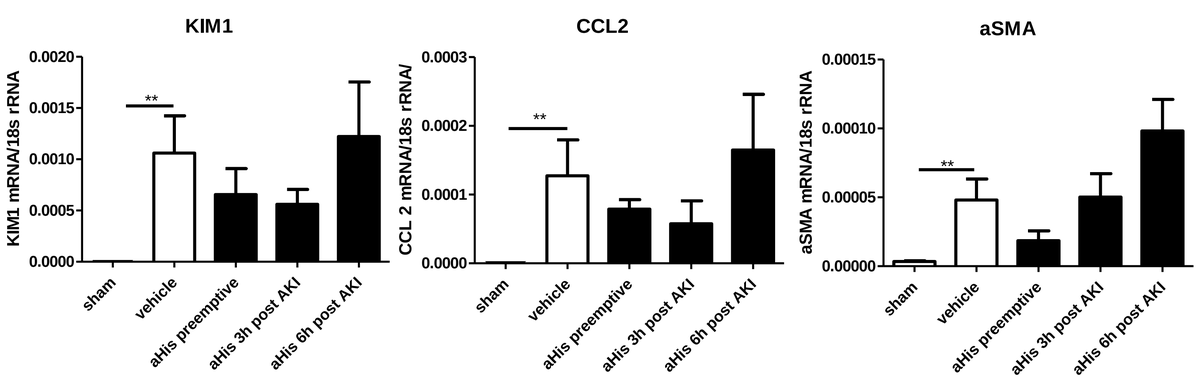
Acknowledgment: funded by DFG AN372/14-3, 23-1 Deutsche Forschungsgemeinschaft

Extracellular histones, plasma levels of IL-6 and TNF-α and gene expression of IL-6 and KIM-1 after preemptive administration of anti-histone IgG 24h post IRI



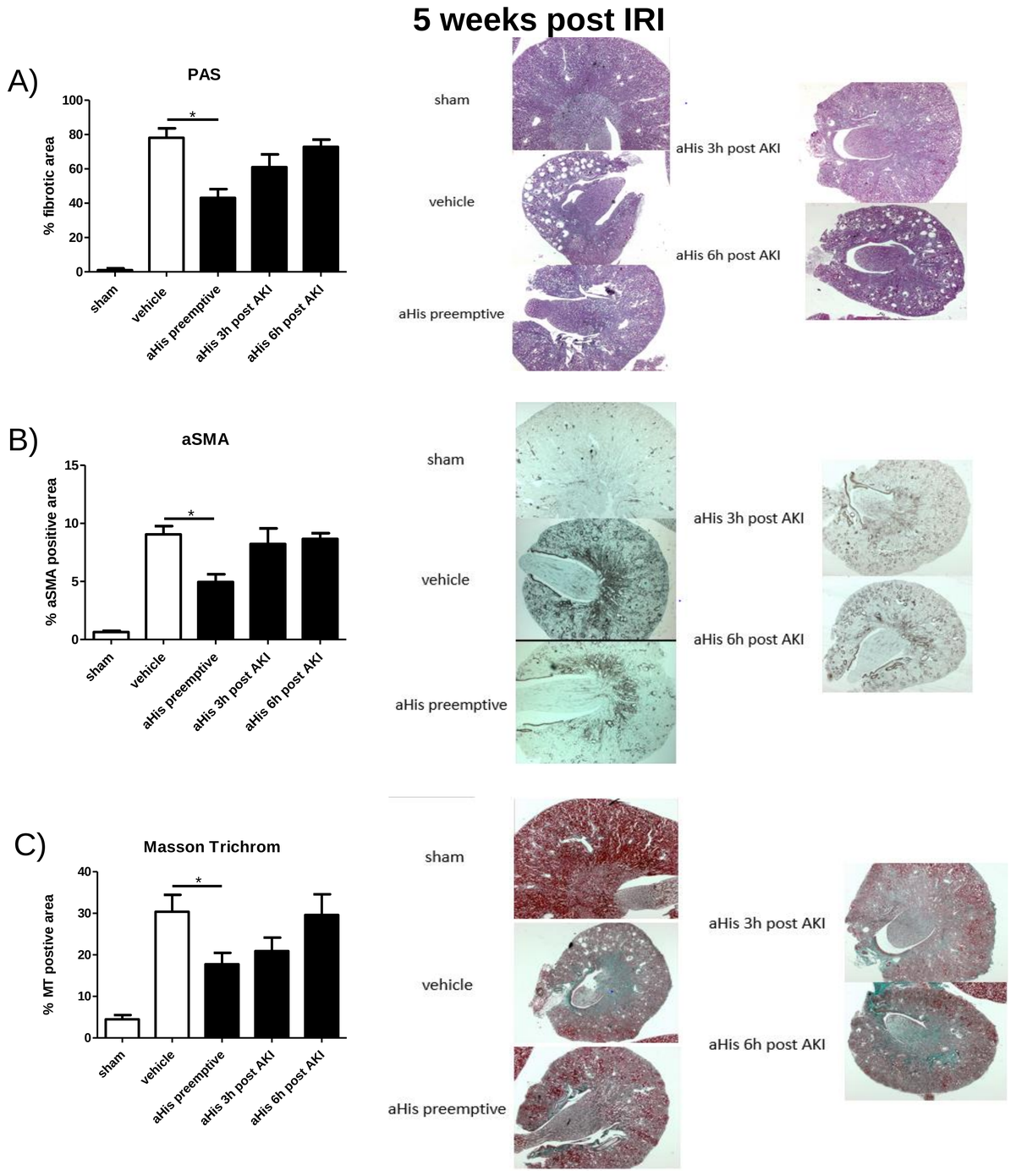
Preemptive neutralization of histones significantly reduced the plasma levels of IL-6 and histones and the gene expression of IL-6 in bilateral IRI (35 min ischemia) and 24h reperfusion compared to the control group (vehicle).

Gene expression of markers for tubular injury, inflammation and fibrosis 5 weeks post IRI



Gene expression of KIM-1, CCL-2 and α-SMA were still significantly upregulated 5 weeks post unilateral IRI (35 min ischemia) indicating persisting inflammation and injury. Neutralization of extracellular histones did not significantly alter the gene expression of theses markers compared to the vehicle group at any time of administration.

Fibrosis after preemptive neutralization of extracellular histones 5 weeks post IRI

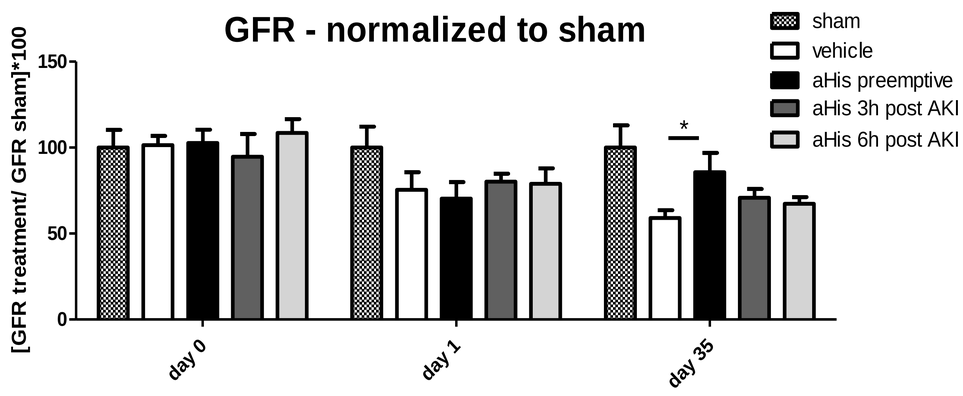


A) Preemptive histone neutralization reduced scarring and glomerular atrophy 5 weeks after unilateral IRI (35 min ischemia).

B) Semi-quantitative analysis showed a 42% reduction of α-SMA deposition in the preemptive treatment group 5 weeks after unilateral IRI (35 min ischemia).

C) Masson Trichrom staining revealed a 35% reduction of collagen induction in the preemptive treatment group 5 weeks after unilateral IRI (35 min ischemia).

Change of GFR normalized to sham 5 weeks post IRI



All treatment groups showed 25-30% loss of GFR 24h after unilateral IRI (35min ischemia). Preemptive histone neutralization significantly protected from loss of GFR 5 weeks after unilateral IRI resulting in a 25% higher GFR compared to the control group.

Summary:

- Preemptive neutralization of extracellular histones reduces the induction of IL-6 and gene expression of IL-6 24h after IRI and attenuates fibrosis and loss of GFR 5 weeks after IRI.
- Delayed neutralization of extracellular histones 3h and 6h post IRI does not improve structural or functional damage 24h or 5 weeks after IRI.

Conclusion:

Extracellular histones contribute to postischemic necrosis and inflammation. Preemptive administration of anti-histone-IgG attenuates inflammation and tubular injury in the early phase after acute tubular necrosis and improves the long term outcome of AKI in mice, probably by limiting irreversible nephron loss.

DOI: 10.3252/pso.eu.54ERA.2017