Intravenous Gemini™ for Prevention of Acute Kidney Injury

Prophylactic Administration of Gemini Reduces Tissue Damage and Improves Kidney Function in a Rat Model of Bilateral Ischemic Reperfusion Induced Acute Kidney Injury

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Abstract

Purpose and Background

The purpose of this study was to evaluate Gemini pretreatment in an ischemia reperfusion model of AKI. Due to its severe nature, AKI represents a significant health problem, especially in patients with co-morbidities such as diabetes. Approximately 1% of all hospitalized patients present with AKI upon admission. Phosphorylated hexaacyl disaccharide (PHAD), the active ingredient in Gemini, is a synthetic small molecule that preconditions the innate immune response via a more selective activation of toll-like receptor 4 (TLR4), characterized by an attenuated pro-inflammatory response relative to traditional TLR4 agonists such as lipopolysaccharide (LPS), while retaining the capacity to engage the innate immune response. We hypothesize that Gemini preconditioning will elicit an attenuated proinflammatory response following ischemic reperfusion (IR).

Methods

Rats were administered vehicle or Gemini intravenously at 0.07 and 0.35mg/kg at 24 and/or 48 hours prior to undergoing bilateral IR to induce acute kidney injury (AKI). A surgical sham group was also included. Blood and urine was collected pre-dose, and post-surgery at 24 and 72 hours (termination), and serum was assessed for BUN and creatinine levels. Urine was evaluated for c-reactive protein (CRP). Kidneys were evaluated for histological changes at 72 hours.

Results

Pretreatment with Gemini reduced serum BUN, serum creatinine and urine CRP in a dose dependent manner at 24 and 48 hours post-dose with significance at 0.35 mg/kg ($p<0.05$) relative to IR control. A single dose of 0.35mg/kg Gemini also significantly reduced the total degree of acute necrosis in cortical and medullary tubules ($p<0.05$). Neutrophil inflammation was significantly reduced ($p<0.05$) with a single pretreatment of Gemini at 0.35mg/kg.

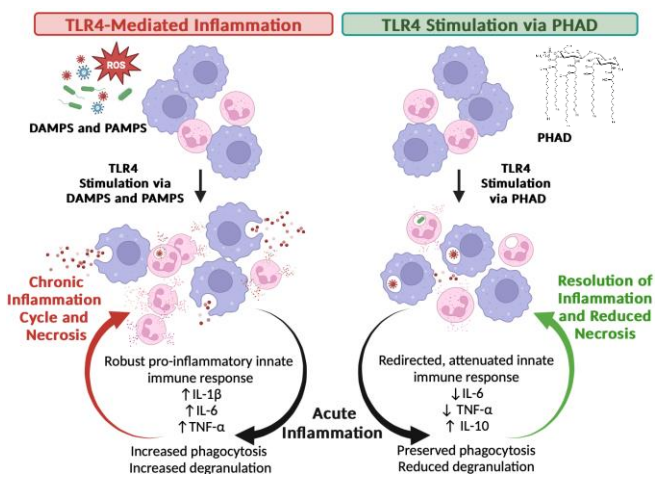
Conclusion

Pretreatment with Gemini significantly improved kidney function as demonstrated by reductions in BUN, creatinine and CRP, and reduced kidney injury as evident in the reductions in cortical and medullary tubular necrosis. Gemini also reduced AKI-related inflammation attributed to neutrophils in the kidney. These results demonstrate the premise of trained immunity as a means of prevention of AKI. The collective improvement in renal function along with the reductions in cellular necrosis and neutrophilic inflammation demonstrate Gemini pretreatment attenuates the severity of IR AKI.

Introduction

- Gemini is a proprietary formulation of phosphorylated hexaacyl disaccharide (PHAD), a toll-like receptor 4 (TLR4) agonist.
- Immunostimulatory preconditioning with Gemini prior to insult prepares the body to harness the characteristic upregulation of multiple pro-inflammatory and microbial gene products and proteins, such as PAMPS (pathogen-associated molecular patterns) and DAMPS (damage-associated molecular proteins).
- This controlled innate immune response facilitates the acute inflammatory process, while simultaneously engaging the activities that promote resolution of inflammation, allowing healing to take place.

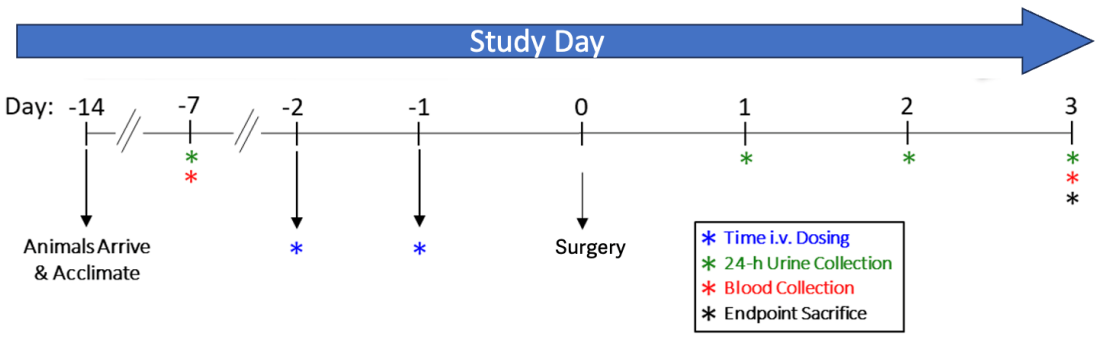
Figure 1. Selective TLR4 Stimulation with Gemini promotes healing.



Study Design and Timeline

- Rats (n=8-28 per group) were administered vehicle or Gemini 24 and/or 48 hours prior to IR surgery (30-minute ischemia) at different dose levels (0.07 and 0.35mg/kg).
- In addition to clinical observations, rats were evaluated for kidney function and functional biomarkers at 24 and 72 hours post surgery, and kidney damage at 72 hours (sacrifice) post surgery.

Figure 2. AKI model study timeline. Animals in treatment groups were dosed at 48 and/or 24 hours prior to IR.



Results

Administration of Gemini 24 hours prior to IR reduced kidney injury as demonstrated in the significantly reduced tissue injury, improved renal function, and attenuation of inflammatory markers. Specifically, as CRP is a general marker of inflammation, the reduction in urinary and serum CRP demonstrate an overall reduction of local and systemic inflammation.

The 0.07 mg/kg dose group also demonstrated non-statistically significant dose dependent reduction in Creatinine and BUN – data not shown .

Figure 3a. Acute Cortical Tubular Necrosis.

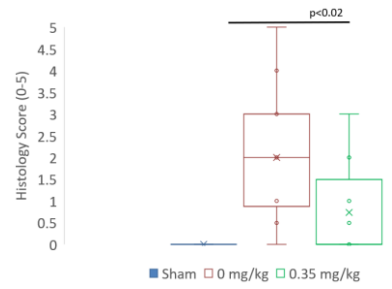


Figure 3b. Acute Medullary Tubular Necrosis.

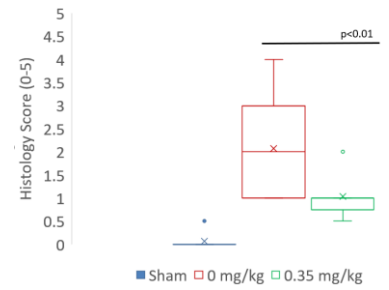


Figure 4a. Change in Serum Creatinine.

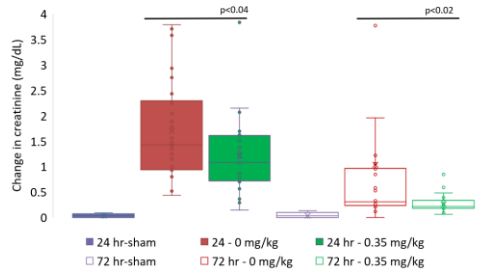


Figure 4b. Change in Serum BUN.

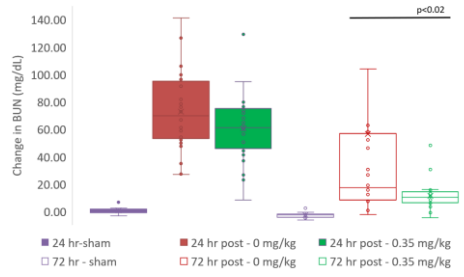


Figure 5a. Urinary CRP at 24 hours.

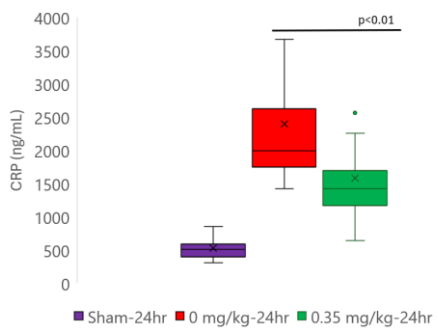


Figure 5b. Serum CRP at 72 hours.

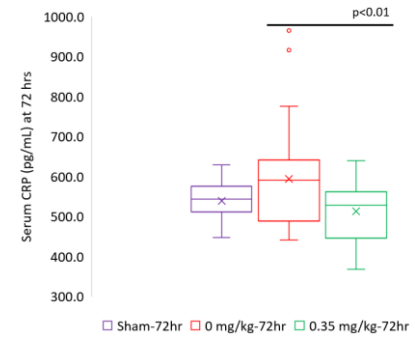


Figure 5c. Urinary IL-6 at 24 hours.

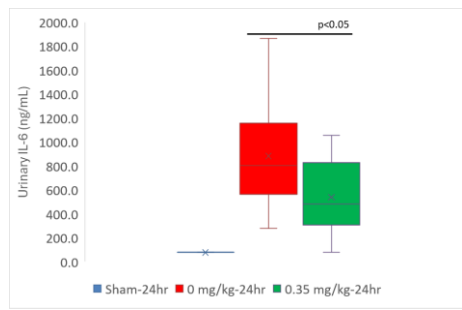
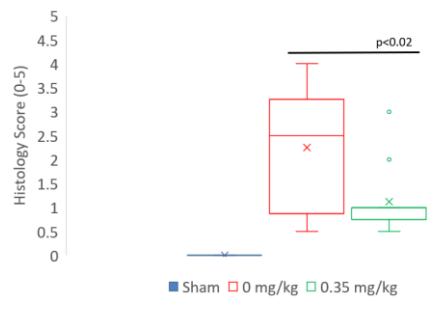


Figure 5d. Neutrophilic Inflammation in Kidney.



Discussion

- Preconditioning with Gemini reduces tissue damage and improves kidney function in a rat model of bilateral ischemic reperfusion induced acute kidney injury
  - Study limitations: rodent to human translation, sample timing and volume (limited), magnitude of effect of treatment corresponds to AKI severity

Conclusions

- Improved kidney function and reduced necrosis likely due to reduction in pro-inflammatory activities, as observed in reduced neutrophil inflammation and reduced local and systemic CRP and IL-6 levels
- Gemini may halt necroinflammation feedback loop by reducing proinflammatory signals that contribute to cellular necrosis, effectively improving organ health
  - Additional indications of improved kidney health include local and systemic changes in NGAL and HO-1 (results not reported here)
- Phase 1 clinical studies with Gemini initiated in February 2024

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