The store-operated calcium channel inhibitor Auxora improved renal function following ischemia induced acute kidney injury in rats

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Abstract

Background. Activation of immune and vasoactive pathways are thought to contribute to the development of acute kidney injury (AKI). Both endothelial cells and CD4+ lymphocytes express the store-operated calcium channel Orai1 and previous studies suggest that Orai1 activity contributes to activation of T-helper 17 cells in AKI of rats and humans. This study was conducted to evaluate the selective Orai1 store-operated calcium channel inhibitor Auxora (CM4620) on the development or recovery from AKI in a rat model of ischemia reperfusion injury.
Methods: *In study I*, male Sprague-Dawley rats (~250-300 g), instrumented with chronic indwelling jugular catheters were subjected to bilateral renal ischemia (40 minutes) and reperfusion (I/R) or sham-surgery. Treatment of rats with Auxora (16 mg/kg over 4 hours i.v.) or placebo was initiated within 30 min I/R and GFR was evaluated 24 h post I/R by transcutaneous clearance of FITC-sinistrin. *Study II* evaluated effects of Auxora on renal function following established loss of GFR, determined by FITC-sinistrin clearance between 2-4 h post I/R. Rats were then randomized and Auxora or placebo treatment initiated at 6 hours post I/R and repeated at 24 and 48 hours. GFR was then evaluated at 72 h post I/R.
Results: *In study I,* GFR was 0.81±0.11 ml/min/100g b.w in sham-controls and markedly reduced by 73% in placebo-treated post I/R rats 24 h post I/R (0.22 ± 0.04 ml/min/100g). The reduction in GFR was significantly attenuated in Auxora-treated rats (0.35 ± 0.04 ml/min/100g b.w.; p<0.05 vs. placebo). The number of Th17 cells (CD4+/IL17+) in kidney was attenuated by approximately 50% in Auxora-treated rats vs. placebo (p<0.05). In *study II*, there was ~50% reduction of GFR between 2-4 h post-I/R rats vs. baseline; there was no difference in GFR was present at time of randomization (0.43±0.02 vs 0.39±0.05 ml/min/100g in placebo vs. Auxora, respectively). GFR recovery was significantly greater in Auxora-treated animals vs. placebo controls (0.81±0.03 vs. 0.47±0.04 ml/min/100g, respectively; p<0.001) 72 h following I/R.
Conclusions: These data suggest Auxora has therapeutically beneficial effects in a rat model of AKI and can hasten recovery of renal function. The basis for improved function may relate to alterations in inflammation and/or improved vascular function formation of TLS, as well as blood pressure control and peripheral vascular responses.

Background

- In ICU patients, the risks of AKI is greater than 50% and is associated with significant mortality. AKI may result from hypoxia/ischemia which can lead to parenchymal tissue injury [1,2].
- Importantly, a significant proportion of patients in the ICU with severe AKI may also have lung injury and require ventilatory assistance[3].
- Studies from several investigators indicate the immune responses to renal ischemia contribute to the degree of AKI severity.[2]
- Our group and others have identified Th17 cells (CD4+/IL17+) as being strongly induced following AKI in rats. This response seems dependent on the activity of the store-operated Ca2+ channel, Orai1 (See Figure 1)[4].
- In addition, the levels of circulating IL17A in ICU patients with AKI was associated with increased mortality and major adverse kidney events.[5]
- In addition, renal ischemia can result in enhanced endothelial activation, which may exacerbate vasoconstriction and/or up-regulate adhesion molecules, contributing to inflammation. Several studies have shown that endothelial activation may also be affected by Store-Operated Ca2+ channel activity[6].
- There are no available therapies for treatment of AKI, which is due in part to the complicated pathophysiology of AKI. It is hypothesized that targeting Store-operated Ca2+ channels may affect the course of AKI by attenuating both vascular and immune cell contributions to AKI

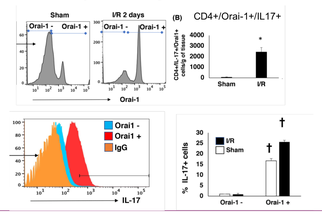


Figure 1. Previous studies have demonstrated the importance of activated adaptive immune cells in the development of AKI. Our group identified the expression of Orai1, a store-operated calcium channel on CD4+ cells which express the cytokine IL17A following experimental AKI in rats. **(from Mehrotra et al, J. Clin Invest 129:4951, 2019)**

Hypothesis (Study Goal)

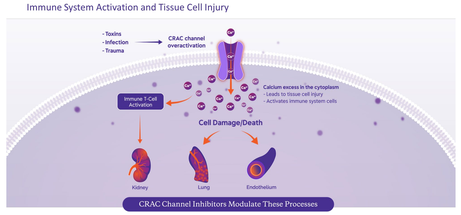


Figure 2. Auxora (zegrocractin) is a store-operated Ca^{2+} channel antagonist with high selectivity for Orai1 and the potential to influence multiple cell targets, including inflammatory cells and vascular cells. This study sought to test the hypothesis that Auxora could improve the recovery of renal function in a rat model of bilateral renal ischemia reperfusion injury.

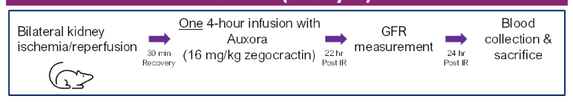
Methods

-Male Sprague Dawley rats were instrumented with chronic indwelling jugular catheters to deliver Auxora or Vehicle/placebo at the indicated times.

Study 1, rats were subjected 40 minutes of bilateral renal ischemia and randomly assigned treatment with a single (4 hour) treatment of Auxora or placebo 30 minutes following reperfusion (*study 1*). Renal function was evaluated by transcutaneous FITC sinistrin clearance.

In *study 2*, determined effect of Auxora following established AKI. GFR was determined between 2-4 hours post ischemia/ reperfusion prior to randomization. Initial treatment was at 6 hours and repeated at 24 and 48 hours and animals euthanized at 72 hours.

Results (Study 1)



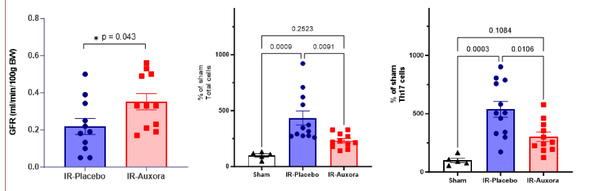


Figure 3. Effects of Auxora on the severity of AKI in rats 24 hours following I/R injury Panel A: GFR is shown from transcutaneous GFR measurements. B) Total infiltrating cells are shown; C) Total CD4+ IL17A+ cells are shown. Data are mean ± SEM; Significance values by Students t-test in A, and by ANOVA in B and C.

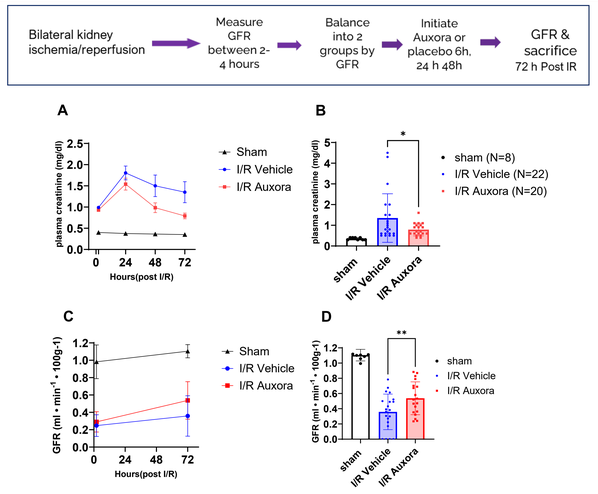


Figure 4. Auxora improves recovery following established AKI. Top Panel Experimental design in which randomization based on GFR at 2-4 hours Post I/R. The plasma creatinine time course (A) and tGFR time course (B) are shown. Final plasma creatinine at 72 hours post I/R (B) and tGFR at 72 hours post I/R is shown, with individual values. Similar tGFR and creatinine values 2-4 hours post I/R indicating good randomization. 4 Vehicle-treated animals died before 72 hours, for animals that died, GFR was set to 0. No Auxora treated rats died. Data are mean ± SE; * indicates P<0.05 and ** indicates P<0.01 on ANOVA and Tukey's post hoc test.

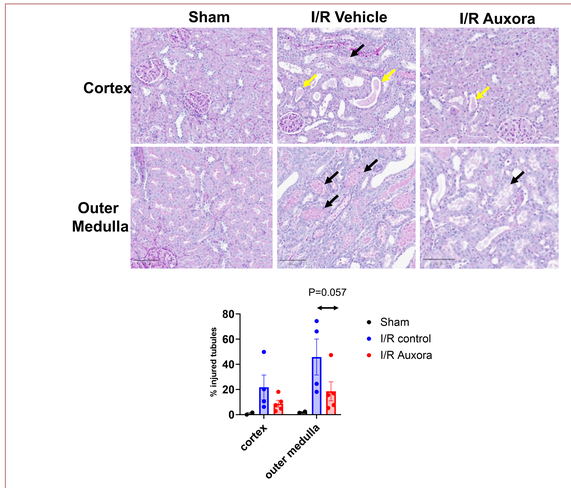


Figure 6. Auxora improves renal structure following severe AKI. Shown are cross sections through renal outer medulla from sham, I/R vehicle and I/R Auxora treated rats (as indicated), 3 days following recovery from surgery. Rat tissues from rats analyzed comprised the most severely initial reductions in GFR prior to randomization (>67% GFR loss). Note prominent necrosis and tubular congestion in I/R vehicle (Black Arrow) and the presence of proteinaceous casts (yellow arrow). The Auxora-treated group illustrated less prominent necrosis and proteinaceous casting. Bar = 100 µm. **Bottom:** Preliminary quantification, illustrating % injured tubules scored by a blinded reviewer (representative of ~ half of the available samples)

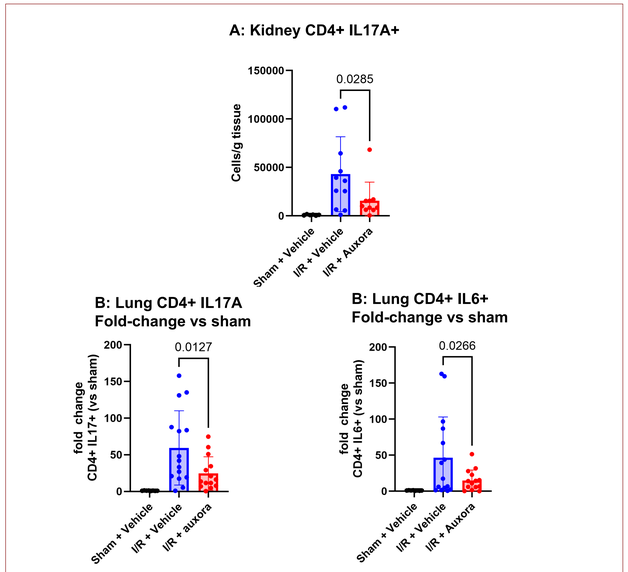


Figure 6. Auxora improves inflammatory lymphocytes in kidney and lung; A) Total number of Th17 (CD4+/IL17A+) cells are shown; B and C Lung CD4+ IL17A + and CD4+ IL6+ cells are shown. For B and C, data were expressed relative to the sham for each experimental run to adjust for variability in total cell recovery in each experiment. Data are mean ± SD. P value shown based on Student's t-test

Conclusion

- Auxora, a store-operated Ca^{2+} channel antagonist with high specificity to Orai1 attenuates the loss of GFR observed 24 hours following AKI and reduces Th17 cell content.
- A reduction of GFR greater than 50% is observed in nearly all rats following 40 min of ischemia reperfusion within 2-4 hours. Approximately 50% of animals manifested a loss of GFR greater than 67%.
- Treatment with Auxora initiated at 6 hours and repeated for 3 days improved GFR and plasma creatinine vs. vehicle treated animals and this was associated with reduced kidney damage.
- Flow cytometry indicated that Auxora attenuated the development of renal inflammation including Th17 cells and reduced the number of Th17 and IL6 expressing cells in lung.

Citations

1) Hoste et al., Intensive Care Med, 41:1411, 2015
2) Basile et al., Comprehensive Physiol 2: 1303, 2012
3) Faubel and Edelstein, Nat Rev Nephrol, 12:48, 2016
4) Mehrotra et al, J. Clin Invest 129: 4951, 2019
5) Collet et al., Critical Care 26:107, 2022
6) Yu et al., BBRC 495, 1864, 2018

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