

## Background

Post-ischemic acute renal failure (ARF) is common and often fatal. Severe blood loss during major operations, sepsis, cardiothoracic surgeries and radiocontrast media application are common causes for ARF. Underlying cellular mechanisms include cell adhesion, cell infiltration with generation of oxygen free radicals, and inflammatory cytokine production. To mimic acute renal failure IR-injury can be induced in a mice model by bilateral renal pedicle clamping for 35 min. This causes ARF with severe loss of renal function and steep rise in creatinine and blood urea nitrogen (BUN) within 24 h post-ischemia. Several experimental ameliorative strategies have been tested. However, there is still a remarkable lack of definitive evidence supporting specific therapies in any setting.

In this study we tested a novel small molecule EA-230, for its ability to improve survival and attenuate loss of kidney function in a clinically relevant model of ARF.

## Study Design

IR injury was induced in male C57Bl/6 mice by clamping both renal pedicles for 35 min. Treatment with saline or different doses of EA-230 (20, 30, 40, 50 mg/kg) twice daily for four consecutive days was initiated 24h post-operatively when acute renal failure was already evident.

- Study groups:**
1. saline
  2. EA-230 20 mg/kg BW
  3. EA-230 30 mg/kg BW
  2. EA-230 40 mg/kg BW
  2. EA-230 20 mg/kg BW

ischemia 35 min



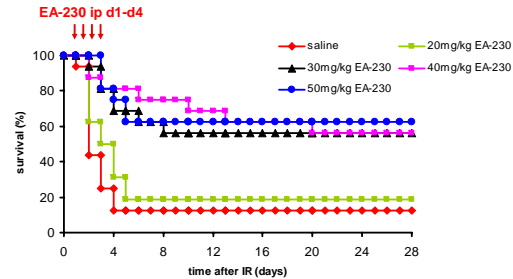
analysis of:

- survival
- renal function
- inflammation
- proliferation

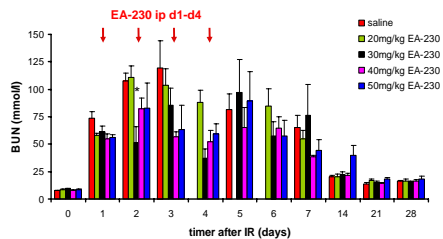
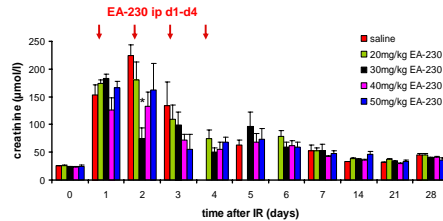
## Survival & Renal Function

### Survival after IR injury:

EA-230 caused dose dependent increase in survival if administered 24h after IR injury. Best results were obtained with doses above 30mg/kg.



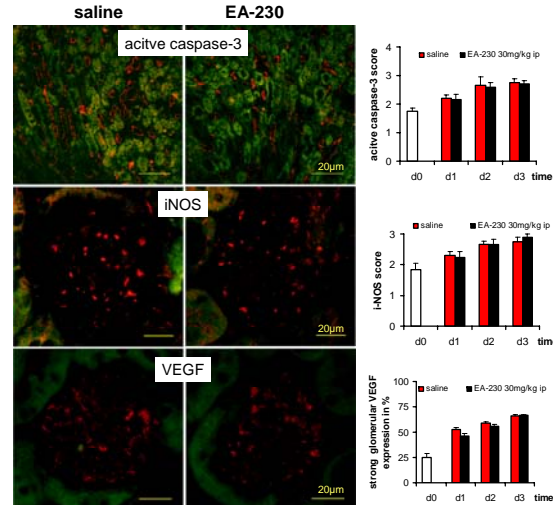
### Renal function after IR injury:



## Apoptosis & Inflammation

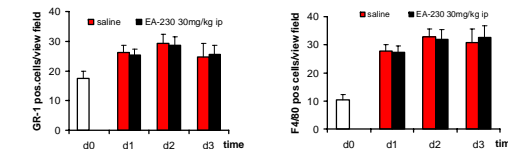
### Active caspase-3, i-NOS, VEGF expression:

No quantitative differences between groups in i-NOS, VEGF and active caspase 3 expression were observed at d1, d2, d3.



### Leukocyte infiltration:

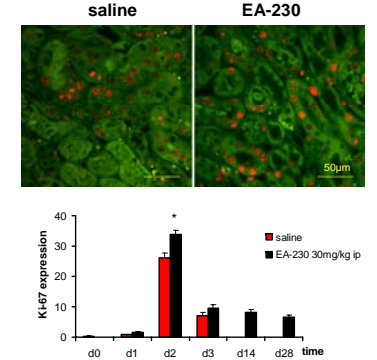
No quantitative differences between groups in infiltration of macrophages (F4/80), granulocytes (GR-1) or T-lymphocytes (CD4, CD8) were observed at d1, d2, d3.



## Proliferation

### Proliferation of tubular epithelial cells (Ki-67 expression):

EA-230 induced significant up-regulation of tubular epithelial cell proliferation at d2 post-ischemia (\*p<0.05 at d2 versus saline).



## Summary & Discussion

EA-230 treatment was initiated 24h after IR injury when ARF (i.e., a substantial loss of renal function) was already evident. Doses of 30, 40 and 50 mg/kg EA-230 were most effective in reducing mortality and in attenuating renal function. EA-230 induced a significant increase in Ki-67 positive tubular epithelial cells two days after the IR injury in EA-230 treated animals. This finding suggests that EA-230 improves regeneration of renal tissue after IR injury

## Conclusion

EA-230 is a novel and promising therapeutic agent for treating acute renal failure. Its beneficial effect is associated with an increase in tubular epithelial cell proliferation.