THE EXPRESSION OF NEURONAL NOS IN AN ACUTE RENAL ISCHEMIA / REPERFUSION INJURY EXPERIMENTAL MODEL: THE EFFECT OF ERYTHROPOIETIN

K. Xanthopoulou[1], I. Tsouchnikas[1], G. Ouzounidis[1], G. Kokaraki[2], R. Lagoudaki[2], C. Simeonidou[2], G. Karkavelas[3], E. Spandou[2], D. Tsakiris[1].

[1]Department of Nephrology, General Hospital of Veria, [2]Department of Physiology and [3]Department of Pathology, Aristotle University of Thessaloniki, Greece.

INTRODUCTION AND AIM

Neuronal NOS (nNOS) is involved in normal renal function regulation, although the individual mechanisms are not fully clarified. nNOS expression is affected under ischemic conditions but its role in acute renal ischemia/reperfusion (I/R) injury remains unknown. Furthermore, it has not been studied whether nNOS is implicated in erythropoietin (EPO) renoprotective action. The aim of this study was to investigate the effect of EPO on the expression of nNOS in an experimental model of acute renal I/R at different time points of reperfusion.

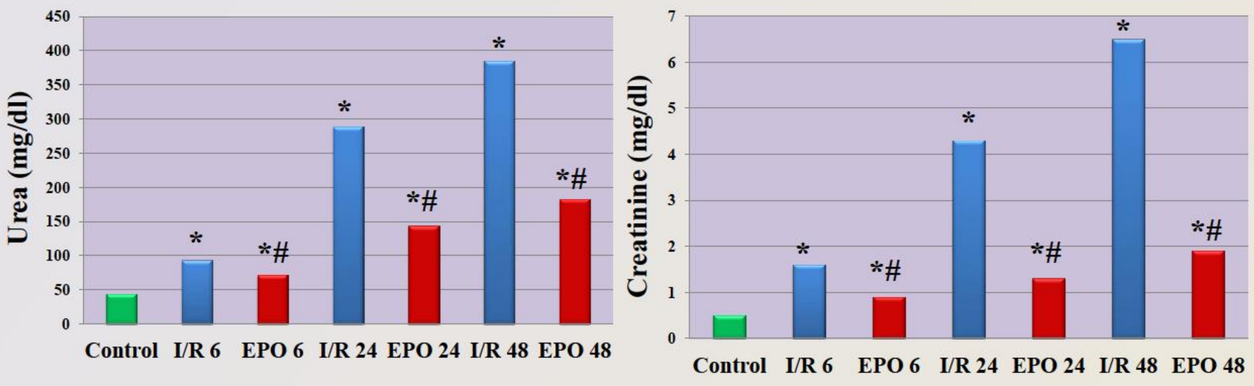
METHODS

Male Wistar rats, randomly divided into two groups: I/R group (n=12) and EPO group (500 U/Kg, i.p, 20 min prior to ischemia, n=15), were subjected to bilateral renal ischemia for 45min. Each group was allocated in three subgroups according to the timing the animals were sacrificed at 6, 24 and 48 hrs of reperfusion. Rats subjected to identical surgical procedure without occlusion of renal pedicles were used as sham-operated group (n=6). Renal injury was assessed by measurement of serum biochemical markers (urea and creatinine) and histological grading. Expression of nNOS was evaluated by RT-PCR and immunohistochemistry.

RESULTS

EPO group had significantly lower serum biochemical markers compared to I/R group at 6, 24 and 48 hrs of reperfusion ($p<0.05$), (Figure 1). Histological evaluation revealed significantly less tubular damage in the EPO compared to I/R group ($p<0.001$), (Figure 2).

Acute renal I/R decreased nNOS mRNA at all time points of reperfusion compared to sham-operated group ($p<0.01$), (Figure 3). A slight increase was observed in the later stages without restoring to normal levels even at 48hrs. EPO pretreatment delayed nNOS mRNA reduction as the onset of the reduction was detected at 24hrs ($p<0.01$). Comparison of nNOS mRNA expression between groups revealed significantly higher mRNA expression in the EPO group at 6hrs ($p=0.02$), (Figure 3). nNOS protein expression was observed in normal kidneys, mainly in macula densa regions and diffusely in renal tubules (Figure 4). Following I/R an obvious decrease in nNOS staining was detected in the early stages of reperfusion, which seemed to be gradually restored at 48hrs. EPO administration resulted in higher nNOS protein expression at 6 and 24hrs compared to I/R group. Immunoreactivity was similar in both groups at 48hrs of reperfusion regardless of the administration of EPO (Figure 5).



*Control vs I/R and EPO, $p<0.05$, # I/R vs EPO, $p<0.05$ at all time points of reperfusion.

Figure 1. Serum levels of urea and creatinine at 6, 24 and 48h in I/R and EPO groups.

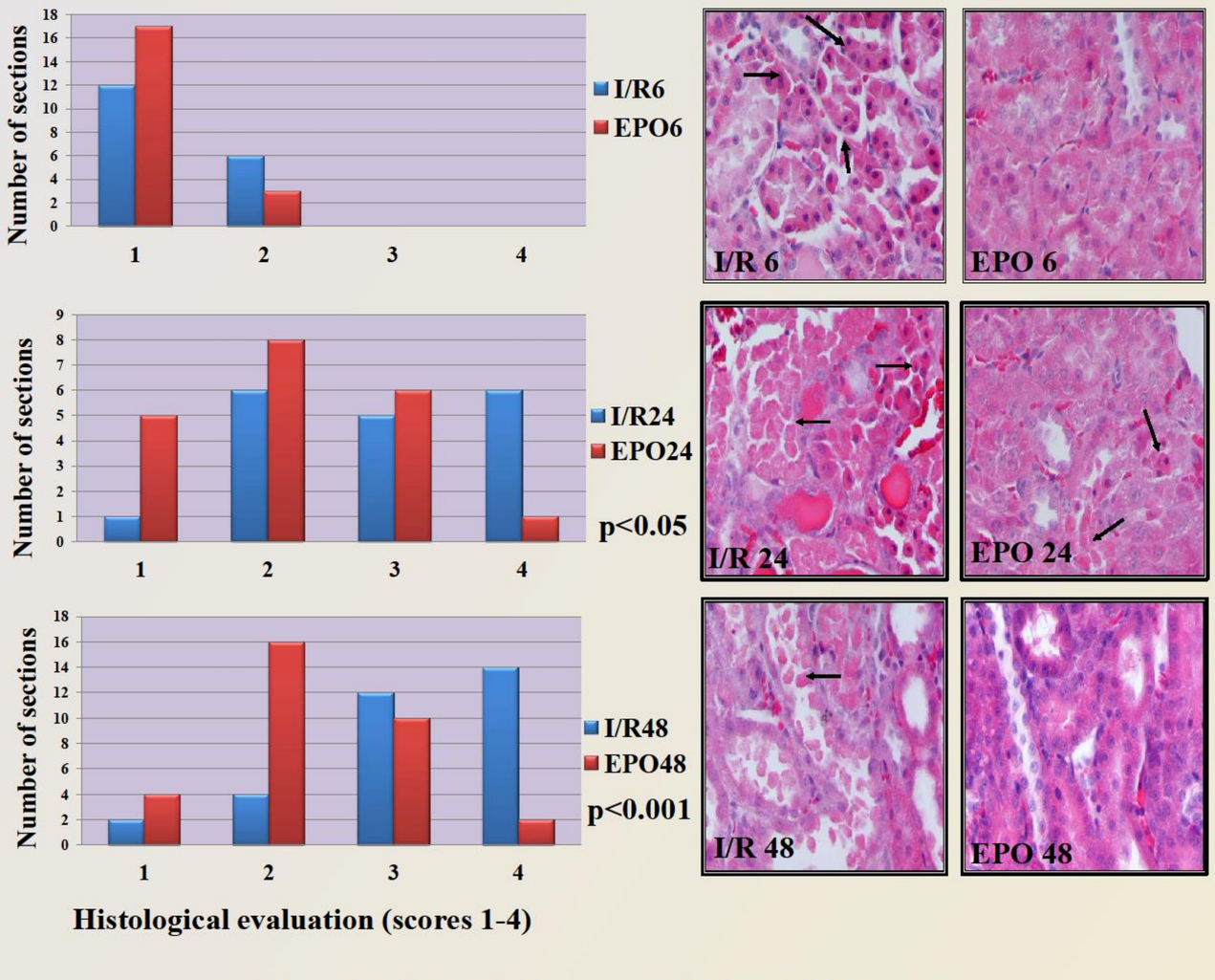
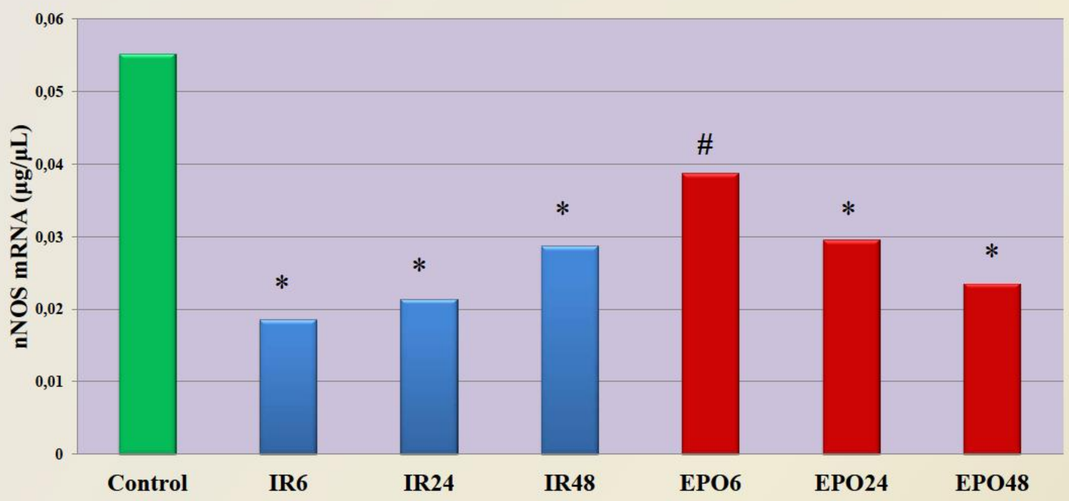


Figure 2. Histological scoring in I/R and EPO groups.



*Control vs I/R EPO 24 and EPO 48, $p<0.01$, # I/R6 vs EPO 6, $p=0.02$.

Figure 3. The expression of nNOS mRNA in I/R and EPO groups.

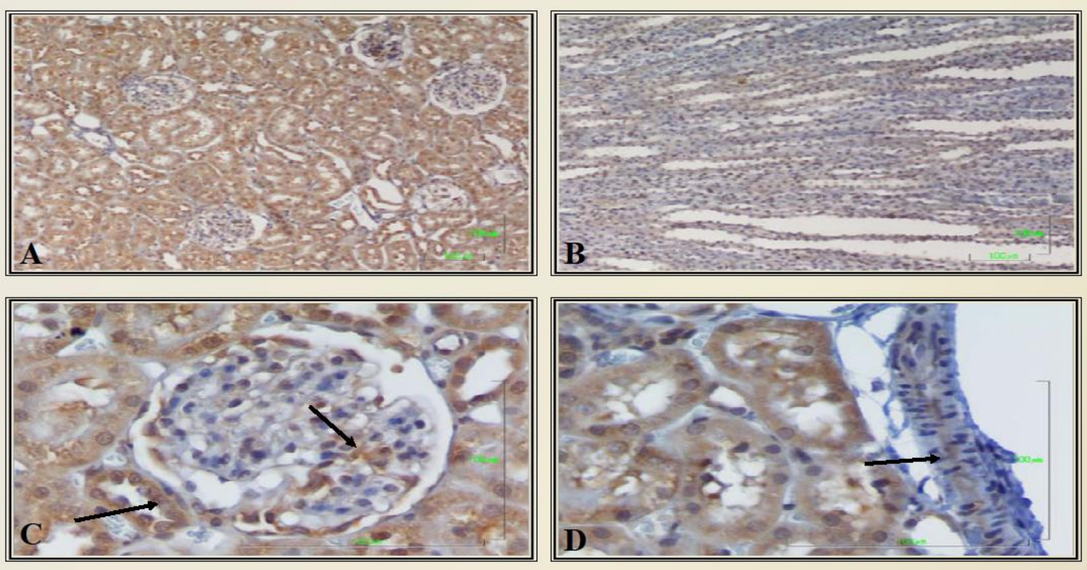


Figure 4. The expression of nNOS in normal kidneys (A cortex, B medulla, C macula densa and corpuscle, D vessel).

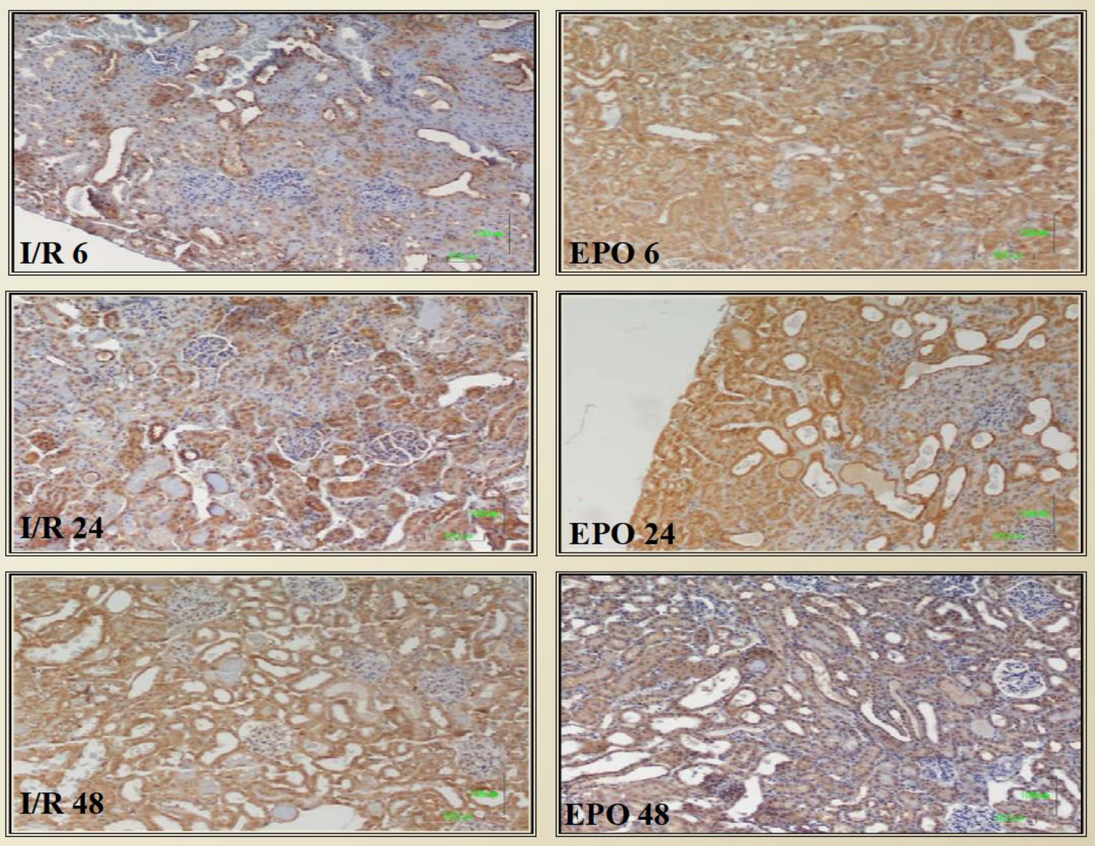


Figure 5. The expression of nNOS in renal I/R and EPO groups.

CONCLUSIONS

- ✓ EPO pretreatment reduced I/R-induced tubular injury and ameliorated renal function
- ✓ nNOS is expressed in normal kidneys
- ✓ Severely damaged renal regions exhibited a weak expression of nNOS indicating that nNOS reduction could be involved in renal injury
- ✓ A gradual increase in nNOS expression in the later stages of reperfusion might be correlated with the recovery of renal function
- ✓ As EPO pretreatment resulted in higher levels of nNOS expression compared to I/R group in the early stages of reperfusion, it could be suggested that restoration of nNOS levels is a possible contributory mechanism of EPO renoprotective effect in acute renal I/R injury

51ERA-EDTA
72--SP
G) Acute Renal Failure. G1) Experimental models.
Kyriaki Xanthopoulou
DOI: 10.3252/pso.eu.51era.2014
Poster presented at: 51 ERA-EDTA CONGRESS AMSTERDAM 2014
PosterSessionOnline.com