SUPPLEMENTAL DIGITAL CONTENT (SDC)

Supplemental Materials and Methods

SOD-1 and SOD-2 levels in kidney tissue were assessed by Western blot using anti-SOD-1 antibody (ab16831, 1/2000 dilution) and anti-SOD-2 antibody (ab11889, 1/2000 dilution), both from Abcam (Cambridge, UK). Nuclear factor \( \kappa B \) (NF-\( \kappa B \)) activation was assessed by Western blot analysis of the amount of NF-\( \kappa B \) p65 subunit phosphorylation in the Ser536 residue (p-p65) and the free I\( \kappa B \)-\( \alpha \) levels in kidney extracts, as previously described (1). Antibodies used were anti-NF-\( \kappa B \) p65 (1:1000, Cell Signaling Technology), anti-p-p65 NF-\( \kappa B \) (1:1000, Cell Signaling Technology) and anti I\( \kappa B \)-\( \alpha \) (Cell Signaling Technology). Renal ICAM-1, VCAM-1, PECAM-1, P-selectin and iNOS abundance in renal tissue was assessed by Western blot. Antibodies used were: anti-ICAM-1, (1:500); anti-VCAM-1, (1:1000); anti-PECAM-1, (1:500); anti-P-selectin, (1:200) from Santa Cruz Biotechnology, CA, USA and anti-iNOS/NOS-2, (1:1000) from Cell Signaling Technology as previously reported (2). Caspase-3 activation was assessed by western blot analysis of cleaved caspase-3 expression (anti-cleaved caspase-3, (1:500, Cell Signaling Technology) as detailed elsewhere (2). Then, membranes were incubated with secondary HRP-conjugated HRP conjugated anti-goat IgG or anti-rabbit IgG (depending of the primary antibody) for 1 hour (dilution 1:10000; Bio-Rad Laboratories). After washing, the immune complexes were detected with chemiluminiscent (ECL) HRP substrate using an image reader (ImageQuant RT ECL, GE Healthcare Europe GmbH, Freiburg, Germany) and the intensity of the bands were measured using the reader software (ImageQuant TL®). Membranes were also reproved with anti-rat tubulin antibody (1:20000; Sigma-Aldrich) to verify equal loading of protein in each lane.

Immunohistochemistry was performed on buffered formalin fixed, paraffin-embedded tissues as previously described (2, 3). Antibodies used were: anti-iNOS (Cell Signaling Technology), and anti-cleaved caspase-3 (Cell Signaling Technology). To evaluate the degree of tubular necrosis, tissue sections stained with hematoxylin-eosin were scored in a blinded fashion, using a previously described semiquantitative scale (4). Each parameter was assessed in five high-power fields (\( \times 40 \)) in the inner
cortex and outer medullary regions (where the tubular damage was most evident), and an average was determined for each section. The parameters included tubule dilatation, tubule cast formation, and tubule cell necrosis. Each parameter was scored on a scale of 0 to 4, ranging from none (value: 0), mild (value: 1), moderate (value: 2), severe (value: 3), to very severe/extensive (value: 4).

References


Figure S1. Representative hematoxilin-eosin staining in cortex and outer medulla of kidneys from rats after 24 (a,b) or 48 hours (c,d) after I/R, treated (b,d) or not (a,c) with cardiotrophin-1. Arrows signal obstructed tubuli. Closed arrows heads mark vacuolized tubular cells. Open arrow heads mark infiltration cells. Asterisks mark denudated tubuli.
**Figure S2.** Representative western blot (a), western blot quantification (b) and immunohistochemistry (c) for iNOS in kidneys from rats subjected to renal ischemia/reperfusion (I/R) treated (CT-1 group) or not (Control group) with cardiotrophin-1 (CT-1), and in sham-operated rats (Sham group). Data are mean ± S.E.M. of 10 rats per group. *: p < 0.05 vs sham group; #: p < 0.05 vs control group.
Figure S3.- Representative western blot (a), western blot quantification (b) and immunohistochemistry (c) for cleaved caspase 3 in kidneys from rats subjected to renal ischemia/reperfusion (I/R) treated (CT-1 group) or not (Control group) with cardiotrophin-1 (CT-1), and in sham-operated rats (Sham group). Data are mean ± S.E.M. of 10 rats per group. *: p < 0.05 vs sham group; #: p < 0.05 vs control group.