HISTOLOGICAL KIDNEY CHANGES ASSOCIATED WITH LOW AND HIGH RECOMBINANT HUMAN ERYTHROPOIETIN DOSES IN A RAT MODEL OF CHRONIC KIDNEY DISEASE

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Background and Aims

Recombinant human erythropoietin (rhEPO) is intensively used to treat anemia of chronic renal failure (CRF) patients under hemodialysis; some patients develop resistance to rhEPO, requiring high rhEPO doses to achieved target hemoglobin levels. However, there is some controversial about the benefic/deleterious effect of rhEPO therapy on kidney tissue.

Our aim was to evaluate renal tissue damage associated to therapy with rhEPO in a rat model of CRF induced by 5/6 nephrectomy.

Groups and Methods

Groups:

Four groups (n=7 each) of male Wistar rats (280 g), were studied during 12 weeks: Sham (without nephrectomy and without rhEPO treatment); CRF (5/6 nephrectomy); CRF+rhEPO(50) and CRF+rhEPO(200): CRF treated with 50 and 200 IU/kg/week (s.c.) of beta-EPO (Recormon®).

Assays:

At weeks 0, 3, 6, 9 and 12, hematological data and renal function was assessed. Body weight, blood pressure and trophy indexes were measured. Anti-EPO antibodies were measured by ELISA. Kidney lesions were analyzed by hematoxylin and eosin, and periodic acid of schiff staining's. Protein expression of NF-kB and CTGF was performed by immunohistochemistry. Histological lesions and immunorectivity was scored.

Statistics:

Results are presented as means ± standard error of means (SEM). Comparisons between groups were performed using ANOVA and the Post hoc Tukey test.

Results

Renal function and haematological profile

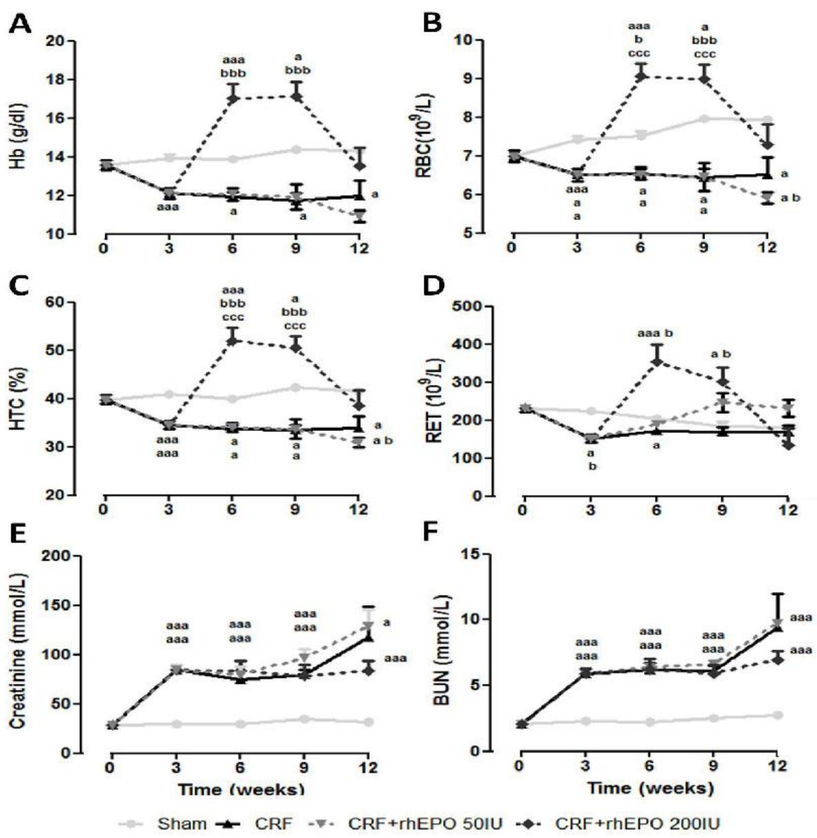

Figure 1.
Hematological renal data throughout the follow-up period of 12 weeks. Hemoglobin (A), red blood cells (B), hematocrit (C), reticulocytes (D), creatinine (E) and BUN (F). Results are means ± SEM (7 rats per group): a- $p < 0.05$, aa- $p < 0.01$, and aaa- $p < 0.001$ vs Sham; b- $p < 0.05$, bb- $p < 0.01$, and bbb- $p < 0.001$ vs CRF; c- $p < 0.05$, cc- $p < 0.01$, and ccc- $p < 0.001$ vs CRF+rhEPO50. BUN, blood urea nitrogen.

Immunostaining analysis

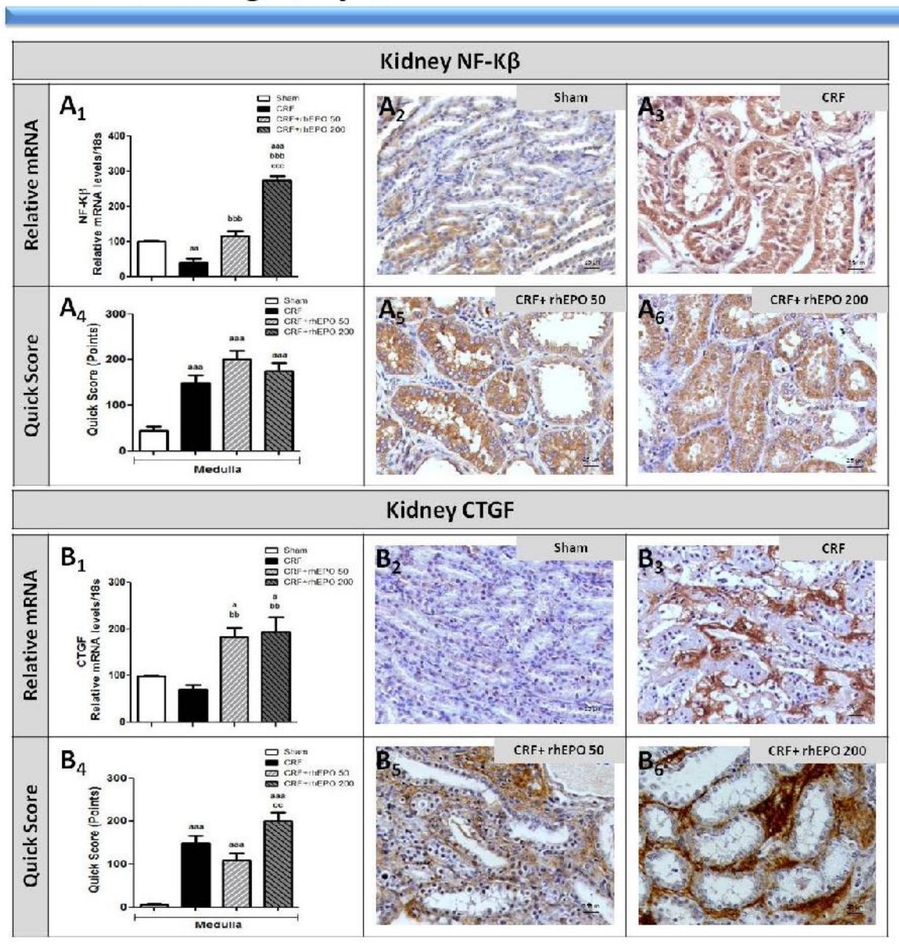

Figure 2.
RHuEPO effects on relative gene expression mRNA levels/18s and immunohistochemical expression of NF-kB (A1-A6) and CTGF (B1-B6) in renal medulla (original magnification, x400). Results are means ± SEM (7 rats per group): a- $p < 0.05$, aa- $p < 0.01$, and aaa- $p < 0.001$ vs Sham; b- $p < 0.05$, bb- $p < 0.01$, and bbb- $p < 0.001$ vs CRF; c- $p < 0.05$, cc- $p < 0.01$, and ccc- $p < 0.001$ vs CRF+rhEPO50.

Histological kidney changes

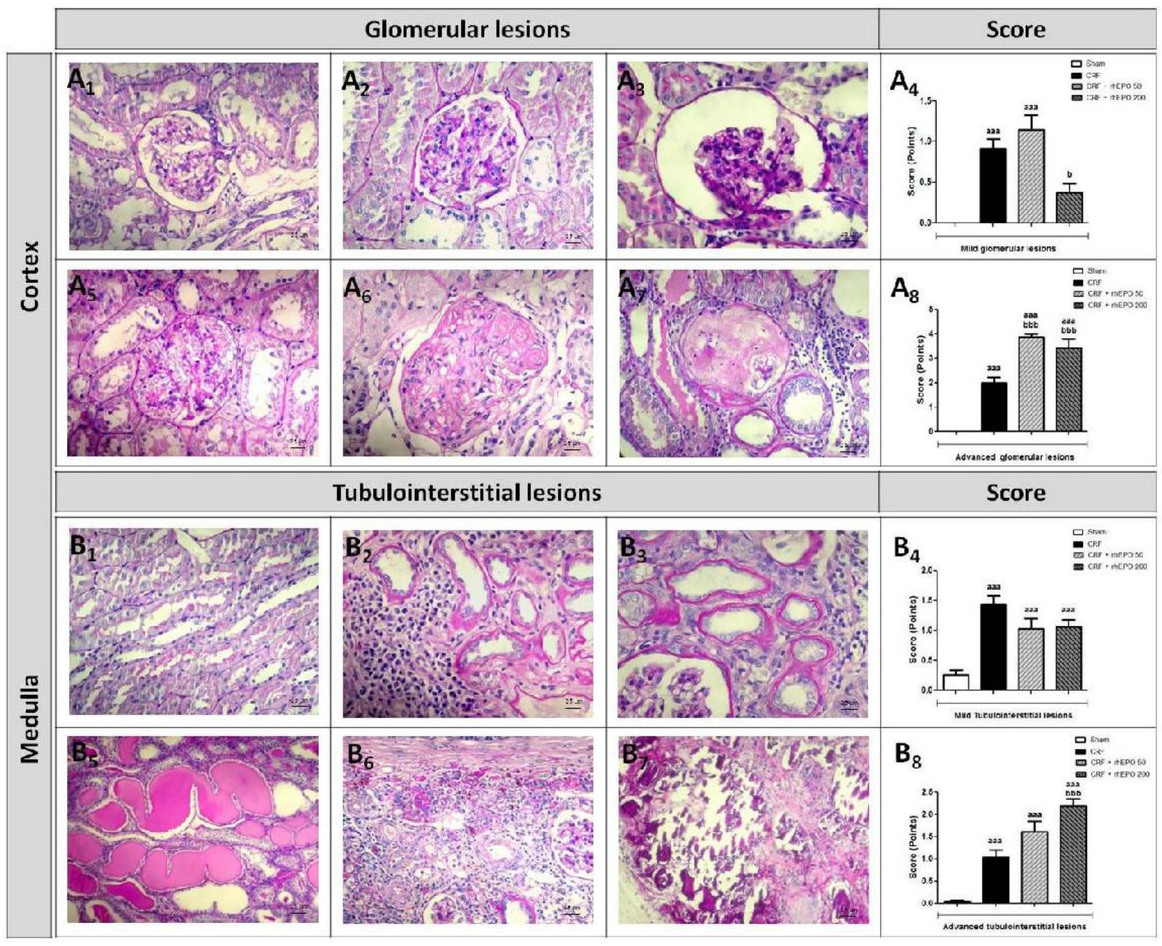

Figure 3.
Representative glomerular (cortex) and tubulointerstitial (medulla) lesions observed in kidneys of rat groups in study, at the final time (PAS staining, original magnification x400): A_1 – normal glomerulus histology in the Sham rats; A_2 – hypercellularity glomerular; A_3 - dilatation of the Bowman's Space and glomerular atrophy; A_4 – Total score of mild glomerular lesions in each rat groups; A_5 - Mesangial expansion; A_6- nodular sclerosis; A_7- Global Glomerulosclerosis; A_8- Total score of advanced glomerular lesions in each rat groups; B_1- normal tubulointerstitial histology in Sham group of rats; B_2- interstitial inflammatory infiltration ; B_3 – Tubular basements membrane irregularity; B_4- Total score of mild tubulointerstitial lesions in lesions in each rat groups. B_5-hyaline cylinders; B_6- IFTA; B_7- tubular calcification; B_8- Total score of advanced tubulointerstitial lesions in each rat groups.

Discussion and Conclusions

Therapy with a high rhEPO dose in CRF rats corrected anemia until the 9th week, after which there was a decline in hematological data (HCT, Hb, RBC and Ret counts). The deleterious effect found on the kidney histology might be due to the presence of anti-EPO antibodies; in addition, rhEPO therapy was associated with increased kidney expression of NF-kB and CTGF, which are markers of inflammation and fibrosis, respectively, and might be induced by anemia-associated hypoxic conditions that should be further elucidated.

Acknowledgement/Financial Support: Roche Pharmaceuticals for providing the rhEPO. FCT Grants: PTDC/SAU-TOX/114253/2009, SFRH/BD/61020/2009 PEst-C/SAU/UI3282/2011 and COMPETE.