Iron metabolism in resistance to rhEPO due to the development of anti-EPO antibodies in a rat model of chronic renal failure

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Background and Aims

Recombinant human erythropoietin (rhEPO) is extensively used to treat anemia of chronic kidney disease (CKD) patients under hemodialysis. Some patients develop resistance to rhEPO, requiring high rhEPO doses to achieve target hemoglobin levels and others may even develop pure red cell aplasia due to the development of anti-rhEPO antibodies. Usually, these patients present an anemia associated with alterations in iron metabolism, which are enhanced in patients who develop resistance to rhEPO therapy.

This study intended to evaluate iron metabolism, at biochemical and molecular levels, in a model of chronic renal failure (CRF) and of resistance to rhEPO therapy.

Groups and Assays

Groups:

Three groups (n=7) of male Wistar rats (280 g), were studied during 12 weeks: Sham – rats subjected to surgery without kidney reduction; CRF – rats subjected to partial (5/6) nephrectomy and CRF + rhEPO 200 – CRF rats treated with 200 IU/kg/week (s.c.) of beta-EPO (*Recormon*®).

Assays:

At weeks 0, 3, 6, 9 and 12, hematological and biochemical data was assessed in blood. At the 12th week, blood and tissues were collected to monitor hematological and biochemical data, including IL-6 and serum iron metabolism markers (iron, transferrin and ferritin). Liver expression of EPO, EPO receptor (EPOR) and iron related genes (by RT-qPCR) was evaluated.

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 data and hematological profile

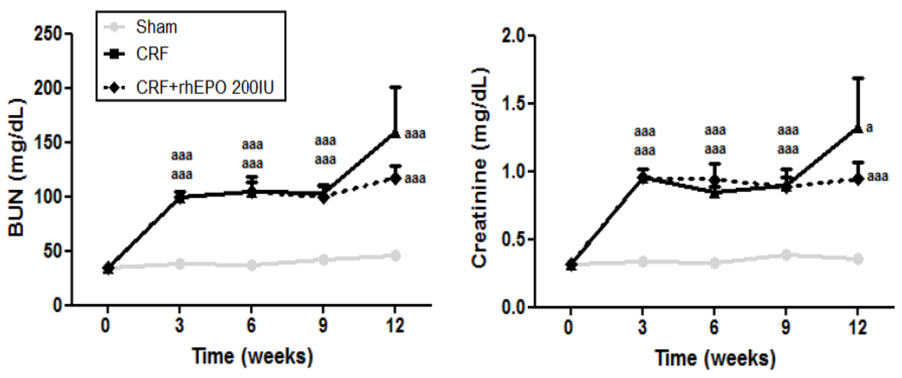

Figure 1.
Evolution of serum renal function data (blood urea nitrogen, BUN, and creatinine) during the 12 weeks follow-up period. Results are means ±SEM (7 rats per group): a- p < 0.05, aa- p < 0.01, and aaa- p < 0.001 vs the Sham group.

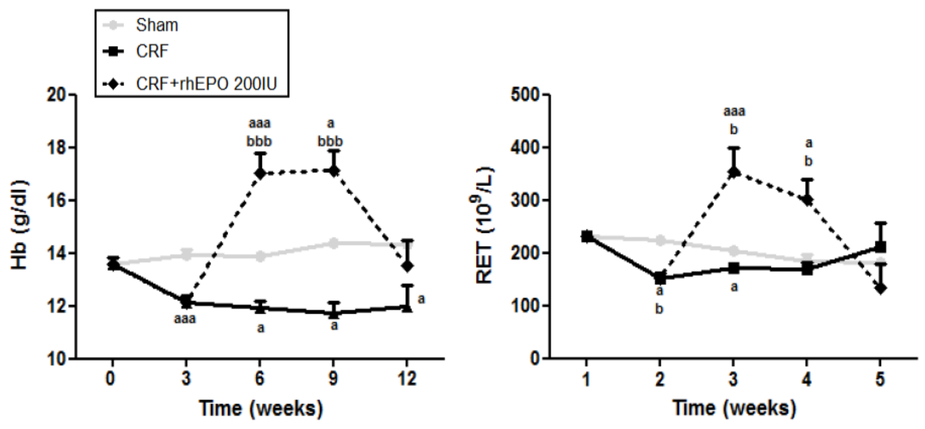

Figure 2.
Evolution of hematological data (hemoglobin and reticulocyte count) during the 12 weeks follow-up period. Results are means ±SEM (7 rats per group): a- p < 0.05, aa- p < 0.01, and aaa- p < 0.001 vs the Sham group; b- p < 0.05, bb- p < 0.01, and bbb- p < 0.001 vs the CRF group.

Serum EPO levels and EPO and EPOR mRNA expression in the liver tissue

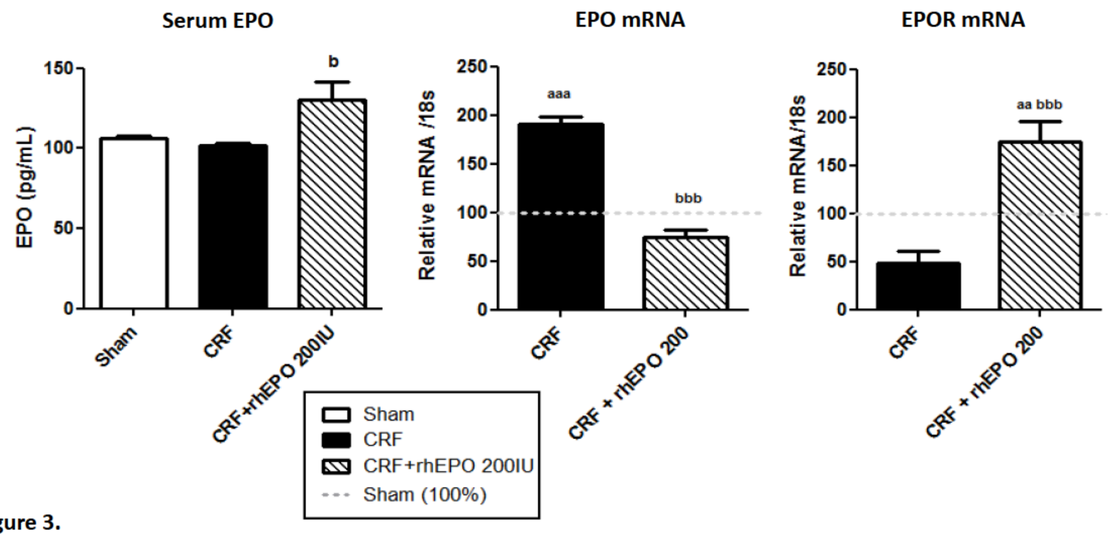

Figure 3.
Serum EPO levels and EPO and EPOR mRNA/18s expression in the liver tissue at the end of the study (12 weeks). Results are means ±SEM (7 rats per group): a- p < 0.05, aa- p < 0.01, and aaa- p < 0.001 vs the Sham group; b- p < 0.05, bb- p < 0.01, and bbb- p < 0.001 vs the CRF group.

Iron homeostasis data (serum and mRNA expression in the liver tissue)

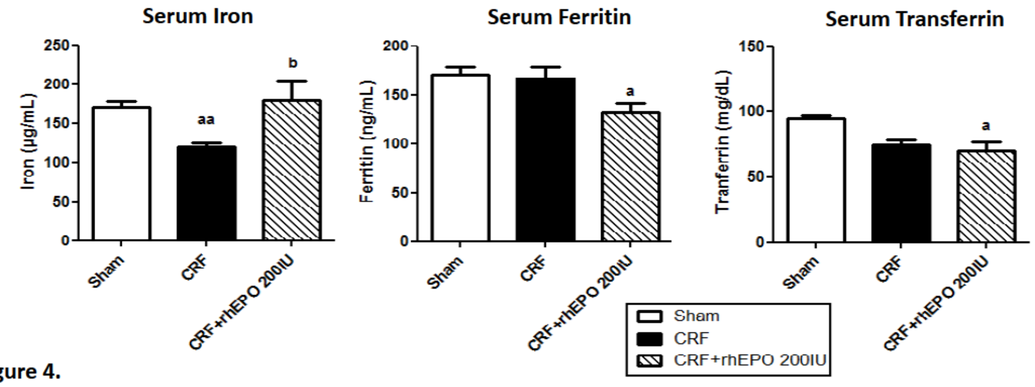

Figure 4.
Serum iron, ferritin and transferrin levels at final time (12 weeks). Results are means ±SEM (7 rats per group): a- p < 0.05, aa- p < 0.01, and aaa- p < 0.001 vs Sham; b- p < 0.05, bb- p < 0.01, and bbb- p < 0.001 vs CRF.

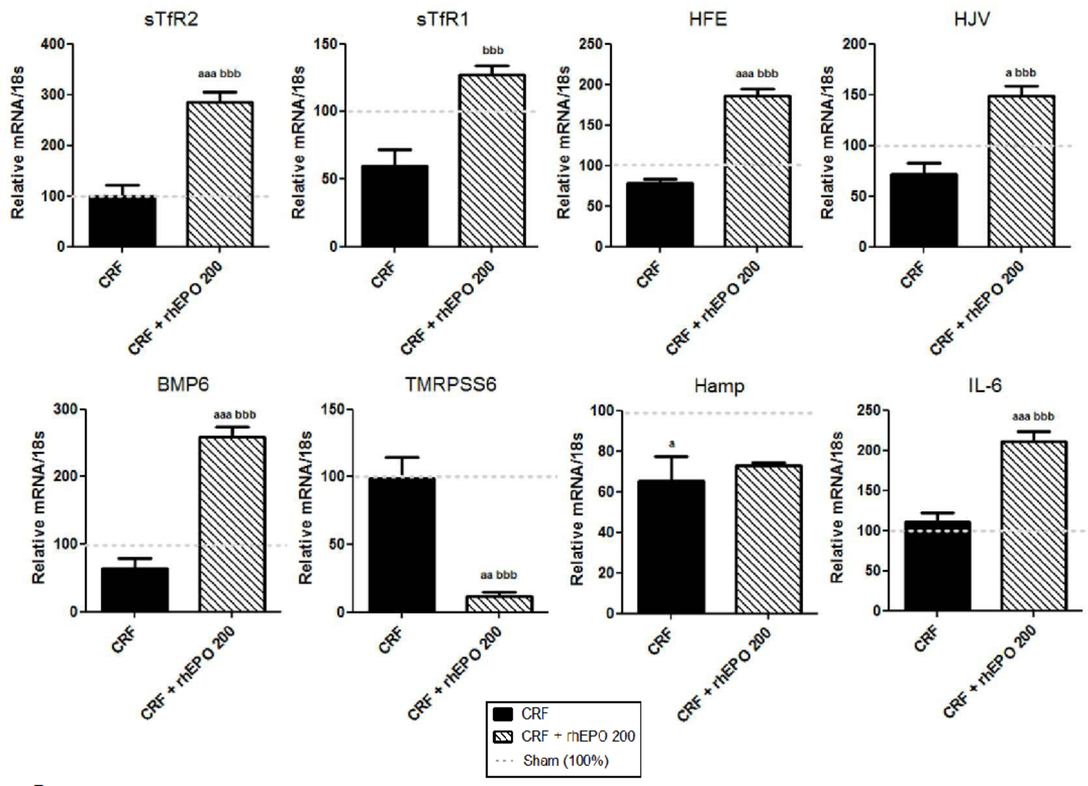

Figure 5.
Liver relative mRNA/18s expression (% of sham) at final time (12 weeks). Results are means ± SEM (7 rats per group): a- p < 0.05, aa- p < 0.01, and aaa- p < 0.001 vs Sham; b- p < 0.05, bb- p < 0.01, and bbb- p < 0.001 vs CRF. Hamp: hepcidin; sTFR: soluble transferrin receptor; BMP6: bone morphogenetic protein 6; HFE: hemachromatosis; HJV: hemojuvelin; TMPRSS6: matripsase-2; SLC40A1: ferroportin; IL-6: interleukin-6.

Conclusions

The formation of anti-rhEPO antibodies between the 9 and 12 week (and pure red cell aplasia) might influence liver mRNA expression of iron-related mediators, thus affecting iron absorption and mobilization to improve serum iron levels, but without proper RBC maturation. The liver expression of Hamp (hepcidin) would result from the balance between stimulatory and inhibitory regulations.

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