TRANSCRIPTOR FACTORS RESPONSIBLE FOR EPO GENE REGULATION, TRIGGERED BY ADMINISTRATION OF rHuEPO HIGH DOSES

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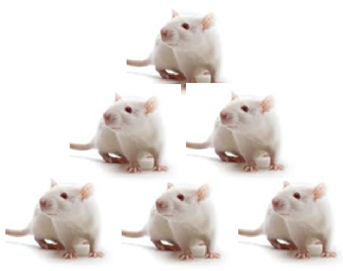
INTRODUCTION

Recombinant human erythropoietin (rHuEPO) therapy in hemodialysis patients corrects anemia. There is a marked variability in rHuEPO sensitivity (up to10-fold variability in dose requirements to correct anemia) and about 5-10% of patients show a marked resistance to rHuEPO therapy. The mechanism underlying the development of rHuEPO resistance remains poorly clarified. Since EPO and EPO receptors are expressed in non-renal tissues, the therapeutic induction of EPO expression pathways in those tissues could be a key step in the treatment of anemia. However, this could only be achieved after the elucidation of the cellular/molecular mechanisms that controls non-renal EPO expression.

Our aim was to clarify the erythropoietin gene regulation and expression in renal and liver tissue, using an animal model treated with high rHuEPO doses, as used in case of resistance to rHuEPO therapy.

METHODOLOGY



16 male Wistar rats, 12 weeks old

Divided in 2 groups

rHuEPO Group

Administrated for 6 weeks with 400 IU/Kg/week, via subcutaneous injection

Control Group

Administrated for 6 weeks with saline solution, via subcutaneous injection

Determinations:

- Hemoglobin (Hg)
- Red Blood Cells (RBCs)
- Retyculocytes (RET)
- Hematocrit (Ht)
- Platelets (PLT)
- Leucocytes (LC)
- …

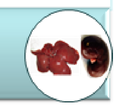
Hematological

- Urea
- Creatinine
- Iron
- Tranferrin
- Erythropoietin (EPO)
- C-reactive protein
- …

Biochemical

- Hypoxia-inducible factors (HIFs-alpha)
- Aryl hydrocarbon receptor nuclear translocator (HIFs-Beta)
- Factor inhibiting HIF (FIH)
- EPO
- Hepatocyte nuclear factor 4 (HNF4)
- …

Gene expression



RESULTS

Erythrocyte and reticulocyte count

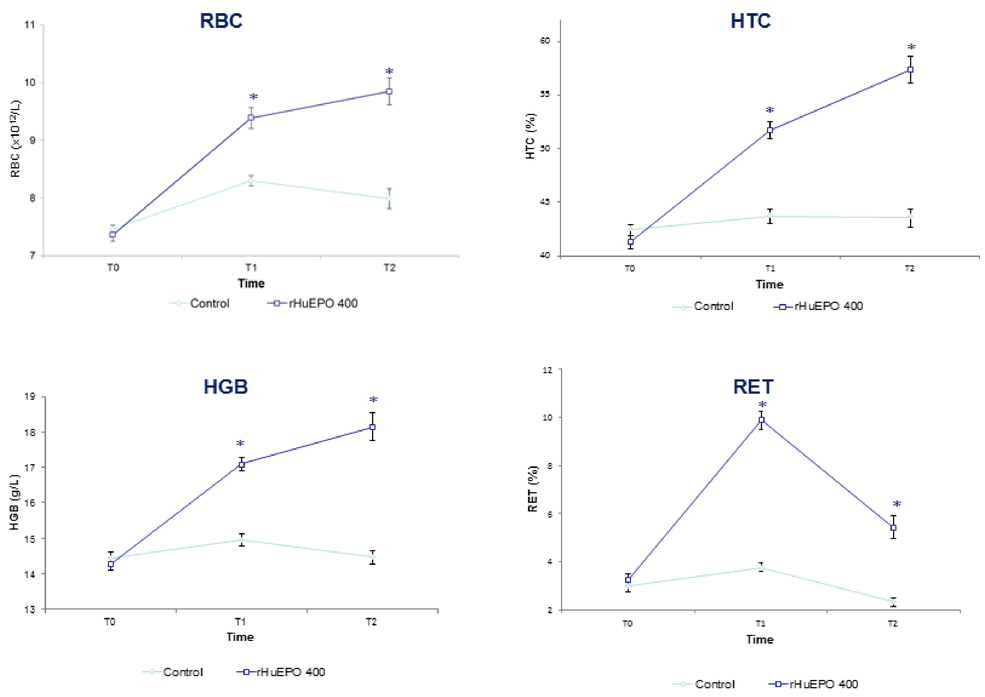


Fig. 1 - Erythrocyte data and reticulocyte count during the follow-up period of 6 weeks under rHuEPO treatment. Results are expressed as mean ± SD. *p<0.005 *vs* control group

Iron, Transferrin and Erythropoietin

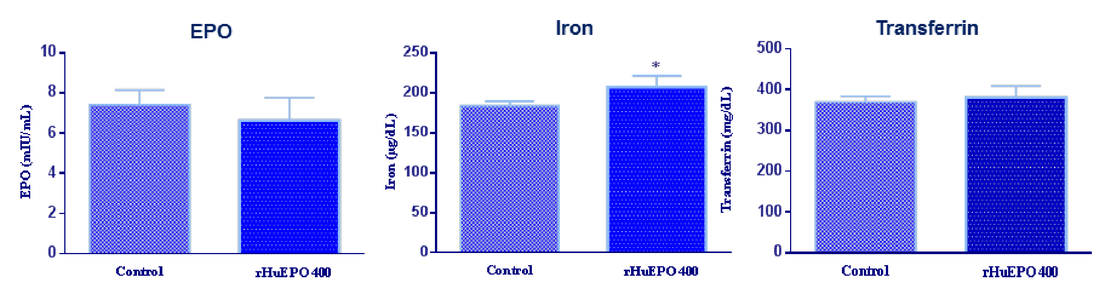


Fig. 2 – Endogenous EPO levels plus iron and transferrin concentration at the end of the 6 week protocol. The plotted data are the mean ± S.D. (n=3). *p<0.05 *vs* control group

Relative mRNA expression of genes involved on EPO regulation on the liver

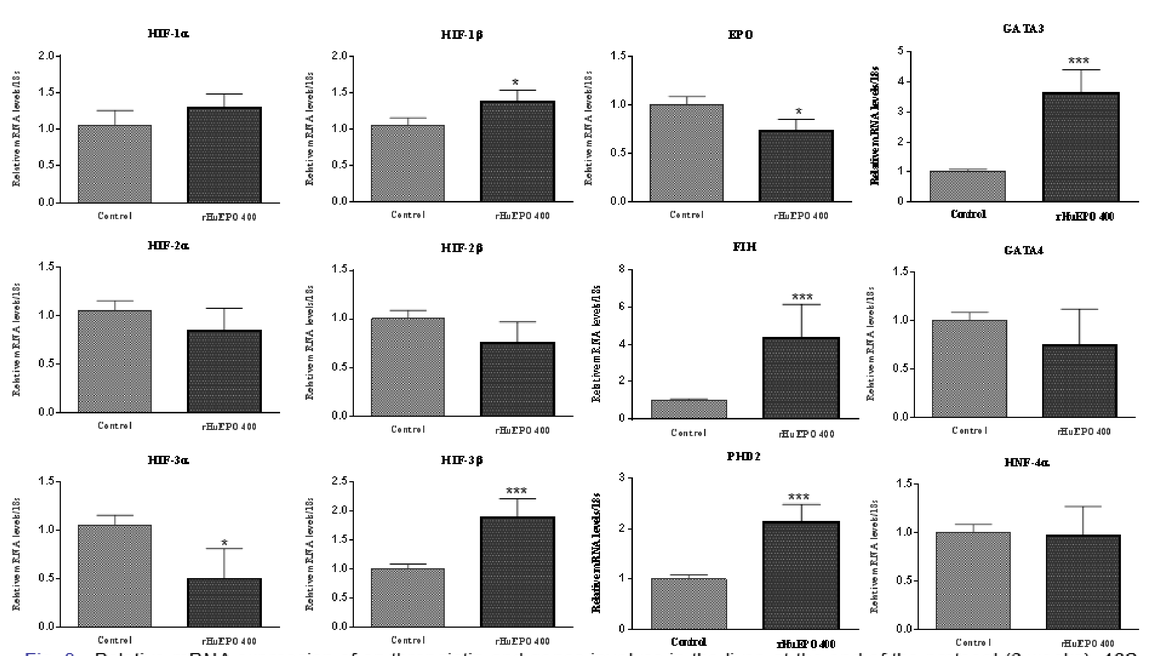


Fig. 3 - Relative mRNA expression of erythropoietin and genes involves in the liver, at the end of the protocol (6 weeks). 18S rRNA was used as reference gene. Results are expressed as mean ± SD. *p<0.05, **p<0.01, and ***p<0.001 *vs* control group.

Relative mRNA expression of genes involved on EPO regulation on the kidney

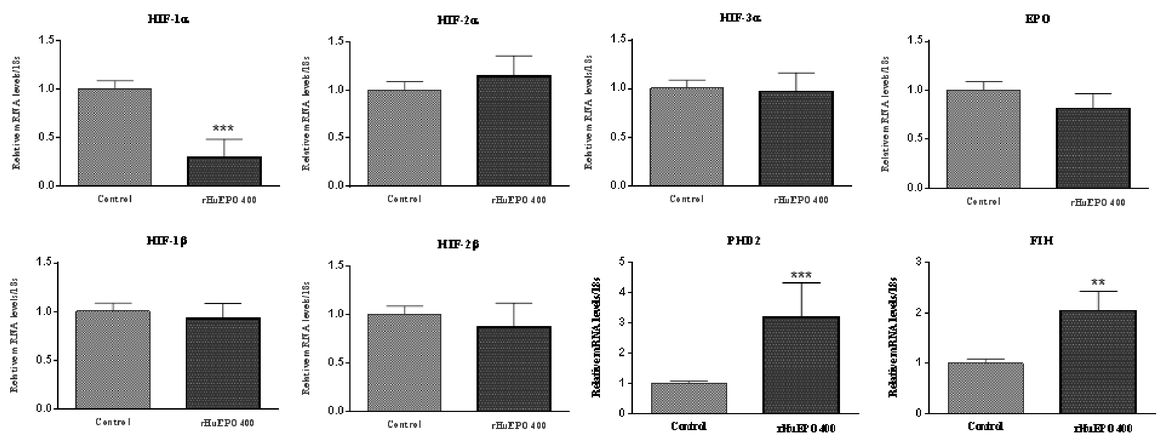


Fig. 4 - Relative mRNA expression of erythropoietin and genes involves in the liver, at the end of the protocol (6 weeks). 18S rRNA was used as reference gene. Results are expressed as mean ± SD. *p<0.05, **p<0.01, and ***p<0.001 *vs* control group

DISCUSSION & CONCLUSION

-rHuEPO led to a increase of hemoglobin and red blood cells and reticulocyte counts along the entire protocol

-The endogenous EPO levels decreased, resulting from a down regulation of EPO gene, observed in both liver and kidney.

-The rHuEPO group didn't show any significant difference in what concerns to *HIF-2α* expression, however a significant down-regulation of *HIF-1α* was seen at kidney level

-*HIF-3α* showed a strong down-regulation in the liver. while *FIH* and *PHD2* were significantly up-regulated in both organs.

The transcriptor factors responsible for the EPO gene modulation seem to change and adjust when high doses of rHuEPO are administrated

EPO gene regulation mechanisms appear to diverge between different organs – kidney *vs*. liver

ACKNOWLEDGEMENTS

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