REGULATION OF LIVER AND KIDNEY ERYTHROPOIETIN GENE EXPRESSION IN A RAT MODEL OF ANEMIA ASSOCIATED WITH CHRONIC RENAL FAILURE

João Fernandes[1,2*], Sandra Ribeiro[2,3], Patrícia Garrido[1], José Sereno[1], Elísio Costa[3,2], Flávio Reis[1], Alice Santos-Silva[2,3]

1. IBILI, Faculty of Medicine, University of Coimbra, Azinhaga Santa Comba, Celas, P–3000-548 Coimbra, Portugal.

2. Institute for Molecular and Cellular Biology, University of Porto, Rua do Campo Alegre 823, P–4150-180 Porto, Portugal.

3. Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira 228, P–4050-313 Porto, Portugal.

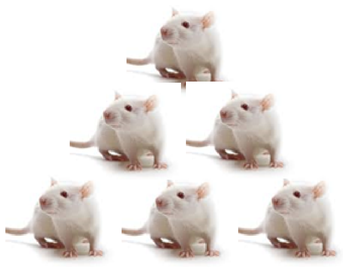
*fernandesjcg@gmail.com

INTRODUCTION

Regulation of erythropoiesis and the maintenance of erythroid homeostasis rely on modulation of erythropoietin (EPO) gene expression in response to tissue oxygen tension. The mechanisms underlying this regulation at renal and non-renal tissues remain to be fully elucidated, in particular in some pathological conditions as is the case of chronic renal failure (CRF). Understanding tissue-specific regulation of EPO production in the case of CRF could be clinically useful in order to identify new targets and therapies.

Our aim was to clarify the regulation of EPO gene, in both kidney and liver, using for that purpose a CRF rat model.

METHODOLOGY



16 male Wistar rats, 12 weeks old

Divided in 2 groups

Sham Group

Rats submitted to surgery without kidney mass reduction

CRF Group

Rats submitted to a two-stage 5/6 nephrectomy

6 weeks later

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
- …

Gene expression

RESULTS

Erythrocyte and reticulocyte count

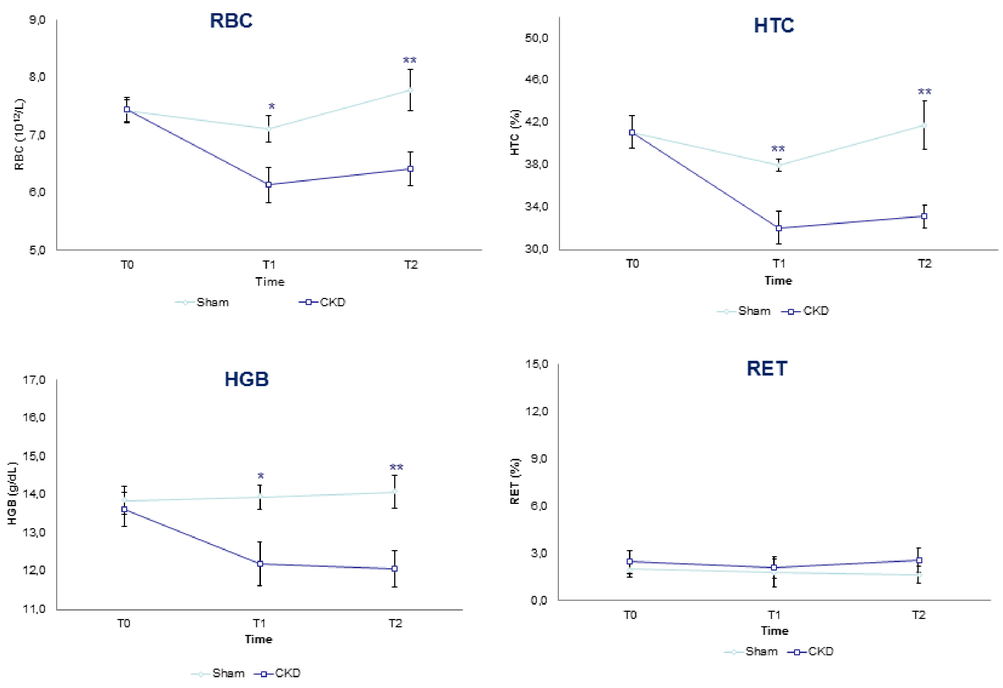


Fig. 1 - Erythrocyte data and reticulocyte count during the follow-up period of 6 weeks. Results are expressed as mean ± SD. *p<0.05, **p<0.005 *vs* Sham group

Iron, Transferrin and Erythropoietin

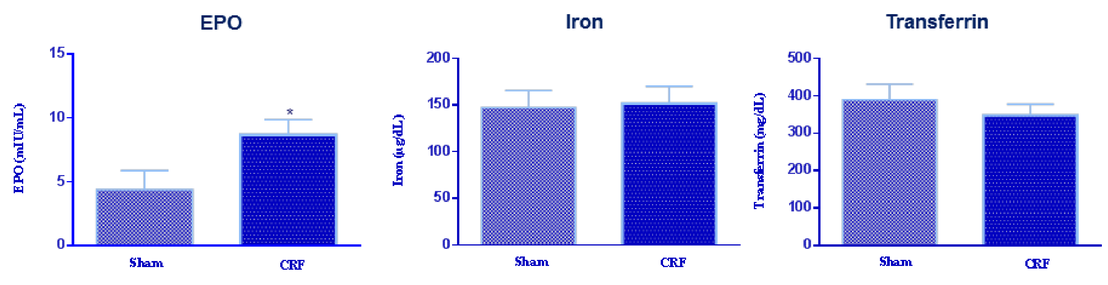


Fig. 2 – Endogenous EPO levels plus iron and transferrin concentration at the end of the 6 week protocol. The plotted data are the mean ± S.D. (n=3). *p<0.05 *vs* Sham group

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

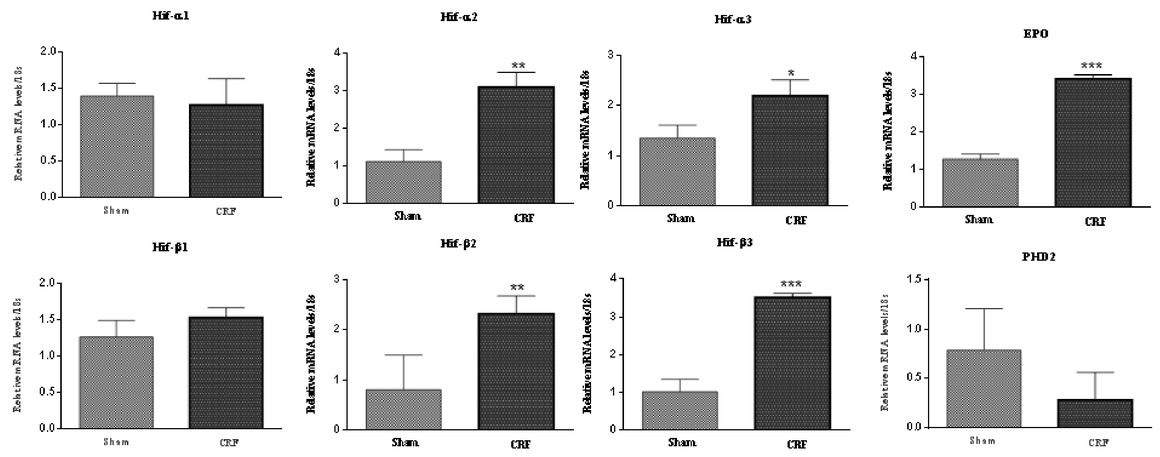


Fig. 3 - Relative mRNA expression of erythropoietin and genes involved in the liver, at the end of the protocol (6 weeks). 18S rRNA was used as reference gene. Results are expressed as mean ± SD. *p<0.05, **p<0.01, and ***p<0.001 *vs* Sham group.

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

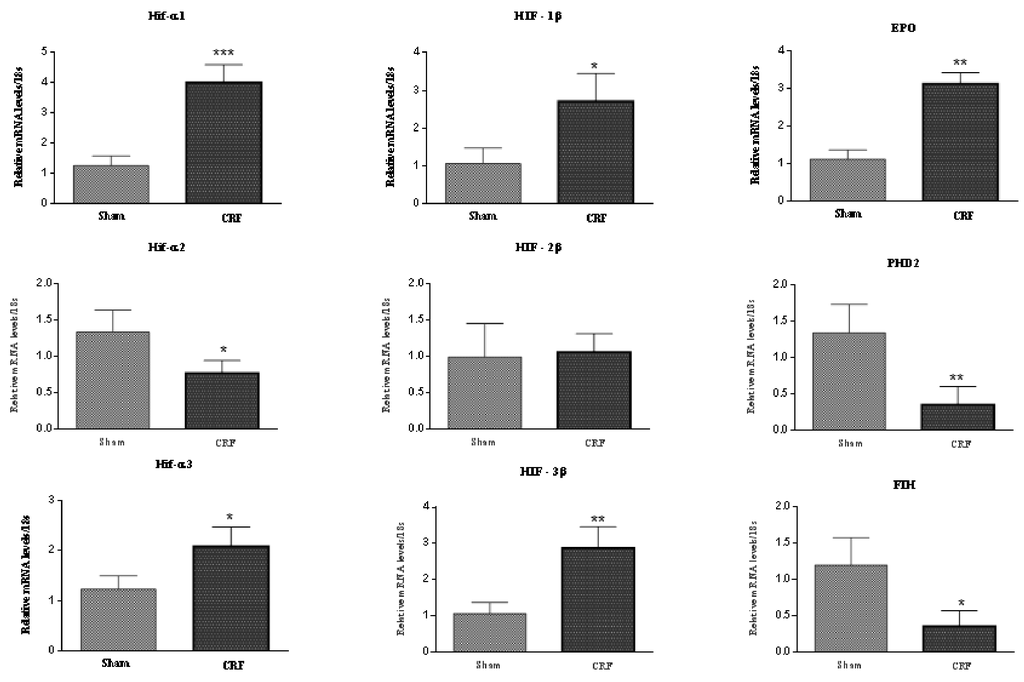


Fig. 4 - Relative mRNA expression of erythropoietin and genes involved in the kidney, at the end of the protocol (6 weeks). 18S rRNA was used as reference gene. Results are expressed as mean ± SD. *p<0.05, **p<0.01, and ***p<0.001 *vs* Sham group

DISCUSSION & CONCLUSION

-After the nephrectomy, hemoglobin levels and red blood cell count decreased significantly; this profile was maintained until the end of the 6 weeks protocol

-Endogenous EPO levels significantly increased in CRF group, resulting from a significant up-regulation of EPO gene seen in the liver and in the kidney.

-The mechanism behind EPO gene up-regulation in the CRF group seems to vary between kidney and liver:
HIF-1α seems to play a main role in the kidney (the 1/6 remnant), while HIF-2α appears to be more preponderant in the liver

-HIF-3α and HIF-3β seem to have an important involvement in CRF, since both genes were significantly increased in both kidney and liver

-FIH showed a strong down-regulation at kidney level, while PHD2 down-regulation occurred in both tissues.

In a CRF situation, the liver seems to try to compensate the associated low EPO levels in circulation by increasing hepatic EPO production.

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

ACKNOWLEDGEMENTS

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