THE CRUCIAL ROLE OF IRON METABOLISM IN ERYHTROID HYPOPLASIA/PURE RED CELL APLASIA DUE TO THE DEVELOPMENT OF ANTI-EPO ANTIBODIES

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Background and Aims

The current therapeutic use of recombinant human erythropoietin (rHuEPO) to correct anemia in chronic renal failure patients has been associated to some cases of pure red cell aplasia (PRCA), due to the development of cross-reactive anti-EPO antibodies. This complication is very rare; however, the use of EPO biogenerics has increased the number of cases in the last years. The mechanisms underlying the break in immune tolerance remains poorly elucidated.

Our aim was to clarify how, facing an erythroid hypoplasia/PRCA condition, the depression of marrow erythroid activity affects iron metabolism, by using an animal model.

Groups and Methods

Groups:

Male Wistar rats (200-250g) were divided into 3 groups (n=8): control - saline (s.c.); two rHuEPO groups, treated respectively with 50 and 200 IU/Kg bw/week of beta-rHuEPO (Recormon®,Roche Pharmaceuticals), during 9 weeks.

Assays:

At starting and along the protocol, blood samples were collected to assess renal function, hematological and biochemical data, and iron metabolism (iron, transferrin and ferritin). At the end of the protocol, kidney, liver and duodenum were collected for RT-qPCR studies. We studied the gene expression of EPO, EPOR, transferrin receptor 2 (TfR2), hepcidin (Hamp), ferroportin (SLC40A1), hemojuvelin (HJV), transferrin (TF), hemochromatosis (Hfe), divalent metal transporter 1 (DMT1), matriptase-2 (TMPRSS6), interleukin-6 (Il-6) and bone morphogenic protein 6 (BMP6), which was normalized to the housekeeping genes beta-actin and glyceraldehyde 3-phosphate dehydrogenase.

Statistics:

Results are presented as means standard deviation (SD). Comparisons between groups were performed using ANOVA and the Post hoc Tukey test. For single comparations we used the Mann-Whitney U-test.

Results

Erythrocyte and reticulocyte count

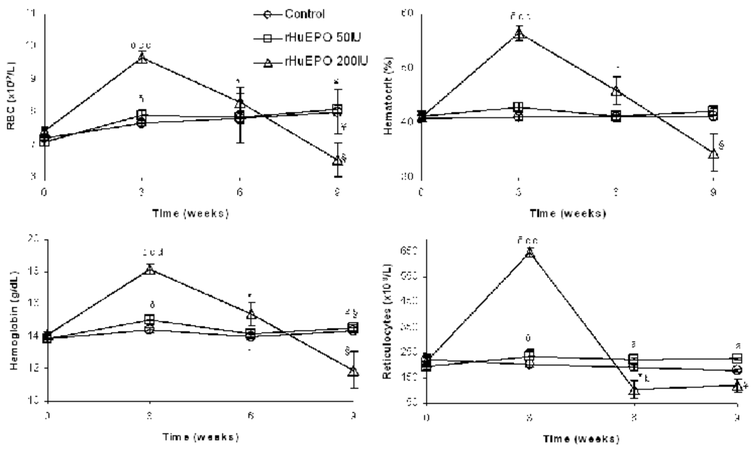

Figure 1. Erythrocyte data and reticulocyte count during the follow-up period of 9 weeks under rHuEPO treatment. Results are expressed as mean SD. ^{a}p <0.05 *vs* control; ^{b}p <0.05 *vs* 50 IU rHuEPO; ^{c}p <0.001 *vs* control; ^{d}p <0.001 *vs* 50 IU rHuEPO; $^{\delta}p$ < 0.005 T0 *vs* T1; $^{*}p$ < 0.05 T1 *vs* T2; p < 0.05 T2 *vs* T3.

Relative mRNA expression of EPO and iron regulatory proteins

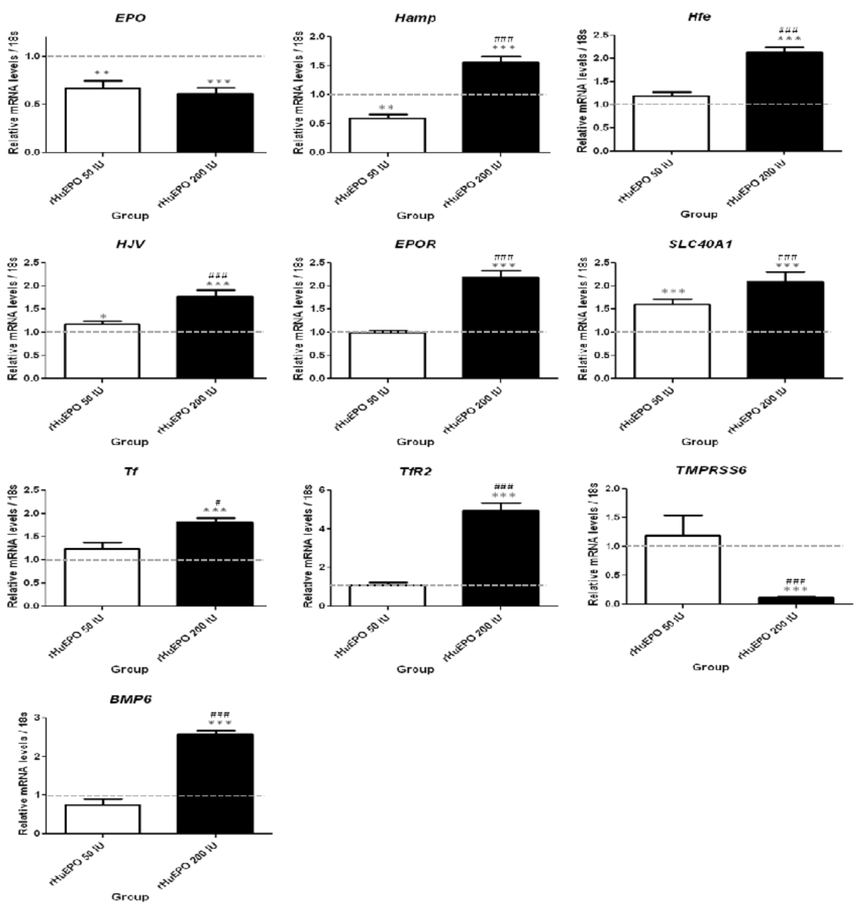

Figure 2. Relative mRNA expression of erythropoietin and iron regulatory proteins in the liver, at the end of the protocol (9 weeks). 18S rRNA was used as reference gene. Results are expressed as mean SD. $^{*}p$ < 0.05, $^{**}p$ < 0.01, and $^{***}p$ < 0.001 *vs* control group; $^{\#}p$ < 0.05, $^{\#\#}p$ < 0.01, and $^{\#\#\#}p$ < 0.001 *vs* 50 IU rHuEPO.

Relative mRNA expression of EPO , DMT1 and SLC40A1

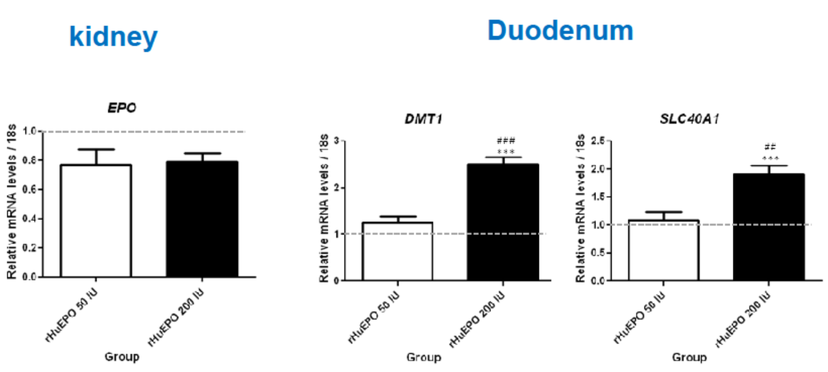

Figure 3. Relative mRNA expression of erythropoietin in the kidney, *DMT1* and *SLC40A1* in the duodenum, at the end of the protocol (9 weeks). 18S rRNA was used as reference gene. Results are expressed as mean SD. . $^{***}p$ < 0.001 *vs* control group; $^{\#\#}p$ < 0.01, and $^{\#\#\#}p$ < 0.001 *vs* rHuEPO50 IU .

- **The hematologic changes (Figure 1) and the detection of anti-EPO antibodies confirmed the development of an antibody-mediated eryhtroid hypoplasia model induced by administration of high rHuEPO doses (200 IU/Kg/week).**
- **The erythropoietic stimuli failed due to inhibition of EPO by anti-EPO Abs, and, thus, serum iron increased.**
- **In accordance, in the liver (Figure 2) we found an overexpression of Tf, TfR2, BMP6, HFE, HJV and, therefore, in hepcidin; matriptase was downregulated further contributing to overexpression of hepcidin.**
- **The increased serum iron, which results from increased absorption and mobilization, is supported by the overexpression of DMT1 and ferroportin in duodenum and liver (Figure 2 and 3).**

Erytrhopoiesis in erythoid antibody-mediated hypoplasia

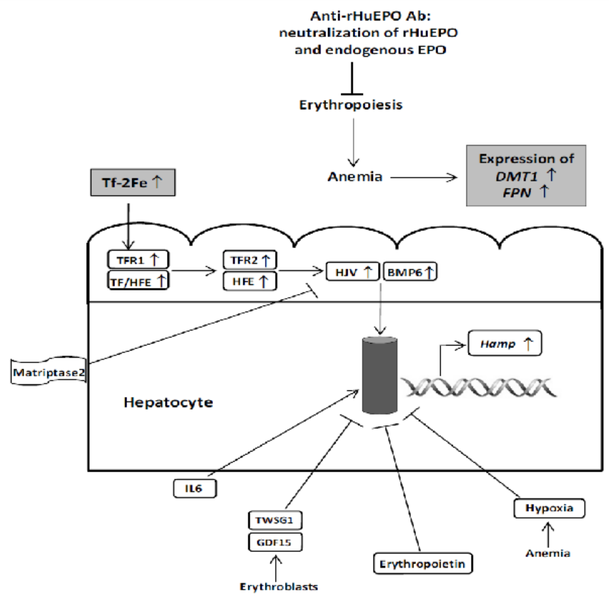

Figure 4. Model proposed for erythropoiesis and iron metabolism in erythroid antibody-mediated hypoplasia. Anti-rHuEPO antibodies inhibit both rHuEPO and endogenous EPO leading to anemia that is further aggravated by increased iron that favours hepcidin expression.

Conclusions

Considering the EPO inhibition through anti-EPO antibodies and the consequent reduction in erythropoiesis, it seems that serum iron concentration is the key modulator in this type of erythroid hypoplasia (Figure 4). Actually, it has been recently reported that iron chelation therapy promotes an improvement in erythropoiesis in PRCA, though the mechanism whereby this is achieved is still unclear.

This study provides new insights on how rHuEPO-induced erythroid hypoplasia affects iron metabolism, which could be useful to find new targets and improve therapeutic strategies against this increasingly common condition.

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