LIVER IRON IS A MAJOR REGULATOR OF HEPCIDIN GENE EXPRESSION VIA BMP/SMAD PATHWAY IN A RAT MODEL OF CHRONIC RENAL FAILURE UNDER TREATMENT WITH HIGH rHuEPO DOSES

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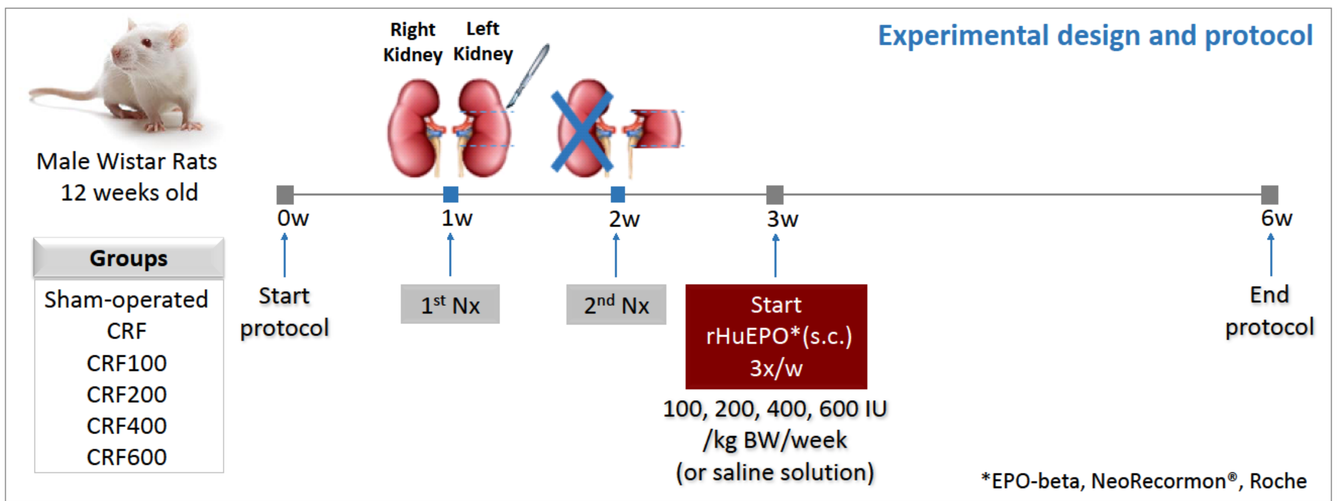
INTRODUCTION

Hepcidin is the major central regulator of iron metabolism, controlling iron absorption and mobilization. Considering its interaction with several factors that are altered in chronic kidney disease (CKD), particularly in hyporesponsive CKD patients under therapy with high recombinant human erythropoietin (rHuEPO) doses.

We aimed to study the impact of increasing rHuEPO doses on the regulation of iron-hepcidin metabolism in a rat model of CKD-anemia.

METHODS



Experimental design and protocol

Determinations

Hematological	Red blood cells (RBC) count; Hemoglobin; Hematocrit; Reticulocytes count
Biochemical	Iron; Ferritin; Transferrin; Transferrin saturation (TSAT)
Gene expression (qPCR)	Liver: Transferrin receptors 1 and 2 (Tfr1/2); Hemojuvelin (HJV); Hepcidin; Bone morphogenetic protein 6 (BMP6); Matriptase-2; Hypoxia inducible factors (HIF1α/2α) Erythropoietin (EPO); Duodenum: Divalent metal transporter 1 (DMT1)
Protein (Western Blot)	Liver: Hepcidin; pERk1/2:ERK1/2; pSMAD1/5/9:SMD1/5/9; SMAD4 Duodenum: Ferroportin
Staining	Perls' Prussian Blue (hemosiderin staining)
Statistical analysis	Results are presented as mean ± standard error of the mean(SEM). For comparison between groups Wilcoxon and Mann–Whitney U tests were performed.

RESULTS

Table 1 - Hematological data at 0 (before treatment), 1 and 3 weeks (end of protocol) of rHuEPO treatment

Parameters		Sham	CRF	CRF+rHuEPO 100IU	CRF+rHuEPO 200IU	CRF+rHuEPO 400IU	CRF+rHuEPO 600IU
RBC (x 10^{12}/L)	0w	7.41±0.07	7.43±0.09	7.03±0.12	7.53±0.11	7.60±0.09	7.03±0.07
	1w	7.11±0.08	6.11±0.12***a***	7.02±0.21***b***	6.80±0.14***b***	7.62±0.06***abd***	8.08±0.11***abcd***
	3w	7.77±0.13	6.41±0.11***a***	7.92±0.40***b***	7.48±0.17***b***	8.39±0.21***bd***	10.53±0.20***abcde***
Hemoglobin (g/L)	0w	13.85±0.13	13.63±0.18	13.55±0.13	13.54±0.17	13.68±0.13	13.66±0.18
	1w	13.94±0.11	12.26±0.22***a***	13.58±0.37***b***	13.86±0.20***b***	14.55±0.14***b***	16.33±0.29***abcde***
	3w	14.07±0.15	12.06±0.18***a***	14.65±0.52***b***	13.89±0.32***b***	15.45±0.37***abd***	20.08±0.37***abcde***
Hematocrit (%)	0w	41.12+0.54	41.34+0.59	38.40+0.31	40.90+0.55	41.25+0.45	39.23+0.41
	1w	38.02±0.20	32.13±0.63***a***	38.73±1.19***b***	36.21±0.92***b***	41.88±0.49***abcd***	50.11±0.93***abcde***
	3w	41.77±0.80	33.56±0.57***a***	43.37±2.09***b***	38.03±0.87	46.54±1.43***bd***	66.16±1.16***abcde***
Reticulocytes (x 10^{9}/L)	0w	144.39±12.38	183.41±20.89	80.37±8.20	177.06±18.25	151.09±10.19	108.08±19.72
	1w	122.47±22.47	134.11±15.26	215.92±23.86	358.17±25.05***ab***	701.81±39.67***abcd***	520.08±45.99***abcde***
	3w	124.77±14.56	161.67±17.87	258.28±13.61***a***	158.84±14.67	119.90±12.17***c***	252.18±48.78***ae***

Results are presented as Mean ± SEM. ***a*** p<0.05 vs Sham group; ***b*** p<0.05 vs CRF group; ***c*** p<0.05 vs CRF+rHuEPO 100IU; ***d*** p<0.05 vs CRF+rHuEPO 200IU; ***e*** p<0.05 vs CRF+rHuEPO 400IU (Mann-Whitney U test).
RBC – Red blood cells; 0w –start of protocol; 1w – 1 week after the start of rHuEPO treatment; 3w – 3 weeks after the start of rHuEPO treatment (end of protocol); CRF – chronic renal failure; rHuEPO – recombinant human erythropoietin.

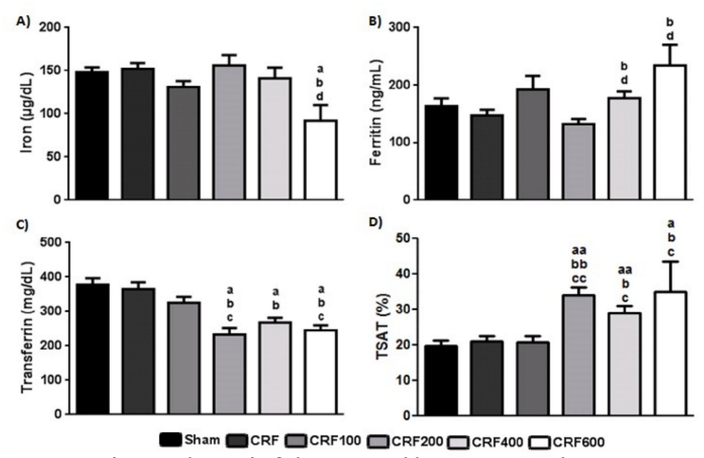


Fig. 1 - Iron data at the end of the protocol by group. Results are expressed as mean6SEM. (a) P<0.05 versus Sham group, (b) P<0.05 versus CRF group, (c) P<0.05 versus CRF100 group, (d) P<0.05 versus CRF200 group (Mann–Whitney U test).

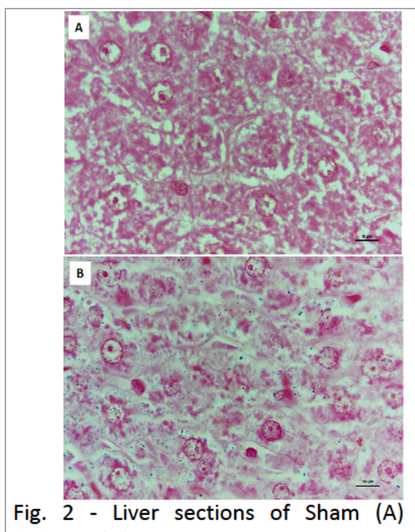


Fig. 2 - Liver sections of Sham (A) and CRF600 (B) groups demonstrating staining for hemosiderin (Perls Stain 100x).

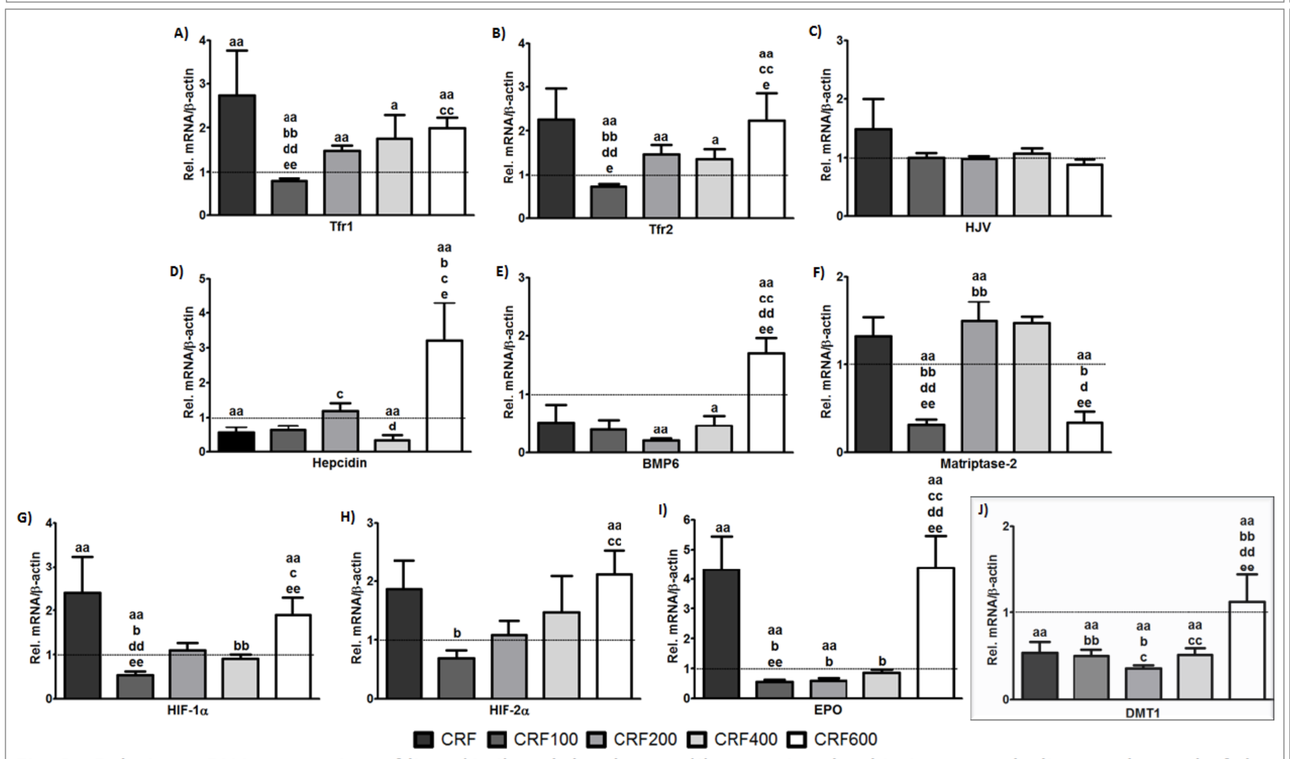


Fig. 3 - Relative mRNA expression of liver (A–I) and duodenum (J) genes involved in iron metabolism at the end of the protocol. Results are expressed as mean6SEM. (a) P<0.05 versus Sham group, (b) P<0.05 versus CRF group, (c) P<0.05 versus CRF100 group, (d) P<0.05 versus CRF200 group, (e) P<0.05 versus CRF400 group, (f) P<0.05 versus CRF600 (Mann–Whitney U test).

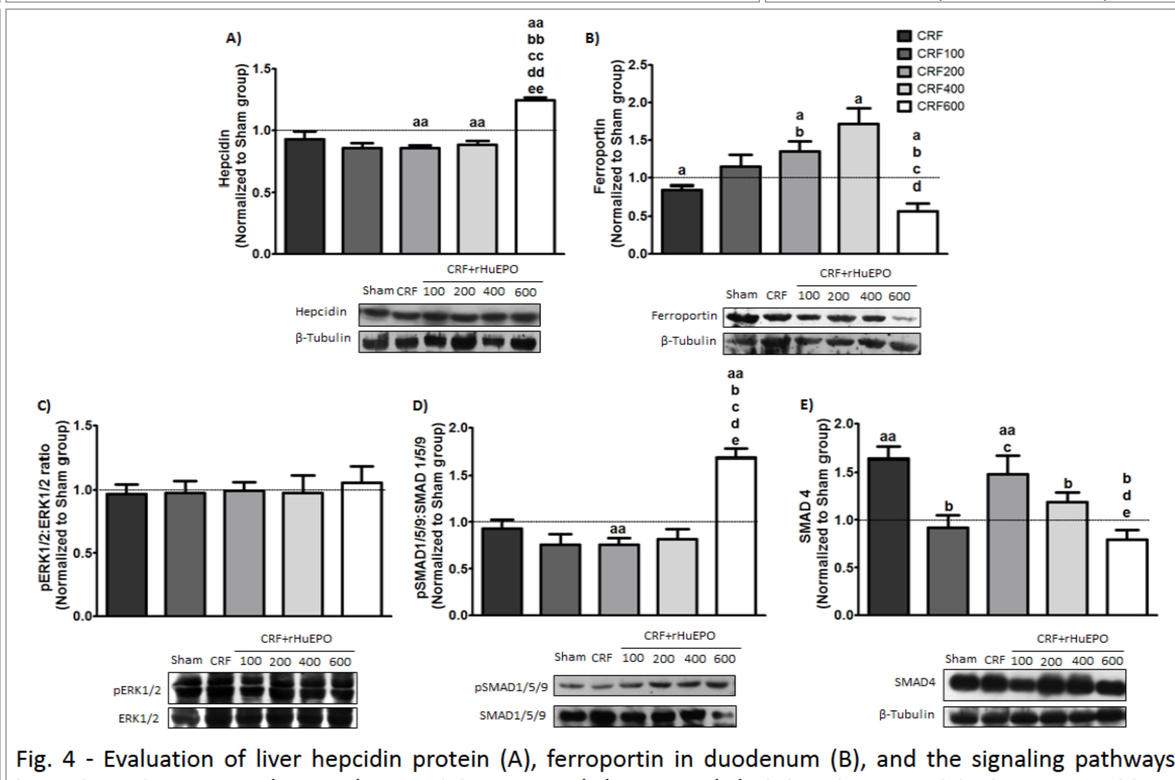


Fig. 4 - Evaluation of liver hepcidin protein (A), ferroportin in duodenum (B), and the signaling pathways of hepcidin in liver-pERK1/2:ERK1/2 ratio (C), pSMAD1/5/8:SMAD1/5/8 (D) and SMAD4 (E)—by western blotting. Results are expressed as mean6SEM. (a) P<0.05 versus Sham group, (b) P<0.05 versus CRF group, (c) P<0.05 versus CRF100 group, (d) P<0.05 versus CRF200 group, (e) P<0.05 versus CRF400 group (Mann–Whitney U test).

DISCUSSION/ CONCLUSIONS

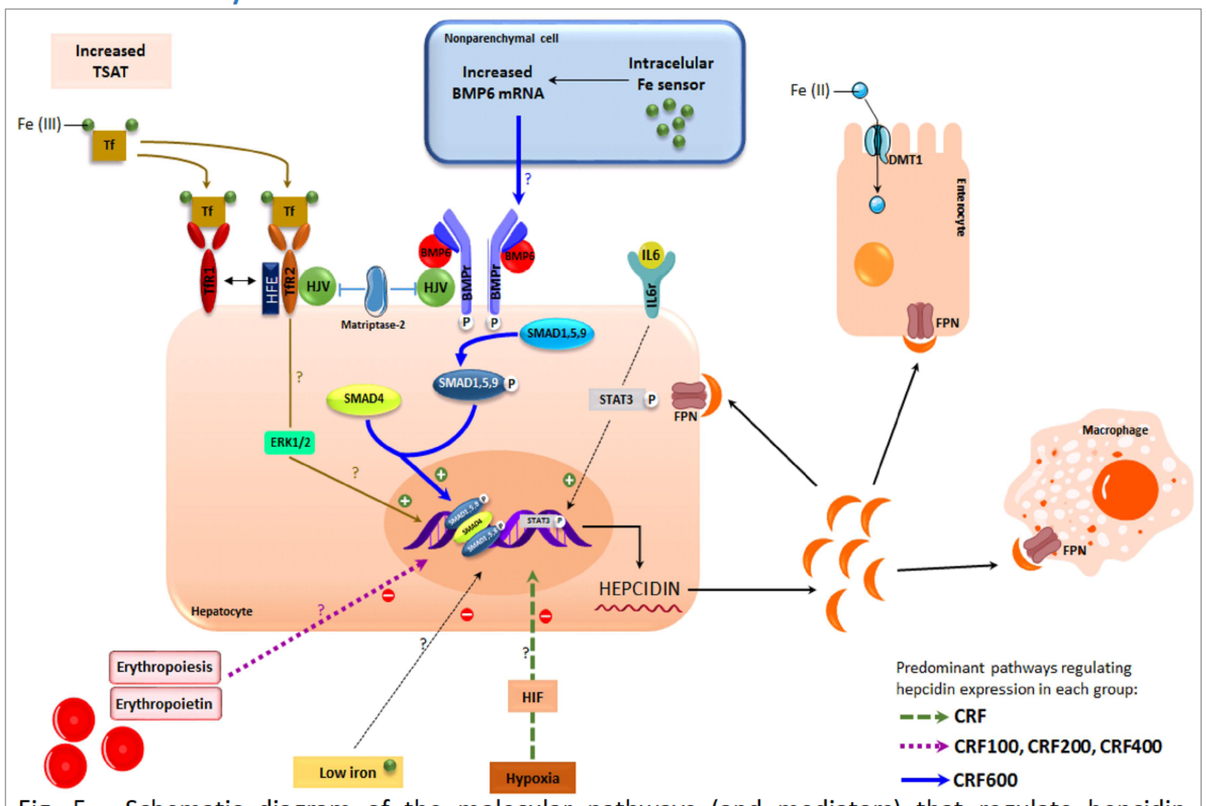


Fig. 5 - Schematic diagram of the molecular pathways (and mediators) that regulate hepcidin expression in liver.

- The CRF animals without rHuEPO treatment developed anemia, when compared to the sham group, that was corrected in a dose-dependent manner in the CRF animals treated with rHuEPO (Table 1);
- Serum iron concentration was reduced in the CRF600 treated group, ferritin levels were increased in the CRF400 and CRF600 treated groups and TSAT increased in the CRF200, CRF400 and CRF 600 treated groups, compared to CRF group (Fig. 1);
- Liver histological sections stained with Perls' Prussian blue showed iron deposition as hemosiderin in the CRF600 group (Fig. 2);
- Hepcidin and BMP6 mRNA levels in the liver were down-regulated in all groups, except in the CRF600 treated that presented a significant up-regulation (Fig. 3), confirmed by Western blot (Fig. 4);
- Protein liver pSMAD1/5/9:SMAD1/5/9 ratio was increased in CRF600 treated group (Fig. 4);
- Our data suggests that liver iron overload is an important stimuli for hepcidin synthesis, stronger than the inhibitory effect of high rHuEPO doses (Fig. 5);
- Our findings raise the hypothesis that when high inflammation (triggering hepcidin expression) is associated with increased iron stores in hemodialysis patients, hepcidin expression is also up-regulated via BMP6, enhancing hepcidin synthesis, leading, therefore, to worsening of anemia and, eventually, to a hyporesponse/resistance to rHuEPO therapy.

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