IMPAIRED RENAL ENDOTHELIAL NITRIC OXIDE SYNTHASE AND RETICULOCYTE PRODUCTION AS MODULATORS OF HYPERTENSION INDUCED BY RECOMBINANT HUMAN ERYTHROPOIETIN IN THE RAT

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INTRODUCTION AND AIMS

Hypertension is a common side effect of recombinant human erythropoietin (rHuEPO) therapy; however, the exact pathways remain to be elucidated. The discovery of non-hematopoietic actions of rHuEPO increased the number of patients that could putatively benefit from this therapy, but to achieve those effects higher doses are usually needed, which increase the risk and incidence of adverse events.

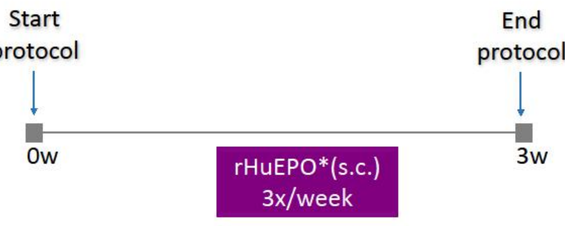
Our aim was to study the effect of a broad range of rHuEPO doses on hematological and biochemical parameters, blood pressure and renal function and damage in the rat (healthy animals), focusing on endothelial nitric oxide synthase (eNOS) and hypoxia-inducible factors (HIFs).

METHODS

Experimental design and protocol

Male Wistar Rats
12 weeks old

Groups
Control
rHuEPO100
rHuEPO200
rHuEPO400
rHuEPO600



Hematological	Red blood cells (RBC) count; Hemoglobin; Hematocrit ; Reticulocytes count
Biochemical	Serum: BUN; Creatinine; Vascular endothelial growth factor (VEGF) 24h urine: Urea; Creatinine and clearance (CC); Albumin excretion (AE); GFR
Blood pressure	Systolic blood pressure (SBP): tailcuff method
Gene expression (qPCR)	Hipoxia inducible factors (HIF-1α, HIF-2α); EPO; VEGF
Western Blot	HIF-2α; Beta common receptor (βcR); eNOS; AKT
Histopathological analysis	Periodic acid of Schiff (PAS) staining; Masson's Trichrome staining
Statistical analysis	For comparison between groups Wilcoxon and Mann-Whitney U tests were performed (SPSS version 22.0)

RESULTS

Table 1 - Hematological, biochemical and blood pressure data at the end of study protocol

Parameters	Control	rHuEPO100	rHuEPO200	rHuEPO400	rHuEPO600
Hematological data					
RBC (x 10^{12}/L)	7.99 ± 0.17	8.20 ± 0.13	9.41 ± 0.12***ab***	9.85 ± 0.23***ab***	10.53 ± 0.39***abc***
Hemoglobin (g/L)	14.47 ± 0.19	14.74 ± 0.30	17.06 ± 0.32***ab***	18.14 ± 0.40***ab***	19.60 ± 0.60***abc***
Hematocrit (%)	43.57 ± 0.85	44.89 ± 1.19	53.21 ± 1.16***ab***	57.37 ± 1.20***abc***	66.56 ± 1.03***abcd***
Reticulocytes (x 10^9/L)	205.68 ± 16.16	215.01 ± 26.67	179.34 ± 26.07	535.60 ± 52.38***abc***	469.55 ± 31.74***abc***
Serum biochemical data					
BUN (mg/dL)	21.02 ± 0.33	22.36 ± 0.51	21.70 ± 0.42	18.36 ± 0.38***abc***	23.06 ± 0.70***ad***
Creatinine (mg/dL)	0.37 ± 0.02	0.32 ± 0.02	0.36 ± 0.03	0.39 ± 0.02	0.40 ± 0.02
VEGF (pg/mL)	321.97 ± 29.54	340.35 ± 33.51	826.64 ± 39.17***ab***	306.50 ± 19.61***c***	697.57 ± 68.02***abd***
Urine biochemical data					
Urea (mg/dL)	5280.00 ± 617.13	5950.00 ± 575.39	6525.00 ± 359.94	5457.00 ± 340.07	5400.00 ± 733.03
Creatinine (mg/dL)	83.00 ± 7.16	82.86 ± 5.65	97.50 ± 7.96	90.00 ± 6.17	90.00 ± 15.92
AE (mg/L)	3.00 ± 0.33	3.00 ± 0.65	2.00 ± 0.31	3.71 ± 0.84	2.17 ± 0.48
CC (mL/h/rat)	108.75 ± 5.24	113.32 ± 5.38	105.13 ± 8.49	107.16 ± 8.67	108.06 ± 16.00
GFR (mL/h/rat)	108.76 ± 5.27	111.11 ± 6.21	109.34 ± 6.87	117.68 ± 8.83	110.88 ± 16.21
Blood pressure					
SBP (mmHg)	105.20 ± 0.90	119.06 ± 1.12***a***	129.15 ± 0.85***ab***	118.70 ± 1.9 ***ac***	145.64 ± 1.23***abcd***

Results are presented as mean±SEM: ***a*** p<0.05 vs control group; ***b*** p<0.05 vs rHuEPO100 group; ***c*** p <0.05 vs rHuEPO200 group; ***d*** p<0.05 vs rHuEPO400 group (Mann-Whitney U Test).

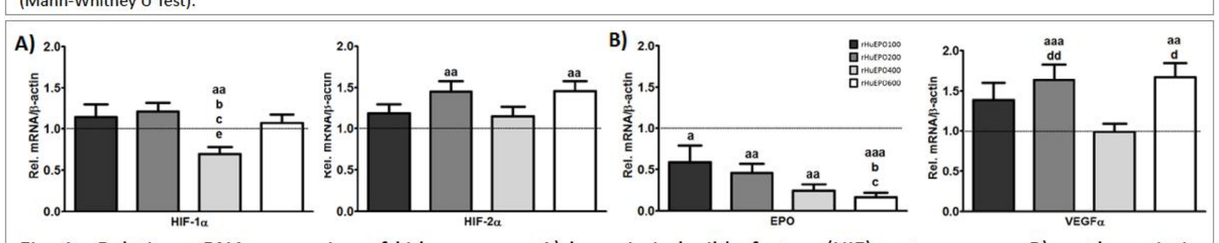


Fig. 1 - Relative mRNA expression of kidney genes. A) hypoxia-inducible factors (HIF) system genes; B) erythropoietin (EPO) and vascular endothelial growth factor alpha (VEGFα) genes. β-actin was used as the reference gene. Results are presented as mean ± SEM: a p<0.05, aa p<0.01, aaa p<0.001 vs control group; b p<0.05 vs rHuEPO100 group; c p<0.05 vs rHuEPO200UI group; d p<0.05, dd p<0.01 vs rHuEPO400 group; e p<0.05 vs rHuEPO600 (Mann-Whitney U test).

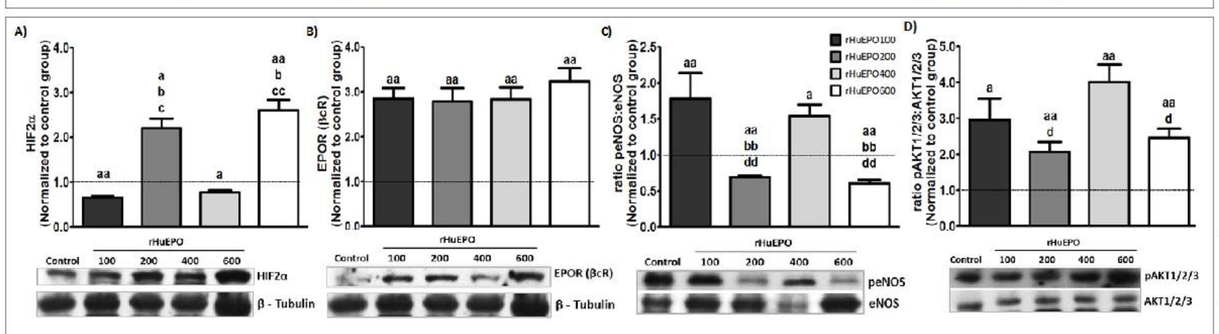


Fig. 2 - Kidney proteins evaluation by Western Blot and representative image for the different groups.. A) hypoxia inducible factor alpha (HIF-2a); B) erythropoietin receptor (EPOR) - β common receptor, C) ratio phosphorilated endotelial nitric oxide (peNOS) to total eNOS; D) pAKT1/2/3:AKT1/2/3 ratio. Results are expressed as mean ± SEM: a p<0.05, aa p<0.01 vs control group; b p< 0.05, bb p <0.01 vs rHuEPO100 group; c p< 0.05, cc p 0.01vs rHuEPO200UI group; d p <0.05, dd p<0.01vs rHuEPO400 group (Mann-Whitney test).

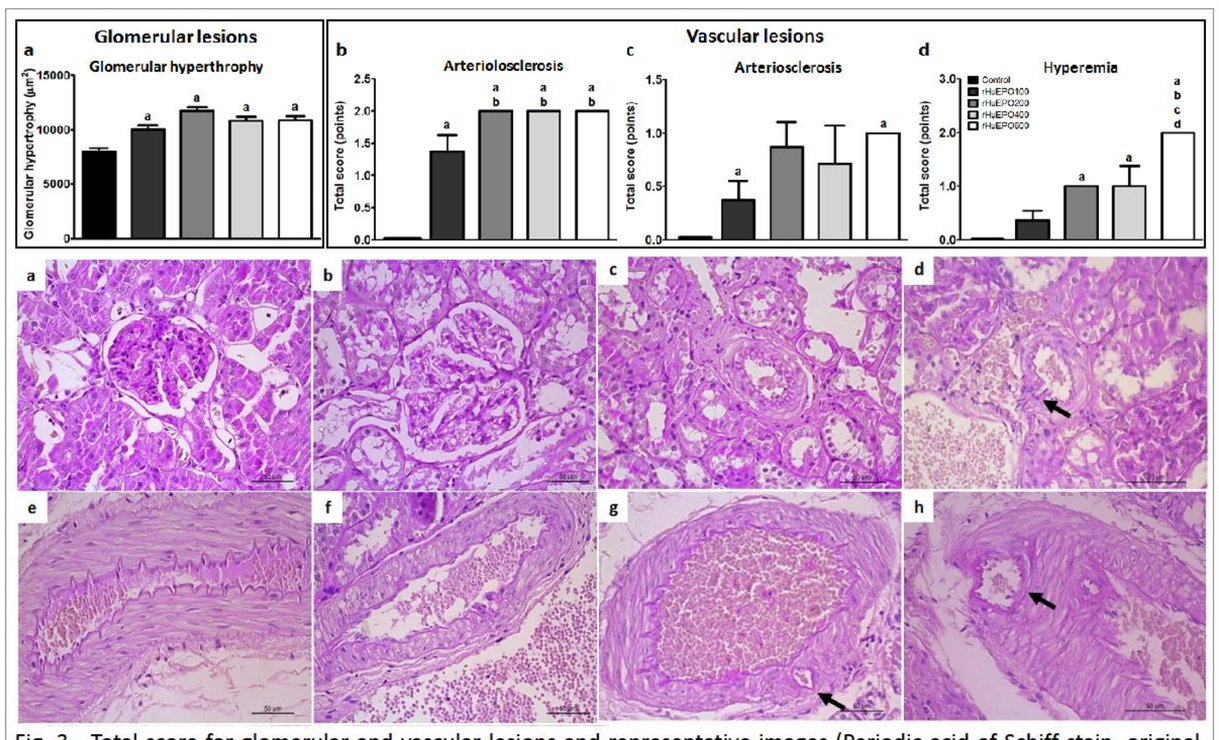
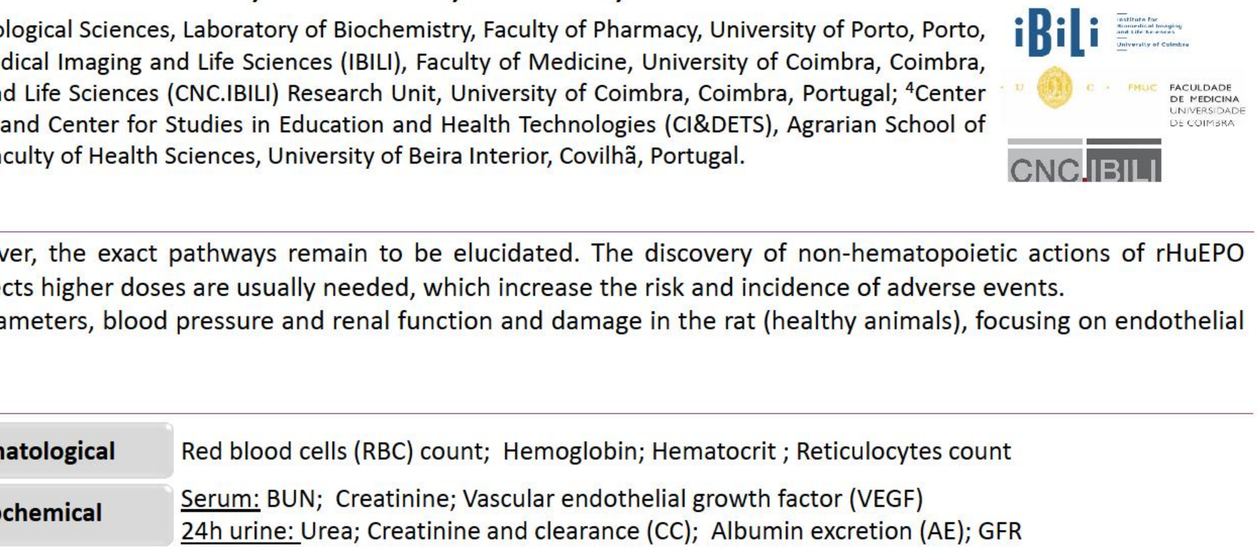


Fig. 3 - Total score for glomerular and vascular lesions and representative images (Periodic acid of Schiff stain, original magnification ×400) at the end of protocol. a) normal glomerulus; b) glomerular hypertrophy; c) normal arteriole; d) arteriolosclerosis (grade 2, arrow), e) normal arteria; f) arteriosclerosis (grade 1); g) hyperemia (grade 2) and neovascularization (arrow); h) neovascularization (arrow) and arteriosclerosis (grade 1). Results are presented as mean ± SEM: a p<0.05 vs control group; b p<0.05 vs rHuEPO100 group; c p<0.05 vs rHuEPO200 group; d p<0.05 vs rHuEPO400 group (Mann-Whitney U Test).



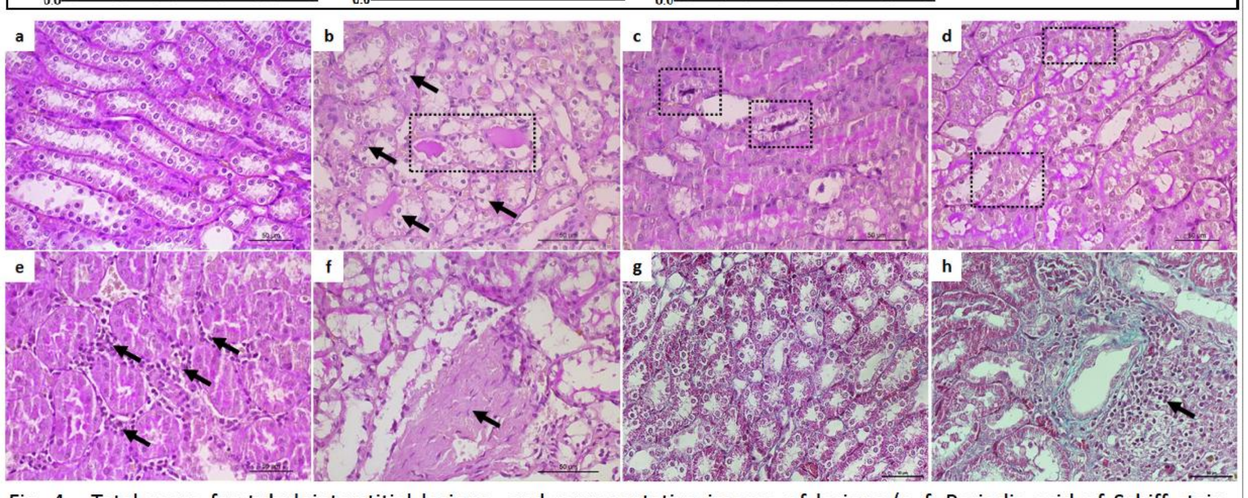


Fig. 4 - Total score for tubulointerstitial lesions and representative images of lesions (a–f, Periodic acid of Schiff stain, original magnification ×400) and collagen fibers deposition (g–h, Masson's Trichrome staining, original magnification ×400) at the end of protocol. a) normal tubules from control group; b) hyaline cylinders (grade 1, square) and tubular vacuolar degeneration (grade 2, arrows); c) granular cylinders (grade 1, arrows); d) tubular hidropic degeneration (grade 1, squares); e) interstitial inflammatory infiltrate (grade 2, arrows); f) interstitial fibrosis and tubular atrophy (IFTA, grade 1, arrow); g) no deposition of collagen fibers in control group; h) deposition of collagen fibers around tubules (grade 1, rHuEPO600 group) and interstitial inflammatory infiltrate (grade 1, arrow).). Results are presented as mean ± SEM: a p<0.05 vs control group; b p<0.05 vs rHuEPO100 group; c p<0.05 vs rHuEPO200 group; d p<0.05 vs rHuEPO400 group (Mann-Whitney U Test).

DISCUSSION/CONCLUSIONS

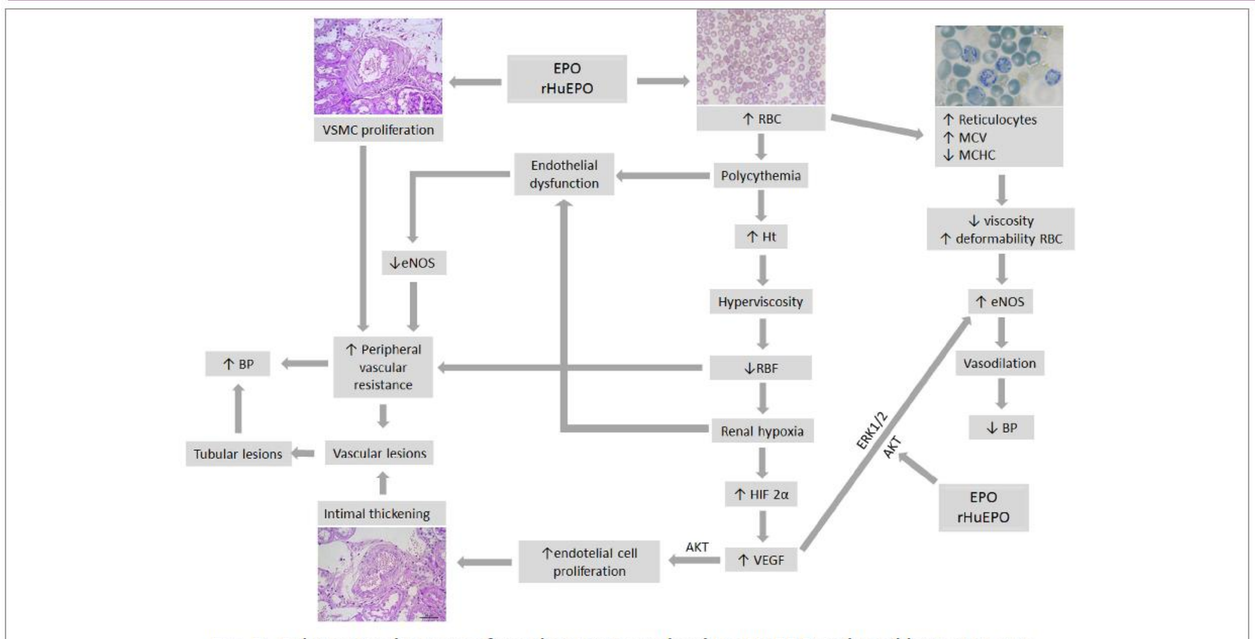


Fig. 5 - Schematic diagram of mechanisms involved in rHuEPO-induced hypertension.

- Our study showed that rHuEPO-induced hypertension might involve indirect (hematological) and direct (renal) effects that varies according to the dose used.
- rHuEPO therapy should be used rationally and under adequate surveillance, as hypertension develops even with lower doses.
- Especial caution with higher doses should be taken, as rHuEPO-induced hypertension leads to early renal damage without alterations in traditional markers of renal function, thus underestimating the serious adverse effects and risks of rHuEPO treatment.
- The need for more sensitive and precocious markers of renal damage is warranted.

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