Recombinant human erythropoietin therapy has beneficial cardio-renal effects on moderate stages of chronic renal failure in the rat

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Summary

This study aimed to assess the cardio-renal effects of rhEPO therapy on an animal model of moderate chronic renal failure (CRF). Four groups (n=7) of male rat were evaluated during a 12-week follow up period: control; rhEPO: 50 IU/Kg/wk; CRF: two-stage $^{3}/_{4}$ nephrectomy; CRF+ rhEPO (start after the 3rd wk of surgery). Renal function, haematology and serum inflammation and redox status were assessed. rhEPO treatment was able to partially attenuate renal function markers, totally correct anaemia, also showing a proliferative and antioxidant action, due to increased serum TGF-β1 and decreased 3-NT. In conclusion, rhEPO therapy might be recommended in moderate CRF stages in order to efficiently correct not only the underlying anaemia but also the deleterious cardio-renal effects, due to a proliferative and antioxidant renoprotective action.

Keywords: moderate chronic renal failure – rhEPO therapy – cardio-renal effects

Introduction

Chronic renal failure (CRF) patients develop anaemia which further promotes cardiovascular insufficiency. This triad of dysfunctions, already known as cardio-renal anaemia syndrome, seems to be favoured by the low production of erythropoietin (EPO) by the kidney (1). The introduction of recombinant human EPO (rhEPO)

therapy has marked a significant advance in the management of anaemia associated with CRF. However, an increasingly growing body of evidence indicates that therapeutic benefits of rhEPO could be far beyond correction of anaemia, namely at the cardiovascular level (2,3).

The aim of this work was accessed the cardio-renal effects of rhEPO therapy in moderate CRF stages, using a rat model of ¾ nephrectomy.

Material and Methods

Animal protocol

Four groups (n=7) of male Wistar rats were evaluated during a 12 week follow-up period: control (without treatment); rhEPO (50 IU/Kg/wk beta Recormon®); CRF and CRF+rhEPO treated with rhEPO after the 3rd week of surgery. At the final time (15 weeks), blood was collected and kidneys were removed, in order to evaluate renal function and renal trophism, haematological data, and serum proliferative, inflammatory and redox status markers.

Assays

Renal function: Serum creatinine, ureia and uric acid levels were assessed through automatic methods/equipments (Hitachi 717 analyser).

Haematological data: Red blood cell (RBC) count, haematocrit (Hct), haemoglobin (Hb) concentration and reticulocyte (RET) count, were assessed in whole blood EDTA by using an automatic Coulter Counter® (Beckman Coulter Inc., USA).

Inflammatory and redox status markers: Serum levels of interleukin 2 (IL-2), IL-1β, transforming growth factor β1 (TGF-β1) and tumour necrosis factor α (TNF-α) were measured by ultrasensitive Quantikine® ELISA kits (R&D Systems, Minneapolis, USA). Serum C-reactive protein (CRP) was determined by using an ELISA kit from Helica Biosystems, Inc. (Fullerton, CA, USA). The thiobarbituric acid reactive-species (TBARs) assay was used to assess serum products of lipid peroxidation (via malondialdehyde: MDA) and ferric reducing antioxidant potential (FRAP) assay was used to estimate total antioxidant status (TAS), as previously described (4). Serum 3-nitrotyrosine (3-NT) was measured by immunoassay (HyCult biotechnology b.v., Uden, Netherlands).

Data Analysis

Results are means ± s.e.m. Data was compared using one-way ANOVA and Fisher's test. Significance was accepted at p less than 0.05.

Results

Renal function

There was a statistically significant increase in serum creatinine and urea 3 weeks after surgery (data not shown), remaining high along the following 12 weeks. In the CRF+rhEPO group no significant effects were found (Table 1). The same group still

Table 1 – Effects of rhEPO treatment in renal function, hematological data, and inflammatory profile and redox status markers in a rat model of moderate CRF

Parameters	Final time (15 weeks)			
	Without ¾ N		With ¾ N	
	Controls	rhEPO	CRF	CRF+rhEPO
Renal function				
Creatinine (μmol/L)	39.78 ± 1.77	38.90 ± 2.64	80.44 ± 5.30 [aa]	64.53 ± 2.65
Urea (mmol/L)	13.93 ± 0.56	13.78 ± 0.61	24.21 ± 1.01 [a]	22.49 ± 0.99
Uric acid (μmol/L)	36.88 ± 9.52	49.37 ± 8.33	28.55 ± 5.35	36.88 ± 5.95
Haematological data				
Hb (g/L)	139.44 ± 3.61	128.72 ± 5.29	134.39 ± 2.01	148.81 ± 17.20
Haematocrit (pp of 0.1)	0.409 ± 0.007	0.361 ± 0.017	0.395 ± 0.006	0.439 ± 0.056
RBC ($x10^{12}$/L)	7.44 ± 0.10	6.84 ± 0.38	6.91 ± 0.14	7.83 ± 0.94
RET ($x10^{9}$/L)	383.98 ± 28.97	286.04 ± 22.01	326.09 ± 27.02	228.08 ± 17.98
Kidney trophism				
KW/BW (g/kg)	2.62 ± 0.05	2.63 ± 0.08	3.96 ± 0.39	5.12 ± 1.49
Inflammatory profile				
CRP (μg/ml)	24.78 ± 1.25	24.47 ± 0.90	25.83 ± 0.66	26.93 ± 0.98
IL-1β (pg/ml)	26.52 ± 0.94	26.19 ± 0.99	23.76 ± 0.99	27.50 ± 0.95
IL-2 (pg/ml)	36.28 ± 8.70	47.35 ± 3.50	49.34 ± 3.43	47.19 ± 3.50
TNF-α (pg/ml)	16.34 ± 1.81	17.56 ± 2.12	15.75 ± 1.96	19.85 ± 2.44
Redox status				
MDA (μmol/l)	0.27 ± 0.05	0.40 ± 0.05	0.34 ± 0.06	0.46 ± 0.10
TAS (μmol/l)	394.72 ± 51.42	475.89 ± 122.14	408.03 ± 23.62	431.19 ± 41.82
MDA/TAS	0.56 ± 0.05	0.99 ± 0.17	0.73 ± 0.21	0.94 ± 0.18

N: nephrectomy. a - $p< 0.05$, aa - $p< 0.01$ and aaa - $p< 0.001$ *vs* the control group.

showed a trend to increased values of KW/BW when compared with CRF group (Table 1).

Haematological data

Three weeks after nephrectomy, the CRF animals showed a significant decrease of RBC, Hct and Hb (data not shown), normalizing after 9 weeks after surgery and remaining stables until the end. The CRF+rhEPO group showed a trend to increased RBC, HTC and Hb (Table 1).

Inflammatory and redox status markers

No significant differences for serum CRP, IL-1β, IL-2 and TNF-α levels were observed, but an increment of TGF-β1 was found in the CRF rats vs the control. The CRF+rhEPO group produced no changes for serum CRP, IL-1β, IL-2 and TNF-α concentration (Table 1), but imposed a further increase in TGF-β1 vs the CRF animals (Fig. 1A).

No statistically significant changes were found between CRF and control group for serum MDA and TAS, but a significantly higher 3-NT was found. In the CRF+rhEPO

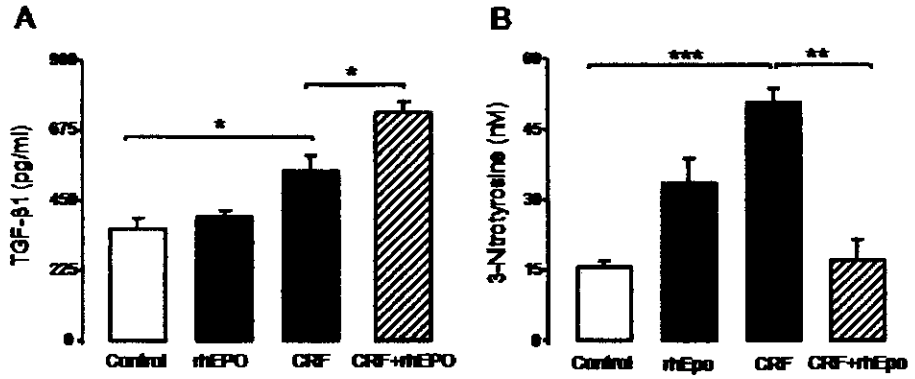

Figure 1- Serum levels of transforming growth factor β1 (TGF-β1) and 3-nitrotyrosine (3-NT) for the groups under study.

rats there was a trend to increased serum MDA and TAS values (Table 1). However, a statistically significant reduction of serum 3-NT levels was found between the group of CRF with rhEPO and those without rhEPO therapy (Fig. 1B).

Conclusions

Our data are consistent with a sustained moderate degree of CRF with development of moderate and corrected anaemia. The remnant kidney showed a proliferative profile, with increased mass (hypertrophism), increased levels of serum TGF-β1 and oxidative stress. rhEPO treatment was able to partially attenuate renal function markers, totally correct anaemia, also showing a proliferative and antioxidant action.

Thus, rhEPO therapy might be recommended in moderate CRF stages in order to efficiently correct not only the underlying anaemia but also the deleterious cardio-renal effects, due to a renoprotective action on the remnant kidney.

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