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The Effect of Recombinant Human Erythropoietin (r-Hu EPO) Administration on the Blood Chemistries and Composition in Uremic Rats

Wpływ podawania rekombinowanej ludzkiej erytropoetyny (r-Hu EPO) na parametry biochemiczne i morfologiczne krwi szczurów z doświadczalną mocznicą

Recombinant human erythropoietin (r-Hu EPO) therapy improves the anaemia of patients on chronic hemodialysis (3, 14), on peritoneal dialysis (6, 12) and those with chronic renal failure who have not yet started any form of renal replacement therapy (8, 9). In the last category there is concern that r-Hu EPO therapy may be associated with deterioration of the reserve renal function (7). But new data showed that even during more prolonged treatment the correction of anaemia does not have a major determinental effect on renal function (4).

The study was undertaken to examine the influence of r-Hu EPO therapy on renal function and rheorogical erythrocytes finding in uremic rats.

MATERIAL AND METHODS

We studied the effect of r-Hu EPO administration in 30 uremic rats and 30 control healthy animals. Male Wistar rats were used. The uremic anaemia in rats was developed as the result of five-sixth nephrectomy during the fourth month after the surgery (10). These nephrectomized rats were divided into three groups, each of them included 8 rats.

The rats of one group were treated with r-Hu EPO 600 U/kg of body mass, the other group was treated with r-Hu EPO 300 U/kg of body mass. Erythropoietin was administered intraperitoneally. Theses doses were given three times per week. Ten nephrectomized rats were not r-Hu EPO treated and served as uremic control. The others 30 male rats were used as r-Hu EPO treated non-uremic control. Ten rats were treated with r-Hu EPO 600 U/kg of body mass, the others were treated with r-Hu EPO 300 U/kg of body mass in similar manner as uremic groups. And other 8 rats were not treated with r-Hu EPO. The blood samples were collected after exsanguination of the animals. The hematocrit, red blood cells, hemoglobin and some indicators as MCHC, MCH and MCV were

determined in the samples using Cell-Dyn 1500 Sequoia-Turner. The urea and creatinine levels were measured using Technicon RA-1000 analyzer and electrolytes Na^+, K^+, Ca^{++} were measured in the serum samples using Ciba Corning Analyzer.

RESULTS

The hemoglobin concentration, hematocrit level and erythrocytes count increased significantly after 300 U/kg r-Hu EPO adminstration (Table 1). In comparison with healthy r-Hu EPO 600 U/kg treated rats, higher values of erythrocytes were obtained in uremic rats treated with similar doses (9.014^6 vs 7.302^6 cells per milimeter^3). After two weeks of 600 U/kg r-Hu EPO treatment the MCV increase was statistically significant and reached values 59.17 $\pm$2.2 in healthy rats and 55.38 $\pm$2.5 in uremic rats. There was only slight increase of white blood cells which did not reach significance. Table 2 shows the middle value of sodium, kalium and calcium levels in the investigated groups of rats. There was statistically significant decrease in the kalium and calcium levels after r-Hu EPO treatment of healthy rats.

Table 3 shows the urea and creatinine levels in healthy and uremic rats after r-Hu EPO administration. There were no differences in the levels of urea and creatinine in healthy rats after r-Hu EPO treatment. Whereas significant increase in creatinine and urea levels was observed in uremic rats after r-Hu EPO administration, both in 300 U/kg and 600 U/kg treated rats. The urea level in uremic rats was 135.4 $\pm$17.5 mg/dl and we observed the increase to 254.14 mg/dl in 300 U/kg treated uremic rats 261.41 $\pm$54.0 mg/dl in 600 U/kg r-Hu EPO treated rats.

The creatinine level was 2.55 $\pm$0.09 mg/dl in uremic non r-Hu EPO treated rats. Two fold increased level was found in 300 U/kg r-Hu EPO treated rats, it reached values 4.3 $\pm$0.37 mg/dl and 4.0 $\pm$0.7 mg/dl in 600 U/kg treated rats.

DISCUSSION

The beneficial effect of human recombinant erythropoietin (r-Hu EPO) on erythrocyte and reticulocyte count and on transfusion requiremens, has repeatedly been demonstrated in maintenance hemodialysis patients. This has been related to the disappearance of anemic symptoms and the improvement of the overal quality of life and physical function and correction of immunological deficiency associated with the uremic state (13). Multicenter investigations are focused on the establishment of optimal model of erythropoietin treatment in maintenance hemodialysis anemic patients. During these investigations there are numerous additional factors influencing r-Hu EPO treatment effects.

We studied rHuEPO adminstration in congenital groups of rats with developed uremic anemia after five-sixth nephretoctomy and in similar groups of healthy rats. The suitable doses were administered peritoneally, the erythropoietin is almost completly absorbed from the empty peritoneal cavity after

Table 1. The blood cells findings of healthy and uremic rats after rHU EPO therapy

	Healthy rats			Uremic rats		
	control	300 U/kg r-Hu EPO	600 U/kg r-Hu EPO	control	300 U/kg r-Hu EPO	600 U/kg r-Hu EPO
Hematocrit	37.2 ±2.4	41.5 ±3.0*	43.6 ±2.8**	34.6 ±1.5	40.3 ±1.3**	49.3 ±1.4**
Hemoglobin	13.9 ±0.8	16.3 ±0.8*	15.3 ±1.4**	12.1 ±0.3	15.0 ±1.04**	18.5 ±0.6**
Erythrocytes	6.857 ±600	7.294 ±193*	7.302 ±2.3**	5.879 ±652	7.142 ±1.45	9.014 ±612
Leukocytes	9.900 ±1.155	11.992 ±2.343	8.814 ±4.428	10.685 ±2.492	12.714 ±3.484	11.27 ±1.048
MCHC	37.4 ±1.0	39.5 ±1.1**	35.0 ±0.96*	37.2 ±0.6	39.2 ±0.9**	36.5 ±1.0
MCH	20.3 ±1.1	22.5 ±0.7**	20.9 ±0.8	19.1 ±0.9	21.4 ±1.0**	19.9 ±0.8
MCV	54.4 ±3.4	56.9 ±2.5	59.1 ±2.2**	51.3 ±2.1	54.4 ±2.3	55.3 ±2.5**

* $p < 0.05$ comparison to control rats
** $p < 0.001$ comparison to control rats

Table 2. The comparison of electrolytes levels in the healthy and uremic rats treated with r-Hu EPO

	Healthy rats			Uremic rats		
	control	300 U/kg r-Hu EPO	600 U/kg r-Hu EPO	control	300 U/kg r-Hu EPO	600 U/kg r-Hu EPO
Na^+ mmol/L	143.4 ±2.9	142.7 ±2.5	144.8 ±2.6	144.2 ±1.6	144.5 ±5.1	142.2 ±2.8
K^+ mmol/L	6.1 ±0.4	6.2 ±0.4	5.6 ±0.6	6.8 ±0.3	7.6 ±0.9	6.7 ±0.1
Ca^{++} mmol/L	1.43 ±0.02	1.38 ±0.05	11.39 ±0.08	1.42 ±0.03	1.24 ±0.12	1.4 ±0.1

Table 3. The urea and creatinine levels in healthy and uremic rats treated r-Hu EPO

	Healthy rats			Uremic rats		
	control	300 U/kg r-Hu EPO	600 U/kg r-Hu EPO	control	300 U/kg r-Hu EPO	600 U/kg r-Hu EPO
Urea mg/%	31.14 ±2.3	36.5 ±11	32.0 ±1.4	135.4 ±17.5	254.1 ±18**	261.4 ±54**
Creatinine mg/%	0.47 ±0.04	0.61 ±0.1	0.54 ±0.07	2.15 ±0.09	4.3 ±0.37**	4.0 ±0.7**

* statistical significance $p < 0.05$ comparison with control rats

** statistical significance $p < 0.001$ comparison with control rats

24 hours in treated animals. We observed the correction of uremic anemia to normal values during two weeks adminstration of r-Hu EPO 300 U/kg of body mass.

Administration of r-Hu EPO 600 U/kg of body mass to uremic rats caused abnormal higher values of erythrocytes count (9.014^6 cells per mm^3) with hemoglobin concentration range 18.5 ± 0.6 mg/dl. In the control healthy groups of rats we did not observe dose-depending raise in erythrocyte count. In accordance with other authors only insignificant changes of leukocyte count, serum sodium and potassium levels were observed (1, 7). The founded decreased level of ionized calcium in r-Hu EPO 300 U/kg of body mass treatments is difficult to explain. Obtained values of MCV were increased to 55.88 ± 2.5 under 600 U/kg r-Hu EPO treatment and these results can be related to raised reticulocyte and young erytrocytes counts. The risk of the acceleration of the renal disease progression under r-Hu EPO therapy is widely investigated (1, 2, 11). Most of the authors declare the opinion that there is no change in the progression rate of renal disease when judged by slope of the reciprocal creatinine curve. We found the statistically significant increase of urea and creatinine levels in rats treatment r-Hu EPO. Experimental studies performed on the r-Hu EPO treatment rats (2) suggested risk of the progression of the renal function involving the hypertension, glomerular hyperfiltration and accelerated glomerulosclerosis. Considering the safety of r-Hu EPO therapy in predialysis patients it is necessary to control some biochemicals parameters and blood pressure to avoid side effects and progression of renal disease.

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STRESZCZENIE

Badano wpływ podawania rekombinowanej ludzkiej erytropoetyny na zachowanie się parametrów biochemicznych i morfologię krwi u szczurów z wywołaną anemią mocznicową w wyniku 5/6 nefrektomii. Stwierdzono statystycznie istotny wzrost wartości mocznika i kreatyniny u szczurów, którym podawano erytropoetynę, co sugeruje ryzyko progresji niewydolności nerek podczas leczenia erytropoetyną.