ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XLVIII, 5 SECTIO D 1993

Klinika Nefrologii, Instytut Chorób Wewnętrznych, Akademia Medyczna im. K. Marcinkowskiego w Poznaniu
Kierownik: prof. dr hab. med. Kazimierz Bączyk

Irena PIETRZAK, Kazimierz BĄCZYK, Włodzimierz KUBIAK

Recombinant Human Erythropoietin Administration Improves Thiamine Content in Blood and Erythrocytes Transketolase Activity in Pre-Dialyzed Patients

Podawanie rekombinowanej ludzkiej erytropoetyny (r-Hu EPO) poprawia zawartość tiaminy we krwi oraz aktywność transketolazy w erytrocytach u chorych w okresie przeddializacyjnym

Recombinant human erythropoietin (r-Hu EPO) has proved to be effective in correcting anemia of dialyzed patients. Since 1987 reports have been published announcing the introduction of this hormone to the treatment of anemia in pre-dialyzed patients, too (2, 6, 7, 8, 12, 13). Besides successful correction of anemia no evidence of acceleration of the progression of renal failure has been noted (2, 7, 8, 9). The question of whether r-Hu EPO can also correct metabolic disturbances in predialzyed patients is to be answered. These metabolic disturbances comprises, among others, the decrease in thiamine content in blood and the deterioration of erythrocytes transketolase activity (ETKA) we described in another paper (11). However, as to the present we did not notice any publication concerning the impact, of r-Hu EPO administration on thiamine content in blood and on the ETKA.

The purpose of our study was therefore to determine the influence of r-Hu EPO therapy in pre-dialyzed patients on thiamine level in plasma and erythrocytes and on the ETKA. Additionally, we wanted to answer the question if there was any parallel change in hematocrit index, thiamine level in blood and the ETKA in patients we observed during r-Hu EPO therapy.

MATERIAL AND METHODS

Twenty normal persons and twelve patients with primary glomerulopathies in the final stage of renal disease (ESRD) participated in the sutdy. (Table 1).

Serum iron levels and TIBC were found within normal limits. There were no signs and symptoms of vitamin B_{12} and folic acid deficiency or aluminium intoxication. During the study all our patients received ferrous sulphate supplementation (100 mg up to 2 times/day) intramuscularly of orally. Thiamine hydrochloride amounting to 10-15 mg/day was given to the patients two months prior and throughout the whole study.

The whole study, lasting 20 weeks, comprised three periods; r-Hu EPO was given intravenously or subcutaneously as depicted in Table 2.

Venous blood was drawn before and every fourth week up to the 20th week of r-Hu EPO therapy. Thiamine in plasma and erythrocytes (free and total) was determined using fluorimetric method by Blum and Markel (1). ETKA – was evaluated using the photocolorimetric method by Bruns (3) modified by Dische (4). The hematocrit index and creatinine were measured using routine methods. Statistical analysis was made using Student's test – t for paired and independent groups.

RESULTS

The mean values of total and free thiamine in erythrocytes and plasma in patients studied, were found significantly higher than those of normal persons (Table 3, 4). The mean free and total thiamine in erythrocytes raised significantly during the i. v. period of study. After the next 12 weeks of s.c. r-Hu EPO administration, the free and total thiamine content in erythrocytes showed further increase (Table 3). It is worth to mention that this increase in free and total thiamine content in erythrocytes holds true for all examined patients (Fig. 1 and 2).

Plasma levels of free thiamine increased significantly already in the fourth week of r-Hu EPO administration and this amelioration of its content persisted up to the completion of the study. On the other hand, plasma levels of total thiamine did not change significantly after r-Hu EPO administration through the study except in the 20th week when it rose distinctly (Table 4, Figs. 3 and 4).

ETKA in all pre-dialyzed patients, immediately before starting r-Hu EPO treatment was found to be insignificantly lower than that in normal persons. Similarly to thiamine in erythrocytes the ETKA increased significantly during the i.v. period and this increase persisted up to the completion of study. This increase took place in all patients studied (Table 5 and Fig. 5). Although this increase in ETKA was remarkable it did not result in normalization of its value.

Hematocrit index in pre-dialyzed patients immediately before starting r-Hu EPO treatment was found to be significantly lower than that in normal persons. It rose significantly during the i.v. phase and persisted during the s.c. phase. This increase was observed in all patients studied (Table 6 and Fig. 6).

DISCUSSION

Our study confirmed the reports of other authors as to the beneficial effect of r-Hu EPO therapy on anemia in pre-dialyzed patients without deterioration of renal function (7, 8, 9). Furthermore, our results showed that the correction of anemia was associated with increase in free and total thiamine level in erythrocytes and plasma, with concomitant rise in ETKA, independently of the route of administration (i.v., or s.c.) and the dose of r-Hu EPO.

It is worth mentioning that thiamine is utilized as a co-factor for oxidation of pyruvic acid and alfa-ketoglutaric acid in the TCA cycle and also as

Table 1. (Mean ± SE)

Groups of subject	N	Age (years)	Sex	Pcr mg/dl	Duration of ESRD before enrolling in the study (years)
Normal persons	20	34.3 ±2.05	12 men 8 women	0.90 ±0.04	–
Pre-dialyzed ESRD pts	12	43.0 ±6.67	2 men 10 women	6.85 ±0.53	4.2 ±0.43

Table 2

Period of therapy	Therapy duration (weeks)	Dose UI/kg b.w.	Times in week	Route of administration
I	8	50	3	i.v.
II	4	25	2	s.c.
III	8	25	1	s.c.

Table 3. Free (F) and total (T) erythrocytes thiamine (Th) levels (μmol/l) and statistical evaluation in normal persons and pre-dialyzed patients (PDP) before (mean ± SE) and during 4, 8, 12, 16, 20 weeks of r-Hu EPO treatment (mean values and mean differences between PDP and subsequent periods of study)

Groups		FTh	p	TTh	p
Normal persons	n=20	0.090±0.009	< 0.5	0.125±0.010	< 0.5
PDP	n=12	0.175±0.004		0.222±0.007	
PDP		0.175		0.222	
r-Hu EPO	4	0.182±0.007	< 0.05	0.238±0.016	< 0.01
	8	0.188±0.013	< 0.01	0.249±0.027	< 0.001
	12	0.191±0.016	< 0.001	0.259±0.037	< 0.001
	16	0.195±0.020	< 0.001	0.262±0.040	< 0.001
	20	0.204±0.029	< 0.001	0.267±0.045	< 0.001

Table 4. Free (F) and total (T) plasma thiamine (Th) levels (μmol/l) and statistical evaluation in normal persons and pre-dialyzed patients (PDP) before (mean ± SE) and during 4, 8, 12, 16, 20 weeks of r-Hu EPO treatment (mean values and mean differences between PDP and subsequent periods of study)

Groups		FTh	p	TTh	p
Normal persons	n=20	0.159±0.014	< 0.3	0.279±0.026	< 0.6
PDP	n=12	0.335±0.007		0.407±0.009	
PDP		0.335		0.407	
r-Hu EPO	4	0.344±0.009	< 0.02	0.408±0.001	< 0.8
	8	0.348±0.013	< 0.02	0.411±0.004	< 0.4
	12	0.350±0.015	< 0.01	0.415±0.008	< 0.3
	16	0.356±0.021	< 0.01	0.420±0.013	< 0.1
	20	0.358±0.023	< 0.01	0.424±0.017	< 0.005

Table 5. ETKA (μmol/ml/min) in normal persons and PDP (mean ± SE) and during 4, 8, 12, 16, 20 weeks of r-Hu EPO therapy (mean values and mean differences between PDP and subsequent periods of study)

Groups		ETKA	p
Normal persons	n=20	2.39±0.10	< 0.1
PDP	n=12	1.53±0.10	
PDP		1.53	
r-HuEPO	4	1.75±0.22	< 0.01
	8	1.82±0.29	< 0.001
	12	1.90±0.37	< 0.001
	16	1.93±0.40	< 0.001
	20	2.07±0.54	< 0.001

Table 6. Hematocrit index in normal persons and PDP (mean ± SE) and during 4, 8, 12, 16, 20 weeks of r-Hu EPO therapy (mean values and mean differences between PDP and subsequent periods of study)

Groups		Ht%	p
Normal persons	n=20	42.3± 1.07	< 0.001
PDP	n=12	24.3± 1.03	
PDP		24.3	
r-HuEPO	4	29.8± 5.5	< 0.001
	8	33.4± 9.1	< 0.001
	12	34.8±10.5	< 0.001
	16	36.3±12.0	< 0.001
	20	36.8±12.5	< 0.001

a coenzyme of transketolase in the pentose phosphate pathway of nonoxidative glycolysis (5).

In experimental conditions prolonged thiamine deficiency resulted in decrease in ETKA (10). It is probable that in uremic conditions a diminished utilization of thiamine exists resulting, among others, in decreased ETKA. This is what we have just observed in our study because even increased levels of thiamine in patients receiving this vitamin were accompanied by the decreased ETKA. Such a situation may be regarded as the funcitonal deficiency of thiamine. The functional deficiency of thiamine is known to impair the oxydation and phosphorylation processes in the body.

The increased content of thiamine in plasma and in erythrocytes means that its increment concerns the whole blood. The explanation of this finding comprises two possible factors. One of them may be the release of thiamine stored in the body, the second one – the acceleration of intestinal absorption of this vitamin. We are prone to accept both of these factors because the amount of thiamine stored in the body seems to be too little to explain convincingly the changes we observed. Furthermore, the increased intestinal absorption of thiamine would result from the hematological improvement with subsequent amelioration of the active transport of thiamine across the intestinal wall.

The question how to explain the greater increase of thiamine in erythrocytes than in plasma during r-Hu EPO therapy is very dificult to answer. One of the possible approaches is the assumption that the improved oxygen supply increases the carbohydrate metabolism in erythrocytes. This is stimulative for further influx of thiamine pyrophosphate into erythrocytes. Such explanation is supported by our results indicating significant increase in ETKA in the course of r-Hu EPO therapy.

REFERENCES

1. Blum K. U., Merkel D.: Klin. Biochem. 10, 437, 1974.
2. Brown C. D., Friedman E. A.: Stable Renal Function and Benign Course of Azotemic Diabetics Treated with Erythropoietin (EPO) for One Year. Kidney Int. 35, 198, 1989.
3. Bruns F. H., Dunwald E., Nattman E.: Stoffwechsel von Riboso-5-phosphat in Hamolysaten. III Quantitative Bestimmung von Sedoheptulose-7-phosphat und einige Eingeschaften der Transketolase der Erythrocyten und des Blutserums. Biochem. Z. 330, 497, 1958.
4. Dische Z.: Qualitative and Quantitative Colometric Determination of Heptose. J. Biol. Chem. 204, 983, 1953.
5. Dreyfus P. M.: Clinical Application of Blood Transketolase Determination. The New Engl. J. M. 20, 596, 1962.
6. Eschbach J. W., et al.: Correction of the Anemia of End-Stage Renal Disease with Recombinant Human Erythropoietin. New Engl. J. Med. 316, 73, 1987.
7. Graf H., et al.: Effectiveness and Safety of Recombinant Human Erythropoietin in Predialysis Patients. Nephron, 61, 1, 1992.
8. Kleinman K. S., Schweitzer S.: The Use of Recombinant Human Erythropoietin in the Correction of Anemia in Predialysis Patients and Its Effect on Renal Function: Double Blind Placebo Controlled Trial. Am. J. Kidney Dis. 14, 496, 1989.
9. Lim V. S., et al.: Effect of Recombinant Human Erythropoietin on Renal Function in Humans. Kidney Int. 37, 131, 1990.
10. Lonergan E. T., Semar M., Lange K.: Transketolase Activity in Uremia. Arch. Intern. Med. Vol. 126, 851, 1970.
11. Pietrzak I., Młynarczyk M.: Zawartość tiaminy we krwi oraz katywność transketolazy w erytrocytach u chorych ze schyłkową niewydolnością nerek (submitted for publication).
12. Stone W. J., et al.: Treatment of Anemia of Predialysis Patients with Recombinant Human Erythropoietin: a Renadomized Placebo-Controlled Trial. Am. J. Med. Sci. 296, 171, 1988.
13. Teehan B. P., et al.: Double-Blind. Placebo-Controlled Study of the Therapeutic Use of Recombinant Human Erythropoietin for Anemia Associated With Chronic Renal Failure in Predialysis Patients. Am. J. Kidney Dis. Vol. XVIII, No. 1 (July) 50, 1991.

STRESZCZENIE

U 12 chorych ze schyłkową niewydolnością nerek obserwowano wpływ leczenia erytropoetyną (r-Hu EPO) na zawartość witaminy B1 (tiamina-Th) w plazmie i erytrocytach oraz na aktywność transketolazy w erytrocytach (ETKA). W trakcie dożylnego podawania r-Hu EPO stwierdzono znamienny wzrost stężenia Th w erytrocytach z jednoczesnym wzrostem ETKA. W okresie podskórnego podawania r-Hu EPO obserwowano dalszy wzrost tych wartości. Stężenie Th w plazmie wzrastało mniej wyraźnie w okresie 20 tygodni leczenia. Na podstawie uzyskanych wyników można przyjąć, że stosowanie r-Hu EPO u chorych w okresie przeddializacyjnym poprawia uszkodzoną w mocznicy przemianę wlęglowodanową w której Th i ETKA odgrywają znaczącą rolę.

LIST OF FIGURES

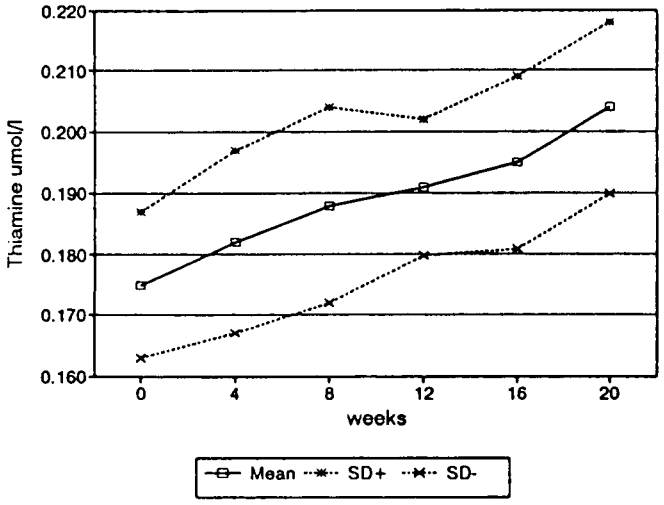

Fig. 1. Influence of r-Hu EPO on free thiamine level in erythrocytes in PDP

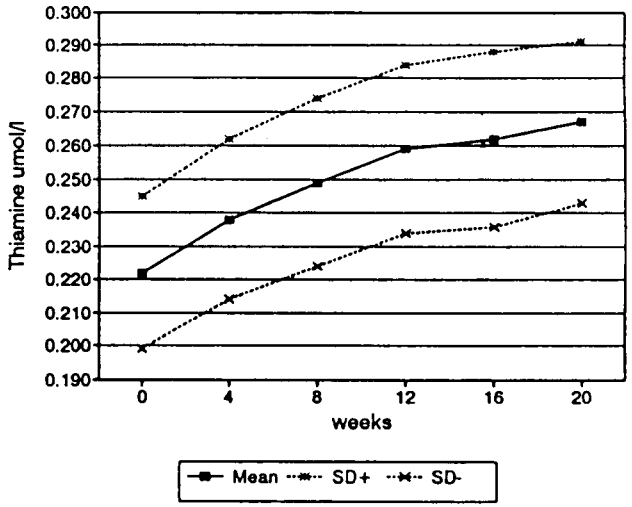

Fig. 2. Influence of r-Hu EPO on total thiamine level in erythrocytes in PDP

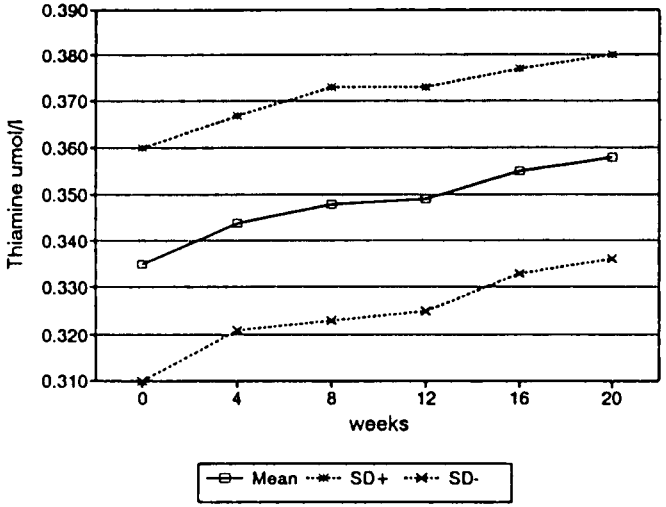

Fig. 3. Influence of r-Hu EPO on free thiamine level in plasma in PDP

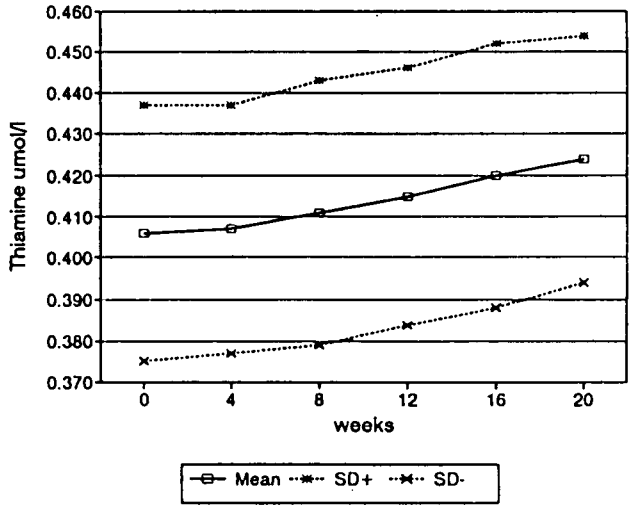

Fig. 4. Influence of r-Hu EPO on total thiamine level in plasma in PDP

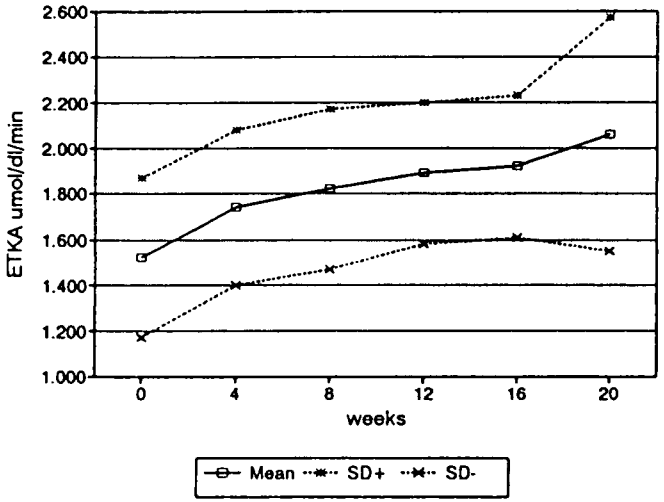

Fig. 5. Effect of r-Hu EPO on ETKA in PDP

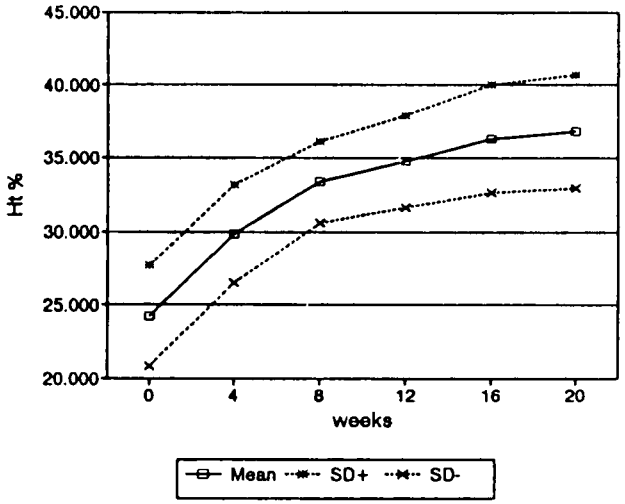

Fig. 6. Effect of r-Hu EPO on Ht in PDP