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Influence of Long-Term Erythropoietin Treatment on Plasma Levels of Calcium-Phosphate Related Hormones in Haemodialyzed Uraemic Patients

Gospodarka wapniowo-fosforanowa u hemodializowanych chorych na przewlekłą niewydolność nerek leczonych ludzką rekombinowaną erytropoetyną

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Exacerbation of secondary hyperparathyroidism (as manifested by calcium deposits and local inflammation in periarticular tissues) has been reported in haemodialyzed (HD) uraemic patients treated with recombinant human erythropoietin (19). However, short-term treatment with recombinant human erythropoietin (r-Hu EPO) did not influence significantly plasma levels of parathyroid hormone (PTH), calcitonin (CT) and 25-hydroxycholecalciferol ($25\text{-}OHD_3$) in uraemic HD patients (9, 10).

The present study aimed to assess the effect of long-term r-Hu EPO therapy for 12 months on plasma PTH, CT, $25\text{-}OHD_3$ and 1,25-dihydroxycholecalciferol ($1,25\text{-}(OH)_2D_3$) in uraemic HD patients.

MATERIAL AND METHODS

This study was performed in a group of 46 HD uraemic patients and in 11 healthy subjects (7 males and 4 females aged 39.4 $\pm$2.43 years). HD patients were divided into three groups.

The first one, so-called „EPO group", consisted of 17 HD patients (11 males and 6 females, aged 44.7 $\pm$2.1 years, mean duration of HD treatment 35.2 $\pm$8.0 months) with chronic renal anaemia (Hct value less than 28%) which was treated with r-Hu EPO for 12 monts.

The second group designed as the „No-EPO I" group comprised 17 HD patients (9 males and 8 females, aged 41.8 $\pm$1.9 years, mean duration of HD therapy 41.8 $\pm$19.1 months) who showed

a similar degree of anaemia as patients of the EPO group but were not treated with r-Hu EPO. Patients of this group were monitored clinically and biochemically as patients of the EPO group.

The third group (so-called „No-EPO II" group) consisted of 12 HD patients (5 males and 7 females, aged 44.0 ±1.7 years, mean duration of HD treatment 80.5 ±15.6 months) not treated with r-Hu EPO, with a Hct value and Hb concentration similar to those observed in EPO group patients after 12 months of the treatment.

In patients of the EPO group the dose of this hormone (Recormon, Boehringer Mannheim) at the beginning of treatment was 20 units/kg body weight three times a week, administered subcutaneously 10 minutes before the subsequent dialysis session. The target haematocrit value was 30–34%. In patients of the EPO and No-EPO I group the following parameters were estimated before (0), after 3, 6, 9 and 12 moths of r-Hu EPO therapy (EPO-group) or intensive clinical monitoring (No-EPO I group), respectively: Hct, Hb, plasma level of calcium (Ca), inorganic phosphate (P), osteocalcin (OC), PTH, CT, 25-OHD_3 and 1,25-$(OH)_2D_3$ and serum alkaline phosphatase (AP) activity. In the No-EPO II group and in healthy subjects all biochemical and hormonal parameters were estimated only once.

PTH (14) and OC (14) were estimated by radioimmunoassay, 25-OHD_3 by a radiocompetitive method (14), 1,25-$(OH)_2D_3$ by HPLC (18) and all other biochemical parameters by routine methods (8). Results obtained in this study were evaluated statistically using Student's T-test for paired and unpaired variables respectively.

RESULTS

As shown on Table I r-Hu EPO treatment was followed by a significant increase in Hct and Hb levels. In patients of the No-EPO I group a statistically significant increase of Hct and Hb levels was also observed during the last 3 months of monitoring. Hct and Hb values in patients of the No-EPO II group and in healthy subjects were 34.9 ±1.1%, 7.4 ±0.3 mmol/l and 41.9 ±1.0% and 8.9 ±0.3 mmol/l, respectively.

Plasma Ca concentration increased significantly after 3 ($p < 0,01$) and 9 ($p < 0,05$) months of r-Hu EPO therapy (EPO group). In contrast in patients of the No-EPO I group plasma Ca did not change significantly during the whole period of monitoring. No significant changes in plasma P were noticed either in patients of the EPO or No-EPO I group (Table I). In patients of the No-EPO II group and in healthy subjects plasma Ca concentrations were 2.23 ±0.06 mmol/l and 2.38 ±0.05 mmol/l and plasma P levels 1.73 ±0.21 mmol/l and 1.17 ±0.05 mmol/l, respectively.

AP activity was significantly higher both in patients of the EPO and No-EPO I group than in healthy subjects (= 827 ±106 nmol/l/s, $p < 0,005$) but did not differ significantly from patients of the No-EPO II group (= 1444 ±260 nmol/l/s). Rhu-EPO treatment was followed by a significant decrease of AP ($p < 0.05$) only after 12 moths of therapy (Table I). During r-Hu EPO therapy a statistically significant increase of plasma OC level ($p < 0.01$) was noticed only after 9 months of treatment (Table I).

In patients of the No-EPO I group plasma OC level did not change significantly. In patients of the No-EPO II group and in healthy subjects plasma

Table 1. Haematocrit (Hct), haemoglobulin (Hb), plasma calcium (Ca), inorganic phosphate (P), osteocalcin (OC), 25-hydroxycholecalciferol ($25\text{-}OHD_3$) levels and plasma alkaline phosphatase activity (AP) in haemodialyzed uraemic patients treated with r-Hu EPO (EPO group) and not treated with r-Hu EPO (No-EPO I group) before (O) and after 3, 6, 9 and 12 months of monitoring. Mean values ±SEM

Months of monitoring		0	3	6	9	12
Hct	EPO	21.8 ±0.9	27.8 ±1.0^z	28.5 ±1.5^y	33.1 ±1.5^z	32.6 ±1.0^z
%	No-EPO I	21.4 ±0.5	21.0 ±0.4	20.6 ±0.5	23.7 ±0.8^x	24.2 ±0.6^y
Hb	EPO	4.54 ±0.16	5.71 ±0.19^z	5.77 ±0.27^y	6.52 ±0.31^z	6.57 ±0.23^z
mmol/l	No-EPO I	4.40 ±0.16	4.27 ±0.11	4.26 ±0.11	4.79 ±0.15	5.00 ±0.17^y
Ca	EPO	2.19 ±0.06	2.34 ±0.08^x	2.23 ±0.05	2.32 ±0.07^w	2.39 ±0.15
mmol/l	No-EPO I	2.27 ±0.05	2.31 ±0.07	2.33 ±0.06	2.26 ±0.07	2.30 ±0.07
P	EPO	1.79 ±0.13	1.81 ±0.16	1.68 ±0.14	1.68 ±0.19	1.73 ±0.14
mmol/l	No-EPO I	1.72 ±0.14	1.71 ±0.10	1.42 ±0.12	1.46 ±0.12	1.65 ±0.13
OC	EPO	48.65 ±3.57	50.47 ±4.00	52.65 ±3.81	65.35 ±4.91^x	57.68 ±2.24
ng/ml	No-EPO I	51.12 ±5.20	59.12 ±3.88	50.59 ±4.79	48.41 ±5.00	46.24 ±3.41
$25\text{-}OHD_3$	EPO	24.4 ±3.6	26.3 ±2.1	23.9 ±2.2	24.5 ±1.4	24.9 ±3.0
ng/ml	No-EPO I	25.4 ±2.9	25.0 ±2.7	23.9 ±1.6	24.9 ±2.3	25.6 ±3.1
AP	EPO	1632.18 ±211.39	1559.88 ±203.09	1576.87 ±270.08	1450.21 ±219.08	1223.30 ±126.98^x
nmol/l/s	No-EPO I	2567.26 ±685.49	2439.92 ±651.99	2939.59 ±896.08	2654.59 ±792.00	2464.95 ±467.00

Statistical significance between "O" and respective values: $w = p < 0.05$, $x = p < 0.01$, $y = p < 0.005$, $z = p < 0.001$.

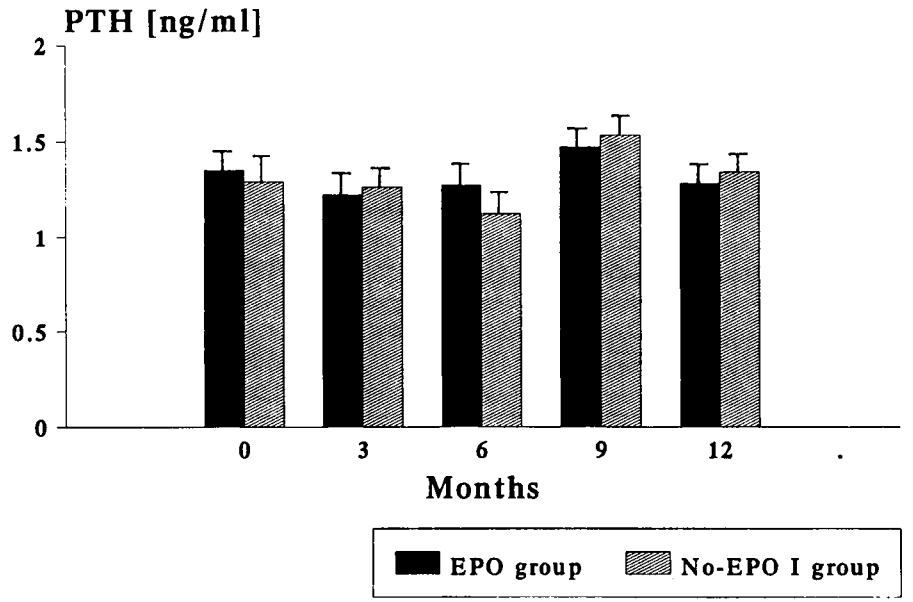

Fig. 1. Plasma parathyroid hormone levels in haemodialyzed uraemic patients treated (EPO group) and not treated (No-EPO I group) with r-Hu EPO (0) and after 3, 6, 9 and 12 moths of observation

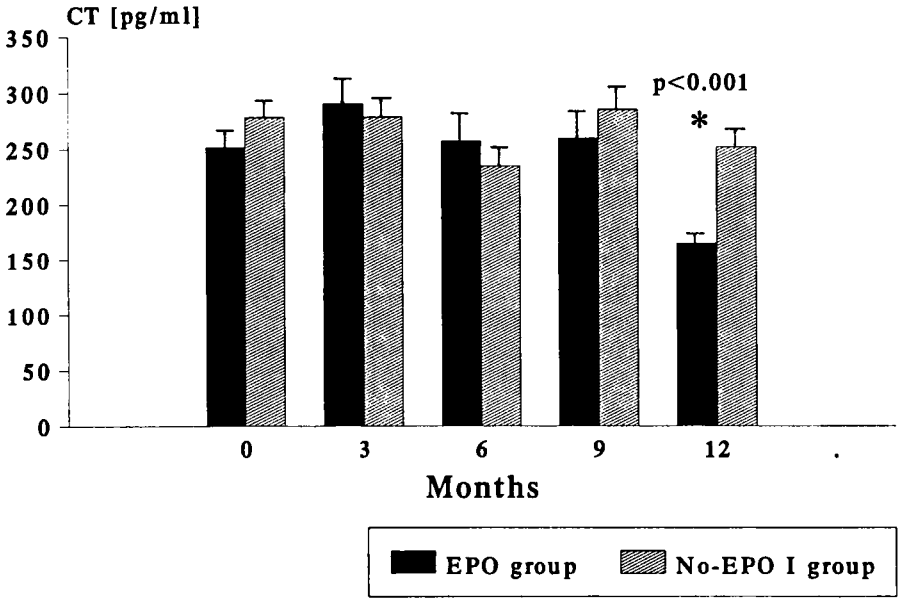

Fig 2. Plasma calcitonin (CT) levels in haemodialyzed uraemic patients treated (EPO group) and not treated (No-EPO I group) with r-Hu EPO before (0) and after 3, 6, 9 and 12 months of observation

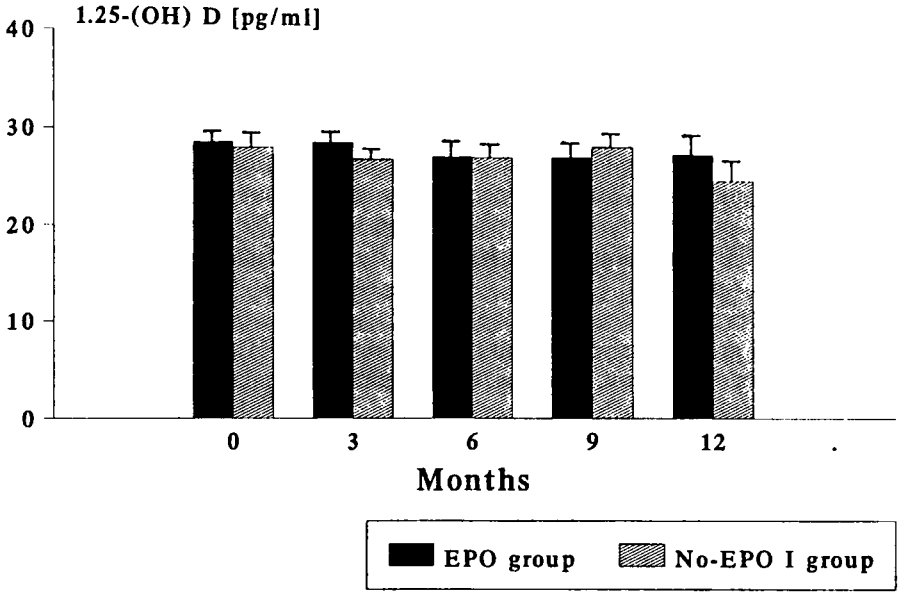

Fig 3. Plasma 1.25-dihydroxycholecalciferol (1.25-$(OH)_2D_3$) levels in haemodialyzed uraemic patients treated (EPO group) and not treated (No-EPO I group) with r-Hu EPO before (0) and after 3, 6, 9 and 12 months of observation

OC levels were 27.0 $\pm$2.6 ng/ml and 24.4 $\pm$5.5 ng/ml, respecitvely, and were significantly lower than those in patients of the EPO and No-EPO I group, respectively, at the beginning of the study ($p < 0.005$).

As it can be seen on Table I plasma levels of 25-OHD_3, did not change significantly either in patients of the EPO or No-EPO I group during 12 months of monitoring and did not differ significantly from values found in patients of the No-EPO II group (= 27.2 $\pm$4.5 ng/ml) and healthy subjects (= 20.6 $\pm$2.9 ng/ml).

One year of r-Hu EPO therapy did not influence significantly plasma PTH levels (Fig. 1). Patients of the No-EPO II group showed similiar PTH values (= 1.61 $\pm$0.23 ng/ml) as patients of the EPO and No-EPO I group, respectively, but higher values than healthy subjects (= 0.49 $\pm$0.09 ng/ml; $p < 0.001$).

Plasma CT levels assessed in patients of the EPO and No-EPO I group were significantly higher than in healthy subjects (= 38.91 $\pm$8.87 pg/ml, $p < 0.001$) but did not differ from those of patients of the No-EPO II group (= 304.12 $\pm$ 52.84 pg/ml). Only after 12 months of r-Hu EPO therapy a significant decrease of plasma CT concentration was noticed (Fig. 2).

As it can be seen in Fig. 3, r-Hu EPO treatment did not influence significantly plasma 1,25-$(OH)_2D_3$ concentration. However, both in patients of the EPO and No-EPO I group as well as in patients of the No-EPO II group (= 23.85 $\pm$2.52 pg/ml) plasma 1,25-$(OH)_2D_3$ levels were significantly lower than in healthy subjects (= 62.21 $\pm$11.81 pg/ml, $p < 0.05$).

DISCUSSION

In order to assess the influence of long-term r-Hu EPO treatment on the haematological status three control groups were studied. Data obtained in patients of the No-EPO I group revealed an unexpected slight but statistically significant increase of the Hct value and Hb concentration during the last three months of monitoring. Thus it seems very likely that improvement of the haematological status observed in patients on r-Hu EPO therapy is caused not only by r-Hu EPO administration but also by other factors such as intensive clinical monitoring.

As plasma levels of calcium-phosphate related hormones found in patients of the No-EPO II group were of similar magnitude as in r-Hu EPO treated patients after 12 months of therapy, it seems, that haematological improvement induced by r-Hu EPO does not influence markedly the calcium-phospate metabolism in haemodialyzed uraemic patients.

As expected, patients of all HD groups showed markedly abnormal humoral and hormonal parameters related to the Ca-P metabolism which are typical changes encountered in uraemic patients. As r-Hu EPO therapy is followed not only by improvement of the haematological status, but also by increased appetite and physical activity (1, 2, 3, 4, 5, 6, 7, 16, 17) that is by factors, which directly (15) or indirecty influence the Ca-P metabolism alterations of Ca-P metabolism related hormones could be expected in r-Hu EPO treated HD patients. As shown in this study in spite of a significant increase of the Hct value already after 3 months of r-Hu EPO treatment only slight changes both of biochemical and hormonal markers of the Ca-P metabolism were noticed in HD patients.

As shown in this study r-Hu EPO treatment was not followed by significant changes in serum P, PTH, 25-OHD_3 and 1,25-$(OH)_2D_3$ concentrations. Only a transitory and moderate elevation of plasma Ca level was noticed. It is to be stressed that patients on r-Hu EPO therapy did not require increased doses of phosphate-binders. These facts clearly show that r-Hu EPO treatment does not influence markedly Ca-P metabolism in uraemic patients. Thus it seems that aggravation of secondary hyperparathyroidism reported by some authors in r-Hu EPO treated patients (20) was rather due to inadequate clinical and biochemical monitoring than to r-Hu EPO therapy per se. As r-Hu EPO therapy shows a significant although transitory effect on secretion of hormones indirectly involved in the Ca-P metabolism such as somatotropin, cortisol (11, 13) and gonadal hormones (testosterone and estradiol) (12, 13) interference of r-Hu EPO therapy with total body Ca-P metabolism can not be excluded. As r-Hu EPO therapy was followed by a significant increase in plasma osteocalcin concentration and a decrease both in plasma CT level and AP activity existence of a direct or indirect influence of r-Hu EPO therapy on bone remodelling seems very likely. For appropriate interpretation of these data morhpometric studies of bone biopsies performed before and after r-Hu EPO treatment seem to be mandatory.

CONCLUSIONS

1. Improvement of haematological parameters observed in r-Hu EPO treated dialyzed patients with chronic renal failure does not seem to be only due to the administration of this hormone.

2. Long-term r-Hu EPO therapy does not influence markedly biochemical and hormonal markers of Ca-P metabolism in patients with chronic uraemia.

3. Intensive clinical, biochemical and hormonal monitoring of patients treated with r-Hu EPO seems to be essential in the prevention of adverse effects related to the Ca-P metabolism and may prevent development of Ca-P metabolism related adverse events in these patients.

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STRESZCZENIE

Celem pracy było określenie wpływu długotrwałej terapii r-Hu EPO na zachowanie się wskaźników biochemicznych i hormonalnych gospodarki wapniowo-fosforanowej u hemodializowanych chorych na przewlekłą niewydolność nerek. Przedmiotem badań było: a) 17 hemodializowanych chorych ze schyłkową niewydolnością nerek leczonych przez 12 miesięcy r-Hu EPO (grupa EPO), b) 17 hemodializowanych chorych ze schyłkową niewydolnością nerek nie leczonych r-Hu EPO (grupa No-EPO I), lecz nadzorowanych klinicznie i biochemicznie jak grupa EPO, c) 12 hemodializowanych chorych ze schyłkową niewydolnością nerek nie leczonych r-Hu EPO, u których wartości hematokrytu i stężenia hemoglobiny były porównywalne do wartości obserwowanych w grupie EPO po dwunastomiesięcznym leczeniu, d) 11 osób zdrowych.

U wszystkich badanych określano wartość hematokrytową (Ht), stężenie hemoglobiny (Hb) we krwi, stężenie w osoczu krwi wapnia całkowitego (Ca), fosfranów nieorganicznych (P), osteokalcyny (OC), parathormonu (PTH), kalcytoniny (CT), 25-hydroksycholekalcyferolu (25-OHD_3) i 1,25 dihydroksycholekalcyferolu (1,25-$(OH)_2D_3$) oraz aktywność fosfatazy zasadowej (FZ). U chorych grupy EPO i No-EPO I oznaczanie wymienionych parametrów powtarzano co 3 miesiące, przez łącznie 12 miesięcy, natomiast u osób pozostałych dwóch grup tylko jednorazowo.

Po leczeniu r-Hu EPO obserwowano typowy wzrost wartości Ht i stężenia Hb. Niewielki, ale statystycznie znamienny wzrost wymienionych wskaźników obserwowano również w grupie No-EPO I. Leczenie r-Hu EPO wykazało tylko niewielki i przejściowy wpływ na stężenie Ca, OC, CT i aktywność FZ nie wpływając istotnie na pozostałe parametry gospodarki wapniowo-fosforanowej.

LIST OF FIGURES

Fig. 1. Plasma parathyroid hormone levels in uraemic haemodialyzed uraemic patients treated (EPO group) and not treated (No-EPO I group) with r-Hu EPO (0) and after 3, 6, 9 and 12 moths of observation.

Fig 2. Plasma calcitonin (CT) levels in haemodialyzed uraemic patients treated (EPO group) and not treated (No-EPO I group) with r-Hu EPO before (0) and after 3, 6, 9 and 12 months of observation.

Fig 3. Plasma 1.25-dihydroxycholecalciferol (1.25-$(OH)_2D_3$) levels in haemodialyzed uraemic patients treated (EPO group) and not treated (No-EPO I group) with r-Hu EPO before (0) and after 3, 6, 9 and 12 months of observation.