Effects of azathioprine on response of renal anaemia to subcutaneous recombinant human erythropoietin

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Abstract

Aims: To determine the effect of concomitant azathioprine treatment on the response of patients with renal failure to treatment with subcutaneous recombinant human erythropoietin (r-HuEPO).

Methods: Two groups of patients with renal failure not receiving haemodialysis were studied. One comprised seven patients receiving erythropoietin alone, the second consisted of nine patients who were also treated with azathioprine. The haematological changes were monitored, and the functional erythropoietic response was studied by two different ferrokinetic models. One analysed the initial, the other the extended plasma iron clearance. Studies were performed before r-HuEPO treatment on all 16 patients, and repeated on 11 of these when the target haemoglobin (10-11 g/dl) was achieved and stabilised. Total erythropoiesis was determined using both techniques. Analysis of the extended plasma iron clearance also permitted calculation of both effective and ineffective erythroid activity.

Results: The haematological response to r-HuEPO was the same for both patient groups. Measurement of total erythropoiesis by both ferrokinetic methods showed good correlation. For those receiving long term azathioprine, the percentage ineffective erythropoiesis was high compared with that of the other patients, and remained so for as long as they continued with azathioprine. For those uncomplicated by azathioprine treatment, r-HuEPO increased levels of both effective and ineffective erythropoiesis by the same degree. A substantial reduction in ineffective erythropoiesis was shown only by those patients who either discontinued or reduced their azathioprine once they started r-HuEPO treatment.

Conclusions: Azathioprine increases ineffective erythropoiesis. In this study, the r-HuEPO dose was sufficient to overcome this effect and promoted effective erythropoiesis so that the anaemia lessened. Measurement of total erythropoiesis provided limited information on the functional changes involved, differentiation of effective from ineffective erythropoiesis being necessary to define the changes after azathioprine reduction or withdrawal.

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Erythropoietin influences the differentiation of committed erythroid progenitor cells into erythroblasts. Its principal effect is to induce the proliferation of erythroid colony forming units (CFU-E) and their subsequent maturation into proerythroblasts.¹ A secondary effect may also include the differentiation of erythroid burst forming units (BFU-E). Inability to produce erythropoietin is the main cause of the characteristic anaemia of end-stage renal disease.^{2,3} Additional contributory factors may be reduced red cell survival and, possibly, the retention of erythropoietic inhibitors.⁴⁻⁷ With the advent of recombinant DNA technology, large quantities of purified human erythropoietin are now available.^{8,9} Previous clinical trials¹⁰⁻¹⁵ have reported the efficacy of recombinant erythropoietin for treatment of the anaemia of renal failure, the functional response being identified by application of ferrokinetic models based on analysis of either the initial or the extended plasma iron clearance lines.^{16,17}

Methods

Pretreatment ferrokinetic studies were performed on 16 patients with end-stage renal disease who were receiving r-HuEPO. Eleven (cases 1-4 and 8-14) did not require dialysis; five (cases 5-7, 15 and 16) were receiving continuous ambulatory peritoneal dialysis (CAPD). For the pre-dialysis group, the initial dose was 120 units/kg body weight/week, administered as two 60 unit subcutaneous injections; doses for the CAPD group comprised 100 units/kg body weight/week in two 50 unit aliquots. For both groups, the drug was self-administered. Doses were adjusted according to response to achieve a stable haemoglobin concentration of between 10 and 11 g/dl.

Patients without iron overload (serum ferritin of < 500 µg/l) received 300 mg elemental iron/day; those with high serum ferritin concentrations were not given supplements unless the serum ferritin dropped to less than 200 µg/l. For patients unable to tolerate oral iron, 250-500 mg iron-dextran complex was administered by slow intravenous injection over 20 to 40 minutes.

When pretreatment ferrokinetic studies were performed, nine patients were also receiving azathioprine (table 1), eight of whom had failing renal transplants and required aza-

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thioprine to suppress chronic rejection of their transplanted kidneys. The maintenance dose was regulated according to both the patient's body weight and any myelosuppressive effects indicated by the blood count parameters. In the case of two patients (cases 12 and 15), azathioprine was withdrawn during the course of r-HuEPO treatment; for four patients (cases 10, 11, 13 and 14), azathioprine concentrations were reduced; the dose was unchanged for the remaining three (cases 8, 9 and 16). Ferrokinetic studies were carried out before r-HuEPO was administered on all 16 patients, and studies after treatment were performed on 11 when the target haemoglobin concentration had been achieved and had remained stable. We were unable to carry out such studies on five patients; three (cases 3, 14, and 15) were poor compliers with the self-administration of r-HuEPO, one (case 2) required dialysis, and one (case 16) died.

^{59}Fe labelled transferrin was prepared,¹⁸ and 4 ml plasma containing between 0.2 and 0.4 MBq of label was injected intravenously. Venous blood samples were collected at timed intervals over the next 14 days. Total erythroid activity was determined from analysis of the initial plasma iron clearance as a measurement of erythron transferrin uptake (ETU),¹⁶ and from analysis of the extended plasma iron clearance as the marrow iron turnover (MIT).¹⁷ The latter technique also permitted calculation of effective erythropoiesis, denoted by the red cell iron turnover (RCIT), and ineffective erythropoiesis, denoted by the ineffective iron turnover (IIT).¹⁷

Red cell volume (RCV) and red cell survival (RCS) were both measured by recommended methods.^{19,20} Plasma volume (PV) was determined by dilution of ^{59}Fe labelled transferrin in the plasma compartment.²¹

Venous blood samples were stained for reticulocytes using a modification of the method of Dacie and Lewis.²² A 50:50 mixture

of whole blood and 0.5% new methylene blue was prepared. After 7 minutes at room temperature, a reticulocyte smear was prepared and counted using a microscope fitted with a graticule in one eyepiece. The reference value shown in table 1 is that routinely used for clinical purposes.

Serum ferritin concentrations were measured using an enzyme linked immunosorbent assay (ELISA).²³

Results

Pre- and post-treatment haematological and ferrokinetic data are shown in tables 1 and 2. For the 11 patients on whom both studies before and after treatment were performed, the red cell volume increased (mean pre-treatment value 0.841; mean post-treatment value 1.431) as r-HuEPO stimulated the effective component of erythropoiesis (mean pre-treatment RCIT 40.1 $\mu\text{mol/l}$ blood/24h; mean post-treatment RCIT 66.2 $\mu\text{mol/l}$ blood/24h). The absolute reticulocyte count, which was regularly monitored throughout the first month following treatment, showed a sustained increase during this time period (mean pre-treatment value $27 \times 10^9/\text{l}$; mean post-treatment value $62 \times 10^9/\text{l}$).

Changes in the level of ineffective erythropoiesis (IIT) were less clearly defined. For patients receiving azathioprine, pre-treatment values for percentage ineffective erythropoiesis (IIT%) were much higher than those observed for patients not receiving this drug. The mean pre-treatment value for IIT% in those taking azathioprine was 58.7% (range 49–69%); for those not receiving azathioprine, this value was 36.0% (range 24–48%).

For those not treated with azathioprine, the pre- and post-r-HuEPO treatment values remained essentially unchanged (mean pre-treatment IIT% 39.2%; mean post-treatment IIT% 38.2%). However, a mixed response was

Table 1 Haematological data

		Reticulocytes ($\times 10/l$)		Treatment period	Haemoglobin (g/dl)		Red cell volume (l)		Plasma volume (l)		Whole blood volume (l)		Serum ferritin ($\mu\text{g/l}$)		Red cell survival (d)	
Case No	Sex	Pre	Post*	(Months)	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
No azathoprine																
1 (P)	F	28	78	4	6.90	10.70	0.92	1.35	3.56	2.17	4.48	3.52	142	29.3	26	24
2 (P)	F	33	60	—	7.60	NA	1.35	NA	3.36	NA	4.71	NA	47	NA	15	NA
3 (P)	F	22	52	—	6.40	NA	0.61	NA	2.03	NA	2.64	NA	72	NA	26	NA
4 (P)	F	28	54	5	7.80	9.50	0.97	1.10	2.35	3.07	3.82	4.17	30	34	23	36
5 (D)	M	28	66	6	8.10	10.90	1.45	2.40	4.88	4.39	6.32	6.79	504	148	24	24
6 (D)	M	23	42	6	6.00	8.10	0.66	0.82	3.55	3.29	4.21	4.11	3278	1024	16	18
7 (D)	F	46	54	10	5.70	8.20	0.99	1.74	4.47	3.70	5.46	5.44	371	46	30	30
Mean values		34	58		6.90	9.50	0.99	1.48	3.46	3.32	4.52	4.81	635	256	23	26
Azathioprine																
8 (P)	M	20	92	6	7.40	11.50	0.81	1.34	3.79	3.76	4.59	5.10	2176	1674	22	24
9 (P)	F	19	88	4.5	7.00	10.40	0.60	1.14	3.70	2.92	4.30	4.06	253	5.5	22	14
10 (P)	M	17	20	7	5.40	7.40	0.76	2.07	4.80	4.34	5.56	6.41	59	107	22	17
11 (P)	F	34	108	2	6.50	11.60	0.62	1.21	3.02	3.21	3.64	4.42	83	41	17	16
12 (P)	F	21	69	7.5	7.40	11.00	0.90	1.34	3.30	2.59	4.20	3.93	110	143	29	35
13 (P)	M	45	63	—	5.90	NA	0.64	NA	4.00	NA	4.64	NA	45	NA	18	NA
14 (P)	F	25	27	—	8.10	NA	0.74	NA	2.49	NA	3.23	NA	81	NA	20	NA
15 (D)	F	20	44	4.5	5.80	9.50	0.55	1.17	3.83	2.93	4.38	4.10	663	659	19	18
16 (D)	F	27	75	—	7.10	NA	0.51	NA	2.70	NA	3.21	NA	45	NA	16	NA
Mean values		25	65		6.70	10.20	0.68	1.38	3.51	3.29	4.19	4.67	391	438	21	21

P = Predialysis patient; * = reticulocyte results following 1 month's treatment; D = CAPD patient; NA = result not available;

reference ranges

Reticulocytes $< 100 (10^9/\text{l})$

Haemoglobin Men 13–18 g/dl
Women 11.5–16.5 g/dl

Serum ferritin Men 15–200 $\mu\text{g/l}$

Women 7–90 $\mu\text{g/l}$

Red cell survival 25–33 d

Table 2 Ferrokinetic data

		Treatment period (months)	ETU ($\mu\text{mol/l}$ blood/24 h)		MIT ($\mu\text{mol/l}$ blood/24 h)		RCIT ($\mu\text{mol/l}$ blood/24 h)		IIT ($\mu\text{mol/l}$ blood/24 h)		IIT (%) ($\mu\text{mol/l}$ blood/24 h)	
Case No	Sex		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
No azathioprine												
1 (P)	F	4	26.70	76.00	87.00	144.00	45.00	78.00	42.00	66.00	48.00	46.00
2 (P)	F	—	27.30	NA	52.00	NA	33.00	NA	19.00	NA	37.00	NA
3 (P)	F	—	45.00	NA	76.00	NA	58.00	NA	18.00	NA	24.00	NA
4 (P)	F	5	20.30	47.70	64.00	72.00	41.00	50.00	23.00	22.00	36.00	31.00
5 (D)	M	6	31.50	58.50	77.00	104.00	54.00	69.00	23.00	35.00	30.00	34.00
6 (D)	M	6	12.70	45.30	34.00	78.00	21.00	51.00	13.00	27.00	33.00	35.00
7 (D)	F	10	23.30	46.80	62.00	87.00	35.00	48.00	27.00	39.00	44.00	45.00
Mean values			26.70	54.90	64.60	97.00	41.00	59.20	23.60	37.80	36.00	38.20
Azathioprine												
8 (P)*	M	6	40.60	47.00	78.00	123.00	32.00	43.00	46.00	80.00	59.00	64.00
9 (P)*	F	4.5	44.00	104.00	60.00	219.00	30.00	78.00	30.00	141.00	49.00	64.00
10 (P)**	M	7	66.00	118.00	99.00	139.00	40.00	85.00	59.00	54.00	60.00	39.00
11 (P)**	F	2	81.00	87.00	115.00	150.00	51.00	83.00	64.00	67.00	56.00	45.00
12 (P)***	F	7.5	66.40	98.00	109.00	123.00	49.00	82.00	60.00	41.00	55.00	33.00
13 (P)	M	—	107.00	NA	150.00	NA	46.00	NA	104.00	NA	69.00	NA
14 (P)	F	—	65.50	NA	129.00	NA	57.00	NA	72.00	NA	56.00	NA
15 (D)***	F	4.5	68.20	72.40	111.00	91.00	42.00	61.00	69.00	30.00	62.00	33.00
16 (D)	F	—	54.00	NA	97.00	NA	37.00	NA	60.00	NA	62.00	NA
Mean values			65.90	87.70	105.00	141.00	42.70	72.00	62.70	68.80	58.70	46.30

P = Predialysis patient; * = Patient maintained on same level of azathioprine; ** = azathioprine dose reduced before post treatment ferrokinetics; *** = azathioprine withdrawn prior to post treatment ferrokinetics; D = CAPD patient; NA = result not available.

Reference ranges

Erythron transferrin uptake (ETU)	36–84 $\mu\text{mol/l blood 24h}$
Marrow iron turnover (MIT)	73–159 $\mu\text{mol/l blood 24h}$
Red cell iron turnover (RCIT)	54–105 $\mu\text{mol/l blood 24h}$
Ineffective iron turnover (IIT)	19–54 $\mu\text{mol/l blood 24h}$
Percentage ineffective iron turnover (IIT%)	13–34%

observed in those taking azathioprine following a change in the azathioprine dose for four patients. Azathioprine was withdrawn from cases 12 and 15. Case 12 was originally receiving 150 mg daily and case 15 50 mg daily. Both doses were withdrawn at six and three months, respectively, before the post-treatment ferrokinetic study was started. Cases 10 and 11 both had their azathioprine dose reduced one month before the post-treatment studies, case 11 from 100 mg to 75 mg daily, and case 10 from 150 mg to 100 mg. These changes resulted in a substantial reduction in ineffective erythropoiesis in all four patients.

A close correlation between the ETU and MIT was recorded for all patients (figure). An increase in red cell survival (RCS) was recorded for two patients only (cases 4 and 12).

Discussion

Our results confirm the effectiveness of twice weekly subcutaneous injections of r-HuEPO

for treatment of both pre-dialysis and CAPD patients. Stimulation of erythropoiesis resulted in rapid mobilisation of iron stores, reflected by a sharp fall in serum ferritin concentration. All patients reported improvements in their sense of wellbeing, appetite, and activity levels. The benefits of treatment with subcutaneous r-HuEPO have also been documented in several previous publications.^{24–26}

Ferrokinetic studies showed that, within the time span of our study, expansion of the erythron by r-HuEPO, rather than an alteration in red cell survival, was primarily responsible for the initial improvement in the anaemia of both patient groups. Nevertheless, previous work has indicated that significant improvements in red cell survival may occur after nine to 15 months of treatment, the explanation for this later improvement remaining unclear.¹³

Total erythroid activity was expressed either as erythron transferrin uptake (ETU)¹⁶ or marrow iron turnover (MIT).¹⁷ The close correlation observed between the ETU and MIT for the renal patients has also been reported for cases of myelofibrosis and myelodysplasia.²⁷ The advantage of ETU measurements resides in the fact that a one day study period only is required, rather than the 10 to 14 days necessary for MIT measurements. The latter method, however, has the advantage of differentiating effective from ineffective erythropoiesis.

For patients to whom azathioprine was not given, r-HuEPO produced an increase in both effective and ineffective erythropoiesis, the ratio of these two components remaining unaltered. Thus for these patients the resultant changes in erythroid activity were adequately defined by measurements of total erythropoiesis, expressed as either the MIT or ETU.

However, in patients receiving azathioprine these parameters did not provide a complete explanation of the functional changes accompanying r-HuEPO treatment. Azathioprine is metabolised to a purine antagonist which

Correlation between MIT and ETU.

interferes directly with erythroid activity, producing the high level of ineffective erythropoiesis recorded for patients receiving this drug. This increase persisted for as long as the patients took azathioprine. Nevertheless, this group still derived benefit from treatment with r-HuEPO. The erythropoietin dose administered in this study seemed to be sufficient to overcome the inhibitory effect of azathioprine on erythroid activity, and promoted effective erythropoiesis to the level required for an improvement in anaemic state. A substantial reduction in ineffective erythropoiesis was shown only by those patients who either discontinued or reduced their azathioprine dose once they started r-HuEPO treatment. In such cases measurement of changes in total erythropoiesis provided only limited information on the functional erythropoietic activity. This was particularly evident when the increase in effective erythropoiesis was accompanied by a substantial decrease in ineffective erythropoiesis. As total erythropoiesis is the summation of effective and ineffective erythropoiesis, the resultant changes recorded for total erythroid activity in such cases may not accurately reflect the improvement indicated by the increase in red cell volume.

Ferrokinetic studies provide an insight into the functional changes following r-HuEPO treatment. However, to obviate any misinterpretation of data, stringent attention must always be paid to the effect of additional treatment which may itself influence erythroid activity.

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