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Clinical Evaluation of Heme Iron Polypeptide: Sustaining a Response to rHuEPO in Hemodialysis Patients

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• **Background:** Optimizing iron and recombinant human erythropoietin (rHuEPO) therapy is necessary to achieve target hemoglobin levels and minimize costs as the end-stage renal disease (ESRD) population expands. Oral iron products in patients with ESRD have been largely abandoned, and the safety of intravenous (IV) iron preparations has improved with the introduction of new-generation compounds that have little allergenicity. Recent work suggests oral heme iron may be an effective supplement for hemodialysis (HD) patients because it is absorbed by patients with high ferritin levels, has fewer side effects, and its absorption is stimulated by erythropoietin administration. **Methods:** We performed an open, 6-month, prospective evaluation of heme iron in HD patients who had been on maintenance IV iron therapy. IV iron was discontinued and replaced with oral heme iron. Serum iron level, hematocrit (Hct), and erythropoietin and IV iron dose were monitored. **Results:** During 6 months, 4 of 37 patients (11%) dropped out because of insufficient iron supplementation or intolerance and 5 patients (14%) were dropped because of unrelated complications or protocol violation. A slight reduction in average transferrin saturation (TSAT) was seen early, but reversed, and no significant changes were seen in TSAT or Hct. A significant reduction in average serum ferritin level was seen at months 4 through 6 ($P < 0.01$). **Conclusion:** During the 6-month study period, heme iron polypeptide successfully replaced IV iron therapy in a majority of HD patients and maintained target Hcts with no concomitant use of IV iron. This treatment was associated with a significant increase in rHuEPO efficiency ($P = 0.04$). *Am J Kidney Dis* 42:325-330.

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INDEX WORDS: Anemia; oral; heme; iron; erythropoietin (EPO); ferritin; hematocrit (Hct).

RECENTLY, intravenous (IV) iron has been the preferred method for delivering iron to hemodialysis (HD) patients to sustain an optimal response to recombinant human erythropoietin (rHuEPO). The introduction of newer generation IV iron preparations associated with a lower risk for anaphylactoid and other reactions has accelerated physician acceptance of this therapy. Oral iron preparations were largely abandoned in the last decade because of their failure to deliver sufficient iron to maintain target hemoglobin (Hgb) levels recommended by national clinical practice guidelines.¹

Inadequacy of oral iron is related to poor compliance, gastrointestinal (GI) side effects, suboptimal GI absorption of iron, and medication costs.² Even with the use of IV iron, not all iron-replete HD patients show an optimal response to rHuEPO therapy. A block in iron utilization caused by an acute-phase inflammatory response may contribute to this phenomenon.³ It would be helpful if there was an oral product that delivered iron in a biologically controlled manner in quantities sufficient to sustain a response to rHuEPO.

A new-generation oral iron product generically called heme iron polypeptide (HIP) was introduced recently. HIP uses the heme porphyrin ring to supply iron to sites of absorption in the

intestinal lumen. Heme is absorbed through a receptor that is different from the absorption mechanism for nonheme (ionic) iron,^{4,5} resulting in different absorption kinetics and GI side-effect profiles compared with ionic iron.⁶ HIP is produced by hydrolysis of bovine Hgb. Amino acids and peptides cleaved by hydrolysis are removed to increase the iron-protein ratio. The extraction technique leaves peptides from the alpha and beta Hgb subunits covalently bound to the heme ring, allowing solubility at pH values far lower than those for heme alone.

Concern has been expressed about the possibility of transmission of bovine spongiform encephalopathy (BSE) through the consumption of bovine tissue. Currently, HIP is manufactured from

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red blood cells of cows of American origin, and both the US Department of Agriculture and Food and Drug Administration currently maintain that the United States is free of BSE. In addition, the putative infectious agents for BSE, conformationally shifted neuronal membrane copper-binding proteins called prions, usually are not found in blood.

Recent work by Seligman et al⁷ evaluated HIP in healthy subjects and saw significantly greater bioavailability of HIP compared with nonheme iron. Work by Hallberg et al⁸ has shown that absorption of heme iron may be more than 10 times greater than that of iron salts in subjects with serum ferritin levels greater than 400 ng/mL (898 pmol/L). Later work by Skikne et al⁹ confirmed the findings of Hallberg et al⁸ and showed that GI absorption of both heme and nonheme iron can be increased dramatically by stimulation with rHuEPO. In a small-scale evaluation, Nissenson¹⁰ presented data that use of HIP in conjunction with IV iron led to significant reductions in the use of both IV iron and rHuEPO in HD patients. We set out to determine if the use of HIP alone (without IV iron) could sustain the response to rHuEPO in HD patients during a 6-month period.

METHODS

HIP was obtained from Colorado Biolabs, Inc (Cozad, NE). We used an open-label, multisite prospective evaluation of stable HD patients. Stable HD patients on renal replacement therapy for a minimum of 6 months were selected. Only subjects administered maintenance IV iron infusions and rHuEPO were selected. Three months of baseline data were obtained to confirm that hematocrit (Hct), serum ferritin, and transferrin saturation (TSAT) values were within Dialysis Outcomes Quality Initiatives (DOQI)-recommended parameters, which at the time were TSAT greater than 20% and ferritin level greater than 100 ng/mL (224 pmol/L).

After receiving institutional review board approval and informed consent, patients either continued treatment with their respective clinic's IV iron protocol or were administered HIP at 21 (low dose) or 36 mg/d (high dose) of elemental iron. Data were collected monthly, including TSAT, Hct, rHuEPO dose, and IV iron dose, with ferritin level determined every third month. Each patient was administered a monthly questionnaire to ascertain GI side effects. Subjects were instructed to take 1 HIP tablet 3 times daily: 1 tablet with breakfast, 1 tablet with lunch, and 1 tablet before bedtime. If the subject's serum iron parameters or Hct decreased to less than the Kidney Disease Outcomes Quality Initiative (K/DOQI)-recommended range for 2 consecutive months, the patient was removed from the study. At all sites,

rHuEPO doses were determined by the respective clinic's dosing algorithm. Nurses and physicians implemented the standard dosing algorithms based on the subject's previous rHuEPO dose and laboratory values for Hgb or Hct. At all sites, the study was supervised by either a dietician or dedicated research nurse so that rHuEPO dosing decisions were determined by standard clinical protocols and not influenced by those running the study.

At 4 study sites, subjects were assigned to their respective treatment by random number generation. At the fifth site, subjects were distributed based on payer source. Fifty percent of patients at that site had no reimbursement plan for IV iron; therefore, patients from this group were assigned to the administration of oral iron. Subjects at that site who had reimbursement plans for IV iron were assigned to continue IV iron administration. This situation clearly allowed for increased patient accrual; overall, 33 patients (15 patients, HIP group; 18 patients, IV iron group) came from this site.

RESULTS

Thirty-seven subjects started on HIP therapy; of these, 9 subjects dropped out. Three subjects discontinued because of GI side effects. In a previous study of healthy subjects by Seligman et al,⁷ no GI side effects were seen with HIP therapy. One subject failed to sustain a TSAT greater than 20% for 2 consecutive months. This patient, in whom prostate cancer was diagnosed, was hospitalized briefly and administered a transfusion after 4 months of HIP therapy. Of the remaining 5 subjects who dropped out, 1 subject did so because of chronic nausea associated with ophthalmic surgery; 2 subjects, because of excessive bleeding during access surgery; 1 subject, because of hospitalization for severe burns and inability to take oral medications; and 1 subject, because of IV iron administered in violation of protocol. These 2 groups were similar for demographics and cause of renal failure (Table 1).

Table 2 lists mean monthly serum iron parameters, and Table 3 lists mean rHuEPO and IV iron doses.

Because of month-to-month variability in iron parameters and because serum ferritin level normally is determined quarterly in the clinical setting, parameters to be evaluated were grouped into 3-month increments. Baseline included months -2, -1, and 0; period I is defined as months 1, 2, and 3 after initiation of therapy; and period II is defined as months 4, 5, and 6 after initiation of therapy.

Comparisons of Hcts were performed both between groups (HIP versus IV iron) by using confidence interval analysis and within group

Table 1. Patient Demographics

	HIP	IV Iron
No. of patients	28	31
Age (y)	60.2 ± 13	62.3 ± 14
Sex		
Men	19 (68)	17 (55)
Women	9 (32)	14 (45)
Primary diagnosis		
Hypertension	13 (46)	18 (58)
Diabetes	6 (21)	5 (16)
Glomerulonephritis	3 (11)	5 (16)
Other	5 (18)	0 (0)
Lupus	1 (4)	0 (0)
Unknown	0 (0)	3 (10)
Ethnicity		
African American	8 (29)	11 (35)
Asian	5 (18)	7 (23)
Caucasian	9 (32)	6 (19)
Hispanic	4 (14)	5 (16)
Pacific Islander	2 (7)	2 (6)

NOTE. Values expressed as number (percent).

(HIP baseline period versus HIP treatment periods) by using paired *t*-test. Between-group analysis was performed to establish adequacy of patient selection. Mean Hct for the IV iron control group during the baseline period was 35.6%, with a 95% confidence interval of 0.873 (range, 34.74% to 36.48%). Mean Hct of the HIP group during the baseline period was 34.8%, which was within this range, thereby indicating adequacy of patient selection (Table 4).

Because our primary end point was maintenance of Hct, we compared average Hcts in the HIP group during periods I and II with baseline. There was no statistically significant change in average Hcts between baseline (34.8%) and pe-

riod I (35.3%; $P = 0.29$) or between baseline and period II (35.4%; $P = 0.19$) in the HIP group (Table 4).

Comparing TSAT values within the HIP group for the 3 time points, there was no statistically significant difference. Comparing serum ferritin values for the 3 time points in the HIP group, there was a significant decrease from baseline (552 ng/mL [1,240 pmol/L]) to period I (510 ng/mL [1,146 pmol/L]; $P = 0.06$) and baseline to period II (447 ng/mL [1,004 pmol/L]; $P = 0.001$; Table 4).

For the combined high- and low-dose HIP groups, average monthly rHuEPO dose during the baseline period while still being administered IV iron was 58,613 U/mo. During period I, a slight decrease of 2,144 U/mo (-3.7%) to 56,469 U/mo ($P = 0.83$) was observed. In period II, average monthly rHuEPO dose decreased again by 8,339 U/mo to 48,130 U/mo (-18.0%) compared with baseline ($P = 0.08$). Although this decrease was not significant, it is interesting to note that rHuEPO dose did not increase in the HIP group to maintain Hct values in the target range of 33% to 36%.

As a secondary evaluation, we compared rHuEPO dose in the IV iron group with the HIP group during the baseline period by means of Student's *t*-test. Baseline average monthly rHuEPO dose in the combined high- and low-dose HIP group was 58,613 U/mo, and baseline average monthly rHuEPO dose in the IV iron control group was 35,271 U/mo, a difference that was significant ($P < 0.02$). One possible explanation for the difference in rHuEPO doses between the 2 groups is that many patients in the HIP

Table 2. Average Monthly Serum Iron Parameters and Hcts

	Month									
	-2	-1	0	1	2	3	4	5	6	
HIP group (n = 28)										
TSAT (%)	34.1	29.0	26.9	29.6	25.3	26.7	26.6	28.6	31.5	
Ferritin (ng/mL)	611	557	597	524	498	520	543	451	462	
Hct (%)	34.4	34.8	35.1	35.0	35.6	35.3	35.7	35.4	35.2	
IV iron control (n = 31)										
TSAT (%)	23.4	31.6	30.9	31.4	33.3	31.2	28.8	33.0	30.5	
Ferritin (ng/ml)	676	544	648	712	716	770	667	737	719	
Hct (%)	35.9	35.5	35.4	35.7	34.9	35.9	35.7	35.9	35.2	

NOTE. To convert ferritin in ng/mL to pmol/L, multiply by 2.247.

Table 3. Average Monthly IV Iron and rHuEPO Doses

	Month									
	-2	-1	0	1	2	3	4	5	6	
HIP group (n = 28)										
IV iron dose (mg)	46	112	18	0	0	0	0	0	0	0
rHuEPO dose (U)	51,686	63,571	60,582	55,914	57,543	55,950	58,061	46,689	39,639	
IV iron control (n = 31)										
IV iron dose (mg)	83	145	152	198	105	202	230	185	80	
rHuEPO dose (U)	37,332	34,029	27,626	32,323	30,794	37,378	38,794	41,258	25,329	

group came from the site that was separated based on payer source; however, even HIP subjects at the randomized sites had greater starting rHuEPO doses (62,062 versus 28,725 U/mo). Comparisons of average monthly IV iron doses also were made during the baseline period between the 2 groups. As might be expected with greater starting rHuEPO doses, during the baseline period, average monthly IV iron dose for the HIP group (58.3 mg/mo) was significantly less than that in the IV iron group (126.6 mg/mo; $P < 0.01$).

rHuEPO efficiency, defined as total weekly rHuEPO dose/Hgb, also was evaluated. Among subjects administered IV iron, no significant change in average monthly rHuEPO dose or efficiency was noted. Conversely, in the HIP group, significant efficiency improvement was observed between baseline and period II (-247; $P = 0.04$; Table 5). Separating the high- and low-dose groups, there was a significant improve-

ment in rHuEPO efficiency in the high-dose HIP group (Table 6). Low-dose group values at periods I and II were improved from baseline, although no significance was seen, possibly because of the small number of subjects ($n = 8$).

However, in the low-dose HIP group, average monthly TSAT decreased from 32.6% to 26.7% ($P = 0.09$) between baseline and period I and continued to decrease in period II to 26.5% ($P = 0.03$) compared with baseline. Even with this small number, this significant decrease suggests low-dose HIP may not be able to sustain a response to rHuEPO therapy for longer periods. Average monthly TSAT in the high-dose group remained relatively constant and showed no statistically significant difference throughout the study (baseline, 28.9%; period I, 27.4%; period II, 29.6%).

For the low-dose HIP group, baseline Hct was 36.2%. This remained relatively constant during period I and then decreased slightly to 35.7% in

Table 4. Statistical Comparison of Grouped Parameter Means

HIP versus IV iron	Baseline	Period I	Period II	<i>P</i> (compared with baseline)
TSAT (%)				
HIP	30.0 ± 1.4	27.2 ± 1.1	28.9 ± 2.0	
IV iron	31.6 ± 1.2	31.9 ± 1.4	30.7 ± 1.3	
Hct (%)				
HIP	34.8 ± 0.4	35.3 ± 0.4	35.4 ± 0.4	
IV iron	35.6 ± 0.3	35.5 ± 0.4	35.6 ± 0.3	
Ferritin (ng/mL)				
HIP	552 ± 107	510 ± 107	446 ± 98	<0.01
IV iron	676 ± 99	737 ± 77	723 ± 75	
rHuEPO (U/mo)				
HIP	58,613 ± 7,754	56,469 ± 6,317	48,130 ± 7,768	
IV iron	32,996 ± 5,998	33,498 ± 5,853	35,127 ± 6,318	

NOTE. Values expressed as mean ± SE. To convert ferritin in ng/mL to pmol/L, multiply by 2.247.

Table 5. rHuEPO Efficiency Compared With Baseline

Group	Baseline	Period I	<i>P</i>	Period II	<i>P</i>
HIP	1,270	1,197	0.55	1023	0.04
IV iron	706	724	0.84	762	0.68

NOTE. rHuEPO efficiency determined by weekly rHuEPO dose (U/mo)/Hgb (g/dL).

period II. In the high-dose HIP group, Hct at baseline was 34.2%. Average Hct in this group then increased to 35.0% during period I ($P = 0.24$) and continued to increase in period II to 35.3%. Compared with baseline, the increase in period 2 was significant ($P = 0.04$).

In the high-dose group, rHuEPO dose was 57,911 U/mo at baseline, 60,656 U/mo in period I ($P = 0.57$ compared with baseline), and 49,343 U/mo in period II ($P < 0.03$ compared with baseline).

DISCUSSION

This study is designed to examine the adequacy of oral heme iron therapy in iron-replete HD patients. New HD patients may present with a wide range of iron stores. Patients who had a dialysis history were chosen for ethical reasons. A study of patients who are not loaded with previous IV iron would better assess the effect of HIP on iron repletion under different circumstances. Only 1 of 29 subjects had to be removed from the study because of a decrease in TSAT values for 2 consecutive months. Also, 3 subjects on HIP therapy (9.7%) dropped out because of GI side effects, primarily flatulence and bloating.

Our primary end point for this study is maintenance of Hct. During the study period, there was no statistically significant change in Hct for the combined HIP groups (baseline, 34.8%; period I, 35.3%; period II, 35.4%) or the IV iron control group (baseline, 35.6%; period I, 35.5%; period II, 35.6%). Maintenance of Hct was even more impressive because the HIP group was administered a significantly lower average monthly IV iron dose during the baseline period. This lower average monthly IV iron dose also may explain why, at baseline, the HIP group was administered greater rHuEPO doses than the IV iron control group.

Because TSAT is a primary indicator of a

patient's ability to respond to rHuEPO,¹¹ it was imperative that average TSAT in the HIP group remain greater than 20% (DOQI practice guidelines). Average monthly TSATs in the combined HIP groups remained greater than this minimum (baseline, 30.0%; period I, 27.2%; period II, 28.9%). The slight decrease in average TSAT in period I, followed by an increase in TSAT in period II, may suggest that patients require an adaptation period after switching to HIP therapy. Heme absorption through the intestines is thought to be receptor mediated and therefore may require a receptor induction and expression period.

As mentioned, serum ferritin level is not an optimal indicator of iron stores in HD patients, and the very high levels commonly seen in HD patients today may be correlated with hyporesponsiveness to rHuEPO.¹¹ Causes of elevated serum ferritin levels in these patients are multifactorial and may include systemic inflammation, administration of IV iron, and/or infection. Even so, DOQI recommends that serum ferritin levels be maintained at greater than 100 ng/mL (224 pmol/L) to prevent the development of absolute iron deficiency. Average serum ferritin levels in the HIP group during baseline, period I, and period II were 552 ng/mL (1,238 pmol/L), 510 ng/mL (1,146 pmol/L), and 446 ng/mL (1,002 pmol/L), respectively, well above the K/DOQI-recommended minimum. The decrease between baseline and period I was not significant ($P = 0.06$); however, the trend continued during period II to 446 ng/mL (1,002 pmol/L), which was significant ($P = 0.0014$). A longer study is needed to determine if this trend would continue. Because Hcts, TSATs, and rHuEPO doses were stable or improved, data suggest that maintenance IV iron therapy contributes to the greater than normal serum ferritin values seen in patients with ESRD. During baseline, period I, and period II, average serum ferritin levels for the IV iron control group were 676 ng/mL (1,519 pmol/L).

Table 6. rHuEPO Efficiency: 21 mg/d in HIP versus 36 mg/d in HIP Groups

HIP (mg/d)	Baseline	Period I	<i>P</i>	Period II	<i>P</i>
21	1227	918	0.31	922	0.44
36	1287	1309	0.85	1063	0.016

NOTE. rHuEPO efficiency determined by weekly rHuEPO dose (U/mo)/Hgb (g/dL).

L), 737 ng/mL (16,45 pmol/L), and 723 ng/mL (1,625 pmol/L) respectively; this corresponds well with published values.¹¹ Thus, HIP treatment may be used as a tool to help determine the relative contribution of various contributors to elevated serum ferritin levels in HD patients.

It appears that HIP is able to sustain a response to rHuEPO in the majority of iron-replete HD patients. Not only did HIP treatment appear to sustain a response to rHuEPO therapy, there was a significant increase in overall rHuEPO efficiency in period II compared with the baseline period. In the 36-mg HIP group, small but significant increases in Hcts and reductions in average monthly rHuEPO doses during a 6-month period also were observed.

These results showing decreased rHuEPO requirements are surprising and suggest that HIP may sustain a rHuEPO response longer than the 6-month study period. However, this study is a preliminary assessment and sets the stage for larger long-term studies that will better elucidate the efficacy of HIP and its relative impact on serum ferritin levels in HD patients.

Together, results indicate that oral HIP may be a viable iron-delivery method for HD patients on rHuEPO therapy. Certain patients may require maintenance IV iron therapy, and others may require IV iron administration after blood loss or if unable to take oral medications. Longer studies will help elucidate the degree to which IV iron needs could be decreased in a population of HD patients administered rHuEPO.

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