Abstract

Background. Preliminary clinical evidence suggests that heme iron polypeptide (HIP) might represent a promising, novel oral iron supplementation strategy in chronic kidney disease. The aim of this multi-centre randomized controlled trial was to determine the ability of HIP administration to augment iron stores in darbepoetin (DPO)-treated patients compared with conventional oral iron supplementation.

Methods. Adult peritoneal dialysis (PD) patients treated with DPO were randomized 1:1 to receive two capsules daily of either HIP or ferrous sulphate per os for 6 months. The primary outcome measure was transferrin saturation (TSAT). Secondary outcomes comprised serum ferritin, haemoglobin, DPO dose and responsiveness, and adverse events.

Results. Sixty-two patients were randomized to HIP (n = 32) or ferrous sulphate (n = 30). On intention-to-treat analysis, the median (inter-quartile range) TSAT was 22% (16–29) in the HIP group compared with 20% (17–26) in controls (P = 0.65). HIP treatment was not significantly associated with TSAT at 6 months on multivariable analysis (P = 0.95). Similar results were found on per-protocol analysis and subgroup analysis in iron-deficient patients. Serum ferritin levels at 6 months were significantly lower in the HIP group (P = 0.003), while the cost of HIP was 7-fold higher than that of ferrous sulphate. No other differences in secondary outcomes were observed.

Conclusions. HIP showed no clear safety or efficacy benefit in PD patients compared with conventional oral iron supplements. The reduction in serum ferritin levels and high costs associated with HIP therapy suggest that this agent is unlikely to have a significant role in iron supplementation in PD patients.

Keywords: anaemia; chronic kidney disease; heme iron polypeptide; iron; randomized controlled trial

Introduction

Treatment of anaemia with erythropoiesis-stimulating agents (ESAs) has resulted in major health benefits for individuals with end-stage kidney disease (ESKD), including improved quality of life, reduced blood transfusion requirements, decreased left ventricular mass, diminished sleep disturbance and enhanced exercise capacity [1–3]. However, ESA treatment results in a substantial increase in the iron demand for erythropoiesis [4, 5], such that as many as 90% of ESA-treated individuals require iron supplementation to sustain an optimal haematological response to ESAs [6, 7].

Numerous studies in haemodialysis populations have shown an advantage of intravenous over oral iron supplementation, as evidenced by enhanced body iron stores,

doi: 10.1093/ndt/gfs372
Advance Access publication 7 September 2012

A randomized controlled trial of oral heme iron polypeptide versus oral iron supplementation for the treatment of anaemia in peritoneal dialysis patients: HEMATOCRIT trial

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augmented haemoglobin levels and reduced ESA requirements [7–10]. Indeed, some trial data have shown that oral iron supplementation in haemodialysis populations is no more effective than placebo or no iron supplementation [11–13]. While comparable detailed data are not available in peritoneal dialysis (PD) patients, a cross-over trial of oral iron versus 2-monthly intravenous iron infusions in 28 PD patients showed that intravenous iron supplementation was associated with a much lower incidence of gastrointestinal disturbances (11 versus 46%, P < 0.05) and superior haemoglobin levels and body iron stores compared with oral iron treatment, albeit at a 6.5-fold higher cost [14]. Other small investigations have shown an inability of oral iron supplements to maintain adequate iron stores in PD patients over medium-to-long-term periods [7], principally because of poor compliance, dose-limiting gastrointestinal side effects and suboptimal gastrointestinal absorption of iron [15]. Despite these data, and because of the greater simplicity of oral iron therapy in PD patients in whom repeated intravenous cannulation is logistically more difficult, a preference exists for oral iron supplementation in PD patients in the first instance [16, 17].

An oral iron supplement able to safely, cheaply and more effectively maintain iron stores and haemoglobin levels in ESA-treated PD patients would therefore be of considerable clinical utility. Preliminary evidence suggests that heme iron polypeptide (HIP) might represent such a promising, novel, therapeutic strategy. HIP is produced by hydrolysis of bovine haemoglobin, resulting in a highly soluble heme moiety that contains more than 1% iron. Since heme is absorbed via a different receptor to that utilized by non-heme (ionic) iron (as is contained in standard oral iron supplements) [18, 19], the absorption kinetics of HIP and ionic iron are dissimilar. Non-heme iron absorption may be negatively affected by the high hepcidin levels seen in patients with ESKD [20], whereas heme iron absorption is not affected by hepcidin levels in other populations such as pregnant women, and may even be inversely correlated [21]. It might therefore be postulated that in ESKD patients with high hepcidin levels, heme iron absorption should be superior to non-heme iron. While studies in healthy individuals have suggested fewer side effects and significantly higher bioavailability of heme iron compared with non-heme iron [22], only very limited data are available regarding the utility of HIP in chronic kidney disease populations, including PD patients.

The aim of this study, therefore, was to determine the ability of HIP administration to augment iron stores in ESA-treated PD patients, when compared with conventional oral iron supplementation.

Materials and methods

Study oversight

The design and methodology of the randomized controlled trial of oral HEMc iron polypeptide Against Treatment with Oral Controlled Release Iron Tablets (HEMATOCRIT) have been described previously [23]. The study was an investigator-initiated, multi-centre, prospective, open-label, randomized Phase 4 controlled trial and was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12609000432213). The study protocol was approved by ethics committees at all participating centres. All patients provided written informed consent prior to trial participation.

Patients

The HEMATOCRIT trial included adult (≥18 years) patients with end-stage renal disease who had been receiving both PD and darbepoetin (DPO) treatment for at least 1 month and were both able and willing to give informed consent. Exclusion criteria included: (i) a history of psychological illness or condition which interfered with the ability to understand or comply with the requirements of the study; (ii) pregnancy or breast-feeding; (iii) known hypersensitivity to, or intolerance of, oral iron, HIP or DPO; (iv) active peptic ulcer disease; (v) vitamin B12 or folate deficiency; (vi) recent (within 1 month) acute infection; (vii) parathyroid hormone level >100 pmol/L; (viii) serum aluminium >2 μmol/L; (ix) presence of systemic haematological disease (including antibody-mediated pure red cell aplasia) or known haemoglobinopathy; (x) major surgical infection, acute myocardial infarction or malignancy within the last 3 months; (xi) intravenous iron therapy, vitamin C therapy, melatonin treatment, androgen therapy or blood transfusion within the previous month; (xii) serum ferritin >500 μg/L or transferrin saturation (TSAT) >50% and (xiii) religious or other objection to consuming product prepared from bovine blood. Patient recruitment occurred between January 2007 and September 2010. The trial stopped in March 2011 after the last recruited patient completed the 6-month study protocol.

Study treatment

Eligible patients were randomized 1:1 to receive oral iron in the form of either HIP (Proferin® ES, Colorado Biolabs, USA; one capsule twice daily equivalent to 240 mg elemental iron daily) or slow-release ferrous sulphate (Ferrogardumet®, Abbott, Sydney, Australia; one capsule twice daily equivalent to 210 mg elemental iron daily). To ensure adequate concealment of allocation, randomization was performed using sequentially numbered, opaque, sealed envelopes. The sequence of interventions was obtained from a computer-generated random number list in permuted blocks provided through the Australasian Kidney Trials network (www.akt.org.au) and was stratified according to centre. Study participants were instructed to take their study medication on an empty stomach at least 2 h apart from phosphate binder ingestion. They were allowed their usual diet. DPO dosages were modified by treating physicians to maintain haemoglobin concentrations between 95 and 125 g/L. Vitamin B and folic acid supplementation were permitted. Vitamin C supplementation, melatonin and androgen therapy were prohibited during the study period. All other care was provided according to standard unit protocols. Demographic and clinical data were collected at the time of participant entry into the study. Blood samples were collected at baseline and then every 2 months for determination of haemoglobin concentration, TSAT and serum ferritin concentration. Patients were reviewed in the outpatient clinic every 2 months to monitor their medications and general well-being. Each patient was followed for 6 months.

Outcome measures

The primary outcome measure was the difference in TSAT between the HIP and ferrous sulphate groups at the end of the 6-month study period. The primary outcome measure of TSAT was chosen in preference to serum ferritin concentration due to the latter being more readily affected by inflammation or infection and therefore potentially a less reliable single measure of iron stores [24, 25]. Haemoglobin concentration was not used as the primary outcome due to the possibility of co-intervention bias due to ESA use.

The secondary outcome measures were the differences between the two groups at the end of the 6-month study period with respect to serum ferritin concentration, proportion of iron-replete patients (TSAT >20% and serum ferritin >100 μg/L), haemoglobin level, DPO dosage, DPO sensitivity index (weekly DPO dose divided by haemoglobin level) and adverse events.

Statistical analysis

Data were expressed as numbers (percentages) for categorical variables, mean ± SD for continuously normally distributed variables and median (inter-quartile range; IQR) for continuous non-normally distributed variables. For univariable comparisons between treatment groups, the χ², Fisher exact test, unpaired t-test and Wilcoxon rank-sum test were used, as appropriate. The effect of the intervention on the primary outcome measure of TSAT at 6 months was measured by analysis of covariance (ANCOVA) with adjustment for baseline TSAT values, patient age, gender, diabetes, body mass index (BMI), cause of ESRF, dialysis duration...
and peritoneal membrane transport status. As malnutrition may have had a potential impact on outcomes, the relationship between serum albumin level and iron indices (TSAT and serum ferritin) was evaluated in post hoc analyses. Similar analyses were performed for the secondary outcomes of serum ferritin, haemoglobin concentration and DPO dosage at 6 months. Because of the non-normal distribution of DPO sensitivity index data at 6 months and inability to find an appropriate transformation, the effect of the intervention on Month 6 DPO sensitivity index tertiles was assessed using multivariate logistic regression. The intention-to-treat population included all randomized patients who provided at least one blood sample for TSAT measurement post-commencement of study medication. If a patient needed to receive intravenous iron during the trial, their last TSAT value prior to its administration was taken as their final TSAT value for the purposes of intention-to-treat analysis. The per-protocol population included only those individuals who took the study drug as prescribed for the entire duration of the study and had blood samples obtained at all specified time-points. The HEMATOCRIT trial aimed to recruit a total of 60 patients (30 in each group) on the basis of 80% power to detect a clinically important difference in TSAT of 7%, assuming a two-sided Type 1 error of 5%, a population standard deviation (SD) of 8% and a 15% drop-out rate over 6 months. Data were analysed using Stata Version 11.1, TX. All P-values were two-sided, and P < 0.05 was considered significant.

Results

Patients

A total of 62 patients were randomized to receive either HIP (n = 32) or ferrous sulphate (control, n = 30) (Figure 1). The two groups were well matched for all baseline characteristics (Table 1).

![Fig. 1. CONSORT diagram showing the number of patients recruited into the HEMATOCRIT study, randomized, followed and analysed.](image-url)
Transferrin saturation

No significant differences in TSAT values were observed between the HIP and control groups at any time point in the intention-to-treat population (Table 2; Figure 2). At 6 months, the median (IQR) TSAT was 22% (16, 29) in the HIP treatment group compared with 20% (17, 26) in controls (P = 0.65). Using ANCOVA, HIP treatment was not significantly associated with TSAT at 6 months (P = 0.95). Serum albumin level was not significantly associated with TSAT on univariable analysis (P = 0.56) and was therefore not incorporated into the multivariable model.

On per-protocol sensitivity analysis, no differences in 6-month TSAT values were observed between the HIP group [27% (20, 25)] and controls [21% (18, 26); P = 0.21; Table 3]. Treatment group was not predictive of TSAT at 6 months using ANCOVA (P = 0.52). Serum albumin level was not significantly associated with TSAT on univariable analysis (P = 0.27).

A further sensitivity analysis was undertaken in a subgroup of trial participants with absolute iron deficiency (serum ferritin <100 µg/L) at baseline (n = 14). In this subpopulation, the median (IQR) TSAT values at 6 months were 19 (13–30.5) in the HIP group and 20 (20.0–31) in controls (P = 0.70). HIP treatment was not associated with the end of study TSAT following multivariable adjustment (P = 0.88). When the subgroup analysis was broadened to include patients with either absolute or relative iron deficiency (TSAT <20% or serum ferritin <100 µg/L) at baseline (n = 28), HIP was not associated with the end of study TSAT following multivariable analysis (P = 0.74).

Serum ferritin

Serum ferritin levels at 6 months were significantly lower in those randomized to HIP compared with ferrous sulphate (P = 0.003, Table 2). Using ANCOVA, HIP treatment was independently predictive of lower serum ferritin levels at 6 months (P < 0.0001). Serum albumin level was not significantly associated with serum ferritin on univariable analysis (P = 0.23).

The proportions of iron-replete patients, defined as TSAT >20% and serum ferritin >100 µg/L, were similar

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**Table 1.** Baseline characteristics of HEMATOCRIT trial study participants according to treatment group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIP (n = 27)</th>
<th>Ferrous sulphate SR (n = 27)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58 (50, 71)</td>
<td>61 (51, 75)</td>
<td>0.33</td>
</tr>
<tr>
<td>Male</td>
<td>12 (44%)</td>
<td>11 (41%)</td>
<td>0.78</td>
</tr>
<tr>
<td>Racial origin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>23 (85%)</td>
<td>21 (78%)</td>
<td>0.84</td>
</tr>
<tr>
<td>Asian</td>
<td>3 (11%)</td>
<td>5 (19%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25 (23, 33)</td>
<td>27 (23, 32)</td>
<td>0.57</td>
</tr>
<tr>
<td>Duration on dialysis (months)</td>
<td>16 (8, 36)</td>
<td>21 (6, 41)</td>
<td>0.78</td>
</tr>
<tr>
<td>ESRF cause</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>12 (44%)</td>
<td>8 (30%)</td>
<td>0.27</td>
</tr>
<tr>
<td>Polycystic kidneys</td>
<td>3 (11%)</td>
<td>3 (11%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>2 (7%)</td>
<td>5 (19%)</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>2 (7%)</td>
<td>6 (22%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>8 (30%)</td>
<td>4 (15%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>0 (0%)</td>
<td>1 (4%)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>0 (0%)</td>
<td>2 (7%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>7 (26%)</td>
<td>5 (19%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Congestive cardiac failure</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>6 (22%)</td>
<td>7 (26%)</td>
<td>0.75</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>1 (4%)</td>
<td>5 (19%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>12 (44%)</td>
<td>10 (37%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Serum albumin level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>34 (31, 35)</td>
<td>33 (31, 34)</td>
<td>0.67</td>
</tr>
<tr>
<td>Month 2</td>
<td>31 (29, 35)</td>
<td>32 (28, 33)</td>
<td>0.42</td>
</tr>
<tr>
<td>Month 4</td>
<td>31 (30, 33)</td>
<td>31 (27, 34)</td>
<td>0.90</td>
</tr>
<tr>
<td>Month 6</td>
<td>31 (28, 33)</td>
<td>31 (28, 35)</td>
<td>0.67</td>
</tr>
<tr>
<td>Peritoneal transport status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>1 (4%)</td>
<td>3 (11%)</td>
<td>0.13</td>
</tr>
<tr>
<td>High average</td>
<td>5 (19%)</td>
<td>9 (33%)</td>
<td></td>
</tr>
<tr>
<td>Low average</td>
<td>20 (74%)</td>
<td>13 (48%)</td>
<td>0.92</td>
</tr>
<tr>
<td>Low</td>
<td>1 (4%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>0 (0%)</td>
<td>2 (7%)</td>
<td></td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>22 (18, 33)</td>
<td>20 (19, 33)</td>
<td>0.92</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>174 (89, 277)</td>
<td>182 (100, 325)</td>
<td>0.59</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>112 ± 13</td>
<td>113 ± 10</td>
<td>0.69</td>
</tr>
<tr>
<td>Darbepoetin dose (units/week)</td>
<td>25 (13, 30)</td>
<td>20 (15, 40)</td>
<td>0.66</td>
</tr>
<tr>
<td>Iron replete (TSAT &gt;20% and serum ferritin &gt;100 µg/L)</td>
<td>12 (44%)</td>
<td>13 (48%)</td>
<td>0.79</td>
</tr>
</tbody>
</table>

HIP, heme iron polypeptide; SR, sustained release.

Values are expressed as number (frequency), median (IQR) or mean ± standard deviation.
in the HIP and control groups at 6 months (52 versus 56%, P = 0.79, Table 2).

**Haemoglobin level**

There were no significant differences in haemoglobin levels at 6 months in those randomized to HIP compared with ferrous sulphate (P = 0.59, Table 2). Using ANCOVA, HIP treatment was not significantly associated with 6-month haemoglobin levels (P = 0.64).

**Darbepoetin dose and responsiveness**

DPO dose was not significantly associated with HIP treatment, either at 6 months (P = 0.66, Table 2) or over the course of the study using ANCOVA (P = 0.54). The DPO sensitivity index was not significantly associated with HIP treatment, either at 6 months (P = 0.96, Table 2) or over the course of the study using multivariate logistic regression (P = 0.30).

**Adverse events**

Adverse event rates (including gastrointestinal adverse events) were not significantly different between the intervention and control groups (Table 4).

**Cost**

The cost (in Australian dollars) of treating one patient with HIP (two tablets daily) for the 6-month study period was $181.44, compared with $26.88 for two tablets daily of ferrous sulphate.

**Discussion**

This study represents the first prospective randomized controlled trial of oral HIP versus ferrous sulphate therapy in patients with chronic kidney disease. It demonstrated a lack of advantage of twice-daily HIP over ferrous sulphate, with the two forms of iron supplementation resulting in similar TSAT values over a 6-month period in the intention-to-treat population, the per-protocol population and subgroups with absolute iron deficiency (with or without relative iron deficiency). HIP treatment did not alter iron-replete status, haemoglobin levels, DPO dose, DPO responsiveness or the frequency of adverse events (including gastrointestinal adverse events), but did result in inferior serum ferritin concentrations and a 6.75-fold increase in iron supplementation costs. The observed high rates of both non-adherence with prescribed therapy and censoring events demonstrate the difficulties associated with oral iron supplementation and conducting clinical trials in this patient group.

The results of the present study contrast with data obtained in healthy individuals. In a double blind-randomized study involving 14 non-anaemic subjects without evidence of inflammatory disease, significantly higher bioavailability was observed following administration of HIP compared with ferrous fumarate or placebo [22]. Specifically, HIP was associated with a 23-fold greater increment in serum iron compared with ferrous fumarate on a milligram-per-milligram basis [22]. Interestingly, however, when subjects in this study were separated into two groups according to their baseline ferritin levels (<50 versus >50 μg/L), the increment in serum iron levels with HIP compared with ferrous fumarate and placebo was only significant in the low ferritin group. Given that the maintenance of serum ferritin >100 μg/L is recommended

### Table 2. Primary and secondary outcomes in the HEMATOCRIT trial intention-to-treat population at 6 months

<table>
<thead>
<tr>
<th>Outcome</th>
<th>HIP (n = 27)</th>
<th>Ferrous sulphate SR (n = 27)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferrin saturation (%)</td>
<td>22 (16, 29)</td>
<td>20 (17, 26)</td>
<td>0.65</td>
</tr>
<tr>
<td>Ferritin (μg/L)</td>
<td>124 (75, 232)</td>
<td>292 (247, 361)</td>
<td>0.003*</td>
</tr>
<tr>
<td>Iron replete (%)</td>
<td>14 (52%)</td>
<td>15 (56%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>111 (105, 125)</td>
<td>113 (106, 122)</td>
<td>0.59</td>
</tr>
<tr>
<td>Darbepoetin dose (μg/week)</td>
<td>20 (10, 50)</td>
<td>20 (15, 40)</td>
<td>0.66</td>
</tr>
<tr>
<td>Darbepoetin sensitivity index (μg L/g Hb/week)</td>
<td>0.14 (0.06, 0.44)</td>
<td>0.16 (0.07, 0.26)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

HIP, heme iron polypeptide; SR, sustained release.
*A significant result.

[Fig. 2. Box and whisker plots of percentage TSAT with HIP (grey bars) compared with ferrous sulphate (checked bars) treatment at Months 0 (Baseline), 2 (M2), 4 (M4) and 6 (M6) post-commencement of study medication. The horizontal lines within the boxes represent median values. The upper and lower limits of each box represent the upper and lower quartiles, respectively. The upper and lower error bars represent the 90th and 10th percentiles, respectively. The solid dots represent outliers.](#)
in dialysis patients to maintain ESA efficacy [16], this suggests a reduced likelihood of observing an advantage of HIP in this patient group. Nevertheless, no benefit of HIP therapy was observed in PD patients with serum ferritin levels <100 µg/L in the HEMATOCRIT trial.

Only one previous trial has investigated the utility of HIP in a chronic kidney disease population. Nissenson et al. [26] performed an open-label, pre-test/post-test trial of HIP (one tablet tds) administered in lieu of intravenous iron supplementation to 37 recombinant human erythropoietin (rHuEPO)-treated haemodialysis patients over a 6-month period. Although 4 (11%) of 37 patients dropped out due to gastrointestinal intolerance (n = 3) or insufficient iron supplementation (n = 1) and 5 patients (14%) were excluded due to unrelated complications or protocol violation, HIP demonstrated the ability to maintain haematocrit targets and iron stores and significantly improve rHuEPO efficiency (mean rHuEPO dose/haemoglobin levels fell from 1270 to 1023 U g/mo/dL, P = 0.04) in the majority of study participants. However, the results of this study were significantly limited by its non-randomized design and the potential for co-intervention and observer biases. Furthermore, failure of study investigators to analyse results on an intention-to-treat basis limits the applicability of these data to ‘real-world’ dialysis populations.

In the present study, similar TSAT values were observed at 6 months irrespective of treatment with HIP versus ferrous sulphate therapy. Similarly, HIP demonstrated no advantage over ferrous sulphate in maintaining haemoglobin levels or reducing DPO utilization. While it is possible that results may have been biased in favour of the null hypothesis due to the low proportion of study participants that completed the trial according to protocol (46%), the majority (66%) of protocol deviations occurred due to non-compliance with study medication or dose reduction or medication cessation due to adverse events. Given that the importance of continuing with all prescribed medications (irrespective of side effects, where at all possible) was heavily emphasized at all clinic visits, it is unlikely that a higher level of adherence could be achieved in a clinical setting. In addition, a similar lack of advantage of HIP over ferrous sulphate for the primary and secondary end points was observed in the per-protocol population.

One possible reason for why HIP failed to improve iron stores compared with ferrous sulphate might relate to the fact that patients were instructed to take their study medication on an empty stomach at least 2 h apart from phosphate binder ingestion. It has been postulated that the increased bioavailability of heme iron compared with ionic iron may be due to less inhibition of gastrointestinal iron absorption by dietary constituents, such as polyphenolic tannins, phytates, soy and dairy products [22]. By administrating both preparations in the fasting state, any potential benefit of HIP may have been minimized.

Interestingly, despite similar serum ferritin levels at baseline, serum ferritin levels at 6 months were significantly lower in individuals treated with HIP than in those treated with ferrous sulphate. This raises the possibility that HIP therapy may actually have constituted an inferior form of oral iron supplementation compared with oral ferrous sulphate. Since serial C-reactive protein measurements were not performed in the HEMATOCRIT trial, a reduction in serum ferritin levels in the HIP group due to an acute phase response cannot be excluded. However, this possibility would seem unlikely, particularly in the light of the comparable frequency of infectious and inflammatory adverse events observed in the HIP and control groups.

A major limitation with oral iron supplementation is impaired compliance due to gastrointestinal intolerance.

### Table 3. Primary and secondary outcomes in the HEMATOCRIT trial per-protocol population at 6 months

<table>
<thead>
<tr>
<th>Outcome</th>
<th>HIP (n = 12)</th>
<th>Ferrous sulphate SR (n = 13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transferrin saturation (%)</td>
<td>27 (20, 35)</td>
<td>21 (18, 26)</td>
<td>0.21</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>127 (98, 302)</td>
<td>268 (220, 346)</td>
<td>0.11</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>113 (107, 124)</td>
<td>112 (103, 124)</td>
<td>0.54</td>
</tr>
<tr>
<td>Darbepoetin dose (µg/week)</td>
<td>30 (13, 60)</td>
<td>20 (10, 20)</td>
<td>0.18</td>
</tr>
<tr>
<td>Darbepoetin sensitivity index (µg L/g Hb/week)</td>
<td>0.22 (0.8, 0.46)</td>
<td>0.16 (0.08, 0.19)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

HIP, heme iron polypeptide; SR, sustained release.

### Table 4. Adverse events and serious adverse events in the HEMATOCRIT trial

<table>
<thead>
<tr>
<th>Outcome</th>
<th>HIP (n = 32)</th>
<th>FESO4 (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total adverse events</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Gastrointestinal adverse events</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Constipation</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhoea and constipation</td>
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<td>1</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Other adverse events</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Restless legs</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Serious adverse events</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cardiac arrest</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Peritoneal catheter exit site</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Dyspnoea</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Abdominal (infected liver cyst)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Peritoneal (infected ulcer)</td>
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<td>1</td>
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<tr>
<td>Peritoneal dialysis membrane leak</td>
<td>1</td>
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<tr>
<td>Fluid overload</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Syncopal episode</td>
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<td>1</td>
</tr>
<tr>
<td>Generalized weakness</td>
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<td>1</td>
</tr>
<tr>
<td>Hyperecalaeemia</td>
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<td>0</td>
</tr>
<tr>
<td>Calciphylaxis</td>
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</table>

HIP, heme iron polypeptide. 
P ≥ 0.05 for all comparisons using the χ² or Fisher exact test.
Because HIP is absorbed via a different receptor to that utilized by ionic iron [18], it has been suggested that HIP might exhibit an improved gastrointestinal side effect profile to that observed with standard oral iron preparations [18, 19, 22]. In the present study, however, gastrointestinal side effects were experienced by 6 of 27 individuals (22%) receiving HIP therapy compared with 4 of 27 (13%) administered ferrous sulphate (P = 0.51). In all cases, symptoms were sufficient to necessitate dose reduction or medication cessation. Given that this study was not powered adequately to explore differences in gastrointestinal toxicity between the two preparations, no definitive conclusions can be drawn from these data. However, they do suggest that a major improvement in gastrointestinal symptomatology with HIP therapy would be unlikely, even in a larger study population.

The principal limitation of this trial was its open-label design, which had the potential to introduce observer and co-intervention bias. However, the primary outcome measure, TSAF, was an objective one and the outcome assessor was blinded to the group assignment. Gastrointestinal adverse events were assessed subjectively and may have been susceptible to placebo or nocebo effects. Although the trial recruited to above the target sample size of 60 and the observed drop-out rate from the intention-to-treat population was less than anticipated (13 instead of 15%), the possibility of a Type 2 statistical error cannot be entirely excluded. Study medication dosage reduction or cessation were relatively common in both groups, primarily related to gastrointestinal intolerance, and were unable to be mitigated against in spite of greater trial-related efforts to monitor and encourage compliance than might be routinely achievable in clinical practice. Nevertheless, given that HIP therapy was associated with a statistically significant and clinically important fall in serum ferritin levels during the HEMATOCRIT trial, a superior haematinic benefit of HIP over ferrous sulphate seems very unlikely.

In conclusion, the HEMATOCRIT trial is the first randomized study to assess the utility of HIP compared with ferrous sulphate therapy in a chronic kidney disease population. Study participants were reflective of PD patients in the developed world, making our results generalizable to this patient group. We conclude no benefit of HIP compared with conventional oral iron supplements in augmenting body iron stores in PD patients. Furthermore, the significant reduction in serum ferritin levels and high costs associated with HIP therapy suggest that it is unlikely to have a significant role in iron supplementation in PD patients.

**Acknowledgements.** The authors gratefully acknowledge the contributions of PD nursing staff, trial coordinators, research staff and patients. K.A.B. is a current recipient of a National Health and Medical Research Council Medical/Dental Post-graduate Research Scholarship. D.W.J. is a current recipient of a Queensland Government Health Research Fellowship.

Funding. The study was funded by Amgen Australia Pty Ltd. The funding body played no role in the study design, collection, analysis and interpretation of data; writing of the manuscript; or the decision to submit the manuscript for publication. The members of the Trial Management Committee attest that the study was performed in accordance with the protocol and the statistical analysis plan and vouch for the accuracy and completeness of the reported analyses.

**Conflict of interest statement.** D.W.J. is a consultant for Amgen, Janssen-Cilag, Roche, Baxter Healthcare and Sigma. He has received research grants, speakers’ honoraria and travel sponsorships from Amgen, Janssen-Cilag, Roche and Baxter Healthcare. He is a current recipient of a Queensland Government Health Research Fellowship. F.B. is a consultant for Baxter and Fresenius. She has received travel sponsorships and speakers’ honoraria from Roche and Janssen-Cilag. C. M.H. has received consultancy fees, research grants, speakers’ honoraria and travel sponsorships from Amgen. The HEMATOCRIT trial was funded by Amgen. The results presented in this paper have not been published previously in whole or part, except in abstract format.

**References**

NGAL in renovascular hypertension


Received for publication: 9.5.2012; Accepted in revised form: 3.7.2012

doi: 10.1093/ndt/gfs370
Advance Access publication 23 August 2012

Chronic renovascular hypertension is associated with elevated levels of neutrophil gelatinase-associated lipocalin

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Abstract

Background. Renovascular hypertension (RVH) is characterized by chronic inflammation of the stenotic kidney and progressive renal dysfunction. Neutrophil gelatinase-associated lipocalin (NGAL), an acute phase protein induced in inflammatory conditions and ischemia, is a novel biomarker for acute kidney injury. We hypothesized that chronic RVH would be associated with increased renal and circulating NGAL levels.

Methods. We prospectively measured renal vein and inferior vena cava (IVC) levels of NGAL and inflammatory cytokines in essential hypertensive (EH) and RVH patients, during constant sodium intake and anti-hypertensive regimens, and compared them with systemic levels in age-matched normotensive subjects (n = 22 each). In addition, we measured urinary NGAL and kidney injury molecule (KIM)-1 in all patients.

Results. Blood pressure, serum creatinine, estimated glomerular filtration rate (eGFR), lipid panels and medications were similar in RVH and EH. Systemic, stenotic and contralateral renal vein levels of NGAL were all similarly elevated in RVH versus normal hypertension and EH (P < 0.05), as were renal vein levels of inflammatory markers like tumor necrosis factor-α. Furthermore, renal vein NGAL levels inversely correlated with eGFR, and directly with renal vein (but not systemic) levels of inflammatory markers. Urinary levels of NGAL and KIM-1 were elevated in both EH and RVH, as were systemic levels of C-reactive protein.

Conclusions. Chronic RVH is associated with elevated NGAL levels, likely due to ongoing kidney and systemic inflammation and ischemia. These findings may also imply the occurrence of the inflammation process in chronic RVH, which might contribute to the poorer outcomes of RVH compared with EH patients.

Keywords: cytokines; inflammation; KIM-1; NGAL; renovascular hypertension

Introduction

Renovascular hypertension (RVH), a common disorder in patients with atherosclerotic vascular occlusive disease of the kidney, is characterized by increased activity of the renin–angiotensin system, reduced renal perfusion pressure and progressive renal dysfunction [1]. Moreover, the combination of RVH and decreased glomerular filtration rate (GFR) predisposes to several complications such as coronary artery disease, cerebrovascular disease and peripheral vascular disease, leading to an increase in cardiovascular mortality [2].

Recent studies have suggested a role for renal inflammation in aggravating renal dysfunction in experimental