Effects of Dietary Factors on the Pharmacokinetics of $^{58}$Fe-labeled Hemin After Oral Administration in Normal Rats and the Iron-deficient Rats

Yongjie Zhang · Di Zhao · Jie Xu · Chunxiang Xu · Can Dong · Qingwang Liu · Shuhua Deng · Jie Zhao · Wei Zhang · Xijing Chen

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Abstract Hemin, iron (III) protoporphyrin chloride (IX), as a stable form of heme iron, has been used in iron absorption studies. The aim of the present study was to elucidate the influences of body iron status and three dietary factors (green tea extract, ascorbic acid, and calcium) on the pharmacokinetics of hemin using stable isotope labeling methods followed by ICP-MS measurement. In this study, a rapid, sensitive, and specific ICP-MS method for the determination of $^{58}$Fe originating from hemin in rat plasma was developed and a rat model of iron deficiency anemia was established. It was found that hemin iron absorption increased significantly under iron deficiency anemia status, with AUC$_{0-t}$ and AUC$_{0-\infty}$ showing significant increase in anemic rats compared to normal ones. Green tea extract strongly inhibited hemin iron absorption in both normal rats and iron-deficient rats. In normal rats administered with green tea extract, $C_{\text{max}}$ resulted significantly reduced, whereas in anemic rats administered with green tea extract both AUC$_{0-t}$ and AUC$_{0-\infty}$ were reduced. On the other hand, ascorbic acid significantly affected hemin iron absorption only in iron-deficient rats, in which $C_{\text{max}}$ showed a significant increase. Interestingly, calcium slowed down the hemin iron absorption rate in normal rats, MRT$_{0-t}$ being significantly different in calcium-treated animals compared to untreated ones. This trend also appeared in the iron-deficient group but it did not reach statistical significance. Our data suggest that the mechanism of hemin iron absorption is regulated by body iron status and dietary factors can influence hemin iron absorption to varying degrees. Moreover, these results may also have general implication in the iron deficiency treatment with iron supplements and fortification of foods.

Keywords Fe · Hemin · Iron-deficient rats · Iron absorption · Pharmacokinetics · Dietary factors · ICP-MS

Abbreviations

ICP-MS Inductively coupled plasma mass spectrometry
AUC Area under concentration–time curve
$C_{\text{max}}$ Maximum plasma concentration
$CL_z$ Clearance
$V_z$ Apparent volume of distribution
$t_{1/2}$ Half life time
MRT Mean retention time
RBC Erythrocyte count
Hb Hemoglobin
Hct Hematocrit
RDW Red cell distribution width
MCV Mean corpuscular volume
MCH Mean corpuscular hemoglobin
MCHC Mean corpuscular hemoglobin concentration

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Introduction

Currently, iron deficiency remains the most common nutritional deficiency in the world; with widespread prevalence among children and women in developing countries [1]. In the wake of this circumstance, many countries introduced varieties of iron supplements and fortification of foods such as iron-fortified corn tortillas and black bean paste [2], breakfast adding ascorbic acid or Na2EDTA [3], and iron-fortified bread adding ascorbyl palmitate [4] to combat iron deficiency. Dietary iron is present as nonheme and heme iron, distinguished by its inorganic or organic form. Nonheme iron accounts for more than 85% of the total iron in the diet, but the low bioavailability limits its contribution to total absorbed iron. Besides, many research showed that nonheme iron is easily affected by many dietary factors: phytic acid, calcium [5] and polyphenols [6] can all inhibit nonheme iron uptake in different degrees. On the contrary, ascorbic acid can enhance nonheme iron absorption [7]. However, heme iron, a kind of iron that is bound to the iron protoporphyrin IX prosthetic groups of proteins, reveals higher bioavailability than nonheme iron. Some studies showed that heme iron is less affected by other components of the diet [8].

Hemin, iron (III) protoporphyrin chloride (IX), is extracted by releasing the globular chain of hemoproteins, such as hemoglobin and myoglobin, and the fifth coordination link of iron atom is occupied by a chloride anion [9]. Compared to heme, in which iron is present in its reduced state (Fe2+), in heme iron exists in its oxidized state (Fe3+), and this makes hemin a more stable molecule. This compound was widely used in the treatment of acute intermittent porphyria through intravenous injection since the early 1970s [10], however, its potential as an iron resource has not been fully studied.

Up until now, 55Fe and 59Fe, two radioisotopes of iron, have been used by many researchers in studies of iron absorption and metabolism [11–13]. However, the main disadvantage of this method is that using 55Fe and 59Fe has a risk of exposure to radiation, and the specially equipped laboratories are required to handle animals with these isotopes treatment. Recently, using stable isotopes of iron (57Fe and 58Fe) in studies of iron absorption was considered as a modified method [14, 15]. In this study, we used 58Fe as a tracer to assess the bioavailability of hemin, and detected by inductively coupled plasma mass spectrometry (ICP-MS), which showed excellent sensitivity and selectivity in the determination of compounds containing metals [16].

Previous research showed that the absorption of iron can be upregulated in the status of iron deficiency anemia, but the absorption of heme iron cannot be upregulated to the same extent as nonheme iron [17, 18]. Other studies investigated the possible effects of variety of dietary factors on iron absorption mostly based on in vitro digestion methods [8, 19], nevertheless in vivo methods were seldom reported. In the present study, we compared the different pharmacokinetic behavior of 58Fe after oral administration of hemin in normal rats and the iron-deficient rats, and elucidated the effects of three dietary factors, green tea extract, ascorbic acid and calcium, on hemin iron absorption in different pathologic status.

Material and Methods

Chemicals and Reagents

Stable isotope, 58Fe, as metal powder (>99.86 %, enrichment), was obtained from ISOFLEX USA (San Francisco, CA, USA). 59Fe-labeled hemin (Fig. 1) (>98 %) was supplied by State Key Laboratory of Natural Medicines, School of Basic Science, China Pharmaceutical University. Germanium Plasma Emission Standard (>99.999 %, purity) was supplied by State Key Laboratory of Natural Medicines, School of Basic Science, China Pharmaceutical University. Green tea extract, ascorbic acid and calcium chloride of analytical grade were from Sinopharm Chemical Reagent Company (Shanghai, China). Triton X-100 of chemical purity, nitric acid of MOS grade, ascorbic acid and calcium chloride of analytical grade were from Shanghai ANPEL Scientific Instrument Co., Ltd. (Shanghai, China). Deionized water was purified using a Milli-Q system (Millipore, Milford, MA, USA). Standard stock solution of 58Fe was prepared in nitric acid (2 %, v/v) at 2 mg/mL and germanium (Ge) was in solution (nitric acid 2 %, v/v) at 1 mg/mL. Green tea extract (>98 %) was purchased from Nanjing Jizheng Medical Technology Co., Ltd. (Nanjing, China).

Establishment of Rat Iron Deficiency Model

Forty 8-week-old male Sprague–Dawley rats (200±20 g) of Specific Pathogen Free-grade were supplied by Shanghai SIPPR/BK Experimental Animal Co. (Shanghai, China). Animals were housed under standard conditions of temperature, humidity and light with food and water provided ad libitum and were acclimatized in the laboratory for seven successive days prior to study. All animal studies were approved by the Animal Ethics Committee of China Pharmaceutical University. Animals were divided into two groups: normal group and model group. Model group rats were fed with an iron-deficiency diet modified according to

<table>
<thead>
<tr>
<th>SI</th>
<th>Serum iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIBC</td>
<td>Total iron-binding capacity</td>
</tr>
<tr>
<td>TS</td>
<td>Transferrin saturation</td>
</tr>
<tr>
<td>SF</td>
<td>Serum ferritin</td>
</tr>
<tr>
<td>TRF</td>
<td>Transferritin</td>
</tr>
<tr>
<td>PCFT/HCP1</td>
<td>Heme carrier protein 1</td>
</tr>
<tr>
<td>DMT1</td>
<td>Divalent metal transporter 1</td>
</tr>
</tbody>
</table>

\(v/v\)
the American Institute of Nutrition 93 Growth Purified Diet (AIN-93G) [20] and purified water. This low-iron diet contains iron no greater than 7.8 mg/kg while the standard rodent chow had an iron concentration of 250 mg/kg. An exsanguination of 1.5 to 2 mL was performed twice a week to model group rats. Normal group rats were supplied with the standard rodent chow and cooled boiled water. According to literature described before, a hemoglobin level of less than 120 g/L and a hematocrit level of less than 36 % were considered as constant anemia condition [21].

Pharmacokinetic Study in Normal Rats and the Iron-deficient Rats

Normal group and model group animals were divided into 4 groups respectively according to their body weight and Hb concentrations: hemin group (n=5), green tea extract+hemin group (n=5), ascorbic acid+hemin group (n=5), and calcium+hemin group (n=5).

Green tea extract, ascorbic acid, and calcium were administered orally to the last three groups at a dose of 100 mg/kg in both normal rats and the iron-deficient rats, respectively. Normal rats and the iron-deficient rats in hemin group received the same volume of purified water simultaneously. $^{58}$Fe-labeled hemin was orally administered to all animals at dosage of 40 mg/kg (equivalent to $^{58}$Fe at 3.48 mg/kg) 20 min later. Plasma samples from normal and model rats (200 μL) were collected into heparinized tubes at the time points of 0, 0.5, 1, 1.5, 2, 4, 8, 15, 24, and 36 h separately. After immediately centrifuged at 12,000×g for 3 min, plasma samples were stored at −20 °C until analysis.

Hematological Measurements

The whole blood of normal rats and the iron-deficient rats were collected to determine the erythrocyte (RBC) count, hemoglobin (Hb) concentration, hematocrit (Hct), red cell distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) by using a hematology analyzer (Beckman Coulter). Serum iron (SI) and total iron-binding capacity (TIBC) were assayed using a kit from Jiancheng Biotech (Nanjing, China). Transferrin saturation (TS) was calculated by (SI÷TIBC)×100 %. Serum ferritin (SF) and transferritin (TRF) were analyzed using commercial ELISA kits (RapidBio, CA, USA).

Sample Preparation and Determination of $^{58}$Fe by ICP-MS

An aliquot of 50 μL rat plasma was diluted to a final volume of 1.0 mL using diluents containing Triton X-100 (0.01 %, v/v), nitric acid (0.05 %, v/v), and N-butyl alcohol (2 %, v/v). After vortex-mixing, the aliquot was injected for analysis by ICP-MS. All the samples were prepared using plastic pipes.

All experiments were carried out on an ICP-MS (Xseries 2, Thermo Scientific), which was operated with the Plasma Screen Plus sensitivity enhancement option fitted, Xt interface cones, and with Peltier cooling of the spray chamber. A standard quartz nebulizer was used, together with a standard quartz impact bead spray chamber and standard single piece, 1.5 mm i.d. injector quartz torch. The instrument was operated using standard instrument operation. Plasma Lab software was applied to instrument control, data acquisition, and analysis. The instrumental and operating conditions were optimized with the commented tune solution.

The operating parameters of ICP-MS instrument were as follows: RF power 1850 W, coolant gas flow 14.3 L/min,

![Fig. 1 The chemical structure of $^{58}$Fe-labeled hemin](image)

Table 1 Hematological parameters of normal group rats and model group rats at the end of inducing anemia experiment (results are means±SD, n=20)

<table>
<thead>
<tr>
<th>Group</th>
<th>RBC (10¹²/L)</th>
<th>Hb (g/L)</th>
<th>Hct (%)</th>
<th>RDW (%)</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>MCHC (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model rats</td>
<td>5.77±0.99</td>
<td>105±21</td>
<td>31.8±6.7</td>
<td>26.2±8.1</td>
<td>54.7±3.6</td>
<td>18.2±1.2</td>
<td>332±10</td>
</tr>
<tr>
<td>Normal rats</td>
<td>7.57±0.77</td>
<td>153±10</td>
<td>44.0±3.2</td>
<td>14.7±0.5</td>
<td>58.6±2.1</td>
<td>20.4±0.8</td>
<td>349±7</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.01 versus normal group
auxiliary gas flow 0.95 L/min, nebulizer gas flow 0.87 L/min, pump rate 1 mL/min, and peak jumping data acquisition mode: dwell time 10 s, duration time 42 s, and three replicates per sample. Ge (20 ng/mL) was added online using of three-way valve as internal standard.

For the detection of iron, the signals of 54Fe, 58Fe, 60Ni, 53Cr, and 72Ge were monitored but only 58Fe were used for calculations. Other elements were used to correct interference, correction formula was as follows:

\[ \frac{58\text{Fe}_{\text{fact}}}{\text{Total counts at mass 58} - 0.05593} = \frac{54\text{Fe} - 0.24921 \times 53\text{Cr}}{2.59021 \times 60\text{Ni}} \]

Data Analysis

The pharmacokinetic parameters were calculated using the pharmacokinetic software WinNonlin Professional Version 5.0.1 (Pharsight Corp., Mountain View, CA, USA) by noncompartmental method, and the dose of 58Fe was used in the calculation of these parameters. All data were presented as means with their standard deviation.

Statistical analysis was performed using SPSS v. 11.5 (SPSS, Chicago, IL). C\text{max} and AUC data were logarithmically transformed to fit a normal distribution. Analysis of variance (one-way ANOVA) with Dunnett for post-hoc analysis was used to compare the pharmacokinetic parameters among different experimental groups. T\text{max} data were analyzed by the Wilcoxon W tests. Differences were considered significant when \( p < 0.05 \).

Results

Validation of Analytical Method

The calibration curve of 58Fe was linear within the ranges from 5 to 1000 ng/mL in rat dilution samples (\( R^2 > 0.999 \)). The relative standard deviation of precision (15 replicate samples) was 7.67, 0.81, and 3.79 % at 10, 100, and 800 ng/mL, respectively. The accuracies determined for intra- and inter-day were all within 100±10 % of the actual values. After dilution, no significant matrix effect occurred. The ICP-MS method described above has been proved to be sensitive, selective, and rapid for determination of 58Fe in rat plasma samples. The optimized method was validated to guarantee the need of the determination.

Preparation of Rat Iron Deficiency Model

In the third week of the start of the experiment, at 12 weeks of age, animals displayed anemia with an RBC of 5.77±0.99×10\(^12\)/L, Hb of 105±21 g/L, and Hct of 31.8±6.7 %. In particular, the hematological parameters of model group rats showed significantly lower than those of normal group rats (Table 1). At the end of the third week, the biochemical parameters associated with iron storage status of model group also varied significantly (Table 2). The level of SI, TS, and SF decreased over 100 % compared with normal group, while TIBC and TRF values increased 26.4 and

<table>
<thead>
<tr>
<th>Group</th>
<th>SI (µmol/L)</th>
<th>TIBC (µmol/L)</th>
<th>TS (%)</th>
<th>SF (ng/mL)</th>
<th>TRF (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model rats</td>
<td>12.7±12.8(^a)</td>
<td>130.9±20.9(^g)</td>
<td>10.1±10.3(^b)</td>
<td>40.5±29.7(^b)</td>
<td>4.81±1.86(^*)</td>
</tr>
<tr>
<td>Normal rats</td>
<td>47.7±18.9</td>
<td>103.6±4.9</td>
<td>46.4±19.3</td>
<td>86.4±16.3</td>
<td>3.02±0.25</td>
</tr>
</tbody>
</table>

\( ^a p<0.05, ^b p<0.01 \) versus normal group

Table 2: Biochemical parameters of normal group rats and model group rats at the end of inducing anemia experiment (results are means±SD, \( n=20 \))

<table>
<thead>
<tr>
<th>Parameters</th>
<th>58Fe originating from hemin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model Rats</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>9.35±3.42</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>295.7±191.99</td>
</tr>
<tr>
<td>( \text{AUC}_{0-\infty} ) (ng/mL h)</td>
<td>2589.05±1027.58(^*)</td>
</tr>
<tr>
<td>( \text{AUC}_{0-\infty} ) (ng/mL h)</td>
<td>2759.62±1100.07(^*)</td>
</tr>
<tr>
<td>( \text{CL}_{d} ) (L/h/kg)</td>
<td>1.13±0.47(^*)</td>
</tr>
<tr>
<td>( V_{z} ) (L/kg)</td>
<td>15.08±8.60</td>
</tr>
<tr>
<td>MRT(_{0-\infty}) (h)</td>
<td>9.74±1.44(^*)</td>
</tr>
</tbody>
</table>

\( ^* p<0.05 \) versus normal group

Table 3: Mean pharmacokinetic parameters of 58Fe-labeled hemin in plasma after oral administration at a dose of 40 mg/kg to normal rats and the iron-deficient rats (results are means±SD, \( n=5 \))
Effects of dietary factors on the pharmacokinetics of $^{58}$Fe Originating from Hemin in Normal Rats

The mean plasma concentration–time curves of $^{58}$Fe originating from hemin after oral administration at a dose of 40 mg/kg to normal rats in the presence and absence of three different dietary factors are shown in Fig. 3. $^{58}$Fe-labeled hemin was administered after 20 min to the pretreatment of green tea extract, ascorbic acid, and calcium in normal rats. The plasma concentrations of $^{58}$Fe originating from hemin in green tea extract+hemin group declined extremely, in ascorbic acid+hemin group increased slightly compared with hemin group. Interestingly, the plasma concentrations of $^{58}$Fe originating from hemin in calcium+hemin group showed a delayed absorption trend.

The mean pharmacokinetic parameters of these four groups were summarized in Table 4. Compared with the control group (hemin group), the $C_{\text{max}}$, $CL_z$, and $V_z$ of green tea extract+hemin group was significantly different by 19.3, 226.6, and 328.3 %, respectively. The AUC$_{0-t}$ and AUC$_{0-\infty}$ of green tea extract+hemin group showed numerically but not significantly lower than the control group ($p=0.117$ and 0.126, respectively). The AUC$_{0-t}$, AUC$_{0-\infty}$, $C_{\text{max}}$, $CL_z$, and $V_z$ of ascorbic acid+hemin group were of 158.9, 166.4, 113.9, 50.5, and 62.9 % of the control group, respectively. No significant difference was observed. In calcium+hemin group, MRT$_{0-t}$ and $T_{\text{max}}$ (data not shown) were significantly different from those of the control group, suggesting that calcium influenced the absorption and elimination rate of hemin in vivo.

Effects of Dietary Factors on the Pharmacokinetics of $^{58}$Fe Originating from Hemin in Iron-deficient Rats

The mean plasma concentration–time curves of $^{58}$Fe originating from hemin after oral administration at a dose of 40 mg/kg to model rats in the presence and absence of three different dietary factors are shown in Fig. 4. $^{58}$Fe-labeled hemin was administered after 20 min to the pre-treatment of green tea extract, ascorbic acid, and calcium in model rats. The plasma concentrations of $^{58}$Fe originating from hemin in calcium+hemin group showed a delayed absorption trend compared with normal rats.

Table 4 Mean pharmacokinetic parameters of $^{58}$Fe-labeled hemin in plasma after oral administration at a dose of 40 mg/kg to normal rats in the presence and absence of green tea extract, ascorbic acid and calcium (results are means±SD, $n=5$)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hemin</th>
<th>Green tea extract+hemin</th>
<th>Ascorbic acid+hemin</th>
<th>Calcium+hemin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>7.29±3.48</td>
<td>11.22±5.63</td>
<td>9.39±0.84</td>
<td>7.92±1.48</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>153.5±74.73</td>
<td>29.63±13.99 $^*$</td>
<td>174.77±129.79</td>
<td>119.58±36.86</td>
</tr>
<tr>
<td>AUC$_{0-t}$ (ng/mL h)</td>
<td>1072.06±720.85</td>
<td>300.36±96.28</td>
<td>1703.19±935.95</td>
<td>1680.22±821.23</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (ng/mL h)</td>
<td>1133.25±698.48</td>
<td>402.78±140.82</td>
<td>1885.51±948.42</td>
<td>1766.13±878.64</td>
</tr>
<tr>
<td>$CL_z$ (L/h/kg)</td>
<td>3.31±1.92</td>
<td>7.50±3.11 $^*$</td>
<td>1.67±0.65</td>
<td>1.78±0.70</td>
</tr>
<tr>
<td>$V_z$ (L/kg)</td>
<td>35.08±27.81</td>
<td>115.17±51.29 $^*$</td>
<td>22.07±7.29</td>
<td>19.38±5.40</td>
</tr>
<tr>
<td>MRT$_{0-t}$ (h)</td>
<td>7.26±1.65</td>
<td>9.68±0.89</td>
<td>9.84±1.54</td>
<td>11.44±1.04 $^*$</td>
</tr>
</tbody>
</table>

*p<0.05, $^*$p<0.01 versus hemin group
green tea extract, ascorbic acid, and calcium in the iron-deficient rats. The plasma concentrations of $^{58}$Fe originating from hemin in green tea extract+hemin group declined extremely, in ascorbic acid+hemin group increased enormously compared with hemin group. Similar to what observed in normal rats, the plasma concentrations of $^{58}$Fe originating from hemin in calcium+hemin group also showed a delayed absorption trend, but slightly higher than hemin group.

Table 5 showed the pharmacokinetic parameters of the four groups of iron-deficient rats. The $AUC_{0,t}$, $AUC_{0,\infty}$, $CL_z$, and $V_z$ in green tea extract+hemin group showed significantly different from those in hemin group. After calculation, the $AUC_{0,t}$, $AUC_{0,\infty}$, and $C_{max}$ in green tea extract+hemin group were only 15.7, 18.0, and 13.7 % of those in hemin group. Ascorbic acid showed strongly enhancement to hemin iron absorption. However, because of the quick elimination of $^{58}$Fe in the model animals, only $C_{max}$ and MRT$_{0-t}$ showed significant different from those of hemin group. The $AUC_{0,t}$, and $AUC_{0,\infty}$ were comparable within the two groups. Although calcium seems to delay the absorption of $^{58}$Fe-labeled hemin, the $AUC_{0-t}$, $AUC_{0,\infty}$, $C_{max}$, $CL_z$, and $V_z$ did not changed apparently in calcium+hemin group compared with hemin group. Nevertheless, the $t_{1/2}$ and MRT$_{0-t}$ were longer by 125.0 and 111.5 % respectively, while the $T_{max}$ (data not shown) showed a certain degree of difference ($p=0.102$).

**Discussion**

In this study, a rapid, sensitive, and specific ICP-MS method for the determination of $^{58}$Fe originating from hemin in rat plasma has been developed. In order to eliminate the interference from background, a correction formula was used. The assay was linear in the concentration range of 5–1,000 ng/mL with the correlation coefficients $R^2=0.999$. And this method was satisfactory enough for the study of plasma pharmacokinetics of $^{58}$Fe originating from hemin.

We established the model of rat iron deficiency anemia to investigate hemin iron absorption under different pathological status. The enormous decreases such as the RBC, Hb, Hct, MCV, MCH, MCHC, SI, TS, and SF values and marked increases in the RDW, TIBC, and TRF values in the model group of rats (Tables 1 and 2) showed that a state of iron deficiency anemia had been developed. All of the monitoring anemic indices of the model group were significantly different from those of the normal group rats and these were consistent with the characteristics of iron deficiency anemia [22, 23], suggesting the reliability of the model and which could be used in the following experiments.

Previous studies less focused on the heme iron absorption under iron deficiency status. In Roberts SK’s study, a method of in vivo intestinal perfusion of $[^{14}$C]heme in groups of rats of differing iron status was developed. Results showed that marked iron deficiency status induced an over fourfold rise in heme absorption compared with iron-replete animals [24]. In this paper, we compared the different pharmacokinetic profiles of $^{58}$Fe originating from hemin after oral administration to normal rats and the iron-deficient rats. Results showed that hemin iron absorption increased enormously under iron deficiency anemia status, with the $AUC_{0,t}$, $AUC_{0,\infty}$, and $C_{max}$ higher by over two times than those of normal group rats (Fig. 2 and Table 3). Moreover, the significant difference of

**Table 5** Mean pharmacokinetic parameters of $^{58}$Fe-labeled hemin in plasma after oral administration at a dose of 40 mg/kg to the iron-deficient rats in the presence and absence of green tea extract, ascorbic acid and calcium (results are means ± SD, $n=5$)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hemin</th>
<th>Green tea extract+hemin</th>
<th>Ascorbic acid+hemin</th>
<th>Calcium+hemin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>9.35±3.42</td>
<td>10.40±8.16</td>
<td>17.96±12.12</td>
<td>11.69±3.95</td>
</tr>
<tr>
<td>$C_{max}$ (ng/mL)</td>
<td>295.72±191.99</td>
<td>40.49±23.12</td>
<td>638.83±219.99*</td>
<td>290.97±270.16</td>
</tr>
<tr>
<td>$AUC_{0,t}$ (ng/mL h)</td>
<td>2589.05±1027.58</td>
<td>406.40±129.31*</td>
<td>2276.20±949.59</td>
<td>2096.65±761.37</td>
</tr>
<tr>
<td>$AUC_{0,\infty}$ (ng/mL h)</td>
<td>2759.62±1100.07</td>
<td>495.84±231.60*</td>
<td>2499.75±864.62</td>
<td>2331.92±874.23</td>
</tr>
<tr>
<td>$CL_z$ (L/h/kg)</td>
<td>1.13±0.47</td>
<td>6.22±2.31*</td>
<td>1.17±0.34</td>
<td>1.27±0.39</td>
</tr>
<tr>
<td>$V_z$ (L/kg)</td>
<td>15.08±8.60</td>
<td>76.50±31.98*</td>
<td>32.39±26.02</td>
<td>19.93±1.36</td>
</tr>
<tr>
<td>MRT$_{0-t}$ (h)</td>
<td>9.74±1.44</td>
<td>10.18±4.09</td>
<td>6.05±0.63*</td>
<td>10.86±2.19</td>
</tr>
</tbody>
</table>

*p<0.05, *p<0.01 versus hemin group
Effects of dietary factors on the pharmacokinetics

CL₂ and MRT₀₋∞ between model rats and normal rats indicate that the elimination rate of absorbed ⁵⁸Fe were significantly lower and the mean retention time were significantly longer under iron deficiency anemia status. We may speculate that the relatively higher amount of absorbed ⁵⁸Fe and the status of body iron lacking lead the weaker elimination of ⁵⁸Fe in the iron-deficient rats compared with normal rats. West et al. summarized that there were two prevailing hypotheses explaining the absorption mechanism of heme iron: one is a heme receptor-mediated endocytosis and the other is heme transporter, heme carrier protein 1 (PCFT/HCP1). Interestingly, his study showed that the divalent metal transporter 1 (DMT1) may also play a role in heme iron absorption [25]. Another study on hephaestin found that dietary iron-deficiency upregulated hephaestin mRNA level in the proximal small intestine of rats, suggesting that hephaestin gene expression in proximal small intestine is upregulated to absorb more iron from diet in dietary iron-deficiency [26]. To clarify the mechanism of the difference of hemin iron absorption in different pathological status, further study focusing on the changes of expression of PCFT/HCP1 and DMT1 in small intestinal mucosa of animals in iron deficiency is needed.

Former studies investigated the possible effects of variety of dietary factors on iron absorption mostly based on in vitro digestion methods. The results were summarized as phytic acid, calcium [5] and polyphenols [6] strongly inhibiting nonheme iron absorption, and ascorbic acid can increase nonheme iron absorption [27], and heme iron were less affected by dietary composition [8, 25]. However, some studies suggested that heme iron absorption can also be reduced by calcium, decreased by dietary polyphenols and ascorbic acid had little influence on its absorption [28–30]. Nevertheless, the results of our study revealed some different phenomenon. Green tea extract, mainly containing polyphenols, enormously inhibited hemin iron absorption in both normal rats and the iron-deficient rats, with Cmax showed significant difference compared with hemin group in normal rats, AUC₀–∞ and AUC₀–∞∞ showed significant difference in the model rats. Ascorbic acid could significantly increase hemin iron absorption in iron deficiency status, in which Cmax showed significant enhancement. And the degree of enhancement was much weaker in normal animals. Interestingly, calcium slowed down the hemin iron absorption rate in normal rats, with MRT₀–∞ showed significant difference compared with hemin group, while no influence on the absorption extent (AUC₀–t, AUC₀–∞∞ and Cmax were similar with those of control groups respectively). This trend also appeared in the iron-deficient rats but with no significance observed. In the first hour after administration, hemin iron was hardly absorbed, and the plasma ⁵⁸Fe concentration started to increase obviously until the 2-h time point (Figs. 3 and 4, Tables 4 and 5). Moreover, calcium seldom influenced the elimination profile of ⁵⁸Fe originating from hemin, with CL₂ showed similar value among these groups. The above results indicated that hemin iron absorption is a complicated process. Previous research demonstrates that the absorbed hemin could be metabolized into ferrous iron in the intestinal enterocytes [26]. Therefore, we may speculate that several transporters and receptors, including HCP1, DMT1, and other membrane receptors, possibly mediate the uptake of hemin iron in the enterocytes. But only measuring the concentrations of ⁵⁸Fe in plasma could not confirm the participation of PCFT/HCP1, DMT1, hephaestin and other intestinal heme/iron transporters. Further investigations centering on the mechanism of heme iron absorption and its possible influencing dietary factors in different pathological status using different labeling and analytical methods would be of great importance.

A weak point of this methodology is the impossibility to discriminate between hemin that can be taken up at the apical side of enterocytes and released at their basolateral side as an intact molecule and ⁵⁸Fe that could be derive from hemin catabolism in duodenal cells or in digestive tract. Labeling hemin in the porphyrin ring would be a possible way to distinguish between these two possibilities.

In conclusion, this research suggested that iron deficiency status can increase hemin iron absorption. Furthermore, green tea extract strongly inhibited hemin iron absorption, ascorbic acid enhanced hemin iron absorption, and calcium slowed down the absorption speed of hemin iron but not affected the absorption extent. The effect of these three dietary factors showed varied trends in both normal rats and the iron-deficient rats. Beyond the increased knowledge of pharmacokinetic profiles for hemin in animals under different pathological status, the present study would contribute to further research.

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References


