Chemical reactions of vitamin C with intravenous-iron formulations

Suxin Wang¹, Gina Geraci¹, Martin K. Kuhlmann², Nathan W. Levin³ and Garry J. Handelman¹,³

¹Department of Health and Clinical Science, University of Massachusetts, Lowell, MA, USA, ²Vivantes Klinikum im Friedrichshain, Berlin, Germany and ³Renal Research Institute, New York, NY, USA

Abstract

Background. Intravenous (IV) iron is widely prescribed for patients on haemodialysis, to replace iron losses during treatment. It releases labile iron, which can induce oxidation of vitamin C and trigger oxidant damage. We examined the stability of vitamin C in the presence of IV iron compounds. We further examined the ability of vitamin C to release iron from these compounds.

Methods. Vitamin C was measured by high-performance liquid chromatography with electrochemical detection. Iron release from iron sucrose (FeSuc) and ferric gluconate (FeGlu) was determined with the ferrozine method.

Results. Vitamin C, in human plasma or fetal calf serum, was oxidized in this order of reactivity: FeSuc > FeGlu > blank reaction. FeSuc and FeGlu also oxidized vitamin C when added to freshly obtained whole human blood. During a 4 h incubation in buffer, vitamin C stimulated the release of 60% of the iron content of FeSuc at pH 4, with lesser amounts at pH 3, 5 and 6, and 5% release at pH 7. Vitamin C also triggered the release of iron from FeGlu, but less release was observed than with FeSuc. Using ferrozine reagent, no iron release was detected to heparinized human plasma, following addition of 500 μM concentrations of iron compounds.

Conclusion. Each IV-iron compound can oxidize substantial amounts of vitamin C when added to plasma or whole blood. The interaction of vitamin C is accompanied by release of iron from the particle at mildly acidic pH, which may explain the ability of high-dose vitamin C to mobilize iron from storage sites for erythropoiesis.

Keywords: ferric gluconate; haemodialysis; intravenous iron; iron sucrose; vitamin C

Introduction

The use of intravenous (IV) iron compounds is an established part of anaemia management in patients receiving dialysis [1–3]. These compounds provide large amounts of iron (as much as 100 mg/week) directly to the haematopoietic system, which allows more efficient use of epoietin (EPO) or other erythropoiesis-stimulating agents (ESAs) [4]. When IV-iron is administered, the clinician has more assurance that the iron needs of the patient are met, than when oral iron is prescribed. These compounds have undergone substantial evolution; iron dextran was widely used from 1950 to 2000, but has since been largely replaced worldwide by iron sucrose (FeSuc) (Venofer®) and ferric gluconate (FeGlu) (Ferrlecit®), since these newer formulations have minimal tendency to cause anaphylaxis [5,6]. However, the newer iron compounds also have a greater percentage of loosely bound iron [7–9], which has led to concern about potential patient injury from exposure to free iron [10].

Previous work with iron dextran led to the conclusion that IV-iron compounds are primarily cleared by sequestration to the reticuloendothelial system (RES) [11]. After storage in the RES, it is assumed that the compounds are degraded intracellularly with slow return of iron to the bloodstream and carriage by transferrin to the bone marrow. However, substantial amounts may remain stored within the RES. Within 1 month following administration of 1 g of these iron compounds to haemodialysis patients, the total haemoglobin (Hb) pool increased by about 30 g [6,12], whereas by contrast 1 g of iron can theoretically lead to synthesis of 150 g of Hb [13]. In chronic kidney disease where patients were given 2.5 g of iron over 1 year, the circulating Hb pool increased by 15% of the theoretical value [14], although EPO was only provided to a portion of these patients. The rest of the iron may be released over a period of several months; in some cases the iron may accumulate to high levels in the RES [15,16]. To track the kinetics of these iron formulations in the bloodstream, total plasma iron is measured by atomic absorption spectrophotometry; by this method, in one study it was reported that
the iron content of FeGlu is cleared within 2 h [17], whereas in another investigation it was reported that the iron content of FeSuc requires 8 h for clearance [18].

There have been several studies of the ‘free iron’ content of FeGlu and FeSuc; this is usually characterized by mixing the compounds with serum, and examining for change in transferrin saturation. FeGlu was observed to contain more ‘free iron’ than FeSuc [7–9]. By this criterion, the ‘free iron’ content of FeGlu has been estimated at 5%, and the free iron content of FeSuc at 2.5% [9]. A typical dose of 60–100 mg of these compounds contains 2–5 mg of ‘unbound’ iron. Because the bloodstream can usually absorb 7.5 mg of iron into transferrin [13], these doses are considered acceptable, and $T_{\text{sat}} > 100\%$ is rarely reported, but there have been some conflicting studies, that found $T_{\text{sat}} > 100\%$ in some patients after doses of this magnitude [19].

The reactions of vitamin C with iron are an important feature of the biochemistry of these compounds [20–23]. The levels of plasma vitamin C in dialysis patients vary over a broad range, from very low levels (ca. 1 μM) to very high levels (ca. 200 μM) [24–26]. The interactions between vitamin C and iron compounds might be affected by the large range in plasma levels in haemodialysis patients.

Since these compounds can remain in the bloodstream for several hours after IV administration, we have examined their interactions with vitamin C for intervals of up to 5 h. The findings reported here indicate the possibility of substantial interaction of these compounds with vitamin C in the bloodstream of patients after administration. Our data further suggest important interactions of these compounds with vitamin C at the mildly acidic pH of the lysosomal vacuole.

Materials and methods

Reagents

FeSuc (Venofer<sup>©</sup>, three separate manufacturer’s lots) was obtained from Watson Pharma, Inc. (Corona, CA, USA) and FeGlu (Ferrlecit<sup>©</sup>, three separate manufacturer’s lots) from American Regent, Inc. (Shirley, NY, USA). L-Ascorbic acid (vitamin C), meta-phosphoric acid (MPA), ferrozine, and octylamine were purchased from Sigma–Aldrich (Milwaukee WI, USA). Other chemicals and high-performance liquid chromatography (HPLC) solvents were reagent grade.

Addition of iron reagents to serum, plasma, whole blood or buffers

Aliquots of FeSuc or FeGlu were diluted with water, and then added to fetal calf serum, human plasma, whole human blood or buffers. For some experiments, additional vitamin C was added to the sample before addition of the iron compound. The initial experiments with addition to plasma were done with a freshly obtained plasma sample, and the further measurements were done with the same sample that had been frozen and thawed. Three separate manufacturer’s lots of each IV-iron formulation (FeSuc and FeGlu) were evaluated in separate experiments, for measurements of degradation of plasma vitamin C, and for determination of iron release after vitamin C addition.

Measurement of vitamin C

Vitamin C was measured by HPLC with an electrochemical detector (Coulochem II, Chelmsford, MA, USA); the mobile phase was 4 mM octylamine as ion-pairing reagent, 0.05 mM sodium acetate at pH 4.8 and 0.25 mM disodium ethylene-diamine-tetraacetic-acid, at 0.6 ml/min flow rate. HPLC separation was accomplished with a Beckman IP C18 column (4.6 × 250 mM, 5 μm particle size). Injections were done manually, with an injector equipped with a PEEK sample loop. This HPLC method is specific for vitamin C, and has a lower detection limit of 1 μM vitamin C.

Sample treatment for vitamin C measurement

All samples were treated with an equal volume of 10% MPA, centrifuged and the supernatant used for analysis of vitamin C.

Determination of iron release with ferrozine reagent

Iron compounds were incubated in plasma, and in acetate, phosphate, or (4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) buffers at several pH values, with varying amounts of vitamin C present. Present in the reaction mixture was ferrozine (20 mg/ml), neocuproine (20 mg/ml) and ammonium acetate (1 mg/ml). Iron released was determined by measuring sample absorbance at 562 nm with a Shimadzu UV-1601 UV-visible spectrophotometer. The amount of iron in the solution was calibrated with a stock solution of ferrous chloride, prepared in buffer and fully reduced with excess vitamin C.

This method is specific for iron, because of the additional neocuproine, which removes potential interference from copper present in the sample. The ferrozine method has a lower detection limit of 2 μM free iron in the sample.

Results

Oxidation of vitamin C in calf serum by addition of 5 mM FeSuc or FeGlu

To investigate the chemistry of the interaction between IV-iron compounds and vitamin C, we conducted initial studies with higher levels of IV-iron than occurs under physiological conditions. As shown in Figure 1, the addition of either 5 mM FeSuc or 5 mM FeGlu to calf serum supplemented with 0.25 mM vitamin C and incubated at 25°C, led to accelerated oxidation of vitamin C. The rate of loss of vitamin C was greater with FeSuc than with FeGlu.
Oxidation of 54 μM Vitamin C in human plasma with 500 μM IV-iron compounds

To determine the behaviour under physiological conditions, we examined plasma (using heparin anticoagulant) from a healthy donor, with vitamin C = 54 μM. This value is typical of vitamin C levels in healthy adults. Iron compounds were added to separate plasma samples, to a concentration of 500 μM, comparable to peak iron concentrations generally attained after IV-iron administration [17,18]. Fresh plasma was used for the first experiment, and frozen/thawed plasma for the additional two experiments. Samples were incubated at 25°C and vitamin C was measured at intervals compared to a control sample without IV-iron.

It was observed that FeSuc oxidized plasma vitamin C more rapidly than FeGlu. Data from each experiment, which compares a separate manufacturer’s lot of FeGlu and FeSuc, are shown in Figure 2. The mean and SDs are reported in Table 1.

Oxidation of vitamin C in freshly obtained whole blood with 500 μM IV-iron compounds

We tested the oxidation of vitamin C with FeSuc and FeGlu in freshly obtained whole blood from a normal donor (plasma vitamin C, 62 μM). Iron compounds (500 μM) were added, samples were incubated with gentle stirring at 25°C and plasma vitamin C was measured at hourly intervals. The same trend as in isolated plasma was observed; the rate of vitamin C oxidation during the initial 2h was FeSuc > FeGlu > whole blood control (data not shown).

Iron release from IV-iron compounds by vitamin C at different pH levels

We examined the iron released from 500 μM FeSuc and 500 μM FeGlu at several pH values: 3, 4, 5, 6 and 7, with 250 μM vitamin C present. Appropriate buffers (acetate, phosphate and HEPES) were used to establish the pH for each reaction. The percentage of iron released, following a 5 h incubation at 25°C, is shown in Figure 3. FeSuc released more iron than FeGlu for all the pH values studied except for pH 7. In pH 7 buffer, both FeSuc and FeGlu only released 4–5% of
their iron content after 5 h incubation with 250 μM vitamin C.

**Rate of iron release, at pH 4, before/after addition of excess vitamin C**

FeSuc and FeGlu (500 μM) released 2–4% of their total iron content, when incubated at pH 4 for 2 h in the absence of vitamin C, with more release detected from FeSuc. After addition of excess vitamin C (1 mM), which exceeded the concentration of the iron compounds (500 μM) there was accelerated release of free iron. Iron release at 4 h incubation from FeSuc reached 60%, and from FeGlu 35% (Figure 4).

**Iron release from IV-iron compounds added to plasma or serum**

Iron release from 500 μM FeSuc or FeGlu added to human plasma (containing 54 μM vitamin C) or to calf serum (containing 50 μM vitamin C) was determined. No ferrozine-chelatable iron was detected after 5 h, using this assay conducted in mammalian plasma or serum. This finding indicates that iron release at physiological pH is of low magnitude, and does not exceed the binding capacity of apo-transferrin.

**Discussion**

The studies reported here found that IV-iron compounds, FeSuc and FeGlu, accelerated the oxidation of vitamin C when added to fetal calf serum, human heparinized plasma from a normal donor, or freshly obtained whole blood. Degradation of vitamin C may be a factor in the low plasma vitamin C levels found in some patients on haemodialysis, since IV-iron administration is the standard treatment for these patients, and the compounds persist for several hours in the bloodstream [17,18]. These interactions occurred in a plasma sample from a normal control that contained 54 μM vitamin C; plasma vitamin C in dialysis patients varies from 1 to 200 μM, or even greater [24–26]. The effects of different plasma vitamin C levels, and potential interaction with uraemia toxins, need to be evaluated in separate investigations. Vitamin C stability was less in plasma that had been frozen and thawed, which may be a necessary procedure for large-scale evaluations of these effects.

FeSuc brought about the oxidation of vitamin C in normal human plasma more rapidly than FeGlu (Figure 2). This finding is in contrast to reports that FeSuc contains less free iron than FeGlu [7–9]. This observation was made with three different manufacturer’s lots of each compound, suggesting that these differences in reaction rate were not due to variation in the manufacturing process.

Free iron was not detected with either IV-iron preparation when the compounds were added directly to plasma at 500 μM concentration, but this is consistent with the total iron-binding capacity (TIBC) of plasma from healthy donors, which exceeds the free iron reported for these compounds [13]. The ferrozine reagent used for this assay does not detect iron which is released and immediately

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**Table 1. Mean and SD for vitamin C after incubation with IV-iron (three manufacturer’s lots of each iron formulation)**

<table>
<thead>
<tr>
<th>Reaction time (Min)</th>
<th>Control (n = 3)</th>
<th>FeGlu (n = 3)</th>
<th>FeSuc (n = 3)</th>
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<tbody>
<tr>
<td></td>
<td>Vit C (μM)</td>
<td>SD</td>
<td>Vit C (μM)</td>
</tr>
<tr>
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<td>54.00</td>
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<tr>
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</tbody>
</table>

**Fig. 3.** Effect of buffer pH on percentage of iron released from 500 μM FeGlu (▲) or 500 μM FeSuc (■), with 250 μM Vitamin C present, during 5 h incubation at 25°C.

**Fig. 4.** Percentage of iron released from 500 μM FeGlu (▲) and FeSuc (■) at pH 4.0 (0.2 M sodium acetate buffer), before and after the addition of 1 mM vitamin C.
complexed with apo-transferrin, and iron release to the bloodstream that exceeds the capacity of transferrin binding is not generally a characteristic of the current IV-iron protocols with standard protocols with FeSuc (100 mg/dose) and FeGlu (65 mg/dose).

Since the transferrin system can usually absorb some additional free iron, doses up to 200 mg of IV-iron are generally well-tolerated [28]. Larger IV doses (400–500 mg) of FeSuc have been associated with side-effects [29], and hepatotoxicity was reported after administration of 16 mg/kg of FeSuc to a child [30], comparable to an adult dose of 1000 mg. In addition to safety aspects, smaller and more frequent doses of FeSuc (50 mg, once/week) may be associated with lower EPO requirements [31].

In both preparations, ferric iron (Fe(III)) is bound to a high molecular weight complex. FeSuc is described as: Fe(III) hydroxide sucrose complex in water at alkaline pH. The polynuclear inner sphere Fe(III) hydroxide is surrounded by a large number of sucrose molecules. FeGlu is described as: ferric oxide saccharate complex chelated by gluconate carboxylic acid groups. The molecular orientation of FeSuc may allow vitamin C to penetrate and access ferric iron (Fe(III)) more easily than with FeGlu, with more rapid oxidation of vitamin C and correspondingly more rapid release of the resulting Fe(II).

This study shows that in conditions of moderate acidity (pH 3–6) vitamin C can trigger the release of large amounts of iron, with more released from FeSuc than FeGlu. These results are in agreement with the report of Sturm et al. [32], although those investigators did not characterize the pH dependency of iron release. When IV iron compounds were directly dissolved in buffer at pH 4.0, we observed limited release (2–5%) of the total iron, and suggest that iron release is not a result of acid pH attack on the particle. Striking release of iron from these preparations (as much as 60% of the total iron content) was observed after addition of vitamin C.

Zager et al. [10] studied the effects of different iron preparations (iron dextran, FeGlu and FeSuc) in mouse proximal tubular segments, cultured human proximal tubular cells, and bovine aortic endothelial cells. They found that lethal cell injury as assessed by lactate dehydrogenase (LDH) release in mouse and human tubular cells was seen only with FeSuc, but not with FeGlu or iron dextran, following a 1 h incubation. After a 16 h incubation, lethal cell injury was more common with FeSuc. The authors showed with a series of experiments that the generation of oxidative stress was dependent on mitochondrial respiration, but not free iron; oxidative stress was not blocked by the iron chelator desferoxamine. These findings are consistent with large-scale release of iron from FeSuc after uptake into the cell and transfer to the lysosomal compartment. Recently, Zager et al. [34] observed that TNF-α induction by IV LPS was greater in magnitude in mice pre-treated with FeSuc (2 mg/kg) than in mice pre-treated with the same dose of FeGlu [34].

These studies indicate a mechanism for the ability of high doses of vitamin C to mobilize iron for erythropoiesis, as has been suggested by several studies in EPO-resistant HD patients [35–38]. These IV-iron compounds are cleared from the bloodstream within several hours of administration, and taken up into lysosomal vacuoles of RES cells [11]. The vitamin C level in some HD patients can reach several hundred μM in plasma with large oral supplements or IV-dosing [24–26]; and this may lead to much higher concentrations of vitamin C in the lysosomal compartment, where the pH is in the range of 4–5 [39]. More rapid mobilization of iron for use in new RBC synthesis could result from vitamin C–iron interactions within the lysosome. More effective utilization of the iron dose might also minimize some of the cardiovascular side effects reported following chronic administration of large amounts of IV-iron [40].

Use of these IV-iron compounds might also result in transient decrease of plasma vitamin C, especially in patients with high plasma vitamin C levels, and vitamin C might not rebound until the iron compounds are cleared from the bloodstream. Dialysis patients also show a drop of vitamin C following dialysis treatment [41,42] (Handelman et al., manuscript in preparation), and the clinical significance of the oxidation of vitamin C in the bloodstream remains to be determined. Chronically, the oxidation of vitamin C after contact with these compounds could be a contributor to vitamin C deficiency.

Each of the interactions with vitamin C described here, the loss of vitamin C from plasma after addition of IV-iron compounds and the release of the iron at mildly acidic pH, highlight the importance of better understanding of the role of vitamin C in management of dialysis patients.

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Conflict of interest statement. None declared.

References

Interaction of IV-iron compounds and vitamin C


24. Handelman GJ. Vitamin C deficiency in dialysis patients – are we perceiving the tip of an iceberg? *Nephrology Dial Transplant* 2006; 22: 328–331


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