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Parenteral Irons Vs. Transfused Red Cells for Treatment of Anemia During Canine Experimental Bacterial Pneumonia

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Abstract

Background—No studies have been performed comparing intravenous iron with transfused red cells (RBC) for treating anemia during infection. In a previous report, transfused older RBC increased free iron release and mortality in infected animals when compared to fresher cells. We hypothesized that treating anemia during infection with transfused fresh RBC, with minimal free iron release, would prove superior to intravenous iron therapy.

Methods—Purpose-bred-beagles (n=42) with experimental *S. aureus* pneumonia rendered anemic were randomized to be transfused RBCs stored for 7-days or one of two intravenous iron preparations (7mg/kg), iron sucrose, a widely-used preparation, or ferumoxytol, a newer formulation that blunts circulating iron levels.

Results—Both irons increased the alveolar-arterial-oxygen gradient at 24–48-hours (p=0.02–0.001), worsened shock at 16-hours (p=0.02–p=0.003 respectively), and reduced survival (transfusion 56%; iron sucrose 8%, p=0.01; ferumoxytol 9%, p=0.04). Compared to fresh RBC transfusion, plasma iron measured by non-transferrin-bound iron (NTBI) levels increased with iron sucrose at 7, 10, 13, 16, 24 and 48-hours, (p=0.04 to p<0.0001) and ferumoxytol at 7, 24 and 48-hours (p=0.04 to p=0.004). No significant differences in cardiac filling pressures or performance, hemoglobin or cell free hemoglobin were observed.

Conclusions—During canine experimental bacterial pneumonia, treatment of mild anemia with intravenous iron significantly increased free iron levels, shock, lung injury, and mortality

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Authorship

Contribution: D.S. performed experiments, drafted and edited the manuscript. W.X. helped perform experiments and contributed to writing. J.S. contributed to the study design and statistical analysis. S.B.S. and J.B.-M. performed experiments and contributed to editing of the manuscript. S.L.B. and A.P. contributed to sample analysis. D.K.S. helped contribute to sample analysis and editing of the manuscript. H.G.K. and C.N. helped design the experiments and contributed to drafting of the manuscript. I.C.-P. helped design, performed experiments and contributed to drafting and editing of the manuscript.

compared to transfusion of fresh RBC. This was true for iron preparations that do or do not blunt circulating free iron level elevations. These findings suggest that treatment of anemia with intravenous iron during infection should be undertaken with caution.

Keywords

Bacterial Pneumonia; Iron Therapy; Transfused Red Blood Cells; Anemia

Introduction

Anemia is common during hospital admission, particularly for patients in intensive care units (ICU). In one prospective study, nearly 75% of patients admitted to the ICU either had or developed anemia.¹ Traditional first-line therapy for anemia during serious illness has been red blood cell (RBC) transfusion. As a result, RBCs are frequently administered to critically ill patients who are at risk for, are developing, or have serious infections. However, transfusion carries well-known acute and chronic risks, which increase with the number of RBC units administered and can result in organ injury and death.^{2–5} In one meta-analysis including 7,456 hospitalized patients, randomization to a liberal compared to a restrictive RBC transfusion strategy was associated with an increased risk of serious infection.⁵ Furthermore, recent evidence from human and animal studies suggests that transfusion of the oldest stored RBC units amplifies transfusion risks, particularly during infection.^{6–10}

To mitigate transfusion risks, alternative therapy with parenteral iron has been advocated for mild to moderate anemia. For many patients, parenteral iron provides an effective means of increasing red cell mass while decreasing transfusion requirements.^{11–13} Recent reports have shown that the intravenous iron used off-label in such settings as orthopedic surgery, post-partum hemorrhage or in patients receiving chemotherapy for gynecologic cancers is successful in reducing transfusions.^{14–19} Intravenous iron has also been used to correct anemia where transfusion is not possible, such as in patients who decline transfusion on religious grounds.²⁰ These data have promoted the notion that many types of hospitalized anemic patients or those at risk for blood loss may benefit from receiving intravenous iron and have increased its off-label use. Thus, current use of parenteral iron includes many types of hospitalized surgical patients as well as patients with anemia and many who are at risk for developing or have infections.

The current intravenous iron preparations are reportedly safer, easier to use and better tolerated than the original intravenous iron dextran formulation.^{21–23} However parenteral iron therapy still has the potential to overwhelm normal iron binding proteins, such as transferrin, releasing free iron into the circulation, and potentially increasing bacterial infection risks.^{24–26} Despite widespread use of intravenous iron therapy to minimize the need for RBC transfusion, the potential comparative risks have not been well established for these two therapies in anemic patients at risk for or with established infection.

In our validated canine model of bacterial pneumonia, transfusion of older RBC (42-day-old) increased hemolysis and release of cell free-hemoglobin and free iron compared with transfusion of fresh (7-day old) RBC. This increase in free iron during infection was associated with increased lung injury and higher mortality.^{27,28} We hypothesized that if free

iron is the mechanism of increased morbidity and mortality, treatment with transfused fresher RBCs, associated with limited hemolysis and minimal release of free iron, should prove superior to intravenous iron therapy in our model of severe bacterial infection and anemia. To test this, we randomized infected animals with mild to moderate anemia to treatment with one of two iron preparations. Either iron sucrose, one of the most commonly used preparations, or ferumoxytol, a newer iron formulation that may limit infection risk because it blunts free iron levels. We compared these two iron preparations to transfusion with fresh RBC with minimal free iron release. The findings should help define the role and potential toxicities of intravenous iron treatment in blood management.

Materials and Methods

Study Design

Purpose-bred beagles (12–24 months old and weighing 9–12.5 kg) were randomized to one of three treatments: 1) commercially available, canine universal donor leukoreduced RBCs units (20 ml/kg, equivalent to transfusion of two to four units of human packed RBCs, n=18) stored for 7-days in CPDA-1 (DEA1.1, American Blood Resources International, Dixon CA), 2) intravenous iron sucrose therapy (7mg/kg, n=13), or 3) intravenous ferumoxytol therapy (7mg/kg, n=11). The dosages and time of administration of the two iron preparations were derived from a weight based weekly maximal dosing recommendation by the manufacturer of each formulation. In each iron preparation, the dosage is expressed as mg of elemental iron per mL. Ferumoxytol contains 30mg of iron per mL and iron sucrose contains 20mg per mL. Four animals were randomized each week of the 96-hour study; one or two animals received transfused RBC and one or two animals received one of the two iron preparations. During one study week, ferumoxytol was unavailable, so more iron sucrose animals were studied overall; but every week at least one transfused RBC animal and at least one animal receiving one of the two iron preparations were randomized to be studied.

The primary endpoint was 96-hour mortality and secondary endpoints included the degree of shock, determined from the level of vasopressor support required to maintain mean pressure and lung injury, as measured by the alveolar-arterial oxygen gradient (AaO₂ gradient) over the course of the study. Although the marked differences in treatment arms precluded effective blinding, technicians caring for the animals over the 96 hours were not involved in designing the study or aware of the hypothesis tested and used set algorithms to guide care.

This model of *S. aureus* pneumonia has been described previously.²⁹ Briefly, on day 0 animals under general anesthesia were instrumented (femoral, external jugular and urinary catheters), underwent tracheostomy, and received a balloon tipped thermodilution pulmonary artery catheter through the external jugular catheter. The animals were then weaned from anesthesia, sedated with intravenous medications, and supported with mechanical ventilation. (See supplementary Methods for details). After achieving steady state, baseline echocardiogram, hemodynamic and laboratory measures were obtained (Figure 1). At 0h *S. aureus* ($0.5\text{--}1.5 \times 10^9$ CFU/kg) was administered into the right lower lobe of the lung. Two hours following bacterial inoculation, the animals underwent controlled hemorrhage removing 25% of total blood volume (20ml/kg over 30 minutes) resulting in mild anemia. To maintain intravascular volume the removed blood was replaced with normal saline in a

2:1 ratio per ml of blood loss (40ml/kg over 30 minutes). At 4-hours, the animals were randomized to receive treatment with RBC transfusion (20mL/kg transfused over 30 minutes), iron sucrose (intravenously over 6 hours, 1mg/ml in normal saline) or ferumoxytol (intravenously over 1 hour, 1mg/mL in normal saline). From 4 to 96-hours, all animals received standard ICU support titrated to standardized physiologic endpoints using algorithms designed to treat fully developed sepsis.²⁹ Laboratory measures including blood cultures, hemogram, metabolic panel and iron quantification including free iron as measured by non-transferrin bound iron (NTBI) levels, cell free hemoglobin (CFH) levels and transferrin bound iron (TBI) levels were completed at the time points shown in Figure 1. Animals alive at 96-hours were considered survivors and were euthanized. The Animal Care and Use Committee at the National Institutes of Health approved this study before animals were enrolled.

Statistical Analysis

Survival times were plotted using Kaplan-Meier survival curves and analyzed using log-rank tests. Survival is the primary endpoint. The analysis of all other endpoints are exploratory and the results are reported without accounting for multiple comparisons. For all other variables, changes from baseline values were analyzed in linear mixed models to account for the correlation of animals studied in each cycle and the repeated measures within each animal. Because the majority of animals died after the 48h time point, we only analyzed the first 48h for all endpoints except survival. All p-values are two-tailed and considered significant if $p \leq 0.05$. For some variables, logarithm-transformation was used when necessary. SAS version 9.3 (Cary, NC) was used for all analyses in this study. A power analysis was not conducted as this was a pilot study.

Results

Survival

Animals randomized to receive transfused stored fresh RBCs had a significantly lower mortality rate (n=18, 44%) compared to animals randomized to ferumoxytol therapy (n=11, 91%, $p=0.04$) or iron sucrose therapy (n=13, 92%, $p=0.01$) (log rank test) (Figure 2).

Shock score and Lung Injury

Shock Score—All animals were treated with vasopressors to normalize blood pressure. The shock score provides a relative index incorporating the amount of vasopressor (norepinephrine) required to normalize the mean arterial pressure. At 4-hours, just before treatment, there were no significant difference in the shock scores in all animals studied ($p=0.22$) (Figure 3, panel A). During the first 48-hours, animals transfused with fresh RBCs remained normotensive with mean shock scores similar to baseline. At 16-hours compared to the transfused fresh RBCs group; animals infused with ferumoxytol or iron sucrose developed significantly worse shock scores ($p=0.003$ and $p=0.02$ respectively). By 24-hours, the shock score continued to worsen ($p=0.0007$) in the iron sucrose group compared to those transfused fresh RBCs. By 48-hours the shock scores in both ferumoxytol and iron sucrose and the transfused fresh RBC animals no longer differed ($p=0.72$ and $p=0.06$ respectfully).

Lung Injury—Animals treated with ferumoxytol or with iron sucrose had greater increases in lung injury as measured by the mean AaO₂ at both 24-hours and 48-hours compared to animals transfused with fresh RBCs ($p=0.007$, 0.001 for ferumoxytol and $p=0.02$, 0.001 for iron sucrose respectively) (Figure 3, panel B).

Pulmonary Artery Occlusion Pressure (PAOP), Cardiac Index (CI) and Hemoglobin (Hgb) levels

There were no significant differences comparing the iron groups and the transfused fresh RBC group in mean cardiac filling pressures, measured by PAOP, or cardiac performance, measured by CI or left ventricular ejection fraction (data not shown), throughout the study (all, $p>0.07$) (Figure 4, panel A and B). There were also no significant differences in mean Hgb concentrations between treatment groups (all, $p=0.2-0.9$) (Figure 4, panel C). In all groups throughout the study, mean Hgb levels remained greater than 10g/dL. Mean values for Hgb concentration were nominally higher in animals transfused fresh RBCs compared to those treated with either of the two iron preparations, but not statistically significantly different.

Blood Level Measures of NTBI, TBI and CFH

NTBI—At 4-hours, prior to iron or fresh RBC transfusion therapy, the mean plasma NTBI concentration was low and not significantly different among the three study groups ($p=0.26$) (Figure 5, panel A). After therapy with iron sucrose, compared to transfused fresh stored RBC, marked significant increases in plasma mean NTBI levels were measured at all time points (7,10,13, 16, 24, and 48-hours, $p=0.04$ to $p<0.0001$). In contrast, compared to fresh RBC transfusion, animals treated with ferumoxytol had significant increases in plasma mean NTBI levels at only 7, 24 and 48-hours ($p=0.04$ to $p=0.004$).

TBI—Similar to NTBI, at 4-hours there was no significant difference in the plasma mean TBI levels at baseline between iron sucrose ($74.2 \pm 6.6 \mu\text{M}$, mean $\pm$ SE) and transfused fresh stored RBC ($75.9 \pm 8.2 \mu\text{M}$, $p=0.82$). From 7 to 48-hours the mean plasma levels of TBI in the iron sucrose group were significantly elevated (mean increase $28.5 \pm 12.0 \mu\text{M}$) compared to those receiving transfused fresh RBCs ($p=0.02$). Due to an interfering excipient in the diluent of ferumoxytol, TBI could not be quantified by electron paramagnetic resonance spectroscopy in this group.

CFH—There were no significant differences in plasma mean CFH throughout the study (Figure 5, panel B) that could explain the significant increase in mortality, shock or lung injury with the two iron therapies.

Other laboratory values

Measurements including those reflecting hepatic, renal and metabolic function showed no significant differences throughout the study among the three groups (See Supplemental Table 1).

Discussion

In a canine model of antibiotic-treated bacterial pneumonia with mild anemia, iron supplementation with either iron sucrose or ferumoxytol was associated with significantly worse outcomes compared to fresh blood transfusion. Animals treated with iron therapy developed more profound lung injury indicated by a greater increase in the AaO₂ gradient, more severe shock demonstrated by increased vasopressor requirements to maintain systemic pressure, and overall worse survival rates. We found marked increases in free iron levels in animals receiving both iron preparations for days after a single intravenous dose as used clinically to provide a weekly supply of iron; there were no significant increases in free iron levels after fresh blood transfusion. There were no significant differences in cardiac filling pressures or performance, hemoglobin levels or CFH levels to explain these findings. These data suggest that supplemental intravenous iron, but not transfusion of fresh blood, can raise plasma free iron levels, thereby worsening the underlying bacterial infection and outcome.

Iron sucrose was studied because it is one of the most commonly utilized and relatively inexpensive parenteral iron formulations used to treat iron deficiency anemia. Preclinical and clinical studies document that administration is associated with elevations of plasma free iron levels.^{30–32} We found that iron sucrose, when administered at the maximum recommended weekly dose, overwhelmed iron binding proteins and led to large increases in free iron at all time points measured during the first 48 hours after administration. Ferumoxytol was also studied because it blunts free iron levels in the circulation. This relatively newer iron oxide nanoparticle-based preparation reportedly has similar efficacy despite the blunting of free iron levels. The decreased free iron release into the circulation is due to direct reticuloendothelial uptake potentially limiting risks of intravenous iron administration during infection.^{33–35} We found that administration of ferumoxytol at the maximum recommended weekly dose resulted in a brief peak of NTBI within 3 hours of infusion, which then normalized until a second increase occurred ~20 to 40h later. The differing free iron kinetics compared to iron sucrose with blunting of levels at ~3 to 20 h after administration did not lead to any measurable clinically relevant decrease in toxicity. The second peak of free iron levels with ferumoxytol at 24 to 48 hours was unexpected and the mechanism remains uncertain. It is possible that with this preparation, iron is released from dysfunctional or apoptotic macrophages.

The ability of iron to worsen infection (“the iron hypothesis”) has been previously investigated. In murine models, with established infection either intraperitoneal *S. typhimurium*²⁴ or *S. aureus* pyelonephritis, outcome was significantly worsened with iron therapy.²⁵ In other murine studies, the presence of circulating free iron decreased the colony numbers of bacteria necessary to cause death.^{36,37} In a study of human volunteers, transfusion with older RBC resulted in measurable increases of circulating free iron and these patient’s sera supported increased proliferation of *E. coli* compared to sera from subjects receiving fresh RBC with normal low levels of free iron.³⁸ A single oral dose of ferrous sulfate was shown in volunteers to increase transferrin saturation and total serum iron levels. Sera from these patients supported marked elevated growth and doubling time of four different bacterial species compared with serum collected prior to iron therapy.³⁹ Oral

iron supplementation in children with anemia living in sub-Saharan Africa was associated with higher incidence of death related to infection.⁴⁰

Most prior clinical studies of iron did not directly compare RBC transfusion with intravenous iron infusion and limited comparisons to parenteral *versus* oral iron or different formulations of parenteral iron or placebo. A meta-analysis comparing parenteral to oral iron including 10,390 hospitalized patients found no increased risk, but another study, including 10,605 hospitalized patients, found a significantly increased risk of infection with intravenous iron administration.^{41,42} So it is possible that intravenous iron increases the risk of infection more than oral preparations. In an investigation of whether iron administration reduces transfusions in anemic critically ill patients, 140 such patients were randomized to receive either parenteral iron or placebo at ICU admission. Infected patients were not enrolled in this study. There was no significant decrease in transfusion requirements, but hemoglobin concentrations were higher in recipients of iron at ICU discharge.⁴³

The current study, which includes antibiotic use, precludes direct evidence of increased bacterial growth in the study animals. In several prior experiments using this same canine pneumonia model with exchange transfusions, we did find an association between changes in free iron levels as measured by NTBI and worsened outcomes in presence of infection. Transfusion with older stored RBCs (42-day old) *versus* fresher (7-day old) stored RBCs units caused marked increases in plasma levels of free iron. With increasing concentrations of bacterial challenges these increases in plasma free iron levels were shown to decrease over time more rapidly and profoundly with the older blood in a dose-dependent fashion. In parallel, older blood produced greater increases in hemodynamic shock, lung injury and mortality,²⁸ consistent with consumption and utilization of the free iron. In uninfected normal canine controls or uninfected canines with hemorrhagic shock, older blood still produced marked increases in plasma free iron compared to fresh blood, but without the more rapid iron clearance or increased morbidity and mortality.²⁷ In a study of infected canines, which altered the age (7,14, 21,28 or 42 days) of exchange transfused RBCs, only 7-day old RBCs produced no increase in free iron and that cohort was associated with the most favorable outcomes.⁵⁴ These data in total further support the hypothesis that increased morbidity and mortality with elevated free iron levels during infection are due to bacterial utilization and worsening infection. Moreover, iron sucrose and ferumoxytol increase NTBI, a readily accessible source of free iron for microorganisms. This was not true for fresh red blood cells, which might explain the improved survival with transfusion.

Interpretations of our findings are subject to limitations. When under metabolic stress or high oxygen demands, large stores of splenic erythrocytes can be recruited in canines increasing the circulating hematocrit or preventing a measurable loss of circulating RBCs.⁴⁴ However, there is no evidence that canine iron kinetics in this circumstance differ from those of human subjects. It is possible that these outcomes might change if canine RBC were stored differently, different iron preparations were used, a different strain of bacterial species was studied, or if a different animal model was used. We present no data here on older blood transfusion compared to fresh blood because previous studies of infection in this model demonstrated that the older RBCs are associated with increased NTBI levels and worsened outcomes. It is also possible that other commercial iron preparations might have different

effects. However we used two markedly different preparations and suggest that any preparation that releases sufficient free iron will have comparable toxicity. Importantly, this study does not rule out, and rather supports the possibility that intravenous iron preparations as treatment of anemia during infection pose risks similar to transfused older stored canine blood, which produces comparable elevations of free iron. Storage properties of canine RBC may not be identical to those of human blood. Iron metabolism in canines may differ and affect iron level measurements.^{45,46} While previous canine studies suggest that bacteria consume and utilize NTBI causing worse infections and outcomes²⁸, due to the use of antibiotics in our model, we are unable to obtain direct, quantitative evidence of increased pulmonary or systemic infection correlating with morbidity or mortality.

In conclusion, in an experimental large animal model, treatment of anemia with supplemental intravenous iron significantly increased morbidity and mortality when compared to transfusion of fresh RBC in a dose equivalent to two to four units of human packed RBCs. These results support the hypothesis that the toxicity from old stored RBCs in the setting of established infection is mediated by increased free iron. Furthermore, if these results can be extrapolated to the clinical setting, treatment of anemic patients with intravenous iron should be undertaken with caution in the setting of established infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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*Compared to transfusion, log rank test

Figure 2.

Kaplan-Meier survival curve throughout the 96h study comparing transfusion of 7-day old RBC (solid line), parenteral iron preparations ferumoxytol (dashed line) and iron sucrose (dotted line). P values are denoted by asterisks and indicate significance in comparison to transfusion and were calculated using log rank tests.

Figure 3.

Mean ($\pm$ SE) alveolar arterial oxygen gradient (panel A) and shock score (panel B). P values are denoted by asterisks and indicate significance in comparison to transfusion.

Figure 4. Mean ($\pm$ SE) pulmonary artery occlusion pressure (panel A), cardiac index (panel B) and hemoglobin concentration (panel C). There were no significant differences between groups for any of these measures.

Figure 5. Mean log ($\pm$ SE) non-transferrin bound plasma iron (panel A) and cell free hemoglobin (panel B). P values are denoted by asterisks and indicate significance in comparison to transfusion.