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Haptoglobin therapy has differential effects depending on severity of canine septic shock and cell-free hemoglobin level

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Abstract

BACKGROUND: During sepsis, higher plasma cell-free hemoglobin (CFH) levels portend worse outcomes. In sepsis models, plasma proteins that bind CFH improve survival. In our canine antibiotic-treated *Staphylococcus aureus* pneumonia model, with and without red blood cell (RBC) exchange transfusion, commercial human haptoglobin (Hp) concentrates bound and compartmentalized CFH intravascularly, increased CFH clearance, and lowered iron levels, improving shock, lung injury, and survival. We now investigate in our model how very high CFH levels and treatment time affect Hp's beneficial effects.

MATERIALS AND METHODS: Two separate canine pneumonia sepsis Hp studies were undertaken: one with exchange transfusion of RBCs after prolonged storage to raise CFH to very high levels and another with rapidly lethal sepsis alone to shorten time to treat. All animals received continuous standard intensive care unit supportive care for 96 hours.

RESULTS: Older RBCs markedly elevated plasma CFH levels and, when combined with Hp therapy, created supraphysiologic CFH-Hp complexes that did not increase CFH or iron clearance or improve lung injury and survival. In a rapidly lethal bacterial challenge model without RBC

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CONFLICT OF INTEREST

KER, ICP, JS, JF, JJL, TR, JK, SB, XL, AP, HGK, CN, and SBS have disclosed no conflicts of interest. DBKS has disclosed that he is a coinventor on patents on use of nitrite salts in cardiovascular disease.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Appendix S1: Supplemental Methods.

transfusion, Hp binding did not increase clearance of complexes or iron or show benefits seen previously in the less lethal model.

DISCUSSION: High-level CFH-Hp complexes may impair clearance mechanisms and eliminate Hp's beneficial effect during sepsis. Rapidly lethal sepsis narrows the therapeutic window for CFH and iron clearance, also decreasing Hp's beneficial effects. In designing clinical trials, dosing and kinetics may be critical factors if Hp infusion is used to treat sepsis.

Sepsis and septic shock are a major cause of morbidity and mortality.[1,2] Over the past half-century, research has focused, without success, on inhibiting host inflammatory mediators.[3,4] Hemolysis with elevated cell-free hemoglobin (CFH) levels has been documented during bacterial sepsis, and higher CFH levels are associated with a worse outcome.[5,6] Preclinical studies have found that infusing plasma proteins that bind to sites on the CFH molecule improve outcomes.[7–10] This observation suggests an alternative pathophysiological approach to treating sepsis by augmenting host clearance of potential toxins using a plasma protein transfusion therapy rather than by inhibiting host defenses.

The therapeutic approach to this alternative hypothesis can be summarized as follows: Virulent bacteria produce hemolysins, which can disrupt RBCs releasing CFH intravascularly.[11,12] CFH degrades into heme and free iron, resulting in injury via multiple mechanisms. CFH can scavenge nitric oxide, causing vasoconstriction and producing endothelial injury.[13,14] Free iron is an essential nutrient promoting bacterial growth and potentially worsening infection.[15] The presence of heme in the extravascular space may cause oxidative tissue injury and/or activate toll-like receptors, increasing inflammatory tissue injury.[16] Haptoglobin (Hp), a naturally occurring plasma protein, forms high-affinity, high-molecular-weight CFH-Hp complexes metabolized by the reticuloendothelial system.[17] We postulated that these CFH-Hp complexes could both compartmentalize CFH to the vascular space and accelerate CFH clearance during bacterial sepsis, minimizing CFH-related injury and improving sepsis survival rates.

To test this hypothesis, we previously studied commercially prepared human Hp concentrates in a lethal *Staphylococcus aureus* pneumonia model of septic shock.[7] This canine experimental shock model is associated with elevations in CFH levels and applies standard clinical treatments employed during human septic shock. Human Hp concentrate infused in this model significantly improved the animals' metabolic profile and reduced the extent of lung injury, degree of shock, and mortality rates.[7] Improvement was associated with the formation of CFH-Hp complexes, compartmentalizing CFH to the intravascular space and increasing CFH clearance, as well as preventing the release of free iron and presumably heme.

The rationale for the present study is as follows: In human septic shock, compared to this preclinical experimental model, a range of lethality and hemolysis with CFH release would likely be found and may alter the Hp beneficial effects. Understanding how the CFH-Hp complex kinetics relate to its beneficial effects could help in designing future clinical trials in patients with sepsis. The current study addresses two critical questions: 1) In a rapidly 100% lethal model, would Hp therapy have enough time to provide beneficial effects by

reducing the availability of CFH; and 2) in a less lethal model, could high levels of CFH result in toxicity from the circulation of large numbers of CFH-Hp complexes?

METHODS

Twenty-four purpose-bred beagles (18 to 30 months old, 9 to 12.5 kg; Covance Inc., Princeton, NJ) were treated with Hp using two different experimental sepsis protocols (Fig. S1, available as supporting information in the online version of this paper). In the first, 18 animals received an intrapulmonary challenge of *S. aureus* (1–2 × 10^9 colony-forming units/kg). In each successive study week, two animals were exchange transfused 4 hours after bacterial challenge with 20 mL/kg of 42-day-old stored canine universal donor leukoreduced red blood cells (RBCs) (DEA 1.1 ABRINT) over 30 minutes as previously described.[18] Exchange transfusion was repeated (20 mL/kg universal donor canine RBCs) three additional times each separated by 3 hours for a total dose of 80 mL/kg RBCs exchange transfused, the equivalent of approximately 70% replacement of blood volume. Following transfusion, animals were randomized to receive intravenous human Hp (2–1 and 2–2) plasma fraction concentrates (800 mg/kg total dose, in two divided 100 mg/kg bolus doses at 4 and 7 hours after bacterial challenge, followed immediately by a 600 mg/kg continuous infusion over 48 h) or an osmotically equivalent volume of 25% human albumin (Talecris Biotherapeutics) as previously described.[7] Animals were initially administered 12 sequential dilutions of Hp intravenously in a desensitization protocol as previously described.[7]

In a second sepsis study, the eight animals were challenged with *S. aureus* (1–2 × 10^9 colony-forming units/kg) but not exchange transfused. Each study week, two of the eight animals were randomized as described above, desensitized, and then administered either intravenous human Hp (2–1 and 2–2) plasma fraction concentrates (800 mg/kg total dose, in two divided 100 mg/kg bolus doses at 4 and 7 h after bacterial challenge, followed immediately by a 600-mg/kg continuous infusion over 48 h) or administered an equivalent volume of phosphate-buffered saline as previously described.[7] Albumin was not used as the control solution in the second set of experiments to avoid binding heme, which is expected to increase after hemolysis.[19,20] This study was stopped early due to the toxicity seen with Hp therapy.

In both studies, to create conditions translatable to the clinical intensive care unit environment, all animals received fluids, vasopressors, mechanical ventilation, and sedation titrated to physiologic endpoints, as well as oxacillin (30 mg/kg every 4 h) starting 4 hours after bacterial challenge for 96 hours as previously described.[18] All animals were treated identically except for the experimental intervention and, if alive after 96 hours, were considered survivors and euthanized. (For further details of methods used, please see Appendix S1, available as supporting information in the online version of this paper).

RESULTS

Mortality

In an *S. aureus* pneumonia model employing exchange transfusion after prolonged storage of RBCs, animals randomized to receive Hp therapy (n = 9) had a similar mortality to

control animals receiving an osmotically equivalent volume of human 25% albumin (n = 9; 44% vs. 55%; p = 0.65 stratified log-rank test) (Fig. 1A). In a 100% rapidly lethal *S. aureus* pneumonia model without exchange transfusion of RBCs, animals randomized to receive Hp (n = 4) had a significantly shorter survival time compared to animals randomized to an equivalent volume of phosphate-buffered saline (n = 4; median time [h] to 50% lethality 31.5 vs. 46.0; p = 0.02 stratified log-rank test) (Fig. 1B).

Shock score

All animals were treated with vasopressors to normalize blood pressure. The shock score incorporates the amount of vasopressor support (norepinephrine) required to normalize the mean arterial pressures as previously described.[18] Positive shock scores reflect hypertension and negative hypotension requiring vasopressors. During *S. aureus* pneumonia and RBC exchange transfusion, there were no significant differences in mean shock scores for animals treated with Hp versus no Hp (septic controls) (Fig. 1B; for individual components of the shock score, see Fig. S2, available as supporting information in the online version of this paper). However, the two study groups had different shock score patterns of significance compared to baseline. Those septic animals randomized to receive Hp did not develop shock with unchanged scores throughout the study compared to baseline (all $p > 0.05$). In contrast, the septic animals that received no Hp (septic controls) developed shock requiring vasopressors at 36 to 60 hours ($p = 0.03$ to $p = 0.006$).

The *S. aureus* pneumonia animals not receiving RBC exchange transfusions treated with Hp had significantly decreased (worse) mean shock scores at 13 to 36 hours ($p = 0.002$ to $p < 0.0001$) (Fig. 1E, compared to baseline; for components of shock score, see Fig. S2, available as supporting information in the online version of this paper). The *S. aureus* pneumonia animals not receiving RBC exchange transfusions and not treated with Hp (septic controls) compared to baseline had significantly worse mean shock scores at 24 to 48 hours ($p = 0.004$ to $p < 0.0001$). The shock score of those receiving Hp became significantly worse 11 hours earlier (13 vs. 24 h, respectively) than that of animals not receiving Hp (septic controls). Septic animals without RBC exchange transfusions receiving Hp required more vasopressors (worse shock scores) compared to septic controls receiving no Hp at 13 to 24 hours ($p = 0.02$ to $p = 0.008$).

Lung injury score

The lung injury score (LIS) incorporates five variables measuring lung function[18] (Fig. 1C; for individual components, see Fig. S3, available as supporting information in the online version of this paper). The *S. aureus* pneumonia animals undergoing RBC exchange transfusion, receiving Hp had significantly increased mean LIS compared to baseline (0 h) (higher scores more injury) at 7 to 96 hours ($p = 0.004$ to $p < 0.0001$). The *S. aureus* pneumonia animals undergoing RBC exchange transfusion without Hp also had significantly increased mean LIS compared to baseline (0 h) at 7 to 96 hours ($p = 0.02$ to $p < 0.0001$). In the *S. aureus* pneumonia animals undergoing RBC exchange transfusion, mean LISs were not significantly different throughout the study comparing animals receiving Hp and septic controls receiving no Hp (all $p > 0.05$).

The *S. aureus* pneumonia animals not receiving RBC exchange transfusions but treated with Hp had significantly increased mean LIS compared to baseline at 7 to 36 hours ($p = 0.04$ to $p < 0.0001$) (Fig. 1F; for individual components, see Fig. S3, available as supporting information in the online version of this paper). The *S. aureus* pneumonia animals not receiving RBC exchange transfusions and not treated with Hp had significantly increased mean LIS compared to baseline at 10 to 48 hours ($p = 0.02$ to $p < 0.0001$). In septic animals without RBC exchange transfusion at 24 hours compared to baseline, the LIS was significantly greater in the Hp group compared to the no Hp group (septic controls) ($p = 0.046$).

Cell-free hemoglobin

During septic shock with RBC exchange transfusion, mean plasma log (CFH) levels via Drabkin's assay, which measures CFH both bound and unbound to haptoglobin, were significantly elevated compared to baseline (0 h) in animals randomized to receive Hp at 10 to 96 hours (all $p < 0.0001$). In septic controls receiving no Hp, mean log (CFH) levels were significantly elevated compared to baseline (0 h) at 10 to 72 hours (all $p < 0.0001$) (Fig. 2A). Mean log (CFH) levels, were significantly higher in animals with septic shock exchange transfused with RBCs receiving Hp compared to animals with septic shock exchange transfused with RBCs receiving no Hp (septic controls) at 36 to 96 hours ($p = 0.048$ to $p = 0.0001$).

During septic shock without RBC exchange transfusion, mean plasma log (CFH) levels were significantly elevated compared to baseline in animals receiving Hp at 13 and 36 hours ($p = 0.04$ to $p < 0.0001$) (Fig. 2C). In septic shock, animals without RBC exchange transfusion and not treated with Hp (septic controls) mean log (CFH) levels were elevated compared to baseline, at 24 to 48 hours ($p = 0.01$ to $p = 0.0005$). Mean log (CFH) levels were significantly higher in septic animals not exchange transfused receiving Hp compared to animals not exchange transfused with RBCs and not receiving Hp (septic controls) at 36 hours ($p = 0.04$).

Non–transferrin-bound iron

In septic shock, animals undergoing exchange-transfusion and treated with Hp, mean non–transferrin-bound iron (NTBI) levels increased significantly compared to baseline (0 h) at 10 to 48 hours ($p = 0.048$ to $p = 0.004$). The mean NTBI levels at these time points were near or above the normal limit for NTBI (<0.2 μM) (Fig. 2B). Animals with septic shock undergoing exchange transfusion not treated with Hp (septic controls) had significantly increased mean NTBI levels compared to baseline at 13 hours only ($p = 0.03$). The mean NTBI levels in this group otherwise remained near or below the normal limit for NTBI levels throughout the study. NTBI levels were significantly higher in animals with septic shock exchange transfused and treated with Hp compared to animals exchange transfused receiving no Hp (septic controls) at 24 ($p = 0.01$) and 48 hours ($p = 0.04$).

Both animals with septic shock not exchange transfused and treated with Hp and septic animals not exchange transfused and not treated with Hp (septic controls) had no significant increases in mean NTBI levels at all time points studied compared to baseline (all $p > 0.05$)

(Fig. 2D). Mean NTBI levels in all septic animals not exchange transfused remained within the normal range (<0.2 μM) throughout the study independent of treatment with Hp.

Mean CFH and NTBI levels in animals undergoing exchange transfusion with 42-day–stored RBCs compared with previously studied 7-day–stored RBCs

We compared, CFH and NTBI levels reported for exchange transfusing 7-day–stored RBCs (previous study) to RBC studied here (42-day storage) with and without Hp therapy (Fig. 3). The apparent loss of benefit from Hp therapy with longer-stored RBCs was associated with delayed clearance of CFH and NTBI observed with very high CFH plasma levels.

No Hp therapy CFH levels (7- vs. 42-day–stored RBCs)

In septic shock, animals receiving longer-storage RBC (42 days) exchange transfusion and not treated with Hp, mean CFH levels significantly increased compared to baseline (0 h) at 10 to 72 hours ($p = 0.0003$ to $p < 0.0001$ (Fig. 3A). Animals previously studied receiving shorter-storage RBC (7 days) exchange transfusion and not treated with Hp, mean CFH levels significantly increased compared to baseline (0 h) at fewer time points than longer-stored RBCs 36 to 60 hours ($p = 0.02$ to $p = 0.0003$). Mean CFH levels were significantly higher in septic shock animals exchange transfused with longer-stored RBCs and not treated with Hp compared to animals exchange transfused with shorter stored RBCs receiving no Hp (septic controls) at 10 to 72 hours ($p = 0.05$ to $p < 0.0001$).

No Hp therapy NTBI levels (7- vs. 42-day–stored RBCs)

In septic shock, animals receiving longer-storage RBC (42 days) exchange transfusions and not treated with Hp, mean NTBI levels increased significantly compared to baseline (0 h) at 10 ($p = 0.04$) and 13 hours ($p = 0.003$). (Fig. 3B). Animals previously studied receiving shorter-storage RBC (7 days) exchange transfusions and not treated with Hp, mean NTBI levels were not significantly increased compared to baseline (0 h) at all time points studied (all $p > 0.05$). Mean NTBI levels were significantly higher in septic shock animals exchange transfused with longer-stored RBCs and not treated with Hp compared to animals exchange transfused with shorter-stored RBCs receiving no Hp (septic controls) at 13 hours ($p = 0.02$).

With Hp therapy CFH levels (7- vs. 42-day–stored RBCs)

In animals with septic shock receiving longer-storage RBC (42 days) exchange transfusions and treated with Hp, mean CFH levels increased significantly compared to baseline (0 h) at 7 to 96 hours ($p = 0.02$ to $p = 0.0001$) (Fig. 3C). For animals receiving shorter-storage RBC (7 days) exchange transfusions and treated with Hp, mean CFH levels increased significantly compared to baseline (0 h) at 13 to 48 hours ($p = 0.01$ to $p = 0.0009$). Mean CFH levels were significantly higher in animals with septic shock exchange transfused with longer-stored RBCs and treated with Hp compared to animals exchange transfused with shorter-stored RBCs receiving Hp (septic controls) at 10 to 72 hours ($p = 0.001$ to $p < 0.0001$).

With Hp therapy NTBI levels (7- vs. 42-day–stored RBCs)

In animals with septic shock receiving longer-storage RBC (42 days) exchange transfusions and treated with Hp, mean NTBI levels increased significantly compared to baseline (0 h) at

10 to 48 hours ($p = 0.004$ to $p < 0.0001$) (Fig. 3D). In animals with septic shock receiving shorter-storage RBC (7 days) exchange transfusions and treated with Hp, mean NTBI levels were not significantly increased compared to baseline (0 h) at all time points studied (all $p > 0.05$). Mean NTBI levels were significantly higher in animals with septic shock exchange transfused with longer-stored RBCs and treated with Hp compared to animals' exchange transfused with shorter-stored RBCs receiving Hp (septic controls) at 10, 13, and 24 hours ($p = 0.04$ to $p = 0.02$).

Hp vs. no Hp therapy CFH and NTBI levels (7- vs. 42-day–stored RBCs)

At 60 and 72 hours for CFH levels and 48 hours for NTBI levels, the increases with Hp versus no Hp (Fig. 3) were greater with longer-stored RBCs than with shorter-stored RBCs (interaction $p \leq 0.05$). The higher levels at these later time points are consistent with the notion that after older stored RBC exchange transfusions associated with higher CFH-Hp complex levels, clearance of these complexes decreases and results in further elevation of circulating CFH-Hp complexes and NTBI levels.

Hp levels

During septic shock with RBC exchange transfusion, mean log-transformed plasma Hp levels were significantly elevated at all time points studied compared to baseline (0 h) in animals randomized to receive Hp (concentrations are reported on a per-heme basis). In contrast, septic controls receiving no Hp had significantly decreased log Hp levels compared to baseline (0 h) at 10 to 16 hours ($p = 0.0006$ to $p < 0.0001$), but later levels increased significantly compared to baseline (0 h) at 72 ($p = 0.003$) and 96 hours ($p < 0.0001$) (Fig. 4A). Mean log Hp levels were significantly higher in animals with septic shock exchange transfused with RBCs receiving Hp compared to animals exchange transfused but not receiving Hp (septic controls) at 7 to 72 hours (all $p < 0.0001$).

During septic shock without exchange transfusion of RBCs, mean plasma log Hp levels were significantly elevated compared to baseline in animals receiving Hp at 4 to 36 hours ($p = 0.03$ to $p = 0.0001$) (Fig. 4B). In animals with septic shock without exchange transfusion not treated with Hp (septic controls), mean log Hp levels were not significantly different compared to baseline at all time points studied when some animals were still alive (all $p > 0.05$) (Fig. 4B). Mean log Hp levels were significantly higher in septic animals not exchange transfused receiving Hp compared to animals not exchange transfused and not receiving Hp (septic controls) at 4 to 36 hours ($p = 0.04$ to $p < 0.0001$). The constancy of the Hp levels over 96 hours suggests that this dosing regimen (100 mg/kg every 3 h × 2 followed by 12.5 mg/kg/h × 48 h) achieved and maintained a steady state. However, during *S. aureus* pneumonia without exchange transfusion versus with exchange transfusion of RBCs, mean Hp levels at 24 hours in those receiving Hp rose significantly higher compared to septic controls not receiving Hp (interaction, $p = 0.01$), likely because previously infused Hp was not removed during the four-exchange transfusion of RBCs.

Cardiac filling pressures and hematocrit; direct/indirect measures of volume status

In septic animals with and without exchange-transfusion, there were no significant differences in change from baseline at any time point for volume status as measured by

mean central venous pressure (Fig. 4C and D), mean hematocrit (Fig. 4E and F), or mean pulmonary artery occlusion pressure (Fig. 4G and H) except at one time point in one group. The mean pulmonary artery occlusion pressure was higher at 4 hours in septic animals exchanged transfused not receiving Hp compared to septic controls treated with Hp ($p = 0.046$).

Metabolic function

Septic animals exchange transfused with RBCs and randomized to receive Hp, and septic controls not receiving Hp had no significant differences throughout the study (change from baseline, from 4 to 96 h) (all $p > 0.05$) in overall metabolic function as measured by mean pH, pCO_2, base excess, and lactate (Fig. 5A, C, E, G).

For renal, hepatic, and cardiac function; electrolytes; glucose; platelets; transferrin-bound iron, circulating blood cells; and cytokine levels, please see Supplemental Tables, available as supporting information in the online version of this paper.

DISCUSSION

In the current study, we found that alterations in the canine *S. aureus* pneumonia models apparently disrupted mechanism of Hp action, resulting in elimination of the previously observed increased clearance of CFH and of the reduction of NTBI levels. These alterations were associated with loss of the previously observed beneficial effects of Hp infusion on sepsis outcomes. Two changes in the model from the previous study were introduced: Instead of transfusing RBCs stored for 7 days for exchange transfusion, we elected to use RBCs stored for 42 days to increase hemolysis and promote release of log-fold higher plasma levels of CFH. In a second study, we used a 100% rapidly lethal model of sepsis that shortened the therapeutic window of Hp. As in previous studies, Hp dose was calculated to exceed the predicted release of CFH.

In the previous transfusion sepsis study with fresher stored RBCs, low levels of CFH were observed early after transfusion, and there was subsequently a greater rise in CFH with than without Hp therapy due to compartmentalization of the complexes. Later, the complexes fell more rapidly and were cleared more quickly from the vascular space with Hp than without Hp. However, in the current study, with much higher plasma CFH levels, CFH rose significantly in the Hp arm both early and late compared to the arm that did not receive Hp (Fig. 5). This observation suggests that in the setting of supraphysiologic levels of CFH-Hp complexes produced with Hp infusion together with very high levels of CFH in the plasma, there was impaired clearance of CFH and NTBI. This finding was associated with a loss of the previously reported Hp beneficial effect on sepsis survival and lung injury. The only apparent beneficial effect of Hp therapy observed with such high CFH levels was a reversal of shock. These data are consistent with previous findings that transfused blood at the end of the storage period results in very high CFH levels. Persistently high levels of CFH and free iron appear to reduce the previously reported beneficial effects of Hp therapy during septic shock.

In the current study employing the *S. aureus* pneumonia model but with increased lethality, and without exchange transfusion, we show an expected mortality of 100% with death often occurring 24 and 36 hours after infection. The previously studied 75% lethal model resulted in both lower and slower death.[7] Because of the early and rapid deaths, there may be a time-based explanation for the apparent failure of Hp infusion to decrease CFH and NTBI levels and to reduce mortality. Administration of Hp compartmentalized the CFH to the intravascular space in both lethality models, so that there was sufficient time for binding CFH. In the lower lethality model, significant falls in CFH-Hp complexes with increased clearance after Hp infusion were observed only after 24 to 48 hours. In the higher-lethality model, most animals had already succumbed to the infection by that time, and not only was no benefit seen with Hp therapy, but evidence of increased mortality (time to death), lung injury, and level of shock was found.

The metabolism of iron within the intravascular space is complex and only partly understood.[21,22] In the current study, high levels of CFH produced over several days because of continuing hemolysis of senescent older RBCs, in combination with Hp therapy, resulted in extremely high levels of CFH-Hp complexes. These levels likely exceeded normal clearance mechanisms by hepatic and splenic macrophages further raising levels.[8] However, it is also possible that higher NTBI levels resulted from release of NTBI from elevated circulating complexes. In any case, this change in clearance of CFH-Hp complexes with elevated complex and free iron levels may have interfered with host defenses, promoted bacterial growth, and resulted in loss of Hp therapy benefits.[15,21,22]

This study emphasizes the importance of subject selection in any proposed sepsis trial of Hp therapy. Caution should be exercised both for subjects suspected of developing rapidly lethal septic shock (death within 24 hours) and for clinical settings of sepsis that may involve marked hemolysis or massive transfusion of banked blood. The rapid lethality model may have minimized the time needed for Hp to effectively bind CFH, clear the complexes from circulation, and reduce available intravascular iron. Haptoglobin therapy, similar to antibiotic therapy, undoubtedly requires both appropriate concentrations and an adequate time course to effect benefit.[23]

In conclusion, the ability of Hp to clear CFH from the circulation and decrease available intravascular iron appears important for improving outcomes during sepsis. Pharmacologic infusions of Hp are a biologically plausible treatment that could be additive or synergistic to the inhibitory effects of antibiotics on bacterial growth. This approach provides a theoretically superior risk–benefit profile compared to previous therapeutic strategies to inhibit inflammation rather than promote host defenses. This study provides additional information on the clearance of CFH-Hp complexes after Hp infusion and its importance to clinical outcome in a model of septic shock. Understanding and accounting for these factors promoting CFH clearance could lead to improved designs of future clinical trials that, if successful, could define the role of transfused haptoglobin concentrates as a therapy for septic shock.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS:

CFH	cell-free hemoglobin
Hp	haptoglobin
LIS	lung injury score
NTBI	non–transferrin-bound iron

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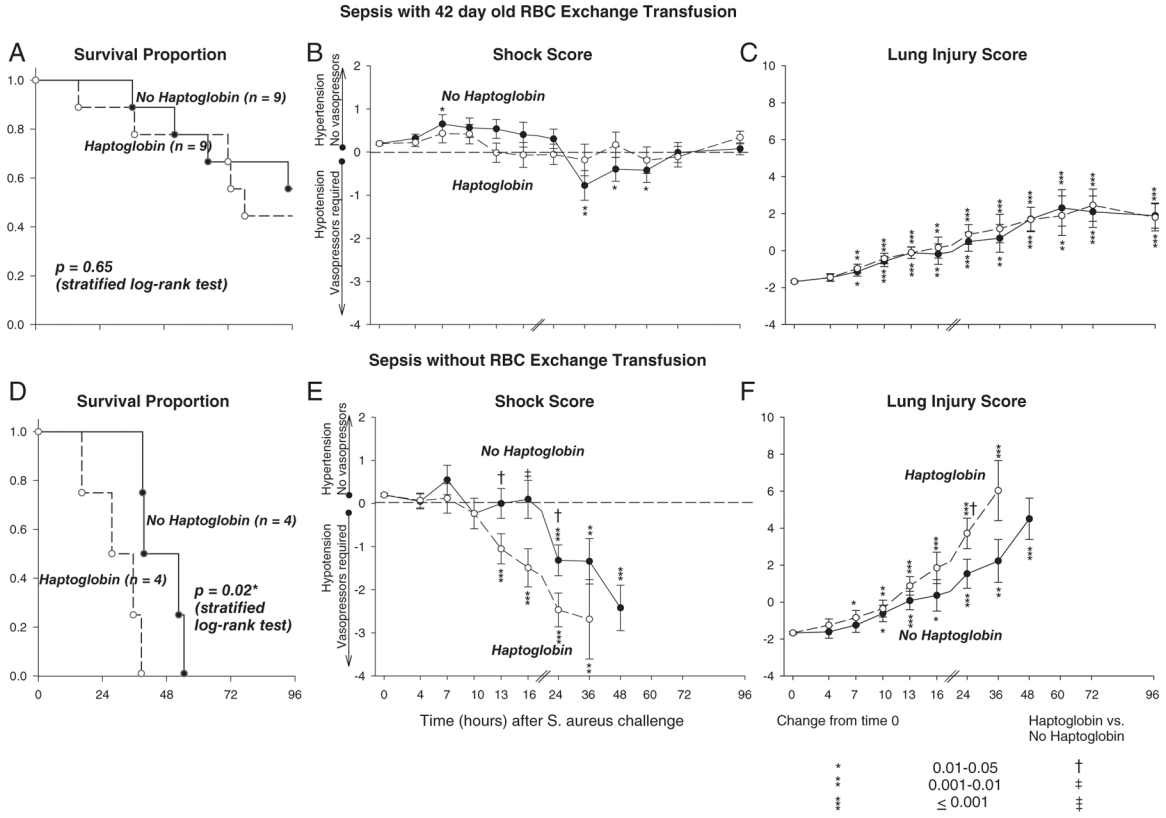

Fig. 1.

(A and D) Kaplan–Meier survival curve for the 96-hour sepsis study. The survival comparison in canines receiving 42-day-old RBC exchange transfusion after lethal *S. aureus* challenge treated with haptoglobin or no haptoglobin (A) or without (D) exchange transfusion after rapidly lethal *S. aureus* challenge treated with haptoglobin or no haptoglobin. P values are denoted by asterisks indicating significance in comparison between each panel group using stratified log-rank tests. (B and E) Mean shock scores (± SE) at serial time points. The shock score accounts for the level of vasopressor support (norepinephrine) needed to maintain the mean arterial pressure at a preset normal level for canines (mean, 80 mmHg). Shock score is compared over 96 hours in canines receiving haptoglobin or no haptoglobin with (B) or without (E) RBC exchange transfusion. Changes from baseline are shown for each study group plotted from a common origin, the mean value for animals at baseline. P values indicate significance in each group comparison in each panel and are denoted by asterisks (for changes over time) or crosses (comparing haptoglobin vs. no haptoglobin at each time point). (C and F) Mean (± SE) LISs at serial time points. The LIS detects pulmonary damage via measurements in mean pulmonary artery pressure, alveolar-arterial oxygen gradient, plateau pressure, oxygen saturation, and respiratory rate. The LIS is plotted over time (*x*-axis) for animals receiving haptoglobin or no haptoglobin with (C) or without (F) RBC exchange transfusion. Changes from baseline are shown for each study group plotted from a common origin, the mean value for animals at baseline. P values indicate significance in each group comparison in each panel and are denoted by asterisks (for changes over time) or crosses (comparing haptoglobin vs. no haptoglobin at each time point).

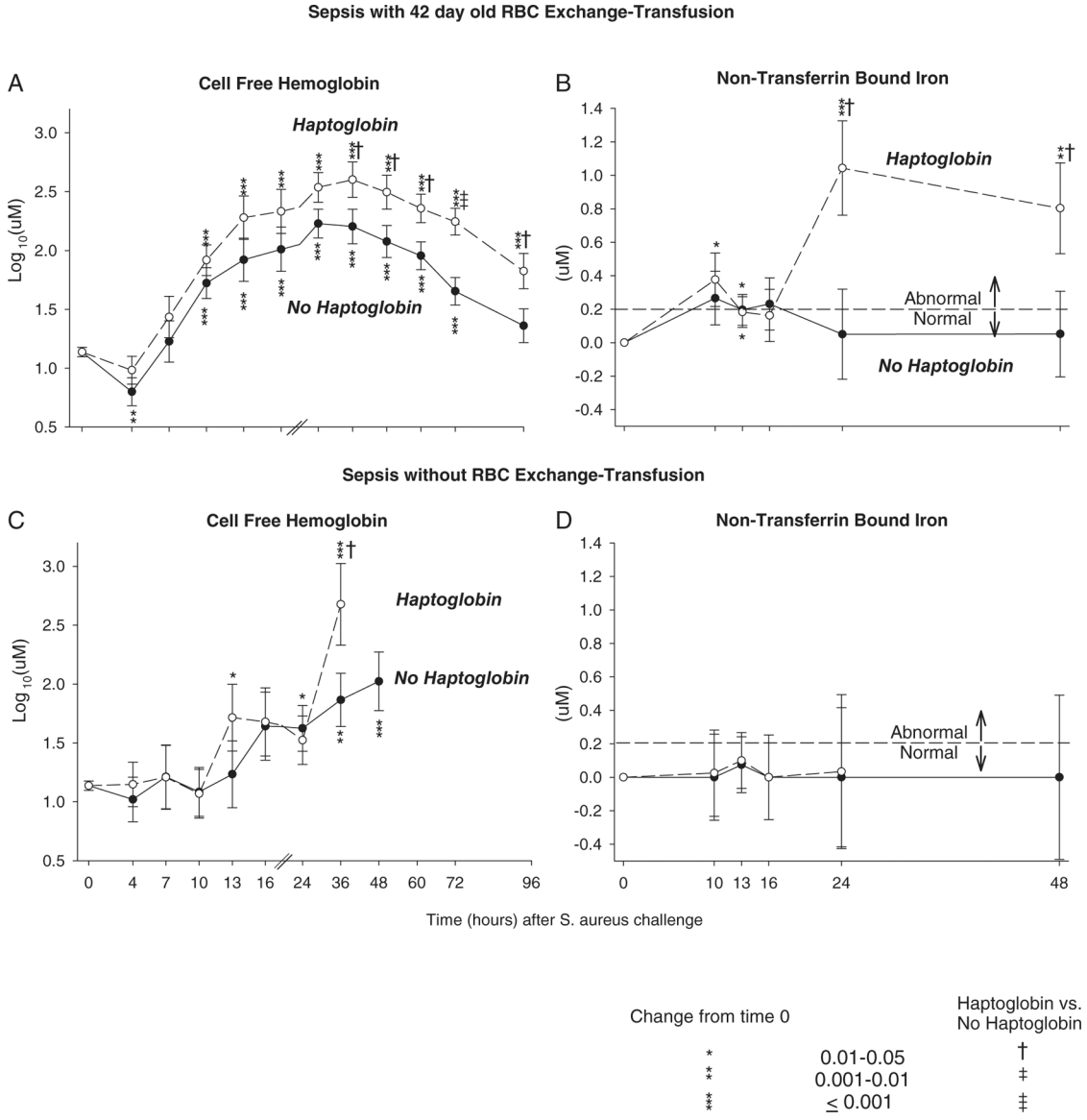

Fig. 2.

Mean CFH and NTBI at serial time points. The format is similar to Fig. 1 except that CFH (A and C) and NTBI (B and D) are shown.

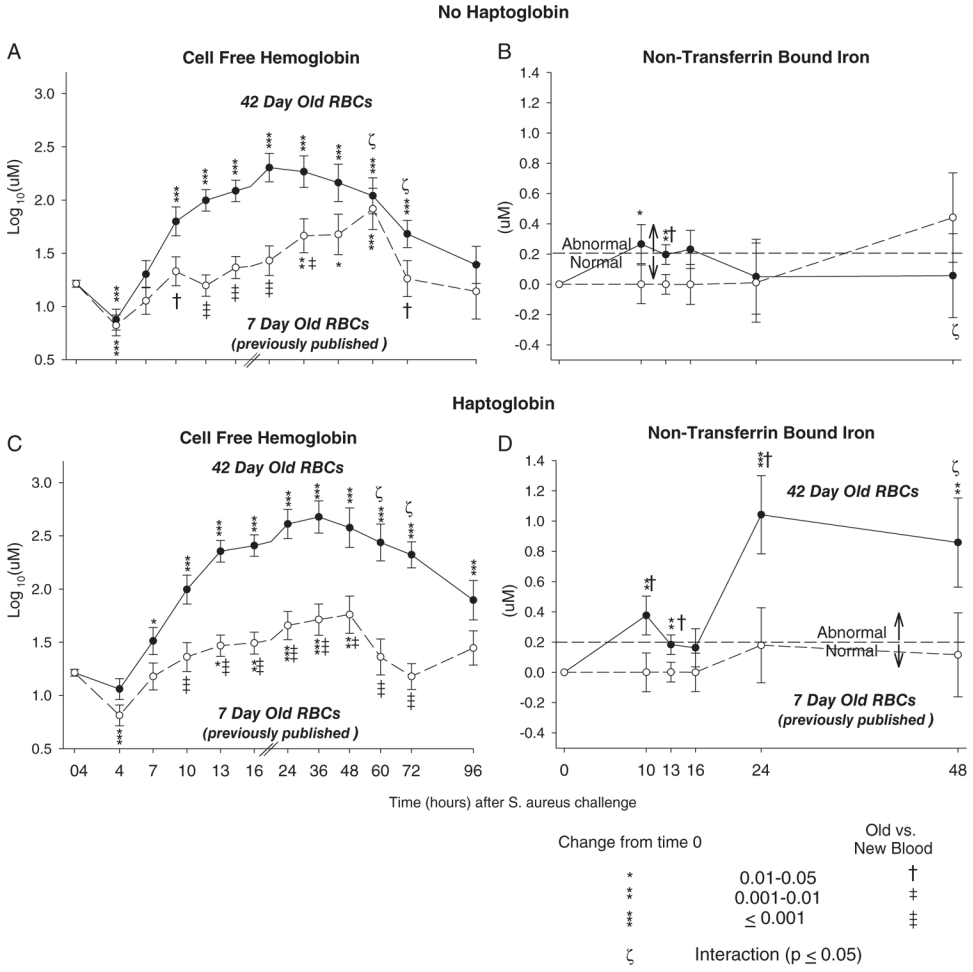

Fig. 3.

Mean CFH and NTBI at serial time points of current 42-day–stored RBC transfusion compared to previously published 7-day–stored RBC transfusion. The format is similar to Fig. 1 except that CFH (A and C) and NTBI (B and D) are shown for the current study of 42-day–stored RBCs for exchange transfusion compared to previously published 7-day–stored RBC exchange transfusion.

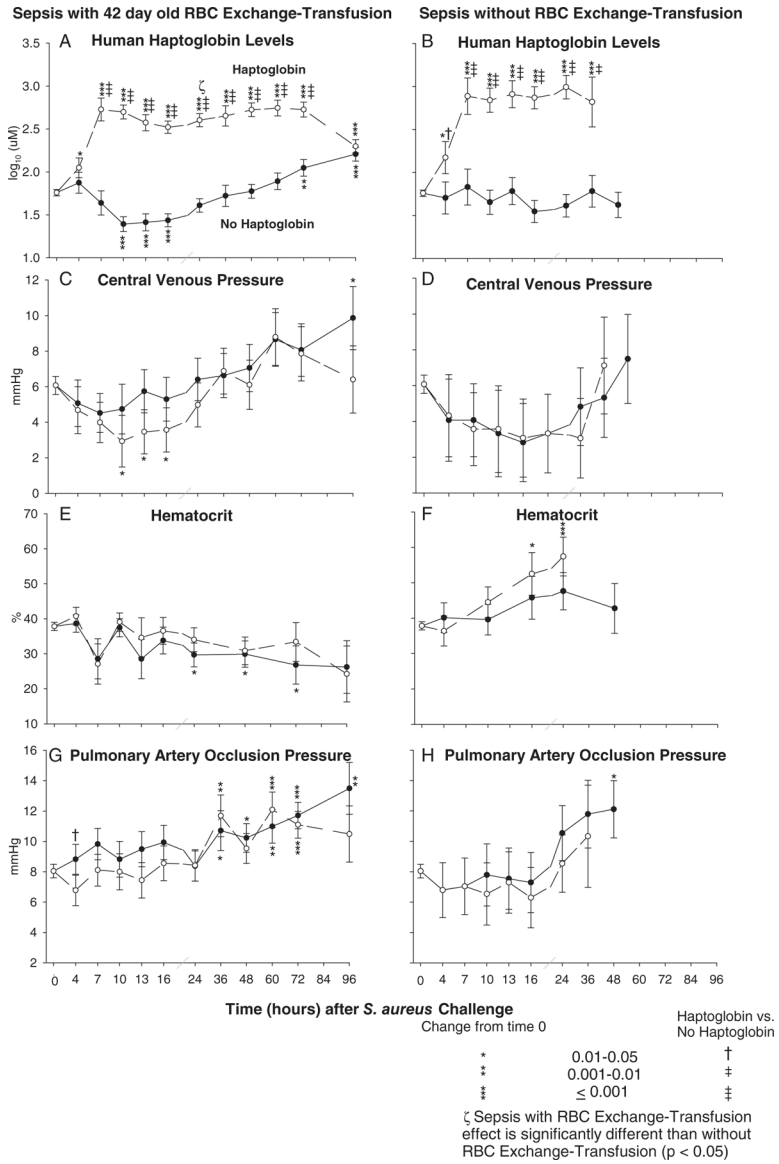

Fig. 4.

Human haptoglobin, central venous pressure, hematocrit, and pulmonary artery occlusion pressure measurements at serial time points. The format is similar to Fig. 1 except that human haptoglobin, central venous pressure, hematocrit, and pulmonary artery occlusion pressure are measured over 96 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with (A, C, E, G) or without RBC exchange transfusion (B, D, F, H).

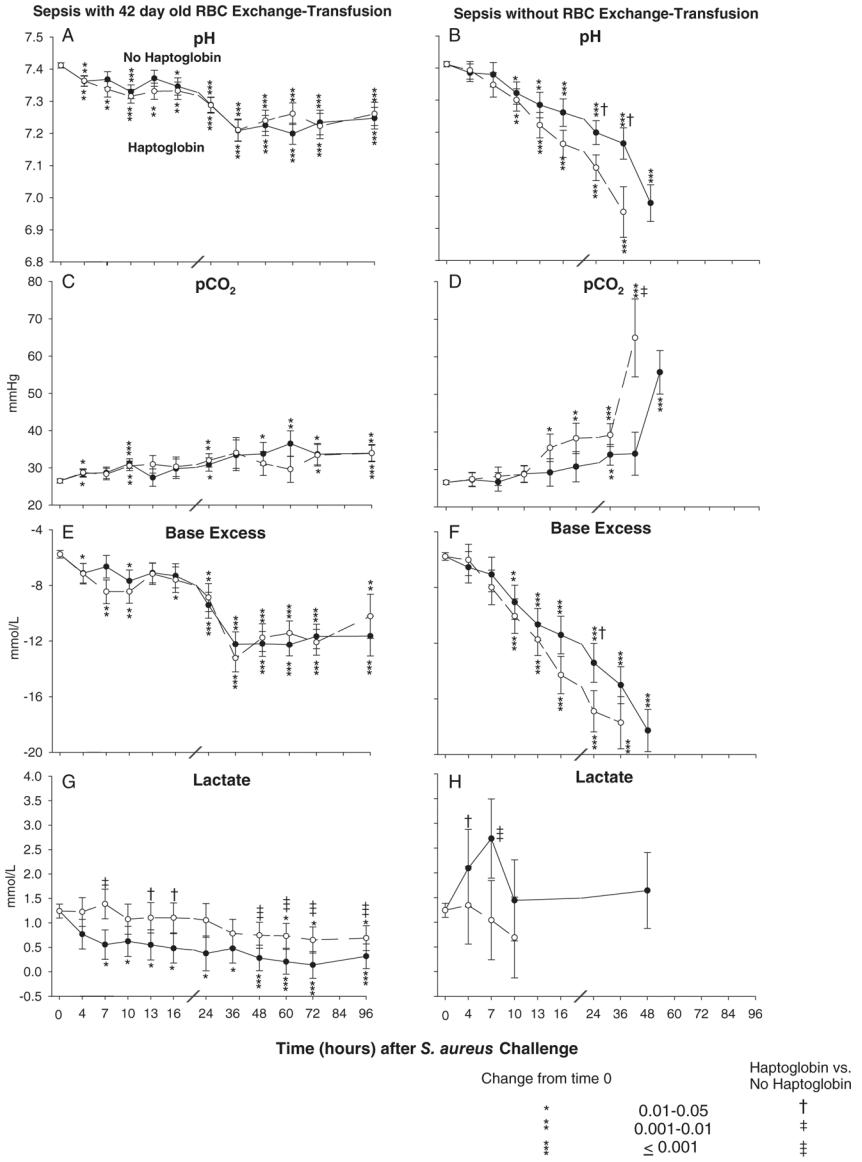

Fig. 5.

Arterial blood gas comparison over the 96-hour duration of the sepsis study. The format is similar to Fig. 1 except that quantitative arterial blood gas measurements (pH, pCO_2, base excess) and lactate levels are compared over 96 hours after *S. aureus* challenge in canines receiving haptoglobin or no haptoglobin with (A, C, E, G) or without RBC exchange transfusion (B, D, F, H).