ANESTHESIOLOGY

# Human Umbilical Cord Mesenchymal Stromal Cells Attenuate Systemic Sepsis in Part by Enhancing Peritoneal Macrophage Bacterial Killing *via* Heme Oxygenase-1 Induction in Rats

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## EDITOR'S PERSPECTIVE

### What We Already Know about This Topic

- Sepsis is a syndrome of life-threatening organ dysfunction caused by a dysregulated host response to infection for which there is no direct treatment
- Mesenchymal stromal cells have potent immunomodulatory, proreparative, and regenerative properties and have potential as therapeutics for sepsis
- Mesenchymal stromal cells enhance bacterial killing by augmenting macrophage function

### What This Article Tells Us That Is New

- In a rat model of fecal peritonitis, human umbilical cord mesenchymal stromal cells improved survival and reduced bacterial load by enhancing peritoneal macrophage function partly through induction of macrophage heme oxygenase-1
- Lipoxin A4 and prostaglandin E2 were found to play important roles in mediating the effect of human umbilical cord mesenchymal stromal cells on peritoneal macrophage heme oxygenase-1 expression

## ABSTRACT

**Background:** Mesenchymal stromal cells have therapeutic potential in sepsis, but the mechanism of action is unclear. We tested the effects, dose-response, and mechanisms of action of cryopreserved, xenogeneic-free human umbilical cord mesenchymal stromal cells in a rat model of fecal peritonitis, and examined the role of heme oxygenase-1 in protection.

**Methods:** Separate *in vivo* experiments evaluated mesenchymal stromal cells in fecal sepsis, established dose response (2, 5, and 10 million cells/kg), and the role of heme oxygenase-1 in mediating human umbilical cord–derived mesenchymal stromal/stem cell effects. *Ex vivo* studies utilized pharmacologic blockers and small inhibitory RNAs to evaluate mechanisms of mesenchymal stromal cell enhanced function in (rodent, healthy and septic human) macrophages.

**Results:** Human umbilical cord mesenchymal stromal cells reduced injury and increased survival (from 48%, 12 of 25 to 88%, 14 of 16, $P = 0.0033$) in fecal sepsis, with dose response studies demonstrating that 10 million cells/kg was the most effective dose. Mesenchymal stromal cells reduced bacterial load and peritoneal leukocyte infiltration (from $9.9 \pm 3.1 \times 10^6$/ml to $6.2 \pm 1.8 \times 10^6$/ml, N = 8 to 10 per group, $P < 0.0001$), and increased heme oxygenase-1 expression in peritoneal macrophages, liver, and spleen. Heme oxygenase-1 blockade abolished the effects of mesenchymal stromal cells (N = 7 or 8 per group). Mesenchymal stromal cells also increased heme oxygenase-1 expression in macrophages from healthy donors and septic patients. Direct *ex vivo* upregulation of macrophage heme oxygenase-1 enhanced macrophage function (phagocytosis, reactive oxygen species production, bacterial killing). Blockade of lipoxin A4 production in mesenchymal stromal cells, and of prostaglandin E2 synthesis in mesenchymal stromal cell/macrophage cocultures, prevented upregulation of heme oxygenase-1 in macrophages (from 9.6 ± 5.5-fold to 2.3 ± 1.3 and 2.4 ± 2.3 respectively, $P = 0.004$). Knockdown of heme oxygenase-1 production in macrophages ablated mesenchymal stromal cell enhancement of macrophage phagocytosis.

**Conclusions:** Human umbilical cord mesenchymal stromal cells attenuate systemic sepsis by enhancing peritoneal macrophage bacterial killing, mediated partly *via* upregulation of peritoneal macrophage heme oxygenase-1. Lipoxin A4 and prostaglandin E2 play key roles in the mesenchymal stromal cell and macrophage interaction.

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Sepsis is a syndrome of life-threatening organ dysfunction caused by a dysregulated host response to infection; there is no direct treatment.[1] It is a major public health burden in terms of mortality, economic cost, and—in those who survive—long-term disability.[1] Intraabdominal infections are among the most common sources of sepsis.[1]

Mesenchymal stromal/stem cells have potent immunomodulatory, proreparative and regenerative properties, and considerable potential as therapeutics for sepsis.[1]

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Mesenchymal stromal/stem cells are effective in preclinical pneumonia and sepsis models, and enhance bacterial killing by augmenting macrophage function[2,3] in adult[3] and neonatal[4] animal models and increasing antimicrobial peptide secretion[2,4]; however, the mechanism of enhanced macrophage function is uncertain. Phase I clinical studies of mesenchymal stromal/stem cells in neutropenic (NCT01849237) and nonneutropenic (NCT02421484)[5] patients with septic shock have reported encouraging results.

Four challenges impede the clinical translation of mesenchymal stromal/stem cells. First, scaling up of mesenchymal stromal/stem cell manufacturing to produce adequate quantities may require repeated cell passage, and this can lead to telomere shortening, mesenchymal stromal/stem cell senescence, and loss of beneficial effect.[6] Second, innovative cryopreservation strategies will be needed for storage and delivery to clinical sites. Third, contamination with viruses or prions, as well as immunogenicity, are concerns with xenogeneic materials; thus, elimination of animal-derived products (*e.g.*, fetal bovine serum, a common growth supplement) is desirable. Finally, our understanding of the mechanisms of action stem cells is incomplete, and this may be especially important in sepsis, where multiple mechanisms are at play and diagnosis is often uncertain.

Compared with cells from other sources, human umbilical cord–derived mesenchymal stromal/stem cells are plentiful and accessible; indeed, they are otherwise a biologic waste product. A single umbilical cord produces 10 times as many early passage mesenchymal stromal/stem cells as a routine bone marrow harvest,[7] obviating the need for repeated culture expansion. In addition, all human umbilical cord–derived mesenchymal stromal/stem cells are of the same "age" and have limited—and identical—environmental exposure, potentially reducing batch-to-batch variability.

We tested the effects of freshly thawed, cryopreserved human umbilical cord–derived mesenchymal stromal/stem cells that were propagated using xenogeneic material-free, serum-free medium in a rodent model of polymicrobial systemic sepsis. We hypothesized that human umbilical cord–derived mesenchymal stromal/stem cells would improve survival and reduce organ injury, and investigated the mechanisms whereby mesenchymal stromal/stem cells enhance bacterial clearance. Because mesenchymal stromal/stem cells enhance macrophage function,[2,3] and heme oxygenase-1 is important in antimicrobial host defense,[8,9] we hypothesized that mesenchymal stromal/stem cells enhance macrophage function by upregulation of heme oxygenase-1. Key mesenchymal stromal/stem cells effects are mediated by secretion of prostaglandin E2[10] or lipoxin A4.[11] In addition, we have recently demonstrated an important role for prostaglandin E2 in mediating the effects of mesenchymal stromal/stem cells on macrophage phenotype.[12] We therefore investigated whether there was a role for prostaglandin E2 or lipoxin A4 in mediating umbilical cord–derived mesenchymal stromal/stem cell—induced heme oxygenase-1 expression in peritoneal macrophages and in monocyte-derived macrophages from human volunteers and patients with sepsis.

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## Materials and Methods

All work was approved by the Animal Care and Use Committee of the Keenan Research Center for Biomedical Science of St. Michael's Hospital, Toronto, Canada (ACC648), and conducted under license from Health Canada, Ottawa, Canada. All studies on human peripheral blood mononuclear cells were approved by the Research Ethics Board of St. Michael's Hospital, Toronto (REB: 14-278). All experiments were carried out in the research laboratories at St. Michael's Hospital, Toronto. All animal experimental procedures (induction of pneumonia; assessment of injury/resolution) were carried out in the morning. More detailed methods are available in the Supplemental Digital Content 1 (http://links.lww.com/ALN/C79).

### Human Mesenchymal Stromal Cells

Human umbilical cord–derived mesenchymal stromal/stem cells were nonenzymatically extracted from full-term human postnatal umbilical cords, cultured in Lonza TheraPEAK MS serum-free complete medium (Cedarlane, Canada) supplemented with antibiotics (28 μM penicillin G, 104 μM gentamycin, 324 nM Amphotericin B) on fibronectin-coated flasks, passaged twice, and cryopreserved as previously described.[13,14] Routine flow cytometry confirmed the presence of mesenchymal stromal/stem cell surface markers. Cryopreserved human umbilical cord–derived mesenchymal stromal/stem cells were thawed, cultured for 3 to 5 days, washed, and resuspended in phosphate buffered saline before administration.

### Rodent Fecal Sepsis Protocol

Specific pathogen–free adult male Sprague Dawley rats (Charles River Laboratories, Canada; 350 to 450 g) were used in all experiments.

*Cecal Slurry Stock Preparation.* Briefly, 20 rats were euthanized, the cecum dissected, and the cecal contents combined, mixed with sterile water, and filtered through sterile meshes (first 380 μm, then 190 μM), and added to an equal volume of 30% glycerol in phosphate buffered saline.[15] The stock was aliquoted into 5-ml cryovials, frozen, and stored at −80°C.

*Fecal Sepsis Induction.* The animals were anesthetized with isoflurane (5%), the lower abdomen was shaved and cleaned, an 18-gauge catheter was inserted through the skin, and cecal slurry (4 ml slurry in 15% glycerol) or vehicle (4 ml 15% glycerol in phosphate buffered saline) was instilled into the peritoneal cavity. The injection site was subsequently cleaned with 3% $H_2O_2$, and the animals were allowed to recover. Buprenorphine (0.05 mg/kg) and lactated Ringer's solution (20 ml/kg) were given subcutaneously before surgery and every 12 h postinstillation.

*Experimental Design.* Preliminary experiments determined the cecal slurry dose required to produce sepsis over a 48-h period. Series 1 determined the effects of human umbilical cord–derived mesenchymal stromal/stem cells in severe fecal sepsis. Animals were randomized to undergo intraperitoneal instillation of cecal slurry (4 ml slurry in 15% glycerol) or sham procedure (4 ml 15% glycerol in phosphate buffered saline). Animals undergoing fecal sepsis were randomized to treatment with 10 million/kg umbilical cord–derived mesenchymal stromal/stem cells or to vehicle administered 4 h after sepsis induction. In series 2, the dose-response characteristics of human umbilical cord–derived mesenchymal stromal/stem cells (low, medium, and high, 2, 5, and 10 million/kg, respectively) were determined. In series 3, the potential for zinc protoporphyrin, an inhibitor of heme oxygenase activity, to block the effects of human umbilical cord–derived mesenchymal stromal/stem cells was examined. Animals were randomized to intraperitoneal injection of zinc protoporphyrin 50 μmol/kg or vehicle 1 day before fecal instillation, and to treatment with umbilical cord–derived mesenchymal stromal/stem cells or vehicle, respectively. In all *in vivo* experiments, umbilical cord–derived mesenchymal stromal/stem cells or vehicle was administered *via* tail vein (in 0.5 ml of sterile phosphate buffered saline) 4 h after sepsis induction.

*Assessment of Septic Injury.* Forty-eight hours later, all animals were reanesthetized, arterial blood pressure and peak airway pressure and lung static compliance and arterial blood gas analysis measured,[16–19] and the animals subsequently euthanized by exsanguination.

## *Ex Vivo* Analyses

Differential leukocyte counts were measured in peritoneal lavage fluid and bronchoalveolar lavage fluid. Bacterial colony-forming unit counts were performed in lung, liver, and spleen tissues using serial dilutions on agar plates. Tissue concentrations of tumor necrosis factor α and interleukin-10 and protein concentrations were assayed in tissue lysates and peritoneal lavage fluid.[20] Western blot analysis of heme oxygenase-1 and nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) total and phospho-activated p65 was performed on whole cell lysates from peritoneal macrophages and tissues.[21]

Macrophage phagocytosis and superoxide production were assessed in peritoneal macrophages from human umbilical cord–derived mesenchymal stromal/stem cell—or vehicle-treated rats, in the presence of heme oxygenase-1 inducers (hemin 10 μM) and inhibitors (zinc protoporphyrin, 10 μM). Phagocytic capacity was assessed using using Alexa-488–conjugated serum opsonized zymosan and enzyme-labeled *Escherichia coli* particles, while reactive oxygen species production was determined using the zymosan/nitroblue tetrazolium assay.[12,22] These assays were also performed in human monocytes isolated from peripheral blood of healthy volunteers and from patients fulfilling clinical criteria for sepsis. Human macrophage phagocytosis and killing of unopsonized *E. coli* were assessed as previously described.[23] The numbers of macrophages were standardized across groups in all experiments.

The roles of prostaglandin E2, lipoxin A4, and heme oxygenase-1 in mediating the effects of human umbilical cord–derived mesenchymal stromal/stem cells on rat and human macrophage phagocytosis and bacterial killing were assessed using exogenous prostaglandin E2 (0.2 μM, CAY14010; Cayman Chemicals, United Kingdom) or lipoxin A4 (0.1 μM, CAY90410; Cayman Chemicals), a prostaglandin E2 inhibitor (10 μM, CAY10526; Cayman Chemicals), and a 15-lipoxygenase inhibitor (2 μM, PD146176; Cayman Chemicals), respectively. Prostaglandin E2 and lipoxin A4 concentrations were assayed in umbilical cord–derived mesenchymal stromal/stem cell/macrophage supernatants. The effect of small inhibiting RNA knockdown of heme oxygenase-1 production in human macrophages on human umbilical cord–derived mesenchymal stromal/stem cell–, lipoxin A4–, and prostaglandin E2–stimulated macrophage phagocytosis and reactive oxygen species production was also assessed.

## Statistical Analysis

The sample size was based on our previous experience with this design and on pilot studies results. We anticipated a mortality in the range of 30% in untreated animals, but the mortality rate in the umbilical cord–derived mesenchymal stromal/stem cell—treated animals was unknown. Our primary measurements were bacterial colony counts, and we estimated (for an ANOVA test, $\alpha = 0.05$, $1\text{-}\beta = 0.8$, effect size $1 \times 10^4$, expected SD $0.75 \times 10^4$) that this required 12 surviving animals for each of three groups. To minimize animal numbers, we randomly allocated 12 animals to each group in series 1, and decided *a priori* to replace nonsurviving animals. For series 2 and 3, we recalculated sample sizes each time, which reduced the numbers of surviving animals needed to eight in each of four groups in series 2, and to five in each of two groups for series 3.

The data are expressed as mean ± SD, or presented as whisker plots (quartile 1 to quartile 3 ± 1.5 interquartile range).

Data were analyzed using GraphPad Prism (GraphPad software, USA). The distribution of all data was tested for normality using Kolmogorov–Smirnov tests. Data were analyzed by one-way ANOVA (using between-subjects factors), or ANOVA on Ranks with *post hoc* testing using

Newmann–Keuls, multiple comparison test, or Dunnett's tests, as appropriate. Chauvenet's (for normal distribution of the data) or Pierce's (nonnormal distribution of the data) criterion was used for data exclusion.[24] The only excluded values are 1 data point per septic animal group for figure 1, D, E, and F, and the values are listed in Supplemental Digital Content 2, tables e1 and e2 (http://links.lww.com/ALN/C80). For animal survival, the log-rank test was used, and there were no censored data during the observation period. A two-tailed $P$ value of less than 0.05 was considered significant.

## Results

### Series 1: Human Umbilical Cord–derived Mesenchymal Stromal/Stem Cells Increase Animal Survival, Decrease Peritoneal Infiltration, and Enhance Bacterial Clearance

*Animal Survival.* Human umbilical cord–derived mesenchymal stromal/stem cell therapy increased animal survival from fecal peritonitis–induced sepsis, increasing the survival rate from 48% (12 of 25) with vehicle to 87.5% (14/16; $P = 0.0033$), while survival was 100% in sham animals (12 of 12; fig. 1A). There were no adverse effects detected in regard to the infusion of umbilical cord–derived mesenchymal stromal/stem cells.

*Peritoneal Infiltration.* Human umbilical cord–derived mesenchymal stromal/stem cell therapy decreased peritoneal inflammatory cell, and particularly neutrophil, infiltration (fig. 1, B and C; $P < 0.0001$) after fecal sepsis.

*Bacterial Burden.* Human umbilical cord–derived mesenchymal stromal/stem cell therapy reduced bacterial load in the liver (fig. 1D; $P = 0.013$) compared to vehicle treatment, while bacterial counts in the spleen (fig. 1E; $P = 0.014$) and peritoneal cavity (fig. 1F; $P = 0.127$) in human umbilical cord–derived mesenchymal stromal/stem cell—treated animals were comparable to sham animals.

### Series 2: Dose-response Characteristics: Highest Effect of 10 Million/kg Umbilical Cord–derived Mesenchymal Stromal/Stem Cells

*Animal Survival.* The rank order of mortality was human umbilical cord–derived mesenchymal stromal/stem cells,

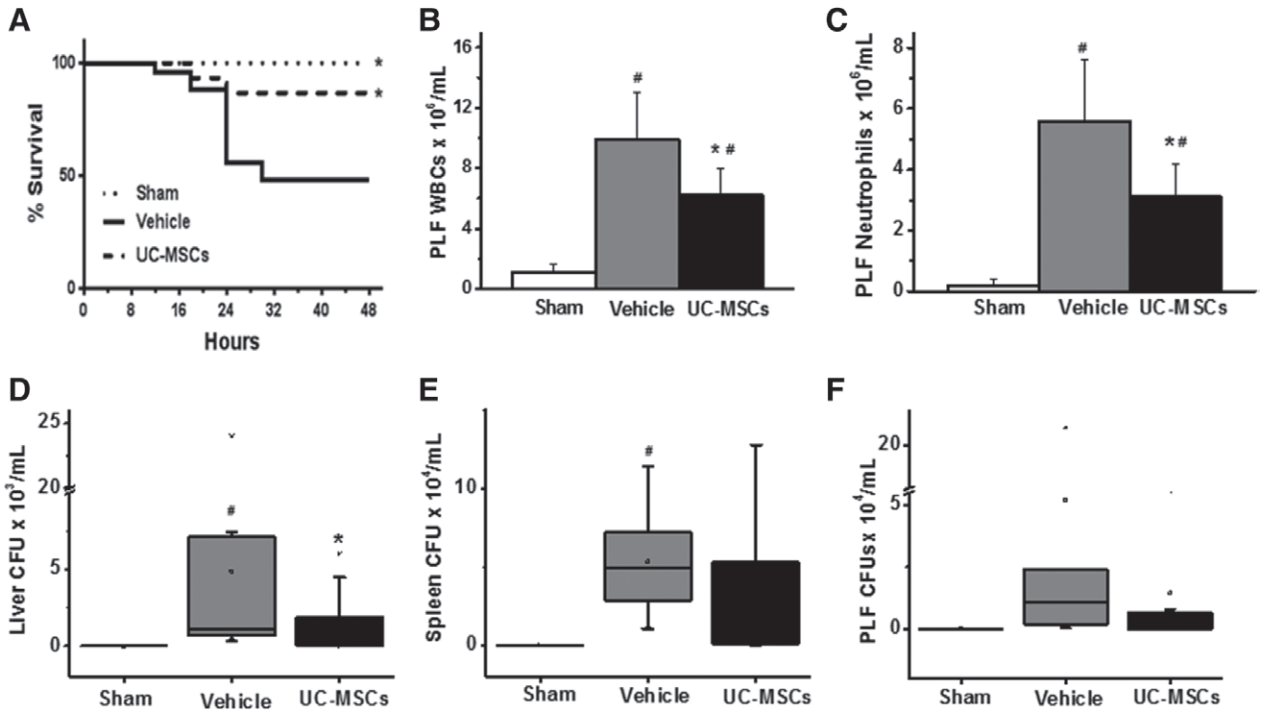


**Fig. 1.** Human umbilical cord mesenchymal stromal cells (hUC-MSCs) reduce the severity of fecal sepsis. (*A*) Kaplan–Meier survival curve demonstrating that hUC-MSCs (10 million/kg) increased survival (14/16) in rodents subjected to fecal peritonitis–induced systemic sepsis compared to treatment with vehicle (phosphate buffered saline; 12/25; $P = 0.0033$). (*B*) hUC-MSC therapy decreased peritoneal lavage fluid (PLF) white blood cell (WBC) count, (*C*) and reduced neutrophil infiltration. (*D*) hUC-MSCs therapy significantly decreased bacterial colony forming units (CFU) in the liver ($P = 0.013$), while (*E*) the reductions in the spleen ($P = 0.014$) and (*F*) PLF ( $P = 0.127$) bacterial counts and were not significantly different from sham animals. The data are expressed as mean ± SD or quartile 1 to quartile 3 ± 1.5 interquartile range. *$P < 0.05$ *versus* vehicle-treated septic rats; #$P < 0.05$ *versus* sham group; N = 12 rats for sham and vehicle-treated septic group, and 14 rats for hUC-MSC–treated group. (*D* and *F*) One value/septic group and (*E*) one value/vehicle-treated septic group were excluded using Chauvenet's criterion (all data provided in Supplemental Digital Content, table e1, http://links.lww.com/ALN/C80).

2 million/kg (50%, 8 of 16) greater than vehicle (44%, 8 of 18) greater than human umbilical cord–derived mesenchymal stromal/stem cells, 5 million/kg (29%, 4 of 14) greater than human umbilical cord–derived mesenchymal stromal/stem cells, 10 million/kg (18%, 2 of 11; $P = 0.036$; Supplemental Digital Content, fig. e1A, http://links.lww.com/ALN/C81).

*Bacterial Burden.* The bacterial load in the liver (fig. 2A; $P = 0.047$) and peritoneal fluid (Supplemental Digital Content, fig. e1C, http://links.lww.com/ALN/C81; $P = 0.049$) was reduced by the 10 million/kg human umbilical cord–derived mesenchymal stromal/stem cell dose, but not with lower doses, while no human umbilical cord–derived mesenchymal stromal/stem cell dose reduced bacterial load in the spleen (fig. 2B; $P = 0.758$).

*Peritoneal Infiltration.* Human umbilical cord–derived mesenchymal stromal/stem cell doses of 5 and 10 million/kg, but not 2 million/kg, significantly reduced peritoneal total white cell (Supplemental Digital Content, fig. e1B, http://links.lww.com/ALN/C81; $P = 0.049$) and neutrophil (fig. 2C; $P = 0.001$) infiltration.

*Inflammation.* Both 5 and 10 million/kg human umbilical cord–derived mesenchymal stromal/stem cells (but not 2 million/kg) significantly reduced peritoneal total white cell (Supplemental Digital Content, fig. e1B, http://links.lww.com/ALN/C81; $P = 0.001$) and neutrophil (fig. 2C; $P = 0.001$) infiltration. Finally, 10 million/kg human umbilical cord–derived mesenchymal stromal/stem cells (but not lower doses) reduced tumor necrosis factor α in the liver ($P < 0.001$), spleen ($P = 0.009$), and lung ($P < 0.0001$, where 5 million/kg was also effective), and increased interleukin-10 in the liver ($P < 0.0001$; fig. 2, D, E, and F; Supplemental Digital Content, fig. e1D, http://links.lww.com/ALN/C81).

*NF-κB Activation.* Human umbilical cord–derived mesenchymal stromal/stem cells decreased activation of NF-κB in the lung ($P = 0.020$), splenic ($P < 0.001$), and hepatic tissues ($P = 0.034$), as assessed by the concentration of the phospho-activated P65 subunit of NF-κB in these organs (Supplemental Digital Content, fig. e2, http://links.lww.com/ALN/C82); these effects of human umbilical cord–derived mesenchymal stromal/stem cells were

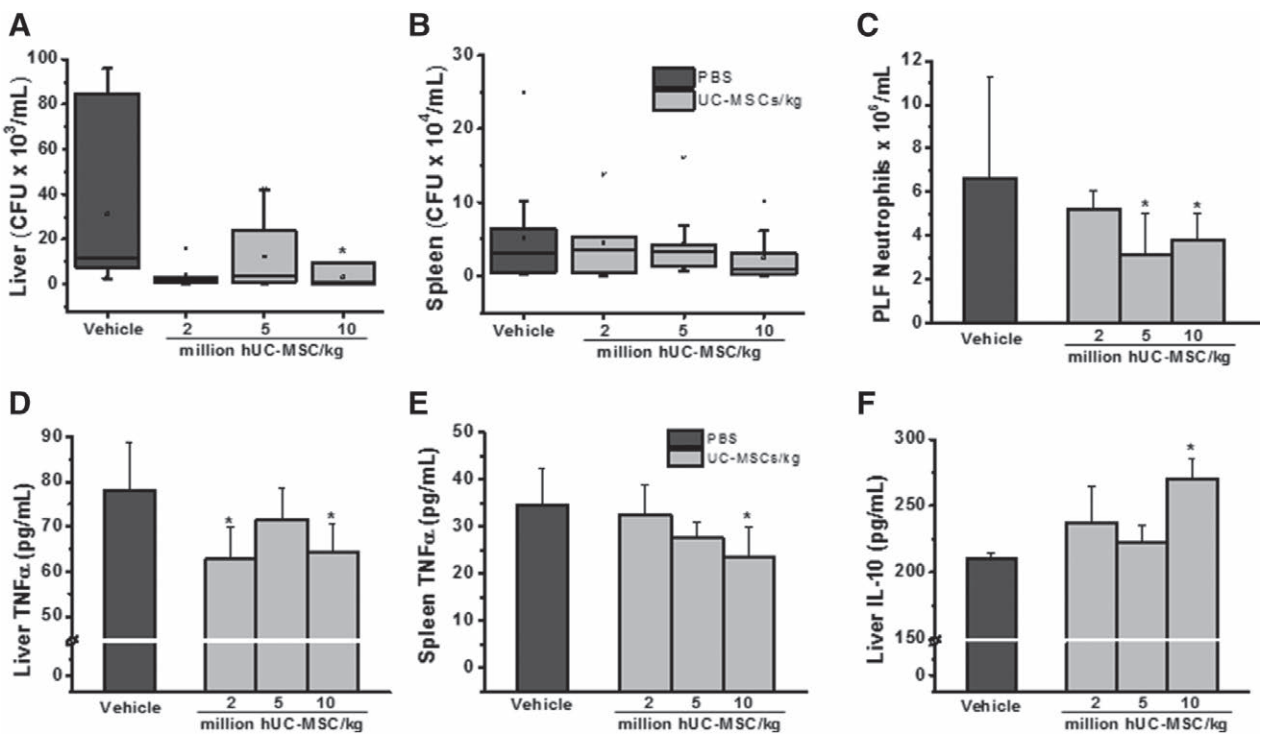


**Fig. 2.** Effects of human umbilical cord mesenchymal stromal cells (hUC-MSCs) in fecal sepsis are dose-dependent. (*A*) hUC-MSC therapy dose-dependently decreased bacterial load in the liver ($P = 0.047$) but (*B*) not in the spleen ($P = 0.758$). (*C*) 10 million/kg (high) and 5 million/kg (medium) hUC-MSC doses, but not 2 million/kg (low) doses, reduce peritoneal neutrophil infiltration ($P = 0.001$) compared to vehicle-treated animals. (*D*) hUC-MSC therapy decreased tumor necrosis factor α (TNFα) in the liver ($P < 0.0001$) and (*E*) spleen ($P = 0.009$) and (*F*) increased interleukin-10 (IL-10) concentrations in the liver ($P < 0.0001$) compared to vehicle-treated animals. The data are expressed as quartile 1 to quartile 3 ± 1.5 interquartile range or mean ± SD. *$P < 0.05$ *versus* vehicle-treated septic rats; N = 8 to 10 per group (N = 8 animals for the treatment with 2 million hUC-MSCs; N = 9 animals for the treatment with 10 million hUC-MSCs, and N = 10 animals for the treatment with 5 million hUC-MSCs and vehicle-treated group). CFU, colony forming units; PBS, phosphate buffered saline; PLF, peritoneal lavage fluid.

dose-dependent, and were greatest with 10 million/kg human umbilical cord–derived mesenchymal stromal/stem cells (Supplemental Digital Content, fig. e2C–E, http://links.lww.com/ALN/C82).

## Mechanisms of Action of Human Umbilical Cord–derived Mesenchymal Stromal/Stem cells: Role of Heme Oxygenase-1

*Heme Oxygenase-1.* The concentration of heme oxygenase-1 in peritoneal macrophages was increased in human umbilical cord–derived mesenchymal stromal/stem cell—treated animals (fig. 3, A and C; $P = 0.015$). Subsequent *ex vivo* studies demonstrated that the magnitude of this increase was comparable to that seen after *ex vivo* coincubation with hemin, a direct inducer of heme oxygenase-1 (fig. 3, B and C; Supplemental Digital Content, fig. e6, http://links.lww.com/ALN/C83; $P = 0.616$). Human umbilical cord–derived mesenchymal stromal/stem cell therapy also increased the concentration of heme oxygenase-1 in the liver (fig. 3D; $P = 0.006$) and in the spleen (fig. 3E; $P < 0.0001$).

*Peritoneal Macrophage Function.* Human umbilical cord–derived mesenchymal stromal/stem cell therapy increased macrophage phagocytic index (fig. 3F), phagosomal reactive oxygen species production (fig. 3G), and fold change of phagocytosis (fig. 3H) in peritoneal macrophages isolated from animals after fecal peritonitis compared to macrophages from vehicle-treated and sham animals. Hemin treatment *ex vivo* increased phagocytosis in macrophages from vehicle-treated (fig. 3, F and H; $P < 0.0001$) but not from human umbilical cord–derived mesenchymal stromal/stem cell—treated animals, suggesting a common pathway. Phagocytic index was not changed by hemin in either vehicle or umbilical cord–derived mesenchymal stromal/stem cell—treated macrophages, suggesting that heme oxygenase-1 induction increases the percentage of macrophages that could perform phagocytosis but not the number of particles they could engulf. Hemin had no effect on reactive oxygen species production, suggesting mediation *via* a non–reactive oxygen species pathway (fig. 3G). The heme oxygenase antagonist zinc protoporphyrin abolished the increase in macrophage phagocytosis induced by human umbilical cord–derived mesenchymal stromal/stem cells, and reduced reactive oxygen species production (fig. 3, F, G, and H). Representative images from these experiments are provided (fig. 3I and Supplemental Digital Content, fig. e3, http://links.lww.com/ALN/C84).

*Heme Oxygenase-1 Inhibition Abolishes In Vivo Human Umbilical Cord–derived Mesenchymal Stromal/Stem Cell Effects.* Human umbilical cord–derived mesenchymal stromal/stem cell (10 million/kg) therapy failed to produce benefit in rats pretreated (N = 7 or 8 per group) with zinc protoporphyrin (50 μmol/kg, intraperitoneal). There was no effect of human umbilical cord–derived mesenchymal stromal/stem cell therapy on animal survival (57%, 4 of 7, *vs.* 62%, 5 of 8), peritoneal neutrophil infiltration (fig. 4A; $P = 0.232$), or bacterial load in the peritoneal lavage fluid, liver, or spleen (fig. 4, B, C, and D; $P = 0.672$, $P = 0.976$, and $P = 0.881$, respectively). Zinc protoporphyrin pretreatment abolished the enhanced peritoneal macrophage phagocytosis induced by human umbilical cord–derived mesenchymal stromal/stem cells (fig. 4, E and F; E, $P = 0.113$; F; $P = 0.265$; N = 4 or 5).

*Umbilical Cord–derived Mesenchymal Stromal/Stem Cell Expression of Heme Oxygenase-1 in Human Macrophages.* Human umbilical cord–derived mesenchymal stromal/stem cells induced heme oxygenase-1 expression in monocyte-derived macrophages isolated from healthy humans ($P = 0.005$), to a comparable degree observed with hemin (fig. 5, A and B). Blockade of lipoxin A4 production, and separately of prostaglandin E2 production, in human umbilical cord–derived mesenchymal stromal/stem cell/macrophage cocultures, each abolished the increase in human macrophage heme oxygenase-1 expression (fig. 5, C and D; $P = 0.004$; N = 4 to 7 for fig. 5A, B, C, and D). Human umbilical cord–derived mesenchymal stromal/stem cells enhanced macrophage phagocytosis of serum-opsonized zymosan, an effect abolished by zinc protoporphyrin, and by inhibition of prostaglandin E2 or lipoxin A4 (fig. 5E; $P < 0.0001$; N = 4 to 6 per group). Human umbilical cord–derived mesenchymal stromal/stem cells enhanced macrophage killing of phagocytosed *E. coli*; this was blocked by zinc protoporphyrin, and by inhibition of prostaglandin E2. Of interest, while lipoxin A4 blockade reduced bacterial phagocytosis, it did not decrease killing of already phagocytosed bacteria (fig. 5F; $P < 0.0001$; N = 4 or 5 per group). Human umbilical cord–derived mesenchymal stromal/stem cells also induced heme oxygenase-1 expression in monocyte-derived macrophages obtained from patients with sepsis (fig. 5, G and H; $P = 0.029$; N = 5).

*Production of Lipoxin A4 and Prostaglandin E2 and Effects in Human Macrophages.* Human umbilical cord–derived mesenchymal stromal/stem cells—but not human macrophages—produced lipoxin A4 in monoculture, while production was increased when human umbilical cord–derived mesenchymal stromal/stem cells and macrophages were cocultured (fig. 6A; $P < 0.0001$). In contrast, both cell types produce prostaglandin E2, while again production was maximal in coculture (fig. 6B; $P = 0.002$). In macrophages, exogenous lipoxin A4 enhanced prostaglandin E2 production in a dose-dependent manner (fig. 6B), but prostaglandin E2 did not increase macrophage production of lipoxin A4 (fig. 6A). Heme oxygenase-1 small inhibiting RNA transfection of macrophages locked the increase of cytoplasmic heme oxygenase-1 by umbilical cord–derived mesenchymal stromal/stem cells, and by lipoxin A4 and prostaglandin E2 (Supplemental Digital Content, fig. e4, http://links.lww.com/ALN/C85; $P < 0.0001$); it abolished the enhancement of macrophage phagocytosis by umbilical cord–derived mesenchymal

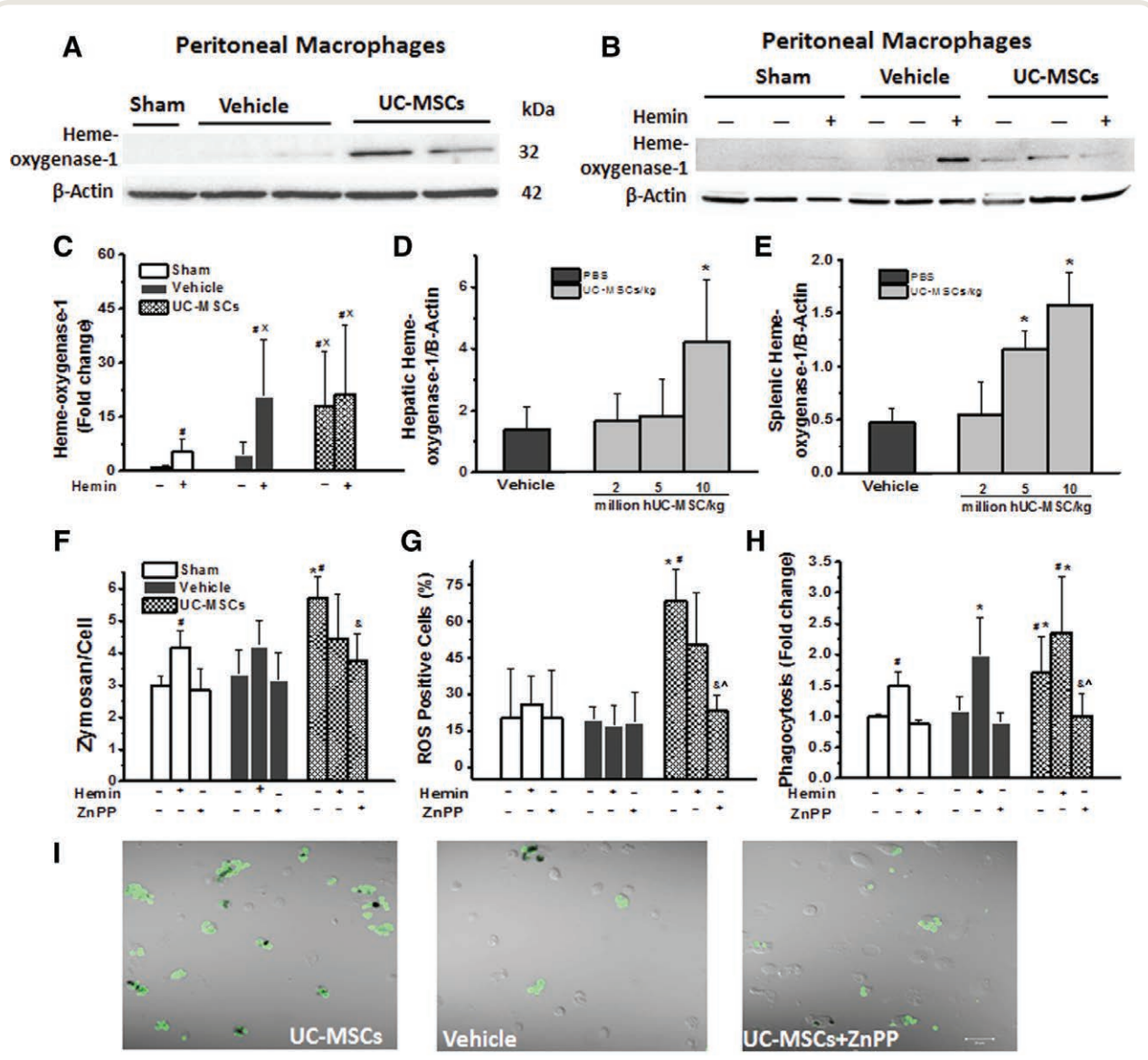


**Fig. 3.** Human umbilical cord mesenchymal stromal cells (hUC-MSCs) dose-dependently increase heme oxygenase-1 expression in peritoneal macrophages, while heme oxygenase-1 blockade ablates hUC-MSC enhancement of macrophage phagocytosis. (*A*) Representative Western blot demonstrating increased heme oxygenase-1 concentrations in peritoneal macrophage lysates from hUC-MSC–treated animals and (*B*) the effect of the heme oxygenase-1 inducer hemin. (*C*) Quantitative densitometry demonstrates the degree of heme oxygenase-1 increase ($P = 0.015$) and the effect of hemin in vehicle but not in umbilical cord mesenchymal stromal cell (UC-MSC)–treated samples ($P = 0.616$). (*D*) Quantitative densitometry of Western blots demonstrating dose-dependent hUC-MSC–induced increase of heme oxygenase-1 concentrations in the liver ($P = 0.006$) and (*E*) the spleen ($P < 0.0001$). (*F*) UC-MSC therapy increased macrophage phagocytic index, (*G*) phagosomal reactive oxygen species (ROS) production, and (*H*) fold change of phagocytosis in peritoneal macrophages isolated from animals after fecal peritonitis compared to macrophages from vehicle-treated and sham animals. (*F* and *H*) The direct heme oxygenase-1 inducer hemin increased phagocytosis, (*G*) but not ROS production in macrophages from vehicle-treated animals. (*F* and *H*) Hemin did not further increase phagocytosis in macrophages from hUC-MSC–treated animals. (*F* and *H*) The heme oxygenase-1 antagonist zinc protoporhyrin (ZnPP) abolished the hUC-MSC induced increase in phagocytosis and (*G*) reduced ROS production in macrophages from hUC-MSC–treated animals. (*I*) Representative images of peritoneal macrophages showing hUC-MSC therapy enhanced phagocytosis of serum opsonized zymosan (*green fluorescence*) and increased phagosomal ROS production (*black dots*) compared to vehicle, while ZnPP blocked these effects. The data are expressed as mean ± SD. All data from a minimum of three independent experiments were done in duplicate. (*C*, *D*, and *E*) #$P < 0.05$ *versus* sham group not treated with hemin; $^{X}P < 0.05$ *versus* vehicle group not treated with hemin; *$P < 0.05$ *versus* vehicle-treated group; N = 4 for sham and 6 for all other groups, referring to the animal number/group. (*F*, *G*, and *H*) *$P < 0.05$ *versus* vehicle control group, #$P < 0.05$ *versus* sham control group, $^{\&}P < 0.05$ *versus* UC-MSC control group, ^$P < 0.05$ *versus* hemin-treated group. N = 4 for sham rats and 5 rats per group in all other groups. Samples of macrophages isolated from peritoneal fluid were taken from each animal and underwent treatment or not (controls). Two slides were prepared from each sample. Images were taken and analyzed in 8 to 10 randomly chosen fields/slide.

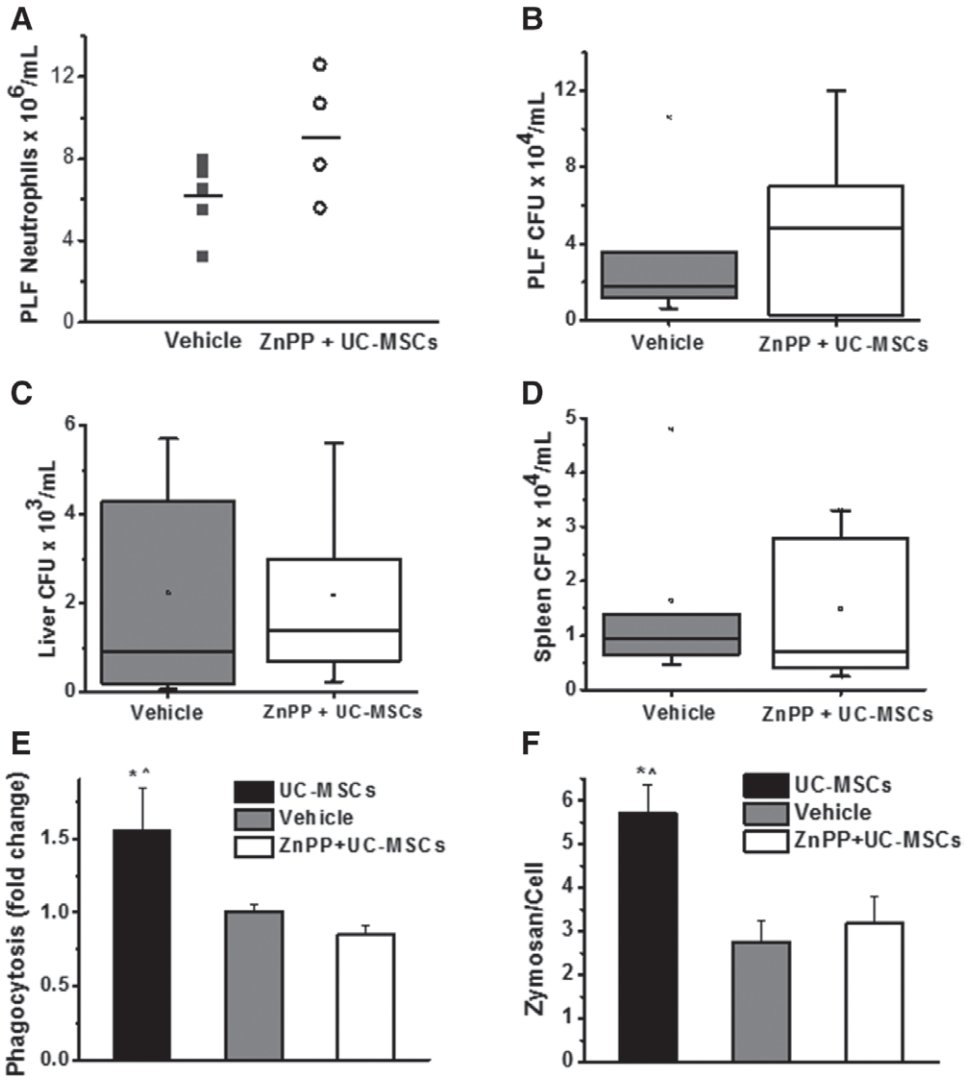


**Fig. 4.** Heme oxygenase-1 blockade abolishes human umbilical cord mesenchymal stromal cell (hUC-MSC) efficacy after *in vivo* fecal sepsis. (*A*) Pretreatment of rats (N = 7 or 8 per group) with zinc protoporphyrin (ZnPP; 50 μmol/kg, intraperitoneally) 1 day before induction of fecal sepsis abolished the effect of hUC-MSC therapy (10 million/kg) on peritoneal neutrophil ($P = 0.232$) infiltration, and on (*B*) bacterial load in the peritoneal lavage fluid, (*C*) the liver ($P = 0.976$) and (*D*) the spleen ($P = 0.881$). (*E*) In subsequent *ex vivo* studies, ZnPP pretreatment of umbilical cord mesenchymal stromal cell (UC-MSC)–treated rats abolished the increase in phagocytosis ($P = 0.113$) and (*F*) the phagocytic index in peritoneal macrophages seen with UC-MSC therapy ($P = 0.265$). The data are expressed as mean ± SD or quartile 1 to quartile 3 ± 1.5 interquartile range. N = 4 rats in ZnPP + UC-MSC rats/group, and 5 rats in vehicle-treated group. (*E* and *F*) Three independent experiments were done: N = 4 rats in ZnPP + UC-MSC rats/group, and 5 rats for both UC-MSCs and vehicle-treated group. Two samples of macrophages isolated from peritoneal fluid were taken from each animal. Slides were prepared from each sample. Images were taken and analyzed in 8 to 10 randomly chosen fields/slide. The data are expressed as mean ± SD. *$P < 0.05$ *versus* vehicle-treated group, ^$P < 0.05$ *versus* ZnPP + hUC-MSC group. CFU, colony forming units; PLF, peritoneal lavage fluid.

stromal/stem cells and by lipoxin A4 and prostaglandin E2; and it abolished the increase in human macrophage reactive oxygen species–positive phagocytes by umbilical cord–derived mesenchymal stromal/stem cells and by prostaglandin E2 (fig. 6, C and D, and Supplemental Digital Content, fig. e5, http://links.lww.com/ALN/C86; $P < 0.001$).

## Discussion

These studies provide several key novel insights regarding the effects and mechanisms of the action of intravenous human umbilical cord–derived mesenchymal stromal/stem cells in a clinically relevant model of systemic sepsis. We demonstrate that umbilical cord–derived mesenchymal

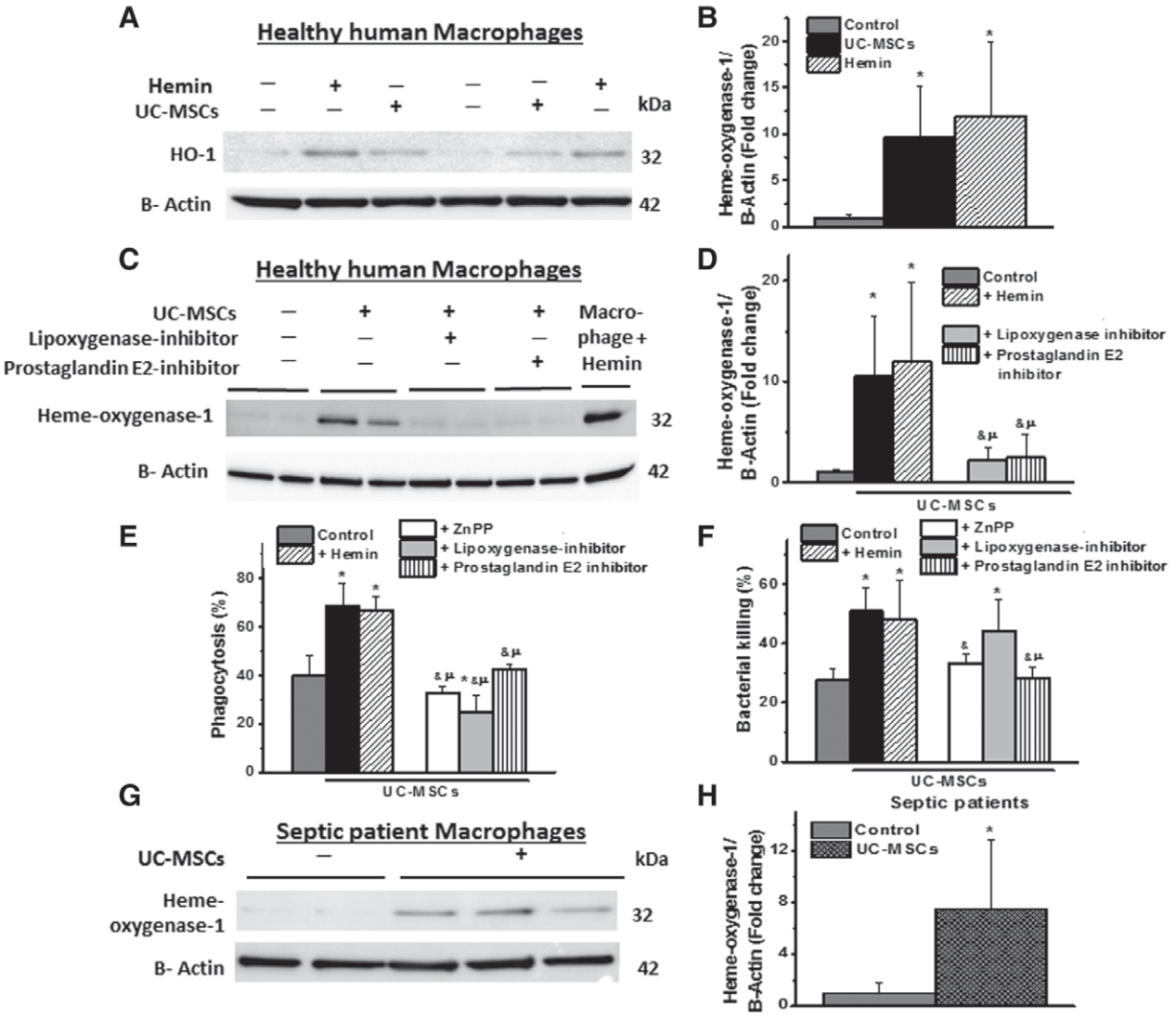


**Fig. 5.** Human umbilical cord mesenchymal stromal cells (hUC-MSCs) enhance heme oxygenase-1 expression in human macrophages from volunteers and septic patients. (*A*) In monocyte-derived macrophages isolated from human volunteers, hUC-MSCs induced heme oxygenase-1 (HO-1) expression similar to that with the HO-1 inducer hemin. (*B*) Quantitative densitometry demonstrates the fold of increase of HO-1 expression in macrophages cultured with hUC-MSCs ($P = 0.005$). (*C* and *D*) The lipoxin A4 inhibitor PD146176 (2 μM) and the prostaglandin E2 inhibitor CAY10526 (10 μM) prevented the increase in macrophage HO-1 expression induced by hUC-MSCs ($P = 0.004$); lysates for Western blot analysis were prepared from two samples of macrophages derived from each of N = 4 healthy volunteers for macrophage + hemin group, N = 6 for umbilical cord mesenchymal stromal cells (UC-MSCs) group, and N = 7 for control group. Three experiments were done. (*E*) hUC-MSCs enhanced monocyte-derived macrophage phagocytosis of serum-opsonized zymosan to a comparable degree to that seen with hemin; this hUC-MSC enhancement of phagocytosis was blocked by zinc protoporphyrin (ZnPP), the prostaglandin E2 inhibitor, and the Lo-inhibitor ($P < 0.0001$). Macrophages derived from blood of healthy volunteers were treated or not (control). N = 4 for hemin group and 6 for all other groups). Two slides were prepared from each sample. Images were taken and analyzed in 8 to 10 randomly chosen fields/slide. (*F*) hUC-MSCs enhanced monocyte derived killing of *E. coli* to a comparable degree to that seen with hemin; this was blocked by ZnPP and prostaglandin E2 inhibitor ($P < 0.0001$; N = 4 for ZnPP group and 5 for all other groups). (*G*) hUC-MSC coincubation induced HO-1 expression in monocyte-derived macrophages from patients with sepsis. Quantitative densitometry demonstrates the fold of increase of HO-1 expression in macrophages cultured with hUC-MSCs on inserts for 48 h ($P = 0.029$; N = 5 septic patients per group, 2 samples per patient). (*H*) $^{*}P < 0.05$ *versus* control macrophages. The data are expressed as mean ± SD. For all multigroup panels, at least three experiments were done; 2 samples/condition in each experiment, samples done at least in duplicate in each of three experiments; $^{*}P < 0.05$ *versus* macrophage control; $^{\&}P < 0.05$ *versus* macrophages + hUC-MSCs; $^{h}P < 0.05$ *versus* macrophages + hemin; $^{\mu}P < 0.05$ *versus* macrophages + hUC-MSCs + hemin.

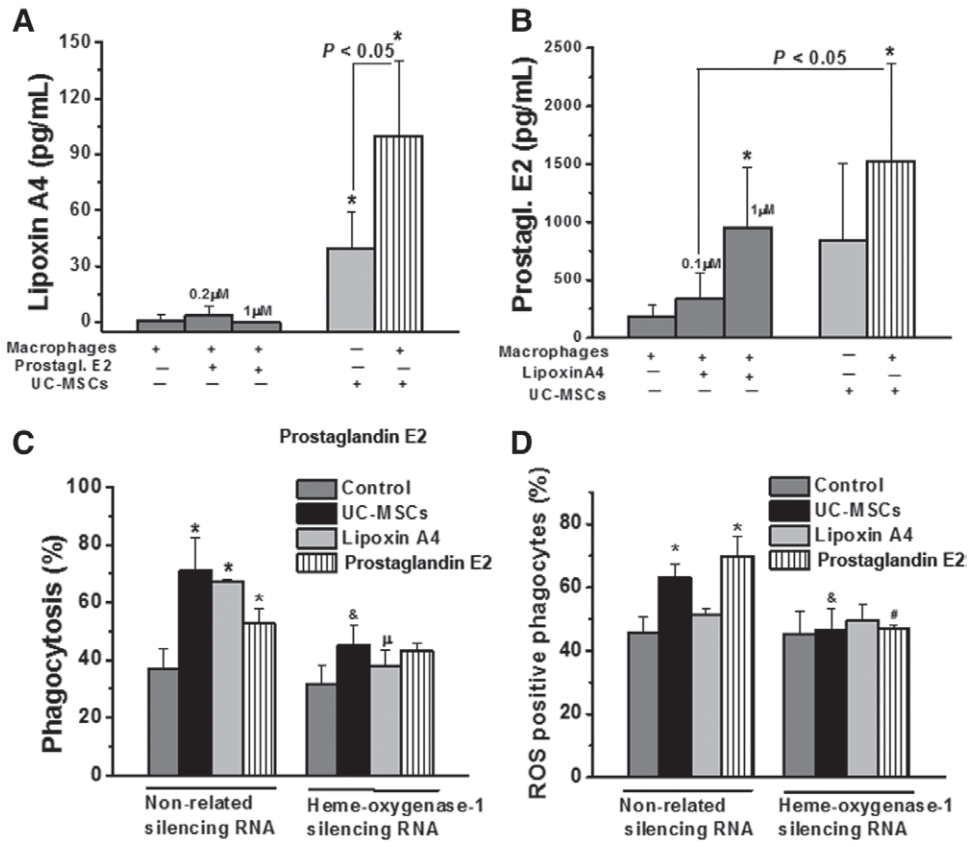


**Fig. 6.** Roles of lipoxin A4, prostaglandin E2, and heme oxygenase-1 in mediating effect of human umbilical cord mesenchymal stromal cells (hUC-MSCs) on macrophage function. (*A*) hUC-MSCs produce lipoxin A4, and this is increased in the presence of human macrophages, while naive and prostaglandin E2–stimulated human macrophages produce minimal lipoxin A4 ($P < 0.0001$; N = 8/group, except for samples stimulated with 1 μM prostaglandin E2, N = 6). (*B*) Human macrophages produce prostaglandin E2, which is enhanced by exogenous lipoxin A4. hUC-MSCs also produce prostaglandin E2, while coculture with human macrophages further enhances prostaglandin E2 production ($P = 0.002$; N = 7/group, except for samples stimulated with 1μM lipoxin A4, N = 5). (*C*) Heme oxygenase-1 silencing RNA transfection of human macrophages abolished the enhancement of human macrophage phagocytosis by umbilical cord mesenchymal stromal cells (UC-MSCs) and by lipoxin A4 and prostaglandin E2 ($P < 0.0001$), and (*D*) it abolished the increase in human macrophage reactive oxygen species (ROS)–positive phagocytes by UC-MSC and by prostaglandin E2 ($P < 0.0001$). (*A* and *B*) *$P < 0.05$ *versus* macrophage control. (*D*) *$P < 0.05$ *versus* nonrelated silencing RNA control; $^{\&}P < 0.05$ *versus* nonrelated silencing + UC-MSCs; $^{\mu}P < 0.05$ *versus* nonrelated silencing RNA + lipoxin A4; #P < 0.05 *versus* nonrelated silencing RNA + prostaglandin E2. (*C* and *D*) N= 6 samples/group, from three different experiments and samples in duplicates. All data are expressed as mean ± SD.

stromal/stem cells improve survival and reduce bacterial load in fecal peritonitis by enhancing peritoneal macrophage function. The mechanism underlying this effect is *via* induction of macrophage heme oxygenase-1. Our *in vivo* and *ex vivo* studies identify key roles for lipoxin A4 and prostaglandin E2 in mediating the effect of human umbilical cord–derived mesenchymal stromal/stem cells on peritoneal macrophage heme oxygenase-1 expression. The ablation of umbilical cord–derived mesenchymal stromal/stem cell actions *via* heme oxygenase-1 blockade demonstrates the pivotal role of heme oxygenase-1 in mediating these effects of umbilical cord–derived mesenchymal stromal/stem cells. Our finding that umbilical cord–derived mesenchymal stromal/stem cells enhance the function of macrophages derived from healthy humans and humans with sepsis *via* a similar mechanism is important from a translational perspective. Furthermore, because a previously cryopreserved xenogeneic material-free cell product was efficacious, this advances the feasibility of early clinical testing.[10,25]

In these studies, human umbilical cord–derived mesenchymal stromal/stem cell therapy reduced mortality, and decreased the bacterial burden induced by fecal peritonitis. NF-κB activation, a key transcriptional regulator of the preinflammatory response, was decreased in the liver, lung, and splenic tissues. A dose-response effect was demonstrated, with greatest (intermediate and zero) effect after 10 (5 and 2) million human umbilical cord–derived mesenchymal stromal/stem cells/kg, respectively. These findings are

relevant because the Cellular Immunotherapy for Septic Shock phase 1 study administered doses of 0.3, 1, and 3 million mesenchymal stromal/stem cells/kg, lower than in the current study and in other preclinical sepsis studies.[10,25] While Cellular Immunotherapy for Septic Shock demonstrated safety, it did not provide a strong biologic signal,[5] and although caution is required in extrapolating between preclinical and clinical settings, the dose-response profile in the current study raise the possibility the higher doses may be appropriate in preliminary clinical tests.

Heme oxygenase-1 is an enzyme that catalyzes the degradation of heme into three biologically active end products, biliverdin/bilirubin, carbon monoxide, and ferrous ion, and is induced by circulating free heme released from hemoglobin during infection.[26] Several biologic effects of heme oxygenase-1 are mediated by carbon monoxide,[9] which promotes phagocytosis and bacterial killing in macrophages by inhibiting the bacterial respiratory chain[27] and by activating macrophage inflammasomes.[28] Heme oxygenase-1 appears to exert multiple actions—potentially beneficial and harmful—in the setting of experimental sepsis. Mice deficient in heme oxygenase-1 have enhanced systemic inflammation, anemia, nephropathy, and depletion of macrophages engaged in recycling of red blood cells. Heme oxygenase-1 appears to play a key role in microbial host defense,[8,9] and exerts protective effects in diverse preclinical organ injury models. Heme oxygenase-1–deficient mice have higher mortality than wild types after polymicrobial sepsis.[9] Hemin, the inducer of heme oxygenase-1, inhibits activation of the NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome and attenuates sepsis-induced lung injury,[29] as well as pancreatitis (and secondary lung injury) induced by L-arginine.[30] Overexpression of heme oxygenase-1 attenuates endotoxin induced acute lung injury,[31] and heme oxygenase-1 may mediate the effects of other potential therapies, such as simvastatin,[32] that attenuate ventilator-induced lung injury. However, Yoon *et al.*[33] demonstrate that hemin induction of heme oxygenase-1 worsens T-cell immunosuppression and increases bacterial burden during late-phase sepsis. In contrast, hemin-induced heme oxygenase-1 expression suppressed sepsis induced skeletal muscle atrophy in mice.[34] In summary, the effects of heme oxygenase-1 in sepsis are complex, they are cell- and tissue-specific and also dependent on the phase of sepsis.

Our data identify a key role for heme oxygenase-1 in the antimicrobial actions of human umbilical cord–derived mesenchymal stromal/stem cell therapy in sepsis. While human umbilical cord–derived mesenchymal stromal/stem cells express very low heme oxygenase-1 (data not shown) they induce heme oxygenase-1 expression in macrophages in the liver, spleen, and peritoneal macrophages of treated animals. Peritoneal macrophages isolated from animals treated with human umbilical cord–derived mesenchymal stromal/stem cells demonstrated increased heme oxygenase-1 expression, as well as enhanced bacterial phagocytosis and killing. Heme oxygenase-1 blockade ablates the increased phagocytosis *in vitro* and *ex vivo*, and abolishes the protective effects of human umbilical cord–derived mesenchymal stromal/stem cells *in vivo*. Finally, the upregulation of heme oxygenase-1 by human umbilical cord–derived mesenchymal stromal/stem cells in human macrophages (volunteers, patients with sepsis) underscores the translational potential of the approach.

Our experiments elucidate key roles for lipoxin A4 and prostaglandin E2 in mediating the human umbilical cord–derived mesenchymal stromal/stem cell—induced increase in peritoneal heme oxygenase-1. Lipoxin A4, produced by human umbilical cord–derived mesenchymal stromal/stem cells, increases macrophage production of prostaglandin E2, which together with prostaglandin E2 produced by umbilical cord–derived mesenchymal stromal/stem cells leads to an increase in macrophage heme oxygenase-1. Blockade of either lipoxin A4 or prostaglandin E2 synthesis in umbilical cord–derived mesenchymal stromal/stem cells ablates the increase in macrophage heme oxygenase-1. Macrophage heme oxygenase-1 knockdown (by small inhibiting RNA) ablated the enhanced macrophage phagocytosis induced by human umbilical cord–derived mesenchymal stromal/stem cells or exogenous lipoxin A4. Macrophage heme oxygenase-1 knockdown also ablated the enhancement of reactive oxygen species production by human umbilical cord–derived mesenchymal stromal/stem cells or exogenous prostaglandin E2. While lipoxin A4 has been reported to mediate resolution of acute lung injury after mesenchymal stromal/stem cells,[11] and to increase heme oxygenase-1 expression in corneal epithelial cells,[35] this is the first report of these actions in macrophages. These novel findings reveal the mechanism by which umbilical cord–derived mesenchymal stromal/stem cells increase heme oxygenase-1 production in macrophages, ultimately improving their function and killing abilities in sepsis. A suggested scheme whereby human umbilical cord–derived mesenchymal stromal/stem cells enhance the macrophage antibacterial effect *via* induction of macrophage heme oxygenase-1 expression through the lipoxin A4 and prostaglandin E2 pathways is illustrated (fig. 7). The exact mechanism by which lipoxin A4 and prostaglandin E2 mediate heme oxygenase-1 production has yet to be explored.

These experiments reveal overlapping but not identical roles for lipoxin A4 and prostaglandin E2 on the human macrophages, suggesting that they mediate some macrophage effects (at least in part) *via* pathways other than heme oxygenase-1. Human umbilical cord mesenchymal stromal/stem cell-derived lipoxin A4 enhances macrophage phagocytosis, but not phagosomal reactive oxygen species production. In fact, killing of phagocytosed bacteria is still enhanced by human umbilical cord–derived mesenchymal stromal/stem cells in the presence of lipoxygenase-inhibitor (fig. 6F), despite effective blockade of heme oxygenase-1

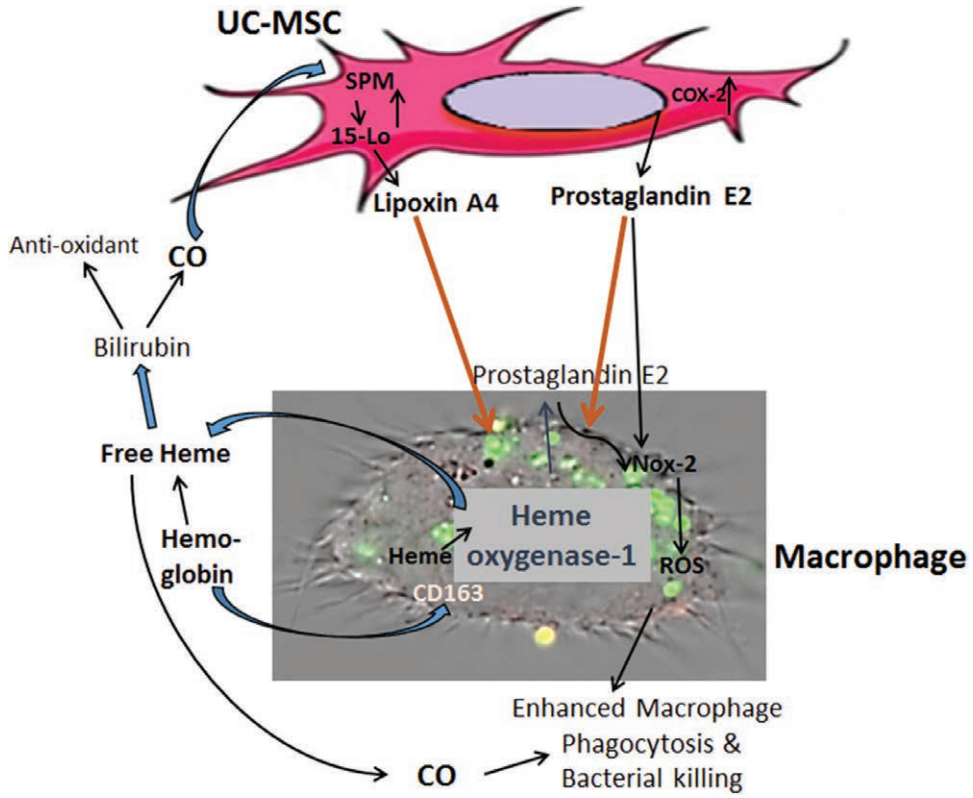


**Fig. 7.** Proposed scheme of molecular mechanisms by which human umbilical cord mesenchymal stromal cells (UC-MSCs) enhance macrophage function. Hemoglobin, accumulated in microbial sepsis upon erythrocyte lysis, gives rise to free circulating heme, with known cytotoxic properties. Hemoglobin is also captured by macrophage receptor CD163, and heme induces heme oxygenase-1 expression in macrophages. Heme oxygenase-1 catalyzes the degradation of heme into biliverdin/bilirubin (with antioxidant properties) and carbon monoxide (CO). CO acts on mesenchymal stromal cells to induce specialized proresolving mediators (*via* 15-Lo stimulation) and increases COX-2 (leading to prostaglandin E2 synthesis), which further induces heme oxygenase-1 expression and activity in macrophages. This positive feedback loop increases CO production, which has direct bacterial killing capabilities. Overall macrophage phagocytic and killing capacity is considerably enhanced, leading to faster and more effective bacterial clearance. Activated macrophages will produce additional prostaglandin E2 and also contribute to the increase of heme oxygenase-1 activity. COX, cyclo-oxygenase; Lo, lipoxygenase, Nox, NADPH oxidase; ROS, reactive oxygen species; SPM, sphingomyelin.

protein induction. In contrast, prostaglandin E2 (derived from human umbilical cord–derived mesenchymal stromal/stem cells and macrophages) increased both phagocytosis and phagosomal reactive oxygen species production, a finding consistent with our previous report that prostaglandin E2 secreted from mesenchymal stromal/stem cells induces phagosomal reactive oxygen species in macrophages.[10,12]

Taken together, our data identify an important mechanism by which human umbilical cord–derived mesenchymal stromal/stem cell therapy decreases the severity of sepsis. Of interest, a role for heme oxygenase-1 in mediating protective effects of mesenchymal stromal/stem cells in liver injury has been recently proposed.[36] Bone marrow mesenchymal stromal/stem cells attenuated acute liver injury caused by endotoxin/D-galactosamine, and increased hepatic heme oxygenase-1 expression; this protection was attenuated by the heme oxygenase antagonist zinc protoporphyrin.[36] In a separate preclinical study, human umbilical cord–derived mesenchymal stromal/stem cells reversed hepatic mitochondrial dysfunction induced by D-galactose, apparently by activation of the nuclear factor-E2–related factor 2/heme oxygenase-1 pathway.[37] Mesenchymal stromal/stem cells transfected with heme oxygenase-1 were more effective than naïve mesenchymal stromal/stem cells in attenuating endotoxin-induced inflammatory and oxidative damage in pulmonary microvascular endothelial cells.[38] In contrast, Hall *et al.* demonstrated that mesenchymal stromal/stem cells enhance neutrophil function in a murine polymicrobial sepsis model *via* mechanisms that were independent of mesenchymal stromal/stem cell heme oxygenase-1.[39] Our findings do not conflict, as they demonstrate that mesenchymal stromal/stem cells modulate heme oxygenase-1 in the host animal cells, *via* mechanisms that do not involve mesenchymal stromal/stem cell heme oxygenase-1. Nevertheless,

these findings reaffirm the fact that the effects of mesenchymal stromal/stem cells are multifactorial and cell-specific.

There are important limitations to the current data. First, our studies were carried out in a rodent model, and caution must be exercised in clinical extrapolation. However, we used a highly clinically relevant model of sepsis—*fecal peritonitis*—and employed thawed cryopreserved xenogeneic material–free umbilical cord–derived human mesenchymal stromal/stem cells that can be rapidly transitioned to clinical testing. These findings suggest that human umbilical cord–derived mesenchymal stromal/stem cells have therapeutic potential for human sepsis, and are a testable therapy. Second, we did not use a control cell group, such as fibroblasts. In our previous experiments, rodent fibroblasts had no benefit[40]; in rodents, human fibroblasts can elicit an independent immune response and confound interpretation,[3] and were therefore not used. Third, zinc protoporphyrin used in our *in vivo* and *in vitro* experiments is not absolutely specific for heme oxygenase-1 inhibition. Although zinc protoporphyrin is a highly selective inhibitor of heme oxygenase, it can also inhibit NO synthases, though at approximately 1,000 times lower affinity. Fourth, human umbilical cord–derived mesenchymal stromal/stem cells did not significantly reduce peritoneal bacterial counts, likely due to the degree of variability in bacterial colony counts. Finally, while heme oxygenase-1 is mainly expressed in macrophages, it is expressed in other cell types (*i.e.*, hepatocytes, Kupffer cells), and we cannot rule out that expression of heme oxygenase-1 by cells other than macrophages could contribute to the beneficial effect of umbilical cord–derived mesenchymal stromal/stem cells in this model.

In conclusion, human umbilical cord–derived mesenchymal stromal/stem cell therapy decreased bacterial burden and improved survival in rat sepsis induced by fecal peritonitis. Umbilical cord–derived mesenchymal stromal/stem cell therapy enhanced peritoneal macrophage phagocytosis and bacterial killing, mediated at least in part *via* upregulation of peritoneal macrophage heme oxygenase-1 concentrations. Lipoxin A4 and prostaglandin E2 play key roles in mediating the umbilical cord–derived mesenchymal stromal/stem cell—induced increase in peritoneal macrophage heme oxygenase-1.

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## Competing Interests

The authors declare no competing interests.

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