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Effects of hemoadsorption on cytokine removal and short-term survival in septic rats

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Abstract

Objective—A broad-spectrum immune-regulating therapy could be beneficial in the treatment of sepsis. Our previous studies have shown that a hemoadsorption device (CytoSorb) removes both pro- and anti-inflammatory cytokines and improves survival in experimental endotoxemia. We sought to determine whether hemoadsorption can also be effective in the treatment of sepsis.

Design—Randomized controlled laboratory experiment.

Setting—University laboratory.

Interventions—Rats were subjected to cecal ligation and puncture (CLP) and 20 hrs later were randomized to receive either hemoadsorption or sham treatment using an arterial-venous circuit. Hemoadsorption was accomplished using a cartridge containing Cytosorb beads. Blood was drawn for cytokine measurements and mean arterial pressure (MAP) was continuously monitored. Cytokines were measured via multiplex bead immunoassays. Survival time was observed for 9 hours after the intervention and assessed by Kaplan–Meier statistics. The overall survival in each group was compared using Fisher’s exact test. Finally, we used a Cox proportional-hazards model to examine the effects of cytokine removal on survival time.

Measurements and Main Results—Baseline plasma cytokine concentrations and MAP were similar between hemoadsorption and sham-treated groups. However, the concentrations of tumor necrosis factor, interleukin (IL)-1 β , IL-6, and IL-10 were significantly lower after hemoadsorption compared to the sham group. Six hours after treatment ended, IL-6 and IL-10 concentrations were still lower in hemoadsorption group. MAP was significantly better in hemoadsorption compared to sham-treated animals ($p < .05$). Finally, mean survival time was significantly longer (720 vs. 381 min, $p < .05$, Mann–Whitney test), and overall survival was significantly better (11/17 vs. 2/16, $p < .01$) with hemoadsorption compared to sham. Combined reduction in both IL-6 and IL-10 was associated with a significantly decreased risk of death (hazard ratio, .11, $p = .005$).

Conclusion—Hemoadsorption reduced circulating cytokines, improved MAP, and resulted in better short-term survival in CLP-induced septic rats.

Keywords

sepsis; aphaeresis; cytokines; inflammation mediators; interleukin-6; interleukin-10; tumor necrosis factor; sorbents

Severe sepsis is a common condition, developing in more than 750,000 people each year in the United States (1) and with similar incidence reported in Europe (2). Despite advances in care, severe sepsis continues to result mortality rates of 25–35% (1–5). Systemic activation of the innate immune response is a prominent feature of sepsis with greater activation in those who fare worst (6). A recent large observational study of community-acquired sepsis secondary to pneumonia noted four aspects of human sepsis that help explain why existing therapies often fail and suggested how potential new therapies might be better designed (6). First, treatments designed to manipulate early cytokine activation are at best impractical, especially for community-acquired sepsis, since the classic cascade occurs before presentation. Second, a “one size fits all” anticytokine approach is likely to be ineffective and potentially dangerous since not all patients mount a cytokine response, and many patients who mount a cytokine response fare well. Third, the highest risk of death was seen in patients who expressed high levels of both pro- and anti-inflammatory cytokines, suggesting that therapy targeted at individual cytokines may not be useful. Finally, typical dosing over two or three days may be too short, since cytokine activation is often persistent.

Blood purification therapy, removing cytokines and other inflammatory mediators from the blood, has the potential to address some or all of these issues (7, 8). By concentration-dependent removal of molecules from the bloodstream, blood purification is a self-tailored therapy and by nonselective (or semiselective) removal, it is broad-spectrum. We have previously reported hemoadsorption using a novel polymer (CytoSorb) that is both highly adsorptive and biocompatible, with pores that allow convection through the material (9). We have shown that in experimental endotoxemia in the rat, CytoSorb removes cytokines, reduces nuclear factor (NF)- κ B DNA binding in liver cells, and improves short-term survival (9). In this study we have tested hemoadsorption therapy begun well after sepsis was initiated by cecal ligation and puncture.

MATERIALS AND METHODS

Surgical Preparation

Following approval by the Animal Care and Use Committee of the University of Pittsburgh, we anesthetized 33 adult (24–28 wks old, weight 400–600g), male, Sprague–Dawley rats with pentobarbital sodium (40 mg/kg intraperitoneally). Cecal ligation and puncture (CLP) was then performed with a predetermined 33% ligated length of cecum and a 20-gauge needle, three-puncture inferior to the ileocecal valve technique. This protocol is associated with a control group mortality of 70–90% at 48 hrs in younger animals (10) and >90% mortality in our experience with older animals. The abdomen was closed and 50 mL/kg lactated Ringers given subcutaneously as fluid resuscitation. Topical anesthetic was applied to the surgical wound. Rats were then returned back to their cages and allowed food and water *ad libitum*. Twenty hours after CLP, the animals were reanesthetized and intubated with a beveled 16-gauge angiocatheter and ventilated with room air using a Harvard rodent ventilator (Holliston, MA) at a tidal volume of 6 mL/kg and a frequency sufficient to maintain an arterial P_{CO_2} between 35–45 mm Hg. The femoral artery and the internal jugular vein were isolated by dissection and cannulated with 1.27 mm polyethylene-90 tubing for use in the extracorporeal circuit.

Experimental Protocol

After the extracorporeal circulation was established, animals were randomized to receive either hemoadsorption ($n = 17$) or sham treatment ($n = 16$) for 3 hrs. The extracorporeal circulation was started from femoral artery and returned back to internal jugular vein. For hemoadsorption a 10 g CytoSorb cartridge (MedaSorb Technologies, Princeton, NJ) with a dead space 3–4 ml, was placed in the circuit while in the sham group the circuit was absent

the cartridge. Blood flow was maintained at approximately 1.0 mL/min and regulated by a stopcock at the precartridge side. All the tubing in the extracorporeal circulation was flushed with heparinized saline (2,000 units/L). After 3 hrs intervention, the treatment was stopped and the rats were observed for another 9 hrs under general anesthesia at which point all surviving animals were killed.

Measurements and Calculations

Mean blood pressure (MAP) was measured every 30 mins during the intervention period and continuously thereafter. Blood (0.8 mL) was drawn from the arterial line at time 0 (20 hr after CLP), 1, 3, and 9 hrs (6 hrs after completing treatment). Whole blood was used for blood gas analysis and the isolated plasma was kept at -80° until thawed for cytokine assays. The cytokine assays included tumor necrosis factor (TNF), interleukin (IL)-1 β , IL-6, and IL-10. To exclude the effects of dilution on the cytokine concentrations or fluid loading on MAP, fluid administration with .9% saline was strictly controlled: 4 ml was given as the animals were placed on the extracorporeal circulation and .8 mL was given as a bolus at time points 1, 3 and 9 hrs. Survival time was recorded in minutes starting from the start of therapy or sham. Cytokines were measured with a multiplex bead immunoassay system (Luminex, BioSource International, Camarillo, CA). Standard curves generated with this assay system extended over several orders of magnitudes of concentrations, while the sensitivity and quantitation of the assays were comparable to the enzyme-linked-immunosorbent assays (ELISAs) (11). For analysis purposes, assay results below the limits of detection (in pg/mL: TNF: 8.7, IL-6: 3.9, IL-10: 16.4, IL-1 β : 14.6) were treated as half of the limit value.

Statistical Analysis

All data (cytokines and MAP) are expressed as mean $\pm$ standard error ($_{SEM}$) and a $p < .05$ was considered to be significant. Normal distribution of the data were confirmed by visual inspection of result histograms and all analyses were repeated after the data were natural log (ln) transformed. Our primary analysis was based on the plasma cytokines concentrations and MAP. Mean differences between the two groups and among different time points were analyzed by analysis of variance for repeated measures (SPSS11.1, Chicago, IL). When a significant F ratio was found, the means at different points were compared to the baseline with posthoc tests. The means between two groups were compared with an unpaired student's t -test. The relationship between changes in MAP and cytokines from baseline until the end of treatment/sham, as well as the relationship between changes in cytokines and survival time were analyzed using Spearman correlation coefficient. The survival analysis between the two groups was based on the time to death and was assessed by Kaplan–Meier statistics, and overall survival in each group was compared using Fisher's exact test. The survival time was compared using the Mann-Whitney test. To explore how cytokine removal was related to survival time we used a Cox proportionalhazards model. We defined the increase or decrease of each cytokine by comparing posttreatment levels to baseline. For the model we included IL-6 and IL-10 patterns as covariates based on a recent large observational study of sepsis (6).

RESULTS

In total, 36 animals were subjected to CLP; three died before 20 hrs and were excluded before randomization. The remaining 33 animals were randomized to hemoadsorption ($n = 17$) and sham ($n = 16$).

Effects of Hemoadsorption on Plasma Cytokines

Differences in circulating plasma cytokine concentrations for animals treated with hemoadsorption or sham are shown in Figure 1. The percentages of assays that fell below the limit of detection for each assay were 40.1% for TNF, 38.5% for IL-1 β , 2.2% for IL-6, and 10.1% for IL-10. Baseline values (20 hrs after CLP) for all cytokines were not different between the two groups. However, the concentrations of TNF, IL-1 β , IL-6, and IL-10 were all significantly lower in the hemoadsorption group after treatment compared to the sham group (Fig. 1). Moreover, after only 1 hr of treatment, the concentrations in IL-6 and IL-10 were significantly lower in hemoadsorption group and even at 9 hrs (6 hrs after treatment ended), IL-6 and IL-10 concentrations were still lower in the hemoadsorption group compared to sham. Importantly, cytokine concentrations increased from 3 hrs to 9 hrs in both hemoadsorption and sham groups.

Effects of Hemoadsorption on Blood Pressure

MAP at each time point is depicted in Figure 2. There was no significant difference in baseline MAP (20 hr after CLP) between the two groups. The MAP was gradually decreased in the sham group, but the MAP in the hemoadsorption group was relatively stable. At 3 hrs and even at 9 hrs, the hemoadsorption group had a significantly higher MAP (71.40 vs. 52.00 mmHg, and 65.25 vs. 36.50 mmHg) compared to the sham group.

Effects of Hemoadsorption on Survival

The survival curves are shown in Figure 3. The mean survival time in the hemoadsorption group was longer compared to the sham group (720 vs. 381 mins, $p = 0.02$). The overall survival (defined at 12 hrs after randomization, 32 hrs after CLP) was also significantly greater in the hemoadsorption group (11/17 vs. 2/16, hazard ratio: 2.87 [95% confidence interval 1.19 to 7.26], $p < .01$).

Association Between Cytokine Concentrations, MAP and Survival Time

Changes in cytokines before and after treatment/sham were negatively correlated with MAP and survival time. Decreases in IL-6 ($r = -.74$, $p < .001$) and IL-10 ($r = -.69$, $p < .01$) were inversely correlated with MAP (Fig. 4). Decreases in TNF and IL-1 β were not significantly correlated with MAP. Similarly, decreases in IL-6 ($r = -.77$, $p < .001$) and IL-10 ($r = -.61$, $p < .01$) were inversely correlated with survival time (Fig. 5). The relationship between decreases in TNF ($r = -.24$, $p = .36$) and IL-1 β ($r = -.48$, $p = .09$) were much weaker. Table 1 shows the results of the Cox proportional hazards model. Combined decreases in both IL-6 and IL-10 were associated with significantly improved survival (hazard ratio, .11, $p < .01$).

DISCUSSION

Our results show that hemoadsorption with the CytoSorb polymer can remove both pro- and antiinflammatory mediators (TNF, IL-1 β , IL-6, IL-10), improve MAP and improve short-term survival in CLP-induced sepsis in the rat. These results are consistent with those of our previous study in acute lethal endotoxemia (9), but extend them in several important ways. First, unlike bolus injection of endotoxin, CLP results in true infection and sepsis (12) and much more closely resembles human sepsis. Second, we began treatment more than 20 hrs following CLP, a time when the cytokine response is fully activated and clinical signs are beginning to become apparent (13); a time that more likely reflects timing of presentation in a clinical situation. Finally, we were also able to characterize the cytokine response several hours after therapy. These data are essential to understanding the kinetics of immune regulation achieved with this therapy.

As seen in Figure 1, cytokines, with the exception of TNF, rebounded after hemoadsorption was terminated. However, by 9 hrs neither IL-6 nor IL-10 levels had returned to baseline and were both significantly lower than controls. A similar pattern was seen with IL-1 β but did not achieve statistical significance. TNF was greatly suppressed by hemoadsorption and did not significantly rebound for the duration of the experiment. Importantly, despite a very short but intense cytokine removal, and despite clear evidence of rebound in other plasma cytokine concentrations, survival was significantly greater with hemoadsorption compared to sham therapy. These results have important implications for the development of sepsis treatments in humans. First, a relative short course of hemoadsorption may be effective as a means to “reset” the host response. Ongoing cytokine production continued or resumed after hemoadsorption was discontinued suggesting that the immune response to infection was not halted by treatment. On the other hand, the rate of rise in cytokines, particularly IL-6 and IL-10, was blunted after hemoadsorption. This may have occurred as a result of down-regulation in cytokine production. Our previous studies in endotoxemia showed that NF- κ B DNA binding was significantly reduced as a result of hemoadsorption (9). As the major transcription factor involved in cytokine gene induction, suppression of NF- κ B would be expected to reduce cytokine production.

Second, not all cytokines tested were affected in the same way. For example, TNF was significantly suppressed by hemoadsorption and failed to recover following its termination. This difference may be due to the relatively short time course of TNF (14, 15) compared to other mediators. However it is notable that TNF remained elevated in the sham-treated animals. Thus, hemoadsorption may have altered the TNF activation pathways differently compared to those of other cytokines. This may not be desirable since some TNF activity is presumably necessary. Additional refinements in the hemoadsorption system may be required to reduce the effect on TNF. Third, results shown in Figures 4 and 5 demonstrate that reduced cytokine levels, especially IL-6 and IL-10, correlated with the improved blood pressure and survival. Moreover, the Cox proportional hazard model showed that the combined decreases in both IL-6 and IL-10 were associated with significantly better survival. Although short-term survival was significantly improved with hemoadsorption, the effect may have been largely due to improvements in MAP compared to sham-treated animals. Improved hemodynamics has been demonstrated in a number of studies of high-volume hemofiltration both in animals (16) and humans (17); yet increased hospital survival has been difficult to demonstrate.

Our results are clearly different from those achieved with conventional hemofiltration, which does not appear to be beneficial in patients without renal failure (18). Indeed our previous work in hemofiltration using AN69 membranes suggests that the main immune modulating effect comes from adsorption of cytokines to the membrane (19). In this regard hollow-fiber dialyzers are extremely limited in terms of available surface area (2.5–3.5 m²) and are easily saturated. By comparison, CytoSorb has a surface area of 850 m² per gram, and the 10 g cartridges we have tested show no evidence of saturation (20).

Our study has several limitations. First, our intervention only included hemoadsorption (or sham) with limited fluid replacement (~10–12 ml/kg). Animals did not receive supplemental oxygen, fluid resuscitation, vasopressors or inotropes, or other therapies. Furthermore, antibiotics or source control surgery were not given. We chose not to treat animals with antibiotics or source control surgery for the important reason that these therapies also alter the inflammatory response and may well have obscured any “signal.” Second, we observed survival time for only a short time, and information concerning long-term survival is required. We intend to examine the effects of hemoadsorption on long-term survival in antibiotic-treated animals in the near future.

In summary, hemoadsorption with this novel, large surface area polymer significantly reduces both pro- and antiinflammatory cytokines and improves short-term survival in rats with CLP-induced sepsis. Building on prior work (9, 20), these results move incrementally toward a feasible blood purification strategy for treatment of sepsis and related disorders of acute inflammation.

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REFERENCES

1. Angus DC, Linde-Zwirble WT, Lidicker J, et al. Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. *Crit Care Med.* 2001; 29:1303–1310. [PubMed: 11445675]
2. Vincent JL, Sakr Y, Sprung CL, et al. Sepsis Occurrence in Acutely Ill Patients Investigators. Sepsis in European intensive care units: results of the SOAP study. *Crit Care Med.* 2006; 34:344–353. [PubMed: 16424713]
3. Bone RC. The pathogenesis of sepsis. *Ann Intern Med.* 1991; 115:457–467. [PubMed: 1872494]
4. Cunneen J, Cartwright M. The puzzle of sepsis: fitting the pieces of the inflammatory response with treatment. *AACN Clin.* 2004; 15:18–44.
5. van der Poll T, Van Deventer SJ. Cytokines and anticytokines in the pathogenesis of sepsis. *Infect Dis Clin North Am.* 1999; 13:413–426. [PubMed: 10340175]
6. Kellum JA, Kong L, Fink MP, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: Results of the Gen- IMS study. *Arch Intern Med.* 2007; 167:1655–1663. [PubMed: 17698689]
7. Kellum JA. Hemoadsorption therapy for sepsis syndromes. *Crit Care Med.* 2003; 31:323–324. [PubMed: 12545045]
8. Kellum JA. Immunomodulation in sepsis: the role of hemofiltration. *Minerva Anesthesiol.* 1999; 65:410–418.
9. Kellum JA, Song M, Venkataraman R. Hemoadsorption removes tumor necrosis factor, interleukin-6, and interleukin-10, reduces nuclear factor-kappaB DNA binding, and improves short-term survival in lethal endotoxemia. *Crit Care Med.* 2004; 32:801–905. [PubMed: 15090965]
10. Singleton KD, Wischmeyer PE. Distance of cecum ligated influences mortality, tumor necrosis factor- α and interleukin-6 expression following cecal ligation and puncture in the rat. *Eur Surg Res.* 2003; 35:486–491. [PubMed: 14593232]
11. Szodoray P, Alex P, Brun JG, et al. Circulating cytokines in primary Sjogren's syndrome determined by a multiplex cytokine array system. *Scand J Immunol.* 2004; 59:592–599. [PubMed: 15182255]
12. Rittirsch D, Hoesel LM, Ward PA. The disconnect between animal models of sepsis and human sepsis. *J Leukoc Biol.* 2007; 81:137–143. [PubMed: 17020929]
13. Villa P, Sartor G, Angelini M, et al. Pattern of cytokines and pharmacomodulation in sepsis induced by cecal ligation and puncture compared with that induced by endotoxin. *Clin Diagn Lab Immunol.* 1995; 2:549–553. [PubMed: 8548533]
14. Neilson D, Kavanagh JP, Rao PN. Kinetics of circulating TNF- α and TNF soluble receptors following surgery in a clinical model of sepsis. *Cytokine.* 1996; 8:938–943. [PubMed: 9050753]
15. Walley KR, Lukacs NW, Standiford TJ, et al. Balance of inflammatory cytokines related to severity and mortality of murine sepsis. *Infect Immun.* 1996; 64:4733–4738. [PubMed: 8890233]
16. Bellomo R, Kellum JA, Gandhi CR, et al. The effect of intensive plasma water exchange by hemofiltration on hemodynamics and soluble mediator in canine endotoxemia. *Am J Respir Crit Care Med.* 2000; 161:1429–1436. [PubMed: 10806135]
17. Uchino S, Bellomo R, Goldsmith D, et al. Super high flux hemofiltration: A new technique for cytokine removal. *Intensive Care Med.* 2002; 28:651–655. [PubMed: 12029417]

18. Cole L, Bellomo R, Hart G, et al. A phase II randomized, controlled trial of continuous hemofiltration in sepsis. *Crit Care Med*. 2002; 30:100–106. [PubMed: 11902250]
19. Kellum JA, Dishart MK. Effect of hemofiltration filter adsorption on circulating IL-6 levels in septic rats. *Crit Care*. 2002; 6:429–433. [PubMed: 12398783]
20. Song M, Winchester J, Albright RL, et al. Cytokine removal with a novel adsorbent polymer. *Blood Purif*. 2004; 22:428–434. [PubMed: 15316198]

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Figure 1.

Effects of hemoabsorption on cytokine removal (mean $\pm$ SEM, pg/mL). Hemoabsorption is shown in solid bars while sham results are shown in open bars. The cytokine data were log-transformed (ln). Each panel shows a separate cytokine: A, tumor necrosis factor (*TNF*); B, interleukin (*IL*)-1 β ; C, IL-6; D, IL-10. All four cytokines showed significant differences over time ($p < .05$) and between groups ($p < .05$). For pair-wise comparisons:

*hemoabsorption vs. sham ($p < .05$) and †compared with baseline ($p < .05$).

Figure 2.

Effects of hemoadsorption on mean arterial pressure (*MAP*) (mean $\pm$ SEM, mmHg). Hemoadsorption is shown in dashed line while sham results are shown in solid line. MAP was significantly different over time and between groups ($p < .01$). For pair-wise comparisons: *hemoadsorption vs. sham ($p < .05$) and †compared with baseline ($p < .05$).

Figure 3.

Effects of hemoadsorption on short-term survival. Survival time (*hr*) was observed from the start of intervention up to 12 hrs. Hemoadsorption results are shown as a dashed line while sham results are shown as a solid line. Survival time was significantly greater in the hemoadsorption group ($p = .02$) and overall survival (until 12 hrs) was significantly improved ($p < .01$).

Figure 4.

Relationship between changes in cytokines (pg/ml) and change in mean arterial pressure (MAP) (mm Hg) before and after hemoadsorption or sham. Interleukin (IL)-6 ($p = -.74$, $p < .001$) and IL-10 ($p = -.69$, $p < .01$) showed inverse correlations with MAP; tumor necrosis factor (TNF) ($p = -.14$, $p = .55$), IL-1 β ($p = -.11$, $p = .69$), were not.

Figure 5.

Relationship between changes in cytokines (pg/mL) before and after hemoadsorption or sham and survival time. Interleukin (IL)-6 ($r = -.77$, $p < .001$) and IL-10 ($r = -.61$, $p < .01$) showed inverse correlations with survival. Neither tumor necrosis factor (TNF) ($r = -.24$, $p = .36$) nor IL-1 β ($r = -.48$, $p = .09$) were significant.

Table 1

Cox proportional hazards model for the association between IL-6 and IL-10 changes and survival

IL-6/IL-10 Changes (frequency)	Hazard Ratio (95% Confidence Interval)	<i>p</i> Value
Increase/increase (40%)	5.21 (1.15–23.76)	.03
Increase/decrease (16%)	1.06 (.10–11.13)	.96
Decrease/increase (8%)	Referent	NA
Decrease/decrease (36%)	.11 (.02–.51)	.005

IL, interleukin.