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Diaspirin cross-linked Hb and norepinephrine prevent the sepsis-induced increase in critical O_2 delivery

ANDREAS W. SIELENKÄMPER, PEI YU, OTTO EICHELBRÖNNER, TAMMY MACDONALD, CLAUDIO M. MARTIN, IAN H. CHIN-YEE, AND WILLIAM J. SIBBALD
The A. C. Burton Vascular Biology Laboratory, Victoria Hospital Research Institute, and The University of Western Ontario, London, Ontario, Canada N6A 4G5

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Sielenkämper, Andreas W., Pei Yu, Otto Eichelbrönner, Tammy MacDonald, Claudio M. Martin, Ian H. Chin-Yee, and William J. Sibbald. Diaspirin cross-linked Hb and norepinephrine prevent the sepsis-induced increase in critical O_2 delivery. *Am J Physiol Heart Circ Physiol* 279: H1922–H1930, 2000.—We hypothesized that support of arterial perfusion pressure with diaspirin cross-linked Hb (DCLHb) would prevent the sepsis-induced attenuation in the systemic O_2 delivery-O_2 uptake relationship. Awake septic rats were treated with a chronic infusion of DCLHb or a reference treatment [norepinephrine (NE)] to increase mean arterial pressure by 10–20% over 18 h. Septic and sham control groups received normal saline. Isovolemic hemodilution to create anemic hypoxia was then performed in a metabolic box during continuous measurement of systemic O_2 uptake. O_2 delivery was calculated from hemodynamic variables, and the critical point of O_2 delivery ($\dot{D}O_{2\,crit}$) was determined using piecewise regression analysis of the O_2 delivery-O_2 uptake relationship. Sepsis increased $\dot{D}O_{2\,crit}$ from 4.99 ± 0.17 to 6.69 ± 0.42 ml·min^{-1}·100 g^{-1} ($P < 0.01$), while O_2 extraction capacity was decreased ($P < 0.05$). DCLHb and NE infusion prevented the sepsis-induced increase in $\dot{D}O_{2\,crit}$ [4.56 ± 0.42 ml·min^{-1}·100 g^{-1} ($P < 0.01$) and 5.04 ± 0.56 ml·min^{-1}·100 g^{-1} ($P < 0.05$), respectively]. This was explained by a 59% increase in O_2 extraction capacity in the DCLHb group compared with septic controls ($P < 0.05$), whereas NE treatment decreased systemic O_2 uptake in anemic hypoxia (1.51 ± 0.08 vs. 1.87 ± 0.1 ml·min^{-1}·100 g^{-1} in septic controls, $P < 0.05$). We conclude that DCLHb ameliorated O_2 extraction capacity in the septic microcirculation, whereas NE decreased the metabolic demands of the tissues.

blood substitute; anemic hypoxia; cardiovascular; rat

SEPSIS IS A SYNDROME that jeopardizes the integrity of many physiological pathways. Besides an activation of inflammatory cascades and a dysfunction of the systemic, regional, and microregional circulations, diffusive and convective O_2 transport are perturbed. Diffusive O_2 transport may be compromised in the lung, for example, because of acute respiratory distress syndrome (6, 18) or in the microcirculation, where tissue edema may increase diffusion distances and therefore compromise uptake of the systemically provided O_2 (16). Convective O_2 delivery ($\dot{D}O_2$) may be impaired when a depression in myocardial contractility interferes with the ability to appropriately increase cardiac output (CO) (10, 25), when vasoplegia of resistance vessels maldistributes blood flow between organs (21), or when microvascular dysfunction causes inadequate capillary perfusion (9, 19). In addition, it has been postulated that mitochondrial dysfunction in sepsis restricts the optimal use of available O_2 (34, 38).

As a consequence of these abnormalities, the normal relationship between systemic $\dot{D}O_2$ and O_2 uptake ($\dot{V}O_2$) is altered in sepsis, and the maximal O_2 extraction capacity of the tissues is thereby decreased (23, 27). Under experimental conditions, this phenomenon becomes manifest as an elevation of the critical $\dot{D}O_2$ ($\dot{D}O_{2\,crit}$), the point where systemic $\dot{V}O_2$ becomes dependent on O_2 supply (23). In a recent study to determine the efficacy of an O_2-carrying, cell-free Hb solution, diaspirin cross-linked Hb (DCLHb) (30), we found that infusing DCLHb improved O_2 extraction capacity in septic rats (30). One possible explanation for this effect was that DCLHb recruited capillaries previously not perfused with red blood cells (RBCs), since a subsequent study demonstrated an increase in the density of RBC-perfused capillaries in the gut mucosa of septic rats after DCLHb infusion (31).

In addition to increasing microvascular perfusion, there are other explanations for the activity of Hb solutions to increase O_2 extraction capacity in sepsis. Because Hb solutions are effective O_2 carriers, but much smaller than RBCs, Hb in solution may access capillaries unavailable to RBCs, because their lumens are narrowed by edema (29). Hb molecules may also facilitate tissue oxygenation, since they are uniformly distributed within the plasma phase and thus reduce diffusion resistance for O_2 (24).

The present study was designed to determine the effect of DCLHb infusion on the systemic $\dot{V}O_2$-$\dot{D}O_2$ relationship and to identify why DCLHb infusion increases the microvascular O_2 extraction in sepsis. We chose to administer DCLHb chronically, in doses that

Address for reprint requests and other correspondence: W. J. Sibbald, The London Health Sciences Centre, Victoria Campus, 375 South St., London, ON, Canada N6A 4G5 (E-mail: wsibbald@julian.uwo.ca).

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provided a moderate increase in mean arterial blood pressure (MAP). Because Hb solutions will increase vascular resistance because of their effect to bind nitric oxide (13, 14, 28), we added a control group (septic rats) in which norepinephrine (NE) was infused to also increase vascular resistance. By this approach, we hoped to isolate any effects of DCLHb infusion on the microcirculation per se, that is, excluding the influence of DCLHb on vascular resistance. The interventions were infused over an 18-h period to allow sufficient time for complete expression of potential effects on the systemic $\dot{V}O_2$-$\dot{D}O_2$ relationship, as well as to enhance the potential generalizability of findings to the clinical situation. When the effects of both treatments on $\dot{D}O_{2\,crit}$ were determined after completion of the treatment phase by use of acute progressive isovolemic hemodilution and on-line measurements of $\dot{V}O_2$, we found that DCLHb and NE were equally effective at preventing the sepsis-induced increase of the $\dot{D}O_{2\,crit}$.

METHODS

The protocol of this study was approved by the Council on Animal Care of the University of Western Ontario (London, ON, Canada).

Animal model. Forty-seven male Sprague-Dawley rats, weighing 320–380 g, were used after a 1-wk acclimatization period in our laboratory. Anesthesia was induced and maintained by halothane inhalation. Catheters were advanced into the left femoral vein, the superior vena cava, and the left carotid artery. A thermodilution CO probe (IT-21 thermocouple, Physiotemp Instruments, Clifton, NJ) was then positioned in the aortic arch via the carotid artery. After cannulation, rats were randomized to undergo sham laparotomy or laparotomy and cecal ligation and perforation (CLP), according to a previously standardized technique (9), to create sepsis. Fluid resuscitation with 0.9% saline (2 ml·100 $g^{-1}\cdot h^{-1}$ iv) was started postoperatively. The carotid line was continuously flushed with heparin solution (45 IU/h) to maintain patency, and fentanyl (2 $\mu g\cdot 100\ g^{-1}\cdot h^{-1}$ iv) was provided to ensure adequate analgesia.

Experimental protocol. Figure 1 shows the experimental design of the study. Twenty-four hours after surgery, MAP and CO were determined, and blood samples were drawn to assess biochemistry, including blood gases. CLP-septic animals (n = 39) were then randomized to receive normal saline (NS) alone (n = 15) or a continuous infusion of DCLHb (n = 14) or NE (n = 10). With both DCLHb and NE, the goal was to administer a dose that increased MAP by 10–20% over the next 18 h. Sham rats (n = 8) received NS. Pilot experiments confirmed that this model of chronic infusion was technically possible and identified the general dose ranges required to achieve target pressures for DCLHb and NE. After 18 h of treatment, measurements were repeated, the animals were placed in a metabolic cage, and the arterial and venous lines were connected to withdrawal and perfusion pumps, respectively. Treatments were continued. After a 30-min acclimatization period, MAP, CO, arterial O_2 content, and systemic $\dot{V}O_2$ were measured. Arterial blood (0.7 ml) was withdrawn to determine Hb concentration, arterial O_2 saturation, and lactate concentration. Isovolemic hemodilution was then carried out (6 ml/h) to determine the systemic $\dot{D}O_2$-$\dot{V}O_2$ relationship. Systemic $\dot{V}O_2$ was measured semicontinuously (see below) while measurements of MAP and CO were repeated after every 2 ml of isovolemic hemodilution. Blood samples for arterial O_2 content, Hb concentration, and lactate were simultaneously obtained. At all times, shed blood was replaced by identical volumes of warmed rat plasma obtained from donor rats.

Animals were excluded if technical failure (e.g., damage or blocking of arterial and venous catheters) occurred before the completion of the treatment phase. After completion of measurements, rats were euthanized with an overdose of pentobarbital sodium (65 mg), and postmortem examination was carried out.

Treatments and isovolemic hemodilution. Twenty-four hours after sepsis was induced, septic animals were randomized to receive a continuous infusion of DCLHb, NE, or placebo (NS). After a bolus infusion of 100 mg of DCLHb solution over 3 min to obtain effective plasma concentrations, DCLHb was infused at a rate of 70–300 $mg\cdot kg^{-1}\cdot h^{-1}$. NE was adjusted to an effective dose within a few minutes and was then infused at a rate of 0.25–1.25 $\mu g\cdot kg^{-1}\cdot min^{-1}$. Doses in the treatment groups were adjusted at 30 min and at 1, 2, 3, 6, and 12 h to maintain the increase in MAP at targeted levels. The femoral line was used for drug infusion, and adjustments for a constant infusion volume were made

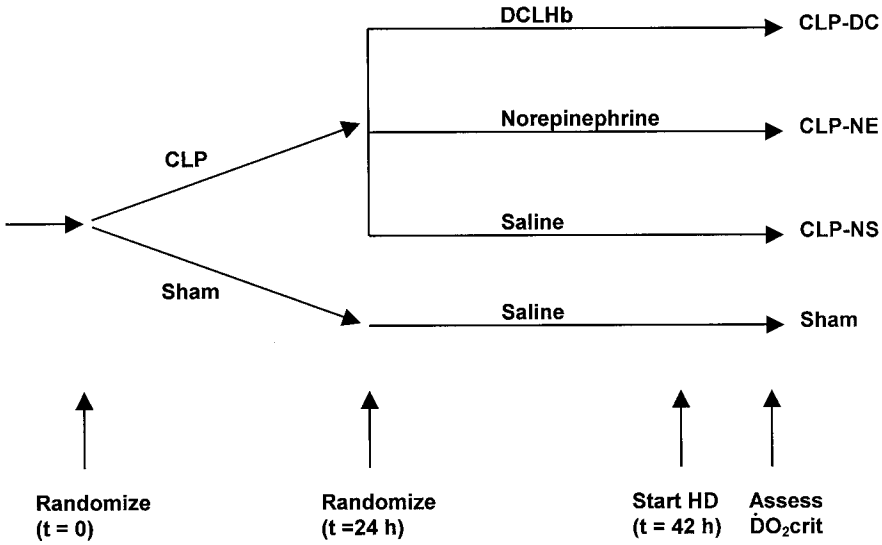

Fig. 1. Experimental design. $\dot{D}O_{2\,crit}$, critical O_2 delivery; t, time; HD, hemodilution; CLP-NS, animals subjected to cecal ligation and perforation (CLP) and treated with normal saline (NS; i.e., septic controls); CLP-DC, septic rats treated with diaspirin cross-linked Hb (DCLHb) infusion; CLP-NE, septic rats treated with norepinephrine (NE) infusion.

via the jugular line. CLP controls and sham rats received NS via both lines (CLP-NS group and sham group, respectively). Total infusion volumes were kept at a rate of 1.5 ml·100 g^{-1}·h^{-1} in all groups. DCLHb was prepared by Baxter Healthcare (Round Lake, IL) as described previously (3, 22) and was formulated at a concentration of 100 g/l in a lactated electrolyte solution. NE was diluted in normal saline and administered at a concentration of 10 μg/ml.

For isovolemic hemodilution, rat plasma obtained from donor rats by use of a previously standardized protocol (30) and warmed to body temperature was filtered through a 40-μm transfusion filter. With the use of syringe pumps (Razel Scientific Instruments, Stamford, CT) set at a rate of 6 ml/h, blood was withdrawn via the arterial line and plasma was infused via the jugular line. In this way, $\dot{D}O_2$ was lowered in a stepwise manner to decrease it beyond the point of $\dot{D}O_{2\,crit}$.

Measurements and calculations. Systemic $\dot{V}O_2$ was measured semicontinuously by means of an Oxymax system (Columbus Instruments, Columbus, OH). A constant flow of room air at a rate of 3.5 l/min was sampled by a paramagnetic O_2 sensor for analysis of O_2 content and then by an infrared CO_2 analyzer. Reference measurements were made by sampling room air every five samples. Systemic $\dot{V}O_2$ was measured from the reduction of air O_2 content within the closed system and displayed on-line. Five consecutive values obtained over a 60-s measurement period were averaged to determine $\dot{V}O_2$ at an individual time point.

MAP was measured with Uniflow disposable transducers (Baxter, Toronto, ON, Canada) and a monitor (model 78353B, Hewlett-Packard, Mississauga, ON, Canada). CO was measured by the thermodilution technique with use of 0.3 ml of NS at room temperature injected via the jugular catheter. The thermocouple output was analyzed with a Cardiotherm 500 AC-R CO computer (Columbus Instruments). Hb and arterial O_2 saturation were assessed using a CO-oximeter (OSM2b hemoximeter, Radiometer, Copenhagen, Denmark), and lactate concentration was determined by means of a quantitative, enzymatic method (Paramax Analytical System, Baxter, Mississauga, ON, Canada). Arterial O_2 content was measured directly using a Lex-O_2-Con O_2 analyzer (Lexington). Systemic $\dot{D}O_2$ was obtained by multiplying arterial O_2 content by CO. Systemic vascular resistance (SVR) and systemic O_2 extraction ratio were calculated using standard formulas. $\dot{D}O_{2\,crit}$ was determined using piecewise regression analysis of the $\dot{V}O_2$-$\dot{D}O_2$ relationship as described by Samsel and Schumacker (26). The whole body $\dot{V}O_2$-$\dot{D}O_2$ relationship is biphasic, with the point where systemic $\dot{V}O_2$ becomes dependent on O_2 supply (the $\dot{D}O_{2\,crit}$) defined at the point of transition from plateau to downslope (27). All possible pairs of regression lines were constructed over all points where $\dot{D}O_2$ and $\dot{V}O_2$ data had been obtained. The pairs of lines were then compared to find the pair with the lowest residual sum of squares of the perpendicular distances from the points to the lines. The $\dot{D}O_{2\,crit}$ was then determined by calculating the intersection point of this pair of lines.

Statistics. For statistical analysis, SigmaStat 2.03 software (Jandel, San Rafael, CA) was used. Mortality was analyzed using Fisher's exact test. ANOVA with post hoc tests and correction for multiple comparisons (Student-Newman-Keuls method) was performed to determine the effects of the treatments in the CLP-septic groups at 18 h and after hemodilution. To determine the effects of sepsis between the sham group and the CLP-septic control group, Student's *t*-test was used. The effects of sepsis and the effects of the treatments on blood pressure during hemodilution were analyzed using two-way ANOVA for repeated measurements with appropriate post hoc comparisons (Student-Newman-Keuls method). For all statistical tests, significance was assumed at $P < 0.05$. Values are means ± SE.

RESULTS

Animal model. Twenty-four hours after the surgical procedures, sham rats had recovered. All animals treated with CLP demonstrated reduced activity, piloerection, and exudation around the eyes and nose. The effects of CLP-sepsis on hemodynamic and biochemical markers are shown in Table 1. Septic rats presented with modest hypotension, an elevated CO, and a decreased SVR. CLP-sepsis was also characterized by leukopenia and thrombocytopenia, whereas the arterial lactate increased only slightly compared with the sham group. On postmortem examination, inspection of the abdominal contents revealed spillage of bowel contents and peritonitis in CLP-septic rats, whereas the aspect of the abdomen was normal in all sham rats.

Effects of DCLHb and NE infusion after 18 h of treatment. Our intention was to increase MAP with DCLHb or NE infusion in CLP-septic rats by 10–20% over 18 h. With either of the treatments, MAP, when averaged across all measurements of the treatment period, was kept in the desired range (Fig. 2, horizontal lines). Average blood pressure was 109 ± 2 mmHg for the sham group, 96 ± 5 mmHg for the CLP-NS rats, and 114 ± 3 and 109 ± 3 mmHg for the DCLHb- and NE-treated groups, respectively. Especially among the animals in the three septic groups, considerable variability in blood pressure was observed independent from treatment (Fig. 2, vertical lines).

Mortality was determined for the treatment period including the time of isovolemic hemodilution before $\dot{D}O_{2\,crit}$ (e.g., O_2 supply dependency) was reached. In the sham group, no mortality was observed. Mortality in the septic groups was 7 of 15 in the CLP-NS group (46.7%), 7 of 14 in the CLP-DC group (50%), and 2 of 10 in the CLP-NE group (20%). Differences in mortality among the treatment groups, and comparing the treatment group with the CLP-NS group, were not significant.

Table 2 summarizes the effects of DCLHb and NE infusion on CO, SVR, O_2 transport, and biochemical

Table 1. *Effects of CLP-sepsis on hemodynamic and biochemical markers at 24 h*

	Sham (n = 8)	CLP (n = 39)	P
MAP, mmHg	115 ± 4.2	99 ± 2.3	<0.01
CO, ml·min^{-1}·100 g^{-1}	53.3 ± 3.9	65.4 ± 1.9	<0.01
SVR, mmHg·ml^{-1}·min·100 g^{-1}	0.16 ± 0.02	0.10 ± 0.01	<0.001
WBC, 10^9/l	9.8 ± 1.1	5.3 ± 0.3	<0.0001
Platelets, 10^9/l	517 ± 131	364 ± 126	<0.05
$\dot{D}O_2$, ml·min^{-1}·100 g^{-1}	9.65 ± 0.7	10.9 ± 0.32	NS
Lactate, mmol/l	0.5 ± 0.03	0.9 ± 0.06	<0.001

Values are means ± SE; n, number of rats. CLP, cecal ligation and perforation; MAP, mean arterial pressure; CO, cardiac output, SVR, systemic vascular resistance; WBC, white blood count; $\dot{D}O_2$, systemic O_2 delivery. P values are from Student's *t*-test; NS, not significant.

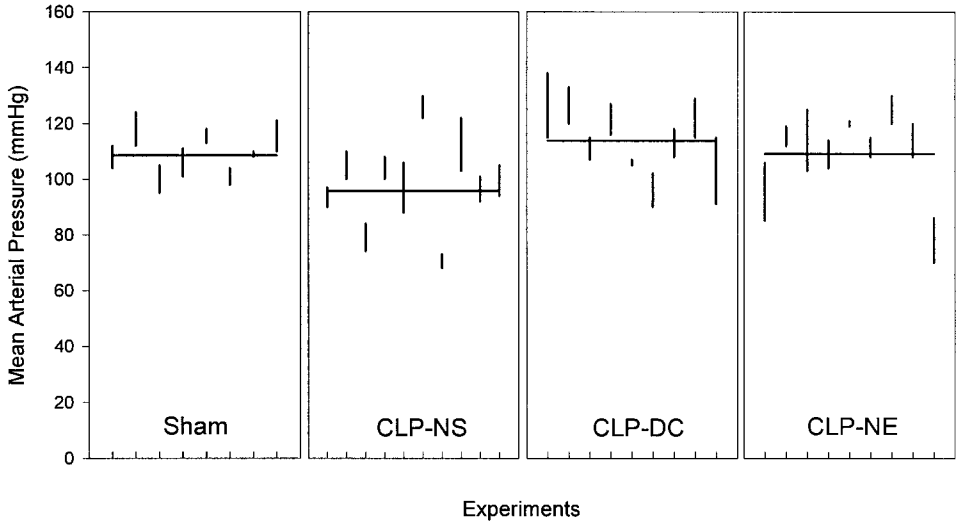

Fig. 2. Goal-directed approach to increase mean arterial pressure (MAP) by 10–20% over 18 h in septic rats. Horizontal lines, MAP across all measured pressures during the 18-h treatment phase; vertical lines, range of pressures for each animal. See Fig. 1 legend for definition of abbreviations.

markers after completion of the 18-h treatment phase. CO and systemic $\dot{D}o_2$ were decreased in the CLP-DC group compared with CLP-NS and CLP-NE groups, and O_2 extraction ratio was higher in the CLP-DC than in the CLP-NE group. SVR was elevated in DCLHb-treated rats, but not in the CLP-NE group. There were no treatment effects on systemic $\dot{V}o_2$, arterial and venous O_2 saturation, Hb concentration, white blood cell count, platelet count, and arterial lactate concentration.

Effects of DCLHb and NE infusion on MAP and O_2 transport during isovolemic hemodilution. Figure 3 shows the changes in MAP during the isovolemic hemodilution procedure in all groups. Compared with baseline, there was a significant decrease in blood pressure during hemodilution in all except the DCLHb group. Compared with the sham group, CLP-septic rats were hypotensive during the hemodilution procedure ($P < 0.05$). Continuing the infusion of DCLHb or NE resulted in higher blood pressure than in untreated septic rats. Toward the end of the experiment, however, blood pressure in the NE-treated rats decreased to the level of the CLP-NS group ($P < 0.05$ vs. DCLHb group).

In the CLP-NS group, $\dot{D}o_{2\,crit}$ was increased compared with the sham group (from 4.99 ± 0.17 to 6.69 ± 0.42 ml·min^{-1}·100 g^{-1}, $P < 0.01$; Fig. 4). At the $\dot{D}o_{2\,crit}$, all the following were also changed compared with the sham rats: *1*) O_2 extraction capacity was depressed (20%, $P < 0.05$); *2*) Hb concentration was greater (67 ± 5 vs. 44 ± 2 g/l, $P < 0.001$); and *3*) systemic $\dot{V}o_2$ was greater (18.5 ± 1 vs. 15.3 ± 0.9 ml·min^{-1}·100 g^{-1}, $P < 0.05$). CO, however, was not different between the sham and CLP septic rats at the $\dot{D}o_{2\,crit}$ (299 ± 24 and 314 ± 22 ml/min, respectively).

In the DCLHb- and NE-infused septic rats, the sepsis-induced increase in $\dot{D}o_{2\,crit}$ was prevented ($P < 0.01$

Table 2. *Effects of 18 h of chronic infusion of diaspirin cross-linked Hb or NE in septic rats*

	CLP-NS (n = 10)	CLP-DC (n = 9)	CLP-NE (n = 9)
CO, ml·min^{-1}·100 g^{-1}	63 ± 3.4	48.1 ± 2.9[b]	60 ± 2.8[c]
SVR, mmHg·ml^{-1}·min·100 g^{-1}	0.1 ± 0.03	0.16 ± 0.05[a]	0.12 ± 0.05
$\dot{D}o_2$, ml·min^{-1}·100 g^{-1}	10.5 ± 0.4	7.7 ± 0.4[d]	9.7 ± 0.4[c]
$\dot{V}o_2$, ml·min^{-1}·100 g^{-1}	20.2 ± 0.9	18.9 ± 0.9	17.8 ± 0.9
O_2E ratio	17.3 ± 0.8	21.4 ± 1.8[e]	15.2 ± 1.7
Sa_{O_2}, %	95.9 ± 2.3	95.3 ± 1.1	91.1 ± 1
Sv_{O_2}, %	66.1 ± 3.5	67 ± 2.5	74.8 ± 1.3
Hb, g/l	118 ± 7	123 ± 5	123 ± 3
WBC, 10^9/l	6.6 ± 0.9	6.3 ± 1	8.1 ± 0.9
Platelets, 10^9/l	218 ± 27	194 ± 38	199 ± 35
Lactate, mmol/l	1 ± 0.1	1.8 ± 0.4	1.3 ± 0.2

Values are means ± SE; n, number of rats. NS, normal saline; DC, diaspirin cross-linked Hb; NE, norepinephrine; $\dot{V}o_2$, systemic O_2 uptake; O_2E, O_2 extraction; Sa_{O_2}, arterial O_2 saturation; Sv_{O_2}, mixed venous O_2 saturation; Hb, arterial Hb concentration. [a]$P < 0.05$ vs. CLP-NS; [b]$P < 0.01$ vs. CLP-NS; [c]$P < 0.01$ vs. CLP-DC; [d]$P < 0.001$ vs. CLP-NS; [e]$P < 0.05$ vs. CLP-NE.

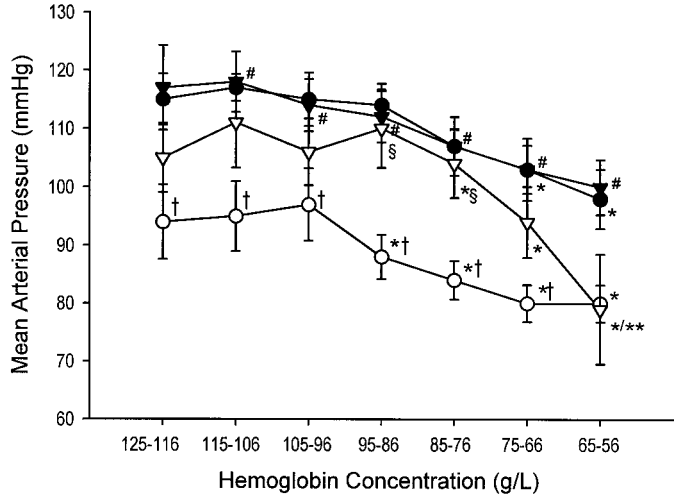

Fig. 3. MAP during progressive hemodilution. DCLHb and NE treatment was continued using the effective doses at the end of the 18-h treatment phase. ●, sham; ○, CLP-NS; ▼, CLP-DC; ▽, CLP-NE. Values are means ± SE. *$P < 0.05$ vs. baseline. †$P < 0.05$, CLP vs. sham. #$P < 0.05$, DCLHb vs. CLP. §$P < 0.05$, NE vs. CLP. **$P < 0.05$, NE vs. DCLHb.

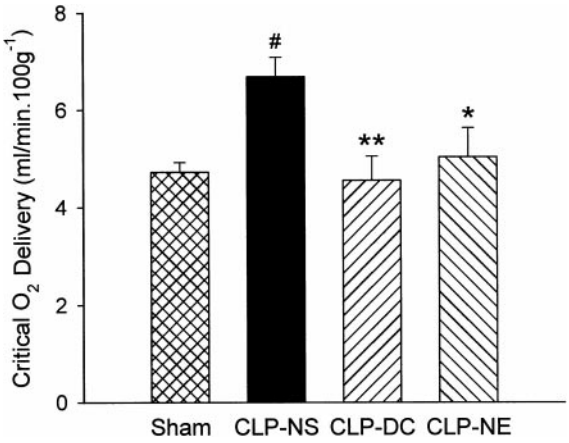

Fig. 4. Effects of DCLHb and NE infusion on $\dot{D}o_{2\,crit}$. Values are means ± SE; n = 7 sham, 8 CLP-NS, 7 CLP-DC, and 7 CLP-NE. #$P < 0.05$ vs. sham. *$P < 0.05$ vs. CLP-NS. **$P < 0.01$ vs. CLP-NS.

and $P < 0.05$ vs. CLP-NS; Fig. 4). At the $\dot{D}o_{2\,crit}$, systemic $\dot{V}o_2$ was decreased in the CLP-NE group (−19% vs. CLP-NS, $P < 0.05$; Fig. 5*A*) but not in the CLP-DC group. The Hb concentration was lower in DCLHb- and NE-treated rats ($P < 0.05$ and $P < 0.001$, respectively; Fig. 5*B*). The O_2 extraction ratio was increased by 59% in DCLHb rats compared with the CLP-NS group ($P < 0.05$), whereas it was the same in the CLP-NE group (Fig. 5*C*). CO at $\dot{D}o_{2\,crit}$ tended to decrease with DCLHb infusion compared with the CLP-NE group ($P = 0.05$; Fig. 5*D*). Body temperature was not different between groups (37.8 ± 0.4, 38 ± 0.4, and 37.7 ± 0.5°C in CLP-NS, CLP-DC, and CLP-NE, respectively).

When the CO-SVR relationship was examined at the completion of the 18-h treatment phase, the CLP-NS group was clearly characterized by a high CO-low SVR ("hyperdynamic") profile compared with the sham group. DCLHb-treated rats, compared with the CLP-NS group, presented with a low CO-high SVR profile. A similar effect was also seen in the CLP-NE group but to a much lesser degree (Fig. 6). Isovolemic hemodilution caused a shift to higher CO and lower SVR in all groups. However, the CLP-DC group, but not the CLP-NE group, maintained a low CO-high SVR profile.

DISCUSSION

This experiment explored the effects of a chronic infusion of Hb solution, DCLHb, on the systemic $\dot{V}o_2$-$\dot{D}o_2$ relationship. With a chronic, 18-h infusion of DCLHb, as well as with NE infusion in a control group, we prevented the usual adverse effect of a sepsis-induced increase in $\dot{D}o_{2\,crit}$. This novel finding supports our conclusion that the disturbance in convective $\dot{D}o_2$ seen in sepsis, which depresses the host's ability to extract O_2, is amenable to treatment.

Approach and animal model. Recent studies suggested that, in the presence of inadequate $\dot{D}o_2$, cell-free Hb solutions may increase the maximal O_2 extraction capacity (24, 30, 32). Therefore, this study aimed at preventing sepsis-induced alterations in the systemic $\dot{V}o_2$-$\dot{D}o_2$ relationship by using the hemodynamic prop-

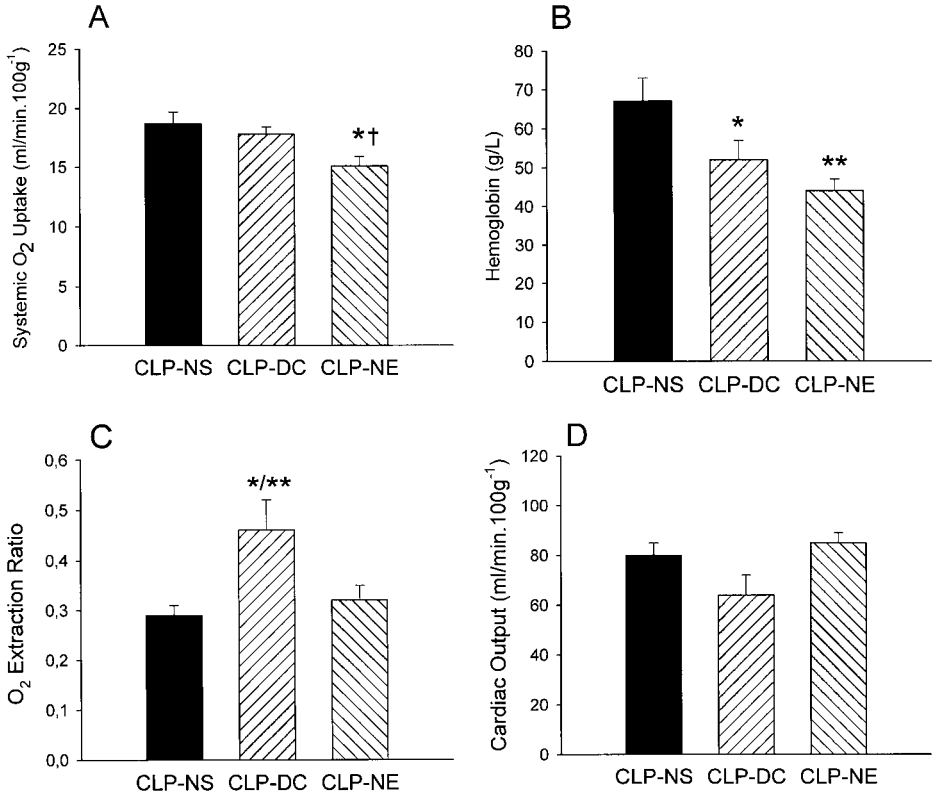

Fig. 5. Effects of DCLHb and NE infusion on key variables of O_2 transport at $\dot{D}o_{2\,crit}$. Values are means ± SE; n = 8 CLP-NS, 7 CLP-DC, and 7 CLP-NE. *A*: systemic O_2 uptake ($\dot{V}o_2$). *$P < 0.05$ vs. CLP-NS; †$P < 0.05$ vs. CLP-DC. *B*: Hb concentration. *$P < 0.05$ vs. CLP-NS; **$P < 0.001$ vs. CLP-NS. *C*: systemic O_2 extraction ratio. *$P < 0.05$ vs. CLP-NS; **$P < 0.05$ vs. CLP-NE. *D*: cardiac output (CO). $P = 0.05$ (not significant), CLP-NE vs. CLP-DC.

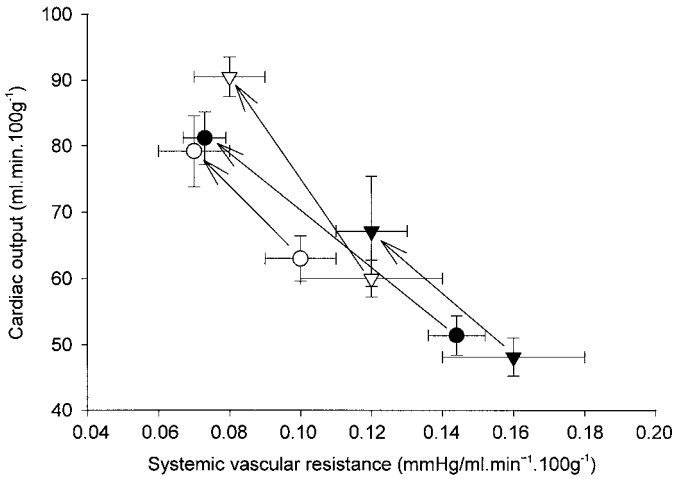

Fig. 6. CO vs. systemic vascular resistance (SVR). ●, sham; ○, CLP-NS; ▼, CLP-DC; ▽, CLP-NE. Arrows, changes in the CO-SVR relationship from the time when the 18-h treatment phase was completed to the point when $\dot{D}o_{2\,crit}$ was reached. Anemic hypoxia caused a shift to higher CO and lower vascular resistances in all 4 groups. DCLHb treatment produced a low CO-high SVR profile compared with the CLP-NS group. Values are means ± SE.

erties of the DCLHb, that is, to support arterial perfusion pressure (28) and to improve microvascular perfusion (31). We also chose this approach to mimic the clinical scenario, where support of blood pressure by chronic infusion of agents that increase vascular resistance and/or CO is a cornerstone of therapies to improve outcome from septic shock (33).

A primary objective of this study was to compare the effects of DCLHb infusion on the systemic $\dot{V}o_2$-$\dot{D}o_2$ relationship with a septic control group, which received only NS to adjust for the infused volume. A sham control group was added to demonstrate the effects of sepsis and to allow an estimation of possible effects of the DCLHb in relation to the insult. A third group that received NE infusion targeted to achieve the same effect on MAP during the 18-h treatment period was intended to control for the vascular effects of the DCLHb solution on arteriolar reactivity and tone. Specifically, our intention was to isolate the effects of the Hb solution on blood pressure from its properties to modify O_2 transport capacity due to altered capillary convective and diffusive O_2 transport (24, 31).

In this study, $\dot{D}o_{2\,crit}$ was the key parameter used to assess the effects of sepsis and the interventions on O_2 transport capacity. The importance of the systemic $\dot{D}o_{2\,crit}$ is that this parameter defines the $\dot{D}o_2$ where O_2 extraction is maximized $\dot{D}o_{2\,crit}$and the systemic $\dot{V}o_2$ becomes dependent on the systemic $\dot{D}o_2$ if the latter is reduced beyond this point. Thus the systemic $\dot{D}o_{2\,crit}$ is the ultimate physiological threshold to the manifestation of tissue hypoxia and shock (27). Two mechanisms have been discussed to explain the presence of a critical value of O_2 supply: diffusion limitation in the microcirculation and physiological arterial-venous shunt (37). It is assumed that, at least on average in the whole body, arterial-venous shunting is more important to determine the maximal value of systemic O_2 extraction (37).

To calculate the $\dot{D}o_{2\,crit}$, we used a hemodilution model that was developed and standardized in our laboratory (11, 30). This model provides direct and online measurements of systemic $\dot{V}o_2$ from awake rats and thus allows the determination of $\dot{D}o_{2\,crit}$ from regression against a larger number of consecutive $\dot{D}o_2$ measurements, as originally described by Samsel and Schumacker (26).

When CLP-septic rats were compared with sham animals 24 h after CLP and before randomization to the treatment protocols, they had developed characteristic signs of sepsis as defined by a consensus conference (2): leukopenia, thrombocytopenia, and mild hypotension. Also, a modest increase in CO and loss of vascular resistance indicated a hyperdynamic cardiovascular response. When O_2 extraction capacity was determined after 42 h, CLP-sepsis was associated with increased $\dot{D}o_{2\,crit}$ and an O_2 extraction deficit compared with the sham group. Myocardial function appeared to be intact, since septic animals reached the same cardiac index at $\dot{D}o_{2\,crit}$. Very similar sepsis-induced changes in O_2 extraction capacity have been demonstrated in dogs by Nelson et al. (23), who proposed that microvascular injury might be the cause of the sepsis-induced attenuation in O_2 transport. For the sepsis model as used in this experiment, alterations in arteriolar vascular reactivity (21), as well as reduced capillary perfusion and attenuation in microvascular blood flow in microvascular networks of different organs, have been demonstrated previously (5, 9, 19, 20).

It is important that chronic infusion of the catecholamine, NE, resulted in the same 10–20% increase in blood pressure that was achieved in Hb-treated septic rats throughout the 18-h treatment period. Therefore, the NE group may be regarded as an appropriate control for the blood pressure component of the effects of DCLHb infusion on the systemic $\dot{V}o_2$-$\dot{D}o_2$ relationship.

Mortality in this study was not significantly different between the septic groups, although the data for the NE group might suggest a decreased mortality compared with septic controls and DCLHb-treated rats. This study, however, was not designed to study effects of DCLHb and NE on mortality. An additional power analysis revealed that a larger sample size would have been required to determine treatment effects on mortality.

Also, it has to be considered that this study presents data from survivors of the septic insult. One cannot exclude that this introduced bias on some of the results. However, because the objective of this study was to determine the effects of chronic DCLHb or NE infusion in sepsis, which is a syndrome with high lethality under experimental and clinical conditions, this was unavoidable.

Effects of interventions. The typical, sepsis-induced alteration of the $\dot{V}o_2$-$\dot{D}o_2$ relationship in CLP-septic rats was prevented by DCLHb, as indicated by a decreased $\dot{D}o_{2\,crit}$ compared with placebo-treated septic

rats. In rats treated with the Hb solution, this effect was associated with an increased ability to extract O_2, suggesting improved diffusive and/or convective O_2 transport in the microcirculation. In the NE group, the sepsis-related increase in $\dot{D}o_{2\,crit}$ was also prevented, but O_2 extraction was not increased at the critical point. However, in this group, a tendency for a decrease in systemic $\dot{V}o_2$ indicated a modulation of the hyperdynamic metabolic response to sepsis.

One possibility is that the only pharmacological property common to DCLHb and NE, that is, to increase vascular resistance, explains the observed effects on $\dot{D}o_{2\,crit}$. Indeed, loss of vascular resistance, also referred to as "septic vasoplegia," is a characteristic consequence of the inflammation process in sepsis as a result of nitric oxide overproduction (17, 21). Septic vasoplegia is followed by decreased perfusion pressures and inappropriate distribution of blood flows (17, 21), which may be the underlying cause for the within-organ, microregional O_2 supply-demand imbalance in sepsis (4, 36). Therefore, improved blood flow distribution and increased perfusion pressure could explain the protective effect of DCLHb and NE infusion against sepsis-induced alterations of the $\dot{V}o_2$-$\dot{D}o_2$ relationship. Moreover, evidence for beneficial effects on the septic microcirculation have been demonstrated previously for DCLHb (31) and NE (40).

It is striking, however, that only DCLHb infusion increased the maximal O_2 extraction capacity, whereas NE infusion, as indicated by a modest fall in $\dot{V}o_2$, preserved a normal $\dot{D}o_{2\,crit}$ via reduction of the metabolic needs. Despite comparable effects on blood pressure, this indicates that the effects of DCLHb and NE on the $\dot{V}o_2$-$\dot{D}o_2$ relationship could be explained, alternatively, by unique properties of each of the two agents.

Aside from cardiovascular effects, DCLHb is characterized by *1*) excellent O_2-carrying properties (8), *2*) a rightward-shifted O_2 dissociation curve [Po_2 at which Hb is half-saturated (P_{50}) = 32.4 mmHg] compared with human blood (8, 30), and *3*) a characteristic distribution in the plasma, outside the RBCs (24). In this study, total Hb concentration was not increased in DCLHb-treated rats, and systemic $\dot{D}o_2$ was decreased, excluding transfusion effects as a cause for increased O_2 extraction capacity. Also, it is unlikely that differences in P_{50} explain the effects of DCLHb on tissue O_2 extraction capabilities, since compared with rat blood, which is characterized by a higher P_{50} of 37–38 mmHg, the O_2 dissociation curve of DCLHb is shifted leftward. Studies on the effects of a leftward-shifted O_2 dissociation curve on the physiological adaptation to acute decreases in $\dot{D}o_2$ reported only unfavorable effects on tissue oxygenation (39). Eventually, the distribution of DCLHb within the plasma compartment is (alternative to the effects on the vasculature) the only other possible explanation for increased O_2 extraction capacity after DCLHb infusion. In a situation where microcirculatory perfusion is impaired, as in sepsis (9, 19), a homogeneous intravascular distribution of DCLHb may increase diffusion capacity and thus improve the abilities of the tissue to extract O_2. For example, DCLHb could serve as a carrier or intermediary vehicle for O_2 released from RBCs. A recent study in which a geometrical model was used, in fact, reported that the presence of Hb molecules outside the RBC decreases the diffusion resistance for O_2 (24).

For the CLP-NE group, the unexpected decrease in systemic $\dot{V}o_2$ may also provide an explanation for the preservation of a normal $\dot{V}o_2$-$\dot{D}o_2$ relationship. This decrease in $\dot{V}o_2$ suggests that NE exhibited anti-inflammatory effects, implying that suppression of the typical systemic inflammatory process in sepsis decreased the O_2 needs of the tissues. This assumption is supported by two recent studies demonstrating that catecholamines modulate monocyte receptor status and cytokine expression during inflammation in a potentially beneficial manner as a result of β_2-adrenorecepter activation (1, 12). In addition, others who studied the effect of NE infusion on O_2 extraction capacity using an endotoxin model where $\dot{D}o_{2\,crit}$ was determined by a progressive decrease in CO also reported a decrease in $\dot{D}o_{2\,crit}$ (40). However, this work included no septic controls to relate this benefit of NE infusion to the extent of the lesion (40).

Alternatively, the decrease in tissue $\dot{V}o_2$ in our study could suggest some degree of tissue ischemia after NE infusion. However, arterial lactate, which has been used as a marker of tissue ischemia (15, 35), was not increased in the NE-treated rats at completion of the treatment phase. Also, it would be expected that, in the presence of baseline ischemia, $\dot{D}o_{2\,crit}$ would be reached at a higher value, opposite to the findings in this study. It therefore appears that no significant compromise of tissue oxygenation occurred during NE infusion.

Intervention effects on cardiac performance. In this study the improvement in the $\dot{V}o_2$-$\dot{D}o_2$ relationship with DCLHb and NE infusion did not only occur in the presence of different effects of the two agents on O_2 transport but was also associated with differences in the hemodynamic profile. After 18 h of treatment, the DCLHb group presented with a decreased systemic $\dot{D}o_2$, most likely to be explained by a reflex fall in CO secondary to the increase in vascular resistance. In NE-treated rats, CO and systemic $\dot{D}o_2$ were not affected, probably since myocardial contractility was supported simultaneously with the increase in blood pressure. From this observation, one can conclude that in the DCLHb group no support of myocardial contractility and systemic $\dot{D}o_2$ was required to increase O_2 extraction capacity. This conclusion is supported by the analysis of the relationship between CO and SVR (Fig. 6), since the low CO-high SVR profile in DCLHb-treated was maintained even when systemic $\dot{D}o_2$ had been diminished to the critical point. The latter observation may also confirm the assumption that the decrease in CO and systemic $\dot{D}o_2$ observed at 18 h of treatment did not reflect a decreased demand of O_2 supply, because, otherwise, isovolemic hemodilution would have caused CO to rise (7).

In summary, this study provides clear evidence for an improved systemic $\dot{V}O_2$-$\dot{D}O_2$ relationship after goal-directed chronic infusion of DCLHb and NE to increase blood pressure in septic rats. The possibility exists that this beneficial effect of DCLHb and NE is the sole consequence of increased perfusion pressure and subsequently improved microvascular perfusion. However, our results show that DCLHb infusion primarily increased O_2 extraction capacity, whereas NE infusion appeared to decrease tissue O_2 demand. Therefore, the observed effects on the sepsis-induced anomalies of the $\dot{V}O_2$-$\dot{D}O_2$ relationship could also be explained by unique, but different, effects of each of the two studied agents: DCLHb may favor O_2 transport in the microcirculation, whereas NE may modulate the inflammatory response to sepsis.

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