Apolipoprotein A-I mimetic peptide treatment inhibits inflammatory responses and improves survival in septic rats

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Am J Physiol Heart Circ Physiol 297: H866–H873, 2009. First published June 26, 2009; doi:10.1152/ajpheart.01232.2008.—Systemic inflammation induces a multiple organ dysfunction syndrome that contributes to morbidity and mortality in septic patients. Since increasing plasma apolipoprotein A-I (apoA-I) and HDL may reduce the complications of sepsis, we tested the hypothesis that the apoA-I mimetic peptide 4F confers similar protective effects in rats undergoing cecal ligation and puncture (CLP) injury. Male Sprague-Dawley rats were randomized to undergo CLP or sham surgery. IL-6 levels were significantly elevated in plasma by 6 h after CLP surgery compared with shams. In subsequent studies, CLP rats were further subdivided to receive vehicle or 4F (10 mg/kg) by intraperitoneal injection, 6 h after sepsis induction. Sham-operated rats received saline. Echocardiographic studies showed a reduction in left ventricular end-diastolic volume, stroke volume, and cardiac output (CO) 24 h after CLP surgery. These changes were associated with reduced blood volume and left ventricular filling pressure. 4F treatment improved blood volume status, increased CO, and reduced plasma IL-6 in CLP rats. Total cholesterol (TC) and HDL were 79 ± 5 and 61 ± 4 mg/dl, respectively, in sham rats. TC was significantly reduced in CLP rats (54 ± 3 mg/dl) due to a reduction in HDL (26 ± 3 mg/dl). 4F administration to CLP rats attenuated the reduction in TC (69 ± 4 mg/dl) and HDL (41 ± 3 mg/dl) and prevented sepsis-induced changes in HDL protein composition. Increased plasma HDL in 4F-treated CLP rats was associated with an improvement in CO and reduced mortality. It is proposed that protective effects of 4F are related to its ability to prevent the sepsis-induced reduction in plasma HDL.

Inflammation and tissue injury in sepsis are caused by multiple factors, including gram-negative and gram-positive bacteria, viruses, and fungi. Lipopolysaccharide (LPS/endotoxin) is a well-characterized component of gram-negative bacteria, and its role in inflammation has been extensively studied (9). Gram-negative sepsis is one of the most common infectious diseases in hospitals, and, in this setting, LPS may play an important role in the induction of inflammatory injury in patients (9, 10). Gram-positive bacteria also release cytotoxic molecules, including exotoxins, peptidoglycan, and lipoteichoic acid, factors that bind to receptors on target cells and activate inflammatory cascades (9).

High-density lipoprotein (HDL) and its major protein component, apolipoprotein A-I (apoA-I), exert prominent anti-inflammatory and antioxidant effects (45). A reduction in serum HDL, which occurs in a majority of septic patients, is a predictor of poor outcome, while increasing HDL concentrations reduces complications associated with endotoxemia in both rodents and humans (38, 45). Thus raising circulating HDL levels represents an important therapeutic goal in the treatment of sepsis. Obtaining therapeutic quantities of HDL, however, is impractical.

Synthetic apoA-I mimetic peptides mimic many of the properties of apoA-I. They act as mediators of cholesterol efflux, reduce proinflammatory properties of low-density lipoprotein (LDL) cholesterol, and inhibit lesion formation in murine models of atherosclerosis (1). Administration of the apoA-I mimetic peptide 4F also promotes the formation of new HDL particles in apoE-null mice (1). Our laboratory previously reported that treatment of cultured endothelial cells with 4F reduced LPS-induced inflammatory responses (18). This study extends our previous observations in rats subjected to cecal ligation and puncture (CLP), a surgical model of polymicrobial sepsis. We present data showing that administration of 4F in vivo, after the induction of sepsis, attenuates changes in lipoprotein profiles and improves cardiac performance and survival in rats subjected to CLP injury. It is proposed that protective effects of 4F are related to its ability to attenuate the sepsis-induced reduction in plasma HDL. An increase in circulating HDL may facilitate neutralization of endotoxin and/or inhibit inflammatory pathways that contribute to blood volume loss and impaired left ventricular (LV) filling in septic rodents.

**METHODS**

**Peptide synthesis.** The apoA-I mimetic peptide 4F, whose amino acid sequence is Ac-DWFKAFYDKVAEKFKEAF-NH2, was synthesized by the solid-phase peptide synthesis method, as previously described (11). Peptide purity was ascertained by mass spectral analysis and analytic HPLC.
Animals. Ten-week-old male Sprague-Dawley rats were purchased from Charles River Breeding Laboratories (Wilmington, MA) and allowed a 1-wk recovery period before experimental protocols were initiated. All protocols were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham (UAB) and were consistent with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 96-01, revised 2002).

CLP surgery and 4F administration. Eleven-week-old rats were randomized to undergo CLP or sham surgery after an overnight (16 h) fast, as previously described (20). All animals received fluid resuscitation (3 ml saline/100 g body wt, subcutaneously) immediately after surgery. CLP rats were randomly assigned to receive 4F (10 mg/kg; n = 45) by intraperitoneal injection or an equivalent volume of saline vehicle (n = 54) 6 h after CLP surgery. The dosage of 4F used in these studies is consistent with a recent clinical study showing that 4F administration at 6 mg/kg significantly improves anti-inflammatory properties of HDL in humans (7). Sham-operated rats (n = 44) underwent the same surgical procedure, but the cecum was neither ligated nor punctured. Animals were assigned to experimental protocols, as described in the following sections.

Plasma measurement of IL-6. Under isoflurane anesthesia, a blood sample (300 μl) was collected from the tail vein at baseline and 2, 6, and 12 h postsurgery. A final sample was collected at 24 h by cardiac puncture. Blood was collected in heparinized tubes and centrifuged at 3,000 rpm for 10 min at 4°C. Plasma was stored in 1-ml aliquots at −80°C for subsequent measurement of IL-6. IL-6 levels were quantified by enzyme-linked immunosorbant assay using an OptEIA rat IL-6 kit (BD Biosciences).

Transthoracic echocardiography. Rats were anesthetized with 2.5% isoflurane, and echocardiographic images were obtained using a 15-MHz ultrasound system (Philips 5500, Andover, MA). Both two-dimensional and M-mode echocardiograms were used to measure the following cardiac dimensions: interventricular septum width (IVS), posterior wall width (PW), LV end-systolic dimension (LVESD), LV end-diastolic dimension (LVEDD), LV end-systolic area, and LV end-diastolic area. End-systolic volume (ESV), end-diastolic volume (EDV), stroke volume (SV) and cardiac output (CO) were derived from LV dimensional measurements using the area-length method, as recommended by the American Society of Echocardiography (23). This calculation method has been previously validated in rats (36). Fractional shortening (FS) was derived from M-mode images. Each animal served as its own control, and data are expressed as both absolute values and percent changes over the 24-h study period.

Blood pressure and blood volume measurements in CLP rats. Since preload and afterload influence cardiac function, we measured mean systemic arterial (MAP) and right atrial pressures (RAP). Polyethylene catheters (PE-50) were implanted in rats 24 h before sham or CLP surgery, as previously described (21). MAP and RAP was measured in conscious rats 24 h after surgery using a polygraph recorder (Grass Instruments, Quincy, MA).

Blood volume was measured in sham-operated and CLP rats, 24 h postsurgery, using the Evans blue dilution method (2). Briefly, a 300-μl blood sample was collected, followed by intravenous infusion of 300 μl of 1% Evans blue. After 5 min, blood was drawn by cardiac puncture. A 50-μl aliquot of plasma was diluted with 950-μl saline, and absorbance was measured at 605 nm. Blood volume was calculated using a standard curve generated by the addition of known amounts of Evans blue to untreated plasma. After euthanasia, hearts and lungs were collected for assessment of tissue edema. Wet weights for each tissue were recorded, followed by incubation in a 70°C oven for 48 h. At this time, dry weight measurements were obtained. Tissue water content was then determined by calculating the wet-to-dry weight ratio.

Lipid and lipoprotein analyses. The lipid hydroperoxide (LOOH) content of plasma collected from sham, CLP, and 4F-treated CLP rats was measured using 2,7′-dichlorodihydrofluorescein (DCF) diacetate, as previously described (30). A 5-μl aliquot of plasma was mixed with 170-μl PBS and 25-μl of DCF (200 μg/ml). The final working concentration of DCF was 50 μM. Samples were incubated at 37°C, and peak DCF fluorescence (excitation: 485-nm wavelength, emission: 530-nm wavelength) was measured after 2 h. Data are expressed as relative fluorescence units (RFU).

Plasma lipoprotein profiles were determined using column chromatography, as previously described (16). Cholesterol profiles were decomposed into component peaks and analyzed for relative area using PeakFit (SPSS Science, Chicago, IL). Absolute values for total cholesterol (TC) and each component peak were determined by comparison with a control sample of known concentration. The apoA-I content of plasma samples from sham, CLP, and CLP + 4F rats was determined by Western blot using a mouse polyclonal apoA-I antibody (Brookwood Biomedical).

Isolation of HDL by density gradient centrifugation. In additional experiments, HDL was isolated from rat in each treatment group by a two-step sequential flotation ultracentrifugation procedure. Plasma density was initially adjusted to 1.06 g/ml with potassium bromide and centrifuged (100,000 rpm) for 24 h at 7°C. The upper layer, containing very low-density lipoprotein (VLDL) and LDL, was removed. The density of the remaining HDL-containing fraction was then adjusted to 1.25 g/ml, mixed, and centrifuged at 100,000 rpm for another 24 h. At the end of this step, HDL, localized in the upper (more buoyant) zone, was collected and dialyzed in 3 liters of buffer containing 1× PBS + 100 μM diethylenetriamine pentaacetaete overnight. On the following day, HDL was collected, and protein concentration was determined using the Bradford protein assay (Bio-Rad). Proteins in isolated HDL fractions (5 μg) were then separated by gradient (4–20%) SDS-PAGE. Bands were visualized by Coomassie blue staining. The relative concentration of each protein in a given sample was then determined and expressed as a percentage of the total protein in each lane. To identify specific HDL-associated proteins, bands were excised and submitted to the UAB Mass Spectrometry Center for identification using a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer.

Statistical methods. All results are reported as means ± SE. Statistical analysis was performed using SigmaStat 3.5 software (Systat Software). Differences between groups were assessed by one-way ANOVA with post hoc testing (Student-Newman-Keuls test) and by Pearson correlation analysis. Survival data were assessed by log-rank analysis (Kaplan-Meier survival method). A difference in survival between individual groups was determined using the Holm-Sidak method. A P value < 0.05 was considered statistically significant.

RESULTS

Plasma IL-6 levels in CLP rats. Plasma IL-6 concentration in sham-operated rats (n = 8) was 200 pg/ml at baseline and did not vary significantly over the 24-h observation period. In contrast, levels of IL-6 were significantly increased in CLP rats (n = 9) within 6 h postsurgery and remained elevated compared with sham-operated controls at time points up to 12 h, then declining to levels not significantly different from sham controls at 24 h (Fig. 1). Since inflammatory pathways, as reflected by an increase in plasma IL-6, were activated within 6 h after CLP surgery, we chose this time point for administration of 4F. Administration of the peptide resulted in a significant reduction in peak IL-6 levels in CLP rats (n = 11) 12 h postsurgery (Fig. 1).

4F attenuates changes in LV dimensions and improves LV filling in CLP rats. Since cardiac dysfunction is a complication of severe sepsis in humans and experimental animals, we monitored cardiac function noninvasively by transthoracic echocardiography.

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surgery (Fig. 2). Collectively, these data suggest that impaired
an index of cardiomyocyte contractile function, was similar in
CLP rats compared with sham-operated controls (Table 1). FS,
postsurgery (Table 1, Fig. 2). HR was significantly increased in
reduced 58
significant changes in SV or CO (Table 1, Fig. 2). A modest, but
dimensional measurements. Sham surgery did not induce sig-
ificant changes in ESV and EDV in sham-operated rats over the 24-h study period.
Changes in cardiac wall thickness in CLP rats corresponded
significantly increased compared with shams at 24 h (Table 1).
It follows
changes in the absolute values for cardiac dimensions in sham-
and 24 h after sham or CLP surgery. There were no significant
echocardiography. Echocardiograms were recorded at baseline
parameters were minimal (Table 1). In contrast to sham-operated animals, dimensional changes in
CLP rats (n = 11) were observed. IVS and PW were signifi-
cantly increased compared with shams at 24 h (Table 1).
Changes in cardiac wall thickness in CLP rats corresponded
with a reduction in LVESD and LVEDD (Table 1). It follows
that ESV and EDV were reduced by 37
with a reduction in LVESD and LVEDD (Table 1). It follows

Parameters of cardiac performance were calculated from
dimensional measurements. Sham surgery did not induce sig-
ificant changes in SV or CO (Table 1, Fig. 2). A modest, but
significant, increase in HR, likely due to surgical stress, was
noted in these animals (Table 1). In contrast, SV and CO were
reduced 58 ± 5 and 47 ± 6%, respectively, in CLP rats 24 h
posturgery (Table 1, Fig. 2). HR was significantly increased in
CLP rats compared with sham-operated controls (Table 1). FS,
an index of cardiomyocyte contractile function, was similar in
both groups at baseline and was not altered by sham or CLP
surgery (Fig. 2). Collectively, these data suggest that impaired
cardiac performance in CLP rats is related to a reduction in LV
filling rather than to a defect in cardiomyocyte function per se.

Treatment of CLP rats with 4F (n = 13), 6 h postsurgery,
blunted changes in cardiac dimensions compared with vehicle-
treated CLP rats (Table 1). 4F attenuated the sepsis-induced
increase in LV wall thickness (i.e., IVS and PW) (Table 1). It follows
that ESV and EDV were reduced by 37
6%, respectively, in CLP rats 24 h
posturgery (Table 1, Fig. 2). Accordingly,
11) CLP (solid bars;
11) rats were measured
by ELISA. Values are means ± SE. *P < 0.05, significant difference compared with sham-operated controls. †P < 0.05, significant difference compared with CLP rats receiving saline vehicle.

Table 1. Effects of CLP surgery and 4F treatment on cardiac dimensional changes

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 11)</th>
<th>CLP (n = 11)</th>
<th>CLP +4F (n = 13)</th>
</tr>
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<tr>
<td></td>
<td>Baseline 24 h %Change</td>
<td>Baseline 24 h %Change</td>
<td>Baseline 24 h %Change</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>1.4±0.1 1.5±0.1 5±3</td>
<td>1.4±0.1 2.1±0.1 53±10*</td>
<td>1.4±0.1 1.7±0.1 21±5‡</td>
</tr>
<tr>
<td>PW, mm</td>
<td>1.4±0.1 1.4±0.1 4±4</td>
<td>1.4±0.1 2.1±0.1 54±8*</td>
<td>1.4±0.1 1.8±0.1 24±5‡</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>4.8±0.1 5.0±0.2 4±5</td>
<td>4.7±0.1 3.3±0.3 29±6*</td>
<td>4.7±0.1 3.6±0.2 18±3*</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>8.3±0.2 8.3±0.1 4±2</td>
<td>8.3±0.1 6.0±0.4 28±4*</td>
<td>8.1±0.1 6.5±0.2 17±2*</td>
</tr>
<tr>
<td>ESV, μl</td>
<td>166±11 162±14 3±7</td>
<td>164±14 101±12 37±6*</td>
<td>168±13 93±6 42±4*</td>
</tr>
<tr>
<td>EDV, μl</td>
<td>453±15 438±22 3±5</td>
<td>486±21 236±23 51±4*</td>
<td>466±15 278±10 40±3†</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>341±4 391±6 12±1</td>
<td>330±6 423±9 29±4*</td>
<td>332±6 430±11 30±4*</td>
</tr>
<tr>
<td>SV, μl</td>
<td>287±12 276±13 2±6</td>
<td>321±9 134±17 58±5*</td>
<td>298±9 185±9 37±4†</td>
</tr>
</tbody>
</table>

Values are means ± SE and are presented as percent changes within each group over the 24-h study period; n, no. of rats. CLP, cecal ligation and puncture; IVS, interventricular septum width; PW, posterior wall width; LVESD, left ventricular end-systolic dimension; LVEDD, left ventricular end-diastolic dimension; ESV, end-systolic volume; EDV, end-diastolic volume; HR, heart rate; SV, stroke volume. *P < 0.05, significant difference compared with sham-operated rats. †P < 0.05 and ‡P < 0.01, significant differences compared with CLP rats.
restore these parameters to levels seen in sham-operated controls (Table 1, Fig. 2). 4F treatment did not alter FS, reinforcing the observation that systolic function is not altered in CLP rats receiving either saline or 4F (Fig. 2). The attenuated changes in cardiac dimensions and performance by 4F in CLP rats, however, underscore the fundamental role of the peptide in improving LV filling.

**Blood pressure and plasma volume changes in CLP rats.** To assess factors influencing LV filling, we measured blood pressure and plasma volume status in control and CLP rats. MAP was similar in conscious, sham-operated (113 ± 3 mmHg; n = 9), CLP (113 ± 2 mmHg; n = 11), and CLP+4F (115 ± 2 mmHg; n = 8) rats 24 h postsurgery. In contrast, there were differences in RAP between groups. RAP was significantly lower in CLP rats at 24 h (1 ± 1 mmHg) compared with sham-operated controls (6 ± 2 mmHg) (Fig. 3). 4F administration in CLP rats was associated with a significant increase in RAP (4 ± 1 mmHg) compared with CLP rats receiving vehicle (Fig. 3). Plasma volume was significantly reduced in CLP rats (8 ± 1 ml; n = 8) compared with shams (12 ± 1 ml; n = 7). 4F treatment was associated with an increase in plasma volume (10 ± 1 ml; n = 8) in CLP rats (Fig. 3). Concurrent with a reduction in plasma volume, there was an increase in the wet-to-dry weight ratios for lung and heart excised from CLP rats (n = 7) compared with shams (n = 7–10), suggesting an increase in tissue edema (Fig. 3). 4F treatment (n = 10–12) was associated with a significant reduction in tissue wet-to-dry ratios in CLP rats (Fig. 3).

**4F treatment improves lipoprotein profiles in CLP rats.** Measurement of plasma LOOH content revealed a significant increase in CLP rats (11,497 RFU, P < 0.05, n = 5) compared with sham-operated controls (8,006 RFU, n = 8). 4F treatment normalized plasma LOOH (8,490 RFU, n = 5) in CLP rats. TC, VLDL, LDL, and HDL were measured by column chromatography in plasma samples obtained from sham-operated, CLP, and 4F-treated CLP rats. Figure 4 depicts a representative elution profile for lipoproteins isolated from rat plasma before surgical intervention. As indicated by Fig. 4, HDL represents the major lipoprotein component of normal rat plasma, with smaller quantities of VLDL and LDL present. A reduction in TC was observed in plasma of CLP rats (n = 9), due principally to a decrease in HDL cholesterol (56% HDL reduction vs. shams) (Fig. 4). Plasma apoa-I content mirrored changes in HDL in each treatment group (Fig. 4). 4F treatment (n = 10) in CLP rats attenuated the reduction in TC and LDL (32% HDL reduction vs. shams) (Fig. 4). LDL levels were elevated in both vehicle- and 4F-treated CLP rats.

Since reports suggest that reduced circulating HDL correlates with increased risk and severity of sepsis complications, we tested whether there was a relationship between changes in HDL and impaired cardiac performance in CLP rats (34, 45). Percent changes in the HDL content of plasma samples from sham-operated (n = 8), CLP (n = 6), and 4F-treated CLP rats (n = 8) were determined for the 24-h study period. These were compared with changes in LVEDV, EDV, SV, and CO over the same time period. There was a positive correlation between reductions in HDL and LVEDD (R = 0.533, P = 0.01, n = 22), EDV (R = 0.599, P = 0.003, n = 22), SV (R = 0.640, P = 0.001, n = 22), and CO (R = 0.622, P = 0.002, n = 22). A scatter plot depicting the relationship between percent changes in HDL and CO is shown in Fig. 5.

**Sepsis-induced changes in HDL protein composition are reversed by 4F.** To determine whether changes in HDL concentration were related to alterations in the protein composition of HDL, we isolated HDL from plasma of sham (n = 6), CLP (n = 6), and CLP+4F (n = 6) rats by ultracentrifugation. Proteins in each isolated HDL fraction were then separated by gradient (4–20%) SDS-PAGE. Staining with Coomassie blue revealed multiple protein bands on these gels (Fig. 6). The bands were excised, and their identity determined by MALDITOF mass spectrometry. Results of these studies confirmed the presence of apoA-I/II, apoE, apoA-IV, apoC-III/II/III, apoM, and serum amyloid A in HDL fractions isolated from each treatment group. The MALDI-TOF characterization of the bands was consistent with the known molecular weights of these proteins. The distribution of each protein in a given
sample was determined by calculating band density as a percentage of total band density for each lane. Results showed that CLP was associated with a significant reduction in HDL-associated apoA-I and apoA-IV (16 and 55% reduction, respectively) and an increase in apoE (50% increase) compared with shams. In contrast, 4F treatment in CLP rats attenuated sepsis-induced changes in these apolipoproteins. apoA-I was increased by 7% in CLP+4F rats compared with controls (Fig. 6). 4F treatment also attenuated changes in apoA-IV (36% reduction) and apoE (66% increase) compared with controls (Fig. 6). apoA-II, apoC-I, and apoC-II resolved on SDS gels at a similar molecular weight. Changes in the proportion of individual proteins cannot be determined under these conditions and will require further immunoblotting studies with specific antibodies. This was also true for apoC-III and serum amyloid A4 (Fig. 6).

4F treatment improves survival in CLP rats. Over a 48-h observation period, there was no mortality in the sham-operated rats (n = 17) (Fig. 7). Significant mortality occurred in rats subjected to CLP (38% at 24 h and 71% at 48 h; n = 21). In contrast, mortality was significantly reduced in 4F-treated CLP rats (n = 14) at each time point (14% at 24 h and 43% at 48 h).

DISCUSSION

Results of the present studies show that 4F treatment reduces inflammation, attenuates changes in HDL, and improves cardiac performance in CLP rats. Circulating levels of proinflammatory mediators increase rapidly after CLP surgery. Plasma endotoxin levels are elevated as early as 1 h after surgery, and peak levels of TNF-α are achieved by 2 h (42). Plasma IL-6 is thought to be an important indicator of outcomes in septic animals and humans (32, 35). In the present study, we observed an increase in IL-6 at 6 h that was sustained through 12 h, confirming previous observations (12, 42) (Fig. 1). Administration of a single dose of 4F, after the induction of sepsis, significantly reduced IL-6 levels at the 12-h time point. By 24 h, IL-6 was reduced in CLP rats and was not significantly different from sham-operated controls or 4F-treated rats.

Since cardiac dysfunction is a component of MODS (10), a major goal of this study was to assess LV function in CLP rats and to determine whether it was influenced by 4F administration. Our data suggest that cardiac contractility per se is not influenced by CLP over the 24-h study period, since FS was similar in CLP and sham-operated rats. In contrast, EDV, SV,
and CO were significantly impaired in CLP rats (Table 1, Fig. 2), suggesting a defect in cardiac performance due to impaired LV filling. Administration of 4F significantly improved these parameters of cardiac function in CLP rats.

Since both preload and afterload influence cardiac function, we assessed pressure differences across the heart in sham-operated and CLP rats. MAP was similar in control, CLP, and 4F-treated CLP rats and ranged from 113 to 115 mmHg. Previous reports show varying blood pressure responses to CLP surgery. Some studies suggest a reduction in MAP (60–100 mmHg) in rats 24 h after CLP surgery (4, 15), while other data show that MAP is unchanged at time points ranging from 24 to 72 h postsurgery (17, 25). Our findings are in agreement with the latter observations. In contrast to MAP, RAP was reduced in CLP rats compared with control rats, revealing a decrease in the filling pressure of the right side of the heart. 4F treatment was associated with a significant increase in RAP (Fig. 3). A previous study suggested that a decrease in CO in CLP rats is associated with a reduction in plasma volume (44). In this regard, our data also show a significant reduction in circulating plasma volume (35% reduction) in CLP rats compared with sham-operated controls that was attenuated by 4F treatment (Fig. 3). Reduced plasma volume was associated with an increase in wet-to-dry weight ratios for lungs and hearts of CLP rats. Collectively, these results suggest that impaired cardiac performance (reduced EDV, SV, and CO) in CLP rats is due to a reduction in circulating blood volume, venous pressure, and LV filling, rather than to an intrinsic defect in the heart itself. These changes may be due to one of two factors. First, there may be a significant increase in unstressed blood volume in CLP rats, resulting in a decrease in venous return. Second, blood volume reduction may be secondary to fluid loss from edema in CLP rats. In support of the latter, lung and heart water content were increased in CLP rats. Extensive fluid accumulation was also consistently observed in the peritoneal cavity of CLP rats.

Defects in lipoprotein metabolism accompany sepsis, and reduced plasma HDL concentration is associated with increased mortality (38, 45). In the present study, a reduction in plasma TC in CLP rats was associated with a prominent reduction in HDL cholesterol. Reduced HDL also correlated with functional cardiac impairment (i.e., reduced EDV, SV, and CO) in CLP rats (Fig. 6). The apolipoprotein content of HDL isolated from CLP rats was altered compared with shams and was characterized, most prominently, by reduction in apoA-I and apoA-IV and an increase in apoE (Fig. 6). These changes are consistent with those observed in patients with systemic inflammatory response syndrome (5). Changes in the apolipoprotein composition of HDL may underlie the reduction in the plasma concentration of the particle in CLP rats. The important role of apoA-I as a mediator of reverse cholesterol transport has been well established, and apoA-I also possesses prominent anti-inflammatory properties (28). Loss of apoA-I is associated with the conversion of HDL from an anti-inflammatory to a proinflammatory particle (22, 39). Incorporation of acute phase proteins in the HDL particle has been linked to an increase in the catabolism of HDL and its apolipoproteins (8, 37). Cytokines may also contribute to a reduction in HDL cholesterol by inhibiting the hepatic synthesis of apoA-I and other apolipoproteins (13). The functional role of apoA-IV is less clearly understood, but data suggest that it plays a role in activation of the HDL-associated enzyme lecithin-cholesterol acyltransferase. apoE is an exchangeable apolipoprotein that acts as a ligand for hepatic receptors that mediate cholesterol.

![Fig. 6. Sepsis-induced changes in HDL-associated proteins are reversed by 4F. HDL from sham-operated rats (n = 6) and CLP rats receiving saline vehicle (n = 6) or 4F (n = 6) was isolated by ultracentrifugation. Top: representative SDS gel showing Coomassie blue staining for separated proteins. The identity of these bands was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Bottom: the density of specific bands was measured to determine changes in the relative concentration of each protein in HDL fractions. Values are means ± SE. *P < 0.05 and **P < 0.01, significant differences compared with sham-operated controls. #P < 0.05 and ##P < 0.01, significant differences compared with CLP rats.](http://ajpheart.physiology.org/)

Fig. 7. 4F treatment improves survival in CLP rats. Sham-operated rats (n = 17) and CLP rats receiving either saline vehicle (n = 21) or 4F (n = 14) were assigned to a 48-h survival study. *P < 0.05, significant difference compared with sham-operated controls. **P < 0.05, significant difference compared with CLP rats receiving saline vehicle. [Fig. 7](http://ajpheart.physiology.org/)
clearance. An increase in the apoE content of HDL isolated from CLP rats may be an additional mechanism to explain HDL clearance from the circulatory system. The observed changes in HDL-associated apolipoproteins were attenuated by 4F treatment and provide proof of concept that circulating HDL in septic rodents is modulated by 4F administration.

Plasma LDL cholesterol was significantly elevated in CLP rats in the presence and absence of 4F treatment (Fig. 4). The proinflammatory nature of LDL has been ascribed to the presence of cytotoxic LOOH (33, 43). There was a significant increase in the LOOH content of plasma from CLP rats that was normalized, however, by 4F treatment. These data are in agreement with previous reports showing that 4F does not directly influence absolute levels of LDL cholesterol in hyperlipidemic mice, but reduces its atherosogenic and/or proinflammatory properties (27, 29). The observation that 4F binds to oxidized lipids with high affinity represents an underlying mechanism of action of this peptide (41). In addition to this, data suggest that 4F induces the formation of new HDL particles that are enriched in paraoxonase, an enzyme that degrades LOOHs (29). Both of these mechanisms of peptide action are thought to reduce inflammatory injury associated with circulating LOOH.

Administration of reconstituted HDL to humans treated with low-dose LPS reduces TNF-α, IL-6, and IL-8 release and decreases expression of monocytic mCD14 (31). Similarly, overexpression of apoA-I in mice results in an increase in circulating HDL levels that confers protection against the administration of exogenous LPS compared with wild-type controls (24). While HDL exerts beneficial effects in the context of sepsis, its protective mechanism(s) of action is unclear. The binding of endotoxin to plasma lipoproteins is associated with reduced LPS toxicity in vivo and in vitro (6, 14). It has been suggested that HDL neutralizes LPS via insertion and masking of the lipid A domain of LPS in the phospholipid leaflet on the surface of the HDL particle (24). Relatively few studies have examined effects of apoA-I mimetic peptides in the context of sepsis. A previous report showed that administration of the apoA-I mimetic peptide 18A, which shares structural similarities with 4F, improved survival in LPS-treated mice (24). Sepsis in CLP rats is associated with 24-h mortality rates that range from 15 to 94% (17, 20). We found that 4F administration, 6 h post-sepsis induction, improved survival on days 1 (86%) and 2 (57%) compared with CLP rats receiving an equivalent volume of saline vehicle (62 and 29%, respectively) (Fig. 7).

It is proposed that the loss of HDL functionality is an early response to sepsis, and that this impacts on LV filling and CO. As sepsis progresses, other pathological mechanisms are induced that contribute to further cardiac impairment. The protective mechanism of 4F action in CLP rats may be due to one or more factors. First, 4F may induce the formation of small, dense, pre-β-HDL-like particles that are enriched in apoA-I and paraoxonase activity, as reported in apoE-null mice (1). Second, 4F may interact with circulating plasma lipoproteins to form HDL-like particles that effectively bind and neutralize bacterial toxins. This is consistent with our observation that injection of [14C]4F in CLP rats results in the localization of 90–95% of radioactive counts in the HDL fraction within 10 min (not shown). Scavenging LPS and other toxins will subsequently reduce inflammation and associated changes in plasma lipoprotein levels. Third, 4F may reduce inflammatory injury by directly inhibiting the synthesis and release of cytokines. In this regard, 4F reduces the release of IL-6 in influenza-infected rodents (40). In the context of CLP-induced inflammatory injury, 4F likely prevents the activation of proinflammatory cascades and improves cardiac performance and survival via one or more of these mechanisms. It is proposed that this apoA-I mimetic peptide, which is currently undergoing clinical evaluation, may be effective in reducing complications associated with sepsis in humans.

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DISCLOSURES

G. M. Anantharamiaiah is a Principal in Bruin Pharma, Inc.

REFERENCES

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