Endotoxin-induced cardiovascular dysfunction in mice: effect of simvastatin

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Endotoxin-induced cardiovascular dysfunction in mice: effect of simvastatin. J Appl Physiol 111: 1118–1124, 2011. First published July 21, 2011; doi:10.1152/japplphysiol.00158.2011.—Lung infections are associated with acute lung injury (ALI), systemic inflammation, and vascular events. Clinical studies suggest that statins improve health outcomes of patients with pneumonia and ALI. The mechanisms by which this occurs are unknown. The aim of this study was to determine whether statins attenuate systemic inflammation and cardiovascular dysfunction related to ALI in mice. Simvastatin (SS; 20 mg/kg) or vehicle solution was instilled intraperitoneally into mice 24 h before and again just prior to intratracheal LPS instillation (1 μg/g). These mice were then anesthetized with 2.0% isoflurane and underwent a short surgical procedure to instill LPS. Four hours later, IL-6 levels in bronchoalveolar lavage fluid and in arterial and venous serum were measured. Cardiac function was evaluated using 2-D echocardiography, and endothelial function was determined using wire myography on aortic sections. LPS induced a significant increase in serum IL-6 levels. SS reduced venous (P = 0.040) but not arterial concentrations of IL-6 (P = 0.112). SS improved the maximal vasodilatory response of the aorta to ACh (P = 0.004) but not to sodium nitroprusside (P = 1.000). SS also improved cardiac output (P = 0.023). Vasodilatory response to ACh was impaired when aorta from untreated mice was incubated with ex vivo IL-6 (P = 0.004), whereas in the aorta from mice pretreated with SS, the vasodilatory response did not change with IL-6 incubation (P = 0.387). SS significantly improved LPS-induced cardiovascular dysfunction possibly by reducing systemic expression of IL-6 and its downstream signaling pathways. These findings may explain how statins improve health outcomes in patients with ALI.

Acute Lung Injury (ALI) related to bacterial infections (e.g., pneumonia) are commonly characterized by a systemic inflammatory response, which, in a dose-dependent fashion, causes extrapulmonary end-organ damage (3, 14, 26, 33). In many cases, the systemic inflammatory response occurs in the absence of overt bacteremia and may progress even after the putative bacterial organisms have been fully treated (33). Epidemiologic and clinical studies suggest that the systemic inflammatory response related to ALI transiently induces endothelial dysfunction and increases the risk of cardiovascular events (20, 22, 31, 38, 40, 47). In clinical studies, serum IL-6 is consistently associated with an increase in the risk of cardiovascular disease (2, 15, 21, 32). Although in humans it is not certain whether IL-6 is mechanistically involved in this process or whether it is acting solely as a biomarker, in animal models, IL-6 appears to be causally involved (25, 39) and downregulation of IL-6 largely prevents the endothelial dysfunction and other cardiovascular endpoints related to acute lung injury (25, 39).

Statins are potent inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (35). Although they were developed to treat hypercholesterolemia and atherosclerosis, there is increasing evidence that they have pleiotropic effects in modulating the inflammatory response (8, 19). Recent clinical studies suggest that treatment with statins can abrogate the systemic inflammatory response associated with ALI and improve health outcomes including survival in patients with sepsis (10, 18, 37). However, the mechanism by which this occurs remains largely unclear. In the present study, we used a murine model to test the hypothesis that short-term pretreatment with simvastatin (SS) attenuates systemic inflammation, improves endothelial dysfunction induced by acute endotoxin exposure to the lungs, and that this process is at least in part mediated by IL-6.

Materials and Methods

Animal Model

Animals. We used C57BL/6j male mice with an average weight of 24.8 ± 1.5 g [mean ± standard deviation (SD)] that were purchased from Jackson Laboratory (Bar Harbor, ME). All animals were 10–12 wk old at the start of the experimental protocol and were maintained on a standard mouse chow and housed in a controlled environment with a 12:12-h light/dark cycle in an animal facility at St. Paul’s Hospital Vancouver, BC. This study was approved by the Animal Care Committee of the University of British Columbia (A06-1494). The care and handling of the animals were in accordance with the policies promulgated by the Canadian Council on Animal Care.

Pretreatment with SS and instillation of LPS. The mice were randomly divided into three groups: 1) those treated with SS followed by intratracheal instillation of LPS (L2630, Sigma-Aldrich, St. Louis, MO; SS + LPS group), see below; 2) those treated with two doses of PBS (Invitrogen, Grand Island, NY) followed by intratracheal LPS instillation (PBS + LPS group); and 3) untreated control group that was not exposed to LPS or treated with SS or PBS (untreated group). In the SS + LPS group, SS (s6196, Sigma Aldrich, St. Louis, MO; 20 mg/kg) suspended in 500 μl of PBS was administered intraperitoneally twice, 24 h before and just prior to LPS instillation as previously described (18, 41). These mice were then anesthetized with 2.0% isoflurane and underwent a short surgical procedure to create a small tracheostomy, which was used to instill 25 μg of LPS mixed in 50 μl of sterile saline through a 28-gauge needle. We chose a relatively low dose of LPS to induce lung inflammation without causing significant structural damages to the lung and to model various insults relevant for human disease (e.g., pneumonia; 13, 16, 17). After the instillation, the incision site was closed and the mice were allowed to recover from

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the anesthetic procedure. The same procedure was used for mice in the PBS+LPS group, except that these mice received PBS instead of SS.

Surgical procedure to collect arterial and central venous blood simultaneously from mice. To determine the arterial-venous (A-V) gradient of IL-6 across the lungs, we collected blood as close as possible to the pulmonary artery at or near the right atrium (the source of central venous blood) and the ascending aorta (the source of arterial blood) simultaneously 4 h after LPS instillation (42). We selected the 4 h time point because it is associated with a brisk inflammatory response in the lungs and systemic circulation related to LPS using this model (42). Full details have been published previously (42).

Measurements of IL-6. The blood samples were centrifuged (400 g, 4°C, 5 min) soon after the blood collection. From the serum, IL-6 was measured using a mouse-specific IL-6 ELISA kit (R&D System, Minneapolis, MN). The lower detection limit of this kit was 7.8 pg/ml.

Evaluation of Pulmonary Inflammation

Cell count, cell differentials, and IL-6 levels in bronchoalveolar lavage. Bronchoalveolar lavage (BAL) was performed just following blood collection. The lungs were harvested from the mice, 1 ml of 0.6 mg/ml EDTA-PBS solution (0.5 M EDTA; Wako, Richmond, VA) was instilled through the trachea using a 24-gauge catheter, the whole lungs were washed once, and then the instilled solution was collected and placed on ice (41). The volume of recovered fluid from each lung was 0.63 ± 0.05 ml (mean ± SD). The BALF was then centrifuged (400 g, 4°C, 5 min) and the supernatants were collected. The total cell count was evaluated with a hemocytometer (41). Cell differentials were evaluated on smears prepared by cytospin and stained with Wright-Giemza’s stain (Miles Scientific, Naperville, IL; 41). Differential cell counts (macrophages, neutrophils, lymphocytes, eosinophils, and basophils) were performed by counting 200 cells selected in random fields of view for each animal. The concentrations of IL-6 were measured as previously described.

Evaluation of Systemic Influence Induced by Experimental Lung Inflammation

Measurement of the vascular responses. To determine the influence of lung inflammation on extrapulmonary tissues, we examined the endothelial function of abdominal aorta using wire myography (5, 29, 30, 43, 49). A concentration-response and maximal endothelium-dependent vasorelaxation and those to SNP were determined using abdominal aorta rings from untreated mice. Briefly, the relaxant responses to ACh were determined to evaluate endothelial function of abdominal aorta using wire myography (5, 29, 30, 43, 49). A concentration-response and maximal endothelium-dependent vasorelaxation and those to SNP were determined using abdominal aorta rings from the untreated mice. Briefly, after euthanasia, the aorta was harvested from the mouse and placed in ice-cold PSS. The vessel was treated as described above, sectioned into two pieces of 2 mm ring, and mounted on a wire myography chamber. We repeated these experiments using aorta harvested from mice pretreated with SS to determine whether SS prevented endothelial dysfunction by altering the inflammatory pathways up- or downstream from IL-6.

Echocardiography. Echocardiography was performed in PBS+LPS and SS+LPS groups at three time points: before IP treatment with SS or vehicle solution (PBS; steady state), 1 h following the second IP treatment with SS or PBS (just prior to LPS instillation), and 4 h following LPS instillation. Nine mice were randomly divided into two groups: PBS+LPS (n = 4) and SS+LPS (n = 5). Echocardiography was conducted while the mice were anesthetized with 2.0% isoflurane and given supplemental oxygen of 1.5 l/min. The mice were kept warm at 37°C using a heating pad. Using VisualSonics vevo 770 (VisualSonics, Toronto, ON, Canada), the heart was imaged in the 2-D and M-modes at the level of papillary muscles (11). End-diastolic left ventricular inner dimension (LVID;d) and end-systolic left ventricular inner dimension (LVID;s) were measured by tracing the inner anterior and posterior walls (11). Diastolic left ventricular volume (LV Vol;d), systolic left ventricular volume (LV Vol;s), stroke volume (SV), cardiac output (CO), and ejection fraction (EF) were calculated using standard formulas: LV Vol;d (μl) = [7.0/(2.4+LVID;d)] × LVID;d³, LV Vol;s (μl) = [7.0/(2.4+LVID;s)] × LVID;s³, SV (μl) = LV Vol;d − LV Vol;s, EF (%) = (LV Vol;d − LV Vol;s)/LV Vol;d × 100, CO (ml/min) = SV (μl) × heart rate(min)/1,000.

Surfactant protein-D, albumin, and other cytokine levels in serum and BALF. To further investigate the effect of SS on lung inflammation, the levels of surfactant protein-D (SP-D), albumin (to evaluate vascular permeability and determine whether translocation occurred from the blood to the lungs), and other cytokines were evaluated in arterial serum and BALF, which were collected 4 h after LPS instillation. For this purpose, 20 mice were randomly divided into the two groups: PBS+LPS (n = 10) and SS+LPS (n = 10). SP-D levels in both arterial serum and BALF were measured with the ELISA kits and albumin levels in BALF were measured with mouse albumin ELISA kits (Immunology Consultants Laboratory, Newberg, OR). The albumin levels were standardized to the total protein levels in BALF, which were measured with Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Rockford, IL). The lower detection limit of the SP-D ELISA kit was 0.89 ng/ml, that of the albumin kit was 7.8 ng/ml, and that of the protein assay kit was 25 μg/ml. The concentrations of the other inflammatory cytokines in BALF were measured using a multiplex suspension bead array immunoassay (Luminex, Austin, TX) with Mouse Cytokine 20-Plex kit (Invitrogen, Carlsbad, CA) according to the manufacturer’s instruction. The panel included IFN-γ-inducible protein of 10 kDa (IP-10), monocyte chemoattractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF), TNF-α, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, keratinocyte-derived chemokine, IL-10, IL-12, IL-13, IL-17, IFN-γ, basic fibroblast growth factor, monokine induced by interferon gamma, macrophage inflammatory protein-1α, and granulocyte-macrophage colony-stimulating factor. We also determined arterial pH and serum lactate acid levels in a subset of these mice 4 h after LPS or saline instillation to determine whether LPS exposure resulted in significant metabolic acidosis.

Table 1. Serum IL-6 levels in arterial and venous blood at 4 h postinstillation

<table>
<thead>
<tr>
<th>Source</th>
<th>Untreated</th>
<th>PBS+LPS</th>
<th>SS+LPS</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial, pg/ml</td>
<td>17.7 ± 5.5*</td>
<td>1280 ± 277†</td>
<td>802 ± 116†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Venous, pg/ml</td>
<td>11.1 ± 3.9</td>
<td>744 ± 156†</td>
<td>414 ± 71†‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6 translocation from the lungs, pg·min⁻¹·g⁻¹</td>
<td>1.8 ± 0.5</td>
<td>71.3 ± 26.1*</td>
<td>85.4 ± 14.9†</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are shown as means ± SE. *The level was significantly higher than the venous level (P < 0.05). †The level was significantly higher compared to that in the untreated group (P < 0.05). ‡The level was significantly lower compared to that in the PBS+LPS group (P < 0.05). Comparisons of the IL-6 levels and arterial-venous (A-V) gradient of IL-6 across the groups were done with ANOVA with post hoc Bonferroni correction.
Table 2. Cell count, cell differentials, and interleukin-6 levels in bronchoalveolar lavage fluid at 4 h postinstillation

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>PBS+LPS</th>
<th>SS+LPS</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell count, ( \times 10^6 )</td>
<td>2.7 ± 0.5</td>
<td>8.1 ± 1.2*</td>
<td>9.2 ± 0.9*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Neutrophil count, ( \times 10^3 )</td>
<td>0.5 ± 0.2</td>
<td>51.9 ± 14.9*</td>
<td>67.4 ± 8.3*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(percentage total cell count)</td>
<td>(2 ± 0.3)</td>
<td>(55 ± 8.4)</td>
<td>(70 ± 3.4)</td>
<td></td>
</tr>
<tr>
<td>Macrophage count, ( \times 10^3 )</td>
<td>25.7 ± 4.8</td>
<td>27.0 ± 5.5</td>
<td>25.4 ± 2.8</td>
<td>P = 0.967</td>
</tr>
<tr>
<td>(percentage total cell count)</td>
<td>(98 ± 0.4)</td>
<td>(44 ± 8.3)</td>
<td>(30 ± 3.5)</td>
<td></td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>1.5 ± 0.24</td>
<td>597 ± 75.3*</td>
<td>637 ± 81.3*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are shown as means ± SE. *The level was significantly higher in comparison with that in untreated group (\( P < 0.05 \)). Comparisons of the data across the groups were done with ANOVA with post hoc Bonferroni correction.

**Statistical Analysis**

All results were expressed as mean value ± SE unless otherwise indicated. Data were analyzed using t-tests (for comparison of 2 independent groups), paired t-tests (for within-group comparison), and ANOVA (for repeated or multiple group comparisons). A Bonferroni correction factor was applied to adjust for multiple comparisons. A Pearson correlation was used for correlation analyses. All analyses were conducted using SPSS 11.0 (SPSS, Chicago, IL) and \( P < 0.05 \) (2 tailed) was considered statistically significant.

**RESULTS**

**Acute Effect of SS on IL-6 Levels in Arterial and Venous Serum**

A total of 60 mice were studied to determine the A-V gradient of IL-6 across the lungs. In 56 of these animals (93% success rate), we were able to collect \( \sim 300 \mu l \) of whole blood from the central vein and ascending aorta combined, making it possible to measure serum IL-6 levels from both the central venous and arterial samples. Serum IL-6 levels in the venous and arterial blood, the A-V gradients, and IL-6 translocation from the lungs to systemic circulation at 4 h following LPS instillation are summarized in Table 1.

In the untreated group, the serum IL-6 levels in both the arterial and venous samples were low (arterial levels: 17.7 ± 5.5 pg/ml vs. venous levels: 11.1 ± 3.9 pg/ml; \( P = 0.002 \)). With LPS instillation (PBS+LPS group), both the arterial and venous levels significantly increased (arterial levels: 1,280 ± 277 pg/ml vs. venous levels: 744 ± 156 pg/ml; \( P = 0.011 \)), with a greater response in the arterial than in the venous samples. In LPS-instilled animals pretreated with SS, the venous but not arterial IL-6 levels decreased significantly (venous sample: SS+LPS: 41 ± 71 pg/ml vs. PBS+LPS: 744 ± 156 pg/ml; \( P = 0.040 \); arterial sample: SS+LPS: 802 ± 116 pg/ml vs. PBS+LPS: 1,280 ± 277 pg/ml; \( P = 0.112 \)), suggesting that SS predominantly attenuated systemic rather than lung inflammation in this model (12, 46).

**Fig. 1.** The effects of pretreatment with simvastatin (SS) or PBS 24 h before and again just before exposure to LPS on endothelial function 4 h following intratracheal instillation of LPS. Vasodilatory response to ACh and that to SNP were evaluated with wire myography. Cumulative concentrations of ACh (10 \(^{-6}\)–10 \(^{-6}\) M) were evaluated to evaluate endothelium-dependent NO-mediated vasorelaxation. An identical protocol was used to study the effects of SNP (10 \(^{-8}\)–10 \(^{-7}\) M) to determine endothelium-independent (NO mediated) relaxation. Tests of significance between concentration-response curves were conducted using 2-way ANOVA. The comparisons of the maximal vasodilatory responses across the 3 groups were made with ANOVA. Bonferroni’s correction factor was applied to adjust for multiple comparisons. A: vasodilatory response to ACh at each concentration; B: maximal vasodilatory response to ACh; C: vasodilatory response to SNP at each concentration. *\( P < 0.05 \); †\( P \) values were >0.05 following adjustment for multiple comparison using Bonferroni’s correction factor.
Cell Count, Cell Differentials, and IL-6 Levels in BALF

BALF was collected in 47 animals at the 4 h time point and these findings are summarized in Table 2. LPS exposure significantly increased total cell count in the BALF (PBS + LPS: 8.1 ± 1.2 × 10⁴ cells vs. untreated: 2.7 ± 0.5 × 10⁴ cells; P < 0.001), and pretreatment with SS did not have a significant impact on the total cell count (SS + LPS: 9.3 ± 0.9 × 10⁴ cells vs. PBS + LPS: 8.1 ± 1.2 × 10⁴ cells; P = 1.000). LPS instillation increased the fraction of neutrophils and decreased that of macrophage with no effect of SS on this response (neutrophils: PBS + LPS: 55 ± 8.4%, SS + LPS: 70 ± 3.4%, untreated: 2 ± 0.3%; macrophages: PBS + LPS: 44 ± 8.3%, SS + LPS: 30 ± 3.5%, untreated: 98 ± 0.4%). LPS induced a significant rise in IL-6 in the BALF (PBS + LPS: 597 ± 75.3 pg/ml vs. untreated: 1.5 ± 0.24 pg/ml; P < 0.001). Pretreatment with SS did not significantly change these levels (PBS + LPS: 597 ± 75.3 pg/ml vs. SS + LPS: 637 ± 81.3 pg/ml; P = 1.000). There was a significant relationship between IL-6 levels in BALF and those in arterial (r = 0.522; P < 0.001) and venous (r = 0.443; P = 0.002).

Association Between Lung and Systemic Inflammation and Endothelial Dysfunction

To determine the association between IL-6 and endothelial dysfunction, we determined the relationship of IL-6 in either BALF or serum with the maximal vasodilatory response of abdominal aorta to ACh. Overall, there was a significant correlation of arterial (r = −0.372; P = 0.030) and venous IL-6 levels (r = −0.375; P = 0.029) with the maximal vasodilatory response achieved with ACh.

Influence of LPS-Induced Lung Inflammation and Pretreatment of SS on Vascular Endothelial Function

LPS exposure decreased the vasodilatory response of abdominal aorta to ACh (P = 0.008; Fig. 1A) but not to SNP (Fig. 1C; PBS + LPS vs. SS + LPS, P = 1.000; PBS + LPS vs. untreated, P = 0.102; SS + LPS vs. untreated, P = 0.105). LPS exposure significantly reduced the maximal vasodilatory response to ACh (PBS + LPS: 78.9 ± 5.5% vs. untreated: 95.7 ± 1.7%, P = 0.010; Fig. 1B). Treatment with SS before the LPS challenge attenuated the impaired response (PBS + LPS: 78.9 ± 5.5% vs. SS + LPS: 95.9 ± 2.1%, P = 0.004; untreated: 95.7 ± 1.7% vs. SS + LPS: 95.9 ± 2.1%, P = 1.000; Fig. 1, A and B). In contrast, SS did not improve the vascular response to SNP following LPS exposure (SS + LPS vs. PBS + LPS, P = 1.000; Fig. 1C), suggesting that SS’s acute vascular effect is mediated by an endothelium-dependent mechanism.

Ex Vivo Effects of IL-6 on Endothelial Function of Aortas from Untreated Mice and SS-Treated Mice

To determine the effect of IL-6 on endothelial dysfunction, we measured the vasodilatory response of aorta from the untreated mice to ACh and SNP prior to as well as following 30 min incubation with IL-6. In the group incubated with IL-6 (n = 8), the maximal vasodilatory response to ACh was significantly attenuated following IL-6 organ bath exposure.
(70.3 ± 10.4%) compared with the baseline response without IL-6 (91.7 ± 3.4%; P = 0.046; Fig. 2A and B). We repeated the above experiment using aorta harvested from mice pretreated with SS. In these mice, the vasodilatory response to ACh was not changed by incubation with IL-6 (P = 0.387; Fig. 2C). In untreated as well as SS-treated mice, there was no change in the vasodilatory response to SNP following incubation with IL-6 (untreated: P = 0.158, SS treated: P = 0.319; see online data supplement). In the time control group, we confirmed that vasoresponsiveness of aorta from untreated and SS-treated mice was not attenuated irrespective of the repeated sequences of ACh and SNP in the timeframe of this experiment.

Influence of LPS Instillation and/or SS Pretreatment on Cardiac Function

We investigated the effects of LPS instillation and the impact of SS pretreatment on cardiac output of these mice using echocardiography. These data are summarized in Fig. 3. LPS instillation significantly attenuated cardiac output (steady state: 1 vs. 4 h following LPS instillation: 0.45 ± 0.07, P = 0.014). With pretreatment with SS, the cardiac output at 4 h following LPS instillation was significantly higher than that in PBS+LPS group (SS+LPS: 0.85 ± 0.11 vs. PBS+LPS: 0.45 ± 0.07, P = 0.023).

Effect of SS on Lung Permeability and Inflammatory Indices Related to LPS Instillation

Pretreatment with SS did not change SP-D levels in arterial serum or BALF (arterial serum: PBS+LPS: 5.0 ± 0.5 ng/ml vs. SS+LPS: 5.0 ± 0.5 ng/ml; P = 0.980; BALF: PBS+LPS: 184 ± 25 ng/ml vs. SS+LPS: 162 ± 23 ng/ml; P = 0.535) and albumin levels in BALF (PBS+LPS: 0.50 ± 0.04 vs. SS+LPS: 0.45 ± 0.04; P = 0.465), suggesting that pretreatment with SS did not modify lung permeability. However, the concentrations in BALF of IP-10 (PBS+LPS: 507 ± 64 pg/ml vs. SS+LPS: 150 ± 11 pg/ml; P = 0.025) were significantly reduced by the pretreatment with SS, suggesting that SS suppresses certain components of lung inflammation induced by LPS instillation. We found that LPS exposure did not result in any significant acidemia (mean pH 7.30 ± 0.04 with LPS exposure vs. 7.26 ± 0.14 with saline exposure; P = 0.052) or lactic acidosis (mean serum lactate level with LPS exposure 1.90 ± 1.20 mM vs. 1.20 ± 0.83 mM with saline exposure; P = 0.375). Thus, at this dose of LPS, there was no evidence of significant metabolic acidosis.

DISCUSSION

The potential beneficial effects of statins on blood vessel function, apart from their well known effects on blood lipids, are controversial (1, 4, 6, 8, 10, 18, 19, 23, 24, 35, 37, 41, 44, 48). The present study has three notable findings regarding the pleiotropic effects of statins using an LPS-lung injury model in mice wherein the extent of lung injury was relatively modest. First, pretreatment with two doses of SS significantly reduced the systemic LPS-induced IL-6 production. Second, the systemic inflammatory response was associated with endothelial dysfunction. Pretreatment with SS “normalized” endothelial dysfunction related to LPS and also prevented the fall in cardiac output related to LPS exposure. Third, ex vivo IL-6 induced endothelial dysfunction, which was significantly attenuated by pretreatment with SS. Collectively, these findings suggested that statins improve vascular function via a non-lipid-mediated pathway. It further showed that statins may downregulate the systemic production of IL-6 and possibly effector molecules and pathways downstream of IL-6 that are involved in the beneficial effects of statins on blood vessels.

Our finding that SS did not materially modify certain components of the lung inflammatory response related to acute exposure of LPS is discordant with results from previous studies (10, 18, 37). Some of the discrepancy may be related to the differences in the doses of LPS that were employed. In the studies by Jacobson et al. (18) and Fessler et al. (10), the LPS dose was 2 and 10 times higher, respectively, than the dose we used for our experiments. Not surprisingly, in these previous studies, the investigators noted impressive morphologic evidence of lung injury and damage. In contrast, there was very little histological evidence of lung injury in our model. Indeed, likely owing to the relatively modest dose of LPS that we used, the mice did not experience significant lactic acidemia and appeared generally healthy at the time of death. Furthermore, the previous studies did not evaluate the effects of statin treatment on endothelial function or other cardiovascular endpoints. Thus the findings of our study may not be directly comparable to the previous murine models of LPS-induced ALI.

The findings of the present study are generally consistent with human studies of Shyamsundar et al. (37), who showed in a group
of 30 healthy volunteers that SS at 40 or 80 mg/day once daily for 4 days can significantly attenuate some of the inflammatory response induced by LPS inhalation (50 μg). Interestingly, in Shyamsundar’s study, SS pretreatment had no significant effect on several lung inflammatory indices such as total cell, macrophage, or lymphocyte counts in the BALF and only a marginal effect on neutrophil count (P = 0.05). However, a significant effect was observed on myeloperoxidase and tumor necrosis factor-α in BALF and plasma C-reactive protein (37).

Our data are also consistent with those from Chalmers et al. (4), who demonstrated that patients with pneumonia on statins prior to their hospitalization had serum C-reactive protein levels (whose expression is regulated by IL-6) that were on average 35% lower than pneumonia patients not on statins prior to their hospitalization. Importantly, the 30-day mortality risk in the statin users was over 50% lower than that of non-users (4), adjusted for various confounders such as age, pneumonia severity score, and comorbidities. Similar findings have been reported by several other groups (23, 24, 44). Other groups have shown that in patients with acute bacterial infection, prior use of statins is associated with a reduced risk of developing severe sepsis and admissions to the intensive care unit (1, 48). However, statin use does not appear to modify the risk of bacteremia (44), suggesting that the benefits of statins are likely by modulating the host immune response to the pathogen.

In the present study, we chose IL-6 as a biomarker of systemic inflammation because in human studies, there is a strong correlation between circulating IL-6 levels and cardiovascular morbidity and mortality (6). Moreover, in human studies, serum IL-6 concentrations are negatively associated with endothelium-dependent vasodilatation (9), suggesting that our murine model has relevance for human disease. There is also increasing evidence that IL-6 is more than just a biomarker and that it may play a causal role in this process. For instance, we (39) and others (36) have shown that mice deficient in IL-6 were protected against cardiovascular dysfunction, which could be reversed with exogenous IL-6. Although the exact pathway by which IL-6 causes endothelial dysfunction has not been fully worked out, studies suggest that it does so by downregulating endogenous nitric oxide (NO) synthase expression (27, 28, 34, 39), disrupting the NO-mediated cyclic guanosine monophosphate regulatory axis (5, 30) and increasing the synthesis of endogenous oxidants such as oxygen radicals and superoxides (36). Statins may “rescue” the endothelium from injury by directly suppressing IL-6 expression and by inhibiting the downstream effector molecules such as Janus kinase/signal transducers and activators of transcription-3 pathway (45). Consistent with these previous observations, we found that SS reduced systemic expression of IL-6 and attenuated endothelial dysfunction related to LPS, which could not be reversed by the addition of exogenous IL-6. We previously demonstrated that IL-6-mediated reduction in cardiac output only 4 h following LPS instillation using IL-6-deficient mice (39). In the present study, pretreatment with SS also prevented the fall in cardiac output, at least in part, possibly by reducing systemic IL-6 expression.

There were several limitations to the present study. First, the SS dose used for this study (20 mg/kg) is higher than those used in clinical practice. We chose a higher dose because mice can become resistant to statins by upregulating their expression of HMG-CoA reductase in response to statins (18), and prior studies have suggested that the biologically effective dose in mice in reducing inflammation is between 10 and 30 mg/kg (7, 18, 50). Second, we administered only two doses of SS prior to LPS exposure. We chose to limit the dosing regimen to determine whether a short course of SS taken during the prodromal period of respiratory infections can abrogate the systemic inflammatory response and prevent the serious complications of sepsis. Additionally, we chose a short treatment period to separate out the acute (anti-inflammatory) effects of SS from its anti-lipid effects. Third, we examined the effects of SS on IL-6 and not other inflammatory cytokines. It is possible that statins may work through non-IL-6 pathways.

CONCLUSIONS

In summary, using a murine model, we demonstrated that short treatment with SS attenuated the LPS-mediated rise in systemic IL-6 and endothelial dysfunction. These data suggest that statins have anti-inflammatory effects that may be useful during the prodromal period in acute infection to attenuate the risk of morbidity and mortality.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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