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HMG-CoA Reductase Inhibitors Decrease Hyperglycemia on Animal Model of Systemic Inflammatory Response Syndrome (SIRS)

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Abstract

Systemic Inflammatory Response Syndrome (SIRS) is a pathophysiologic state associated with trauma and major surgery. Inflammatory cytokines plays an important role in the pathogenesis of SIRS. Cytokines induce the activation of the classic hypothalamic-pituitary stress response that leads to increase of secretion of the stress hormone ACTH and hyperglycemia. HMG-CoA reductase inhibitors, such as statins, have been shown to have anti-inflammatory properties. Recent studies in humans indicate that the perioperative use of statins may decrease morbidity and mortality. Here we tested the hypothesis that statins may decrease the cytokine-induced stress response and hyperglycemia in a murine model of SIRS. SIRS was induced with intraperitoneal injection of 0.1 mg lipopolysaccharide (LPS) per mouse. Mice were pretreated with 0.5 mg of simvastatin or lovastatin 18 hours before the administration of LPS, and plasma levels of Interleukin-2 (IL-2), Tumor Necrosis Factor alpha (TNF-α), the stress hormone adrenocorticotrophic hormone (ACTH), and glucose were determined. We observed that pretreatment of mice with statins nearly completely suppressed the LPS-induced cytokine, ACTH and hyperglycemic responses.

Conclusion: Statins suppress cytokine production in a murine model of SIRS. This decreased cytokine production may lead to suppression of the SIRS-induced stress response and hyperglycemia. We postulate that statins may have an important role as regulators of the SIRS and stress response induced by surgery and trauma, and that akin to β-blockers, statins may become part of the therapeutic arsenal aimed to decrease perioperative morbidity and mortality.

Keywords: Mice; Systemic inflammatory response syndrome (SIRS); Simvastatin; Lovastatin; Statins; Cytokines; Glucose; Stress response; Lipopolysaccharide

Abbreviations: SIRS: Systemic Inflammatory Response Syndrome; LPS: Lipopolysaccharide; IL-2: Interleukin-2; TNF-α: Tumor Necrosis Factor Alpha; ACTH: Adrenocorticotrophic Hormone

Introduction

Systemic Inflammatory Response Syndrome (SIRS) is an often noted complication associated with trauma and major surgery [1-3]. SIRS resembles sepsis in many ways and not coincidentally is associated with major morbidity and mortality [1]. SIRS is initiated by trauma that triggers an increase in pro-inflammatory cytokines, namely tumor necrosis alpha (TNFα), IL-2 and others (1-2). Hypercytokinemia is also associated with activation of the classic stress response mediated by the hypothalamic-pituitary-adrenal axis [1,4-5]. Cytokines have been shown to induce the endogenous secretion of ACTH and corticosteroids [4-5]. Activation of the stress response has many pathophysiological effects, one of which is hyperglycemia [4-5]. It has been clearly shown that perioperative hyperglycemia increases morbidity and mortality in critically ill hospitalized patients, and initiating a practice of strict glycemic control is an important modality to reduce mortality in the ICU [6]. HMG-CoA reductase inhibitors (statins) have unique anti-inflammatory properties [7] that have been suggested to play a role in prolonging survival in a murine model of sepsis and decreasing the progression to sepsis and need for ICU admission in patients with pneumonia [8-9]. Furthermore, stains are associated with decreased perioperative cardiovascular morbidity and mortality in vascular surgery patients [10-12]. The mechanism by which statins decrease perioperative mortality and improve survival in animal models of sepsis/SIRS is not completely understood. We postulate that statins may decrease perioperative morbidity and mortality at least in part by attenuating the inflammatory induced stress response and resultant hyperglycemia observed during and after surgical trauma.

Material and Methods

Materials and methods

Lipopolysaccharide (LPS) was purchased from Sigma-Aldrich Corp. (St. Louis, MO). Simvastatin and lovastatin were obtained from Calbiochem (La Jolla, CA). C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). ACTH ELISA kit was ordered from Research Diagnostics, Inc. (Concord, MA). IL-2 ELISA kits were purchased from R & D Systems (Minneapolis, MN). TNF-α ELISA kit was obtained from Pierce Biotechnology, Inc. (Rockford, IL). Glucose assay kit was purchased from Molecular Probes (Eugene, OR).

Animal care and treatment

All experiments were approved by the Mayo Clinic's Institutional Animal Care and Use Committee. Female C57BL/6J mice (5-8 wk old, 20-25 g) were treated by intraperitoneal injection (IP) with simvastatin

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(0.5mg/animal, 200ml/injection) or vehicle (PBS) 18±2 hours and 3±1 hours prior to IP administration of LPS (0.1mg/animal). Simvastatin was dissolved in ethanol at a concentration of 8-10mg/mL and diluted in PBS to yield the final concentration for injection. Three hours following LPS injection the mice were anesthetized by intramuscular injection of ketamine (150 mg/kg) and xylazine (15 mg/kg) and blood withdrawn from the abdominal vena cava using a heparinized 20 gauge needle. The blood was centrifuged at 100 xg for 5 minutes, the plasma aliquoted into microcentrifuge tubes and samples stored at -80°C until assayed.

ACTH production

ACTH levels were determined by a two-site ELISA according to the manufacture's protocol. It uses affinity purified goat polyclonal antibody and a mouse monoclonal antibody to specific and well defined regions of the ACTH molecule. Calibrators and samples are simultaneously incubated with antibodies in a streptavidin-coated microplate well. At the end of the assay, the microwell plates were washed to remove unbound components and the bound enzyme incubated with the tetramethylbenzidine (TMB). The manufacturer claims sensitivity to 0.46 pg/mL ACTH and cross-reactivity to a-MSH of <5.65% and b-endorphin of < 0.01%.

IL-2 measurement

IL-2 was measured using the quantitative sandwich ELISA technique. Briefly, antibody specific for mouse IL-2 was pre-coated onto a microplate. Standards, controls, and samples were pipette into wells. After washing to remove unbound compounds, an enzyme-linked antibody to IL-2 was added, followed by a second washing, and addition of a substrate solution. The reaction yields a blue color which turns yellow when the Stop Solution was added. The absorbance was then determined on a Molecular Devices Spectramax Plus microplate reader (Sunnyvale, CA) at 450nm wavelength with correction set at 540nM. The sample values were then determined by comparison to the standard curve.

Measurement of TNF-α concentration

TNF-α concentrations were measured using enzyme-linked immunosorbent assay (ELISA). Standards and samples were incubated with anti-mouse TNF-α in a microplate. After incubation, biotinylated antibody was added and incubated for 2 hours. Following wash, streptavidin-HRP and substrate solution were added to each well. Absorbance was measured on an ELISA plate reader set at 450 nm with a correction at 550 nm. The manufacturer claims sensitivity of <9 pg/ml of mouse TNF-α and cross-reactivity with rabbit and cynomologous monkey TNF-α in culture supernatants.

Glucose assay

Glucose levels were determined using the Amplex red glucose/glucose oxidase assay kit. Reactions containing 50μM Amplex Red reagent 0.1 U/mL HRP, 1 U/mL glucose oxidase and known concentrations of glucose in 50 mM sodium phosphate buffer, pH 7.4 were incubated for 30 minutes at room temperature. In this assay, glucose oxidase reacts with D-glucose to form D-gluconolactone and H_2O_2. In the presence of horseradish peroxidase (HRP), the H_2O_2 then reacts with the Amplex Red reagent in a 1:1 stoichiometry to generate the oxidation product, resorufin. The absorbance was then determined spectrophotometrically at 560nm. With this method as little as 3μM D-glucose can be detected.

Data analysis and statistics

The reported experiments were repeated three times and data expressed as mean ± SE. The unpaired t test was used for statistical analysis. P≤0.05 was significant. The figures show the average of 3 independent experiments that include 8 mice in each group. The total number of mice pre group was 24.

Result and Discussion

Effect of simvastatin on LPS-induced pro-inflammatory cytokines

It has been previously shown that during the development of SIRS is associated with an increase in pro-inflammatory cytokine concentrations [1-3]. This increase in cytokines appears to have a major role on the pathogenesis of sepsis and peri-operative SIRS [1-3]. In this investigation the effect of simvastatin on the levels of two pro-inflammatory cytokines (IL-2 and TNF-α after LPS injection in mice was analyzed. IL-2 was measured as it has been shown to be elevated in both animal models of SIRS and in surgical patients [1-3]. Furthermore, systemic administration of IL-2 can lead to a SIRS like syndrome in humans [13]. IL-2 is synthesized primarily by T-helper lymphocytes which have been activated by certain mitogens or by interaction of the T-cell receptor complex [1]. The response of T-helper cells to activation is a release of IL-2 and an induction in expression of IL-2 receptors [1]. Neutrophils and macrophages demonstrate augmented function in the presence of IL-2. IL-2 may be an important modulator of the activity of polymorph nucleated cells (PMN) during SIRS. Statins have been shown to suppress T-helper lymphocytes *in vitro* and *in vivo*, in addition to suppression of IL-2 levels [14].

Another marker of inflammation used in our study was TNF-α. TNF-α is a classic mediator of the sepsis/SIRS [1-3]. Levels of TNF-α increase during sepsis, SIRS and surgical trauma (1-3). TNF-α is produced by many cells but it may be a marker of PMN during SIRS [1-3]. Pre-treatment of mice with simvastatin decreased the LPS-induced elevation in plasma levels of IL-2 and TNF-α (Figure 1).

Statins and the stress response in SIRS

The stress response is an important component of the SIRS [1,4-5]. Activation of the inflammatory cascade leads to activation of the hypothalamic-pituitary-adrenal axis with increased secretion of CRH, ACTH and corticosteroids [1,4-5]. Both IL-2 and TNF-α have been shown to directly and indirectly activate the secretion of ACTH by the pituitary [1,4-5]. ACTH levels are a specific and consistent marker of the inflammatory-induced stress response [4,5,15]. Here, we describe that the pre-treatment of mice with simvastatin can almost completely prevent the increase in ACTH release after LPS treatment (Figure 2). This observation indicates that statins decrease the stress response induced by SIRS. We further explored the effect of statins upon the LPS-induced hyperglycemia. As expected by the effect of simvastatin upon the cytokine levels and ACTH levels, the hyperglycemic response induced by SIRS was completely abrogated by simvastatin (Figure 3).

We also observed that the effect of statins upon the inflammatory and stress response is dependent on the concentration of LPS used to induce the SIRS and on the concentration of the statin. An overwhelming inflammatory response induced by high concentrations of LPS (0.5μg/mice) was less likely to be blocked by simvastatin (data not shown). In conclusion statins can block the stress response in models of SIRS by

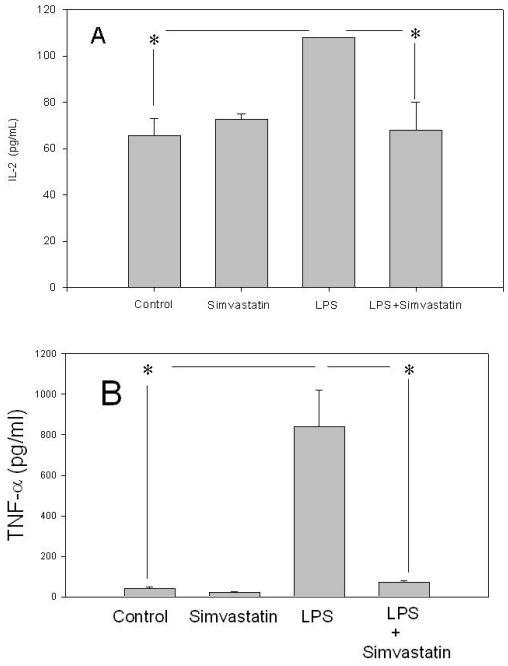

Figure 1: Effect of Simvastatin on LPS-induced IL-2 and TNF-alpha levels. Mice were treated with Simvastatin and LPS as described in Materials and Methods. The inflammatory response was determined be measurement of the cytokines IL-2 and TNF-α. In A Levels of IL-2 were determined 3 hs after treatment with LPS. LPS–induced IL-2 levels were found to be higher in LPS groups than that of LPS+Simvastatin group. In B LPS-induced TNF-α concentration were determined after treatment of mice with simvastatin, LPS, or simvastatin and LPS as described in material and methods. The data is an average of 3 experiments with 8 mice in each group. The differences between control and LPS, and LPS X LPS+ simvastatin were statistically significant (*).

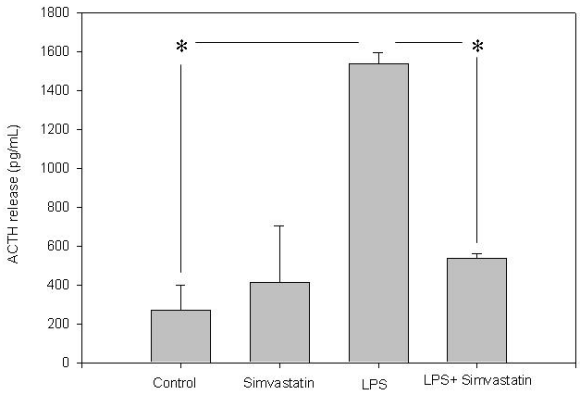

Figure 2: Effect of Simvastatin on LPS-induced ACTH release. ACTH levels were determined after the treatment with simvastatin, LPS or LPS with simvastatin as described above. The data is an average of 3 experiments with 8 mice in each group. The differences between control and LPS, and LPS X LPS+ simvastatin were statistically significant (*).

blocking the generation of cytokines. Statins may have a direct effect on cytokine levels and responsiveness of the hypothalamic pituitary axis.

Clinical implications

HMG Co-A reductase inhibitors are potent inhibitors of cholesterol synthesis and their ability to reduce serum cholesterol and prevent coronary atherosclerosis and acute coronary events has made medications in this class some of the most popular pharmaceuticals prescribed in the world [16-18]. In addition to their lipid lowering effects, statins are potent modulators of the inflammatory response [7,14,19-20]. Statins have been shown to alter endothelial expression of cellular adhesion molecules, alter monocyte migration, macrophage differentiation, and secretion of inflammatory cytokines from macrophages and T cells [7,14]. These anti-inflammatory properties are believed to result in coronary plaque stabilization which may explain the association between statin therapy and reduction in perioperative cardiovascular morbidity shown in a number of recently published manuscripts [10-12].

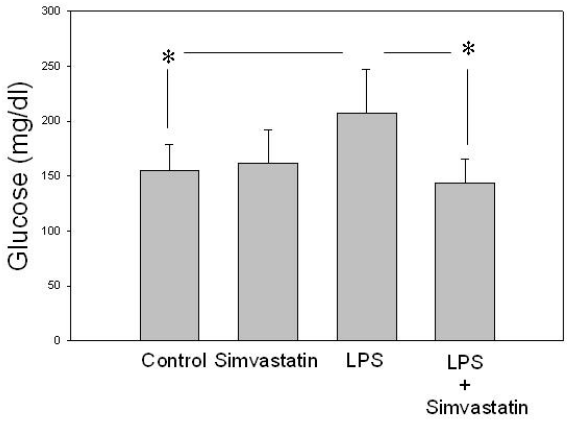

Figure 3: Glucose levels after simvastatin and LPS treatment. Glucose evels were determined after the treatment with simvastatin, LPS or LPS with simvastatin as described above. The data is an average of 3 experiments with 8 mice in each group. The differences between control and LPS, and LPS X LPS+ simvastatin were statistically significant (*).

From animal data, it has been shown that the anti-inflammatory effect of statins is not just a local response at the level of the coronary plaque, but rather a systemic response. Elevated cytokines and hyperglycemia are associated with increased morbidity and mortality in critically ill patients [6-19]. Hyperglycemia is an end product of the systemic stress and inflammatory response. Although it is quite difficult to define a serum concentration of cytokines that places a critically ill individual at risk for morbid events, strict glycemic control improves outcome in this patient population [6]. Improved survival with statin therapy has been show in a murine model of sepsis [8,21-22]. Human studies addressing this subject are few, however statin therapy has been shown to decrease the progression of sepsis and need for ICU admission in patients with pneumonia [9]. One could hypothesize that attenuating the systemic inflammatory response with statins may improve morbidity and mortality associated with SIRS. The ability of statin therapy to decrease IL-2, TNF-a, ACTH, and serum glucose in mice injected with LPS, as shown in this study, should foster support for a prospective study of statins as a potential therapy for surgical and trauma induced SIRS in humans. We propose that statins may be an important adjuvant therapy during the perioperative period to suppress SIRS and stress response. The beneficial effects of statins during the perioperative period may not be only limited to its lipid lowering properties and atherosclerotic plaque stabilization, but also due to their

anti-inflammatory effects. We postulate that akin to β-blockers, statins may become important components of therapies aimed at decreasing perioperative morbidity and mortality.

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