Potential Therapeutic Role of Rosuvastatin and Simvastatin in Acute Lung Injury (Experimental Study)

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Abstract

**Background and Aim:** Acute Lung Injury (ALI) is a common devastating clinical syndrome characterized by life-threatening respiratory failure. Simvastatin and rosuvastatin are well established classes of drugs that effectively decrease serum cholesterol levels. The present work was designed to study the potential therapeutic role of simvastatin and rosuvastatin, in an experimental model of ALI in rats.

**Methods:** 90 male wister rats were subdivided into normal group, Lipopolysaccharide (LPS) treated rats (model of ALI), saline treated group, ALI + simvastatin treated group, ALI + rosuvastatin treated group. Lung tissue TNF α, IL-1 β were assessed at 24 hours, 72 hours and on the 7th day after induction of ALI. Serum AST, ALT, creatinine phosphokinase, creatinine were assessed at the end of the experiment. The effects of simvastatin and rosuvastatin treatment on the aCh induced contractions of tracheal rings isolated from rats with ALI were studied.

**Results:** The LPS induced ALI group showed significant increase in TNF α and IL-1 β. Treatment of rats with ALI with simvastatin, rosuvastatin resulted in significant decrease in these elevated parameter at 24 hours, 72 hours and on the 7th day as compared to the untreated model group. Rosuvastatin was more significantly effective in reducing TNF α, IL-1 β at the end of the present study than simvastatin therapy. Moreover rosuvastatin therapy in ALI did not significantly affect AST, ALT, serum creatinine, or creatinine kinase. Simvastatin and rosuvastatin decreased significantly the aCh induced contractile responses of the isolated tracheal rings when compared to the untreated model group.

**Conclusions:** Simvastatin or rosuvastatin therapy could be beneficial in management of ALI. Rosuvastatin was more safe than simvastatin. These results could offer a new opportunity in management of ALI.

**Key Words:** ALI – Rosuvastatin – Simvastatin – TNF α – IL-1 β.

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Introduction

**ACUTE** Lung Injury (ALI) is a common devastating clinical syndrome characterized by life-threatening respiratory failure requiring mechanical ventilation [1]. ALI is a multifactorial disease process that occurs due to an environmental trigger on the background of a genetic predisposition [2]. The environmental causes of ALI can be classified as direct pulmonary insults (e.g. from pneumonia or aspiration) or indirect pulmonary insults (e.g. from sepsis or severe trauma) and often results in acute hypoxic respiratory failure [3].

Lung inflammation plays a key role in the pathogenesis of this devastating clinical syndrome. The development of Acute Lung Injury (ALI) is characterized by macrophage-mediated [4] and neutrophil-mediated [5] injury associated with the release of inflammatory cytokines and proteases [6]. Pro-inflammatory cytokines such as tumor necrosis factor (TNF α), interleukin (IL)-1 β, (IL)-6, and (IL)-8 are released in response to any of a variety of precipitants [7].

TNFα and interleukin (IL) 1 β are mainly produced by activated interstitial and alveolar cells (primarily macrophages), as well as Endothelial Cells (ECs), and have a major role in the early ALI stage. They act on ECs mainly by inducing a functional program that promotes thrombosis and inflammation [8].

IL-1 β is one of the most biologically active cytokines in the early phases of ALI, which is elevated in plasma and is predictive of clinical outcomes [9]. It is postulated that resident alveolar macrophages stimulated by pathogen recognition generate much of the production of the early cytokines, IL-1 β and TNF-α, which in turn stimu-
late neighbouring cells to produce a battery of chemokines that mediate the recruitment of neutrophils, monocytes and lymphocyte into the alveolar space [10].

There is no effective pharmacological intervention for ALI [11]. Many investigators have focused on therapeutic interventions that might modify or minimize this process. Pharmacological interventions, however, such as treatment with Prostaglandin E 1 (PGE 1), inhaled nitric oxide; surfactant and corticosteroids have not been shown to improve survival of these patients [12].

Statins are a well established class of drugs that effectively decrease serum cholesterol levels by inhibiting the action of 3-Hydroxy-3-Methyl-Glutaryl-CoA (HMG-CoA) reductase [13]. Use of statins for their lipid-lowering properties and beneficial effects on the morbidity and mortality associated with coronary artery disease is now firmly established. Studies have revealed pleiotropic properties of these drugs, including promotion of vasculogenesis, stimulation of bone formation, and anti-inflammatory and immuno-modulatory effects [14].

Atorvastatin, fluvastatin, lovastatin and simvastatin are relatively lipophilic compounds, while pravastatin and rosuvastatin are more hydrophilic as a result of a polar hydroxyl group and methane sulphonamide group, respectively [15]. Differences in structure of statins affect the pharmacological properties, such as: Affinity for the active site of the HMG-COA, rates of entry into hepatic and non-hepatic tissues, availability in the systemic circulation for uptake into non-hepatic tissues and routes and modes of metabolic transformation and elimination [16].

Rosuvastatin has a unique polar methane sulphonamide group, which is quite hydrophilic and confers low lipophilicity [16]. Differences in lipophilicity of statins are related to their penetration into extracellular tissues. Lipophilicity and penetration in nonhepatic tissues have potential clinical implications for muscle toxicity.

The aim of the present work was to study the potential therapeutic role of simvastatin (lipid soluble statin) and rosuvastatin (water soluble statin), in an experimental model of ALI in rats.

Material and Methods

Animals:
Laboratory bred 90 male adult albino rats weighing 150-250g kept in the Animal House of Veterinary College in Beni-Suef University. The study started at 2011 and ended at 2015. They were used for in-vivo and in-vitro experiments. They were maintained under standard laboratory conditions at 25°C, normal light/dark cycle (12 hours dark/12 hours light). They were fed standard chow diet. All animals were handled according to the guidelines of the local ethical committee which comply with the international laws for use and care of laboratory animals.

Drugs and chemicals:
- Rosuvastatin (Crestor) tablet 20mg (Astra-zenca, England).
- Simvastatin (Alkor) tablet 40mg (Alcan, Canada).
- Lipopolysaccharide of Escherichia Coli (055: B5) (Sigma-Aldrich).

Reagents for measurement of biochemical parameters:
- TNF a ELISA kit: (AviBion, Helsinki FINLAND).
- IL-10 ELISA kit: (Sunred Biological Technology Co., Ltd, China).
- AST, ALT (Spectrum diagnostics, Egypt).
- Creatinine phosphokinase (lab biotechnology).
- Creatinine (Spectrum diagnostics, Egypt).

Instruments:
Power Lab Data Acquisition and Analysis Systems (Power Lab 4/30 with Lab Chart Pro, model number ML866/P by ADInstruments) for: Monitoring and recording response from the isolated rat’s trachea.

Experimental design:
The study was divided into 3 time periods (24 hours, 72 hours and 7 days) after the administration of LPS to induce acute lung injury. At each time point, there were 5 groups (6 rats each).

Group 1: Included normal rats and served as control.

Group 2: Rats received lipo-polysaccharide (LPS) of E.Coli in a dose of 100 gg/rat dissolved in 100g1 saline instilled intra-nasally once. This group served as a model of acute lung injury.
Group 3: Rats received 1ml saline, given orally for seven days starting 3 hours after LPS administration and served as control for the groups that receive the tested drugs.

Group 4: Rats received simvastatin dissolved in saline (15mg/kg/day) [17] orally for seven days starting three hours after LPS administration.

Group 5: Rats received rosuvastatin dissolved in saline (15mg/kg/day) orally for seven days starting three hours after they were subjected to LPS.

In- Vivo study:
Induction of ALI:
The rats in the present study were anesthetized, each rat received escherichia coli O55:B5 LPS (Sigma Aldrich) in a dose of 100\(\gamma\)g/rat dissolved in 100\(\gamma\)l saline [18] by intranasal instillation by use of the external nares route until the solution was inhaled. The animals were allowed to recover from anesthesia, returned to their cages, and given free access to water and food. After three hours, treatment with simvastatin, or rosuvastatin, was given to the animals daily and continued for seven days.

Animals in the control groups received 1ml saline orally (simvastatin and rosuvastatin control), daily via the same routes as the corresponding treated groups.

The right lungs of the animals in all the groups were removed and subjected to homogenization. Measurement of the following biochemical parameters in the homogenates were done at 24 hours, 72 hours, and at the end of the expirement.

- TNF-\(\xi\).
- Interleukin- 1 \(\Pi\).

Venous blood samples were withdrawn from the retro-orbital plexus of all the animals in all the groups at the end of the experiment using micro-capillary tubes. The following biochemical parameters were measured in the collected sera:

- Liver tranaminases (AST, ALT).
- Creatinine.
- Creatinine phosphokinase (as a marker of muscle damage).

In- Vitro study:
Isolated tracheal ring preparation in rats [19]:

At the end of the experimental periods, rats were killed by cervical dislocation, the tracheas were dissected and placed on a dish containing physiological solution to remove adhering fat and connective tissue. Next, each trachea was cut transversely into cylindrical rings (with 3-4 cartilage rings). The resultant tissue ring preparation was then suspended in a 10-ml organ bath by two stainless steel wires (0.2mm in diameter) passed through the lumen. For all tissues, one end was fixed to the bottom of the organ bath while the other was connected to a force-displacement transducer for the measurement of isometric force. A resting tension of 1\(g\) was applied. The buffer solution contained modified Krebs-Henseleit solution composed of Sodium chloride 6.9, potassium chloride 0.35, magnesium sulfate 0.14, calcium chloride 0.28, sodium bicarbonate 2.1, sodium acid phosphate 0. 16 and glucose 2.0 [20]. The buffer solution was maintained at 37ºC and oxygenated with 95% O2-5% CO\(_2\). After the equilibration period, intrinsic tone was induced by allowing the rings to equilibrate under an applied tension of 1g for 60min. Ach was added to each preparation in the following concentration 1\(\gamma\)g, 2\(\gamma\)g, 4\(\gamma\)g and 8\(\gamma\)g per 1 0ml organ bath. The tracheal contractile responses to ach were recorded using powerlab. The concentration-response curve to ach was constructed.

Statistical analysis:
The data was collected, coded and entered to computer. The data was analyzed with the program (SPSS) Statistical Package for Social Science Version 16 under Windows 7. Description of quantitative variables were in the form of (mean ± SD). Comparison between quantitative variables was carried by using:

- Student \(t\)-test of two independent samples.
- One way ANOVA of more than two independent samples.
- \(p\)-value <0.05 was significant.

Results

In- Vivo study:

1. Effects of simvastatin and rosuvastatin on the mean (±SD) level of tumour necrosis factor \(\xi\) (TNF\(\xi\)) in lung tissue homogenate:

The mean (±SD) levels of TNF\(\xi\) (ng/gm) in lung tissue homogenate obtained from normal rats were represented in (Table 1) after 24 hours, 72 hours and at the end of the experiment respectively.

Induction of ALI in rats (Group 2) with LPS significantly elevated the TNF\(\xi\) levels in their lung tissue homogenate compared to normal rats (Group
1) after 24 hours, 72 hours and on the 7th day (Table 1).

Oral administration of saline (control Group 3) for rats with ALI did not produce significant change of the elevated levels of TNFα compared to the untreated model (Group 2) all through the experiment (Table 1). Treatment of rats with ALI with simvastatin decreased significantly the high levels of TNFα at 24 hours, 72 hours and at the end of the experiment respectively compared to the untreated model group (Table 1).

Treatment of rats with ALI with rosuvastatin also caused a significant decrease of the mean (±SD) TNFα levels in the lung tissue homogenate. The mean levels of TNFα were significantly reduced at 24 hours, 72 hours and at the end of the experiment respectively, compared to the untreated model group (Table 1).

It was observed that the significant decrease in this parameter was more apparent with time. The percentages of reduction were 33.33%, 46% and 58% with simvastatin treatment, 39%, 50% and 65% with rosuvastatin treatment.

Moreover, the mean level of TNFα was significantly reduced with rosuvastatin therapy than with simvastatin after 72 hours and at the end of the experiment.

Table (1): Effects of treatment with simvastatin and rosuvastatin on the mean (±SD) levels of TNFα (ng/gm) in lung tissue obtained from rats with ALI.

<table>
<thead>
<tr>
<th>Groups</th>
<th>−24 hours after induction of ALI</th>
<th>−72 hours after induction of ALI</th>
<th>−7 days after induction of ALI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats (group 1).</td>
<td>28.66±2.25</td>
<td>28.33±1.03</td>
<td>27.33±1.36</td>
</tr>
<tr>
<td>Model of ALI (group 2).</td>
<td>66±3.03 a</td>
<td>80±9.59 a</td>
<td>81.5±5.46 a</td>
</tr>
<tr>
<td>ALI + saline oral (1ml/day) (group 3).</td>
<td>69±3.03 a</td>
<td>85±7.04 a</td>
<td>84±4.73 a</td>
</tr>
<tr>
<td>ALI + simvastatin (15mg/kg/day) (group 4).</td>
<td>44.16±2.92b</td>
<td>43.66±1.36</td>
<td>34.16±2.56 b</td>
</tr>
<tr>
<td>ALI + rosuvastatin (15mg/kg/day) (group 5).</td>
<td>40.83±3.76 b</td>
<td>40.00±1.41 bc</td>
<td>29.66±2.33 bc</td>
</tr>
</tbody>
</table>

The data expressed as (mean ± S.D) (n=6).
a: Significant as compared to normal (group 1) (p<0.05).
b: Significant as compared to untreated ALI model (group 2) (p<0.05).
c: Significant as compared to simvastatin treated group (group 4) (p<0.05).

2- Effects of simvastatin and rosuvastatin on the mean (±SD) levels of IL-1 β (pg/gm) in lung tissue homogenate:

The mean (±SD) level of IL-1 β in lung tissue homogenate obtained from normal rats were represented in (Table 2) after 24 hours, 72 hours and at the end of the experiment respectively.

Induction of ALI in rats (Group 2) with LPS significantly elevated the IL-1 β levels in their lung tissue homogenate compared to normal rats (Group 1) at all time periods (Table 2).

Oral administration of saline (control Group 3) for rats with ALI did not produce significant change of the elevated levels of IL-1 β compared to the untreated model (Group 2) all through the experiment (Table 2).

The simvastatin treated ALI group showed a significant decrease in the level of IL-1 β at 24 hours, 72 hours and at the end of the experiment respectively compared to the untreated model group (Table 2).

The rosuvastatin treated group showed a significant decrease of the mean (±SD) IL-1 β levels in the lung tissue homogenate at 24 hours, 72 hours and at the end of the experiment respectively, compared to the untreated model group (Table 2). It was noticed that the reduction of this elevated levels was increased with time and the percentages of reduction were 15.3%, 31.2% and 34.3% with simvastatin treatment, 18.6%, 31.9% and 42.5% with rosuvastatin treatment respectively.

The mean level of IL-1 β was significantly reduced with rosuvastatin therapy than with simvastatin at the end of the experiment.
Table (2): The effects of treatment with simvastatin and rosuvastatin on the mean (±SD) levels of IL-1 β (pg/gm) in lung tissue obtained from rats with ALI.

<table>
<thead>
<tr>
<th>Groups</th>
<th>–24 hours after induction of ALI</th>
<th>–72 hours after induction of ALI</th>
<th>–7 days after induction of ALI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats (group 1).</td>
<td>103.00±4.65</td>
<td>105.50±4.72</td>
<td>100±2.90</td>
</tr>
<tr>
<td>ALI (model) (group 2).</td>
<td>241.17±6.52 ^a</td>
<td>266.17±3.06 ^a</td>
<td>268.67±2.07 ^a</td>
</tr>
<tr>
<td>ALI + saline oral (1ml/day) (group 3).</td>
<td>243.67±7.31 ^a</td>
<td>269.00±4.29 ^a</td>
<td>271.50±2.26 ^a</td>
</tr>
<tr>
<td>ALI + simvastatin (15mg/kg/day) (group 4).</td>
<td>204.00±5.06b</td>
<td>183.17±6.49 b</td>
<td>176.67±7.15 b</td>
</tr>
<tr>
<td>ALI + rosuvastatin (15mg/kg/day) (group 5).</td>
<td>196.67±8.66b</td>
<td>181.17±2.14 b</td>
<td>154.67±3.72 bc</td>
</tr>
</tbody>
</table>

The data expressed as (mean ± S.D) (n=6).

a: Significant as compared to normal (group 1) (p<0.05).
b: Significant as compared to untreated ALI model (group 2) (p<0.05).
c: Significant as compared to simvastatin treated group (group 4) (p<0.05).

II- The effects of simvastatin and rosuvastatin on parameters measured in serum:

- Effects on liver function parameters:

  A- The mean level of serum (ALT) (U/L):

  Induction of ALI in rats (Group 2) caused a non significant elevation the ALT in serum compared to normal group at the end of the experiment (Table 3).

  Oral administration of saline (control Group 3) for rats with ALI did not produce significant change of the elevated level of ALT compared to the untreated model (Group 2), at the end of the experiment (Table 3).

  Treatment of rats with ALI with simvastatin elevated significantly the mean level of serum (ALT) at the end of the experiment, compared to the untreated model group (Table 3). The percentages of elevation was 19%.

  On the other hand treatment of rats with ALI with rosuvastatin caused a non significant decrease of the mean (±SD) (ALT) level in serum when compared to the untreated at the end of the experiment (Table 3).

  Moreover, the mean serum (ALT) was significantly increased more with simvastatin therapy than with rosuvastatin at the end of the experiment.

  B- The mean level of Serum Aspartate Transferase (AST) (U/L):

  Induction of ALI in rats (Group 2) resulted in a non significant elevation of the AST in the serum compared to normal group at the end of the experiment (Table 3).

  Oral administration of saline (control Group 3) for rats with ALI did not produce significant change of the elevated level of AST compared to the untreated model (Group 2), at the end of the experiment (Table 3).

  Treatment of rats with ALI with simvastatin elevated significantly the mean level of serum (AST) at the end of the experiment, compared to the untreated model group (Table 3). The percentages of elevation was 24.2%. Meanwhile treatment of rats with ALI with rosuvastatin showed an insignificant decrease of the mean (±SD) (AST) level in serum at the end of the experiment (Table 3) when compared to the untreated model group.

  Moreover, the mean serum (AST) was significantly increased more with simvastatin therapy than with rosuvastatin at the end of the experiment.

- Effects of simvastatin and rosuvastatin on the mean level of serum creatinine (mg/dl):

  Untreated rats with ALI (Group 2) showed an insignificant change in the mean level of serum creatinine at the end of the experiment, compared to the normal group (Table 3).

  Oral administration of saline (control Group 3) for rats with ALI did not produce significant change of the level of serum creatinine compared to the untreated model at the end of the experiment (Table 3).

  Treatment of rats with ALI with simvastatin or with rosuvastatin produced an insignificant change compared to the untreated model group at the end of the experiment (Table 3).

- Effects of the tested drugs on the mean level of serum creatinine phosphoKinase:

  Induction of ALI in rats (Group 2) with LPS resulted in an insignificant change in the CPK in serum compared to normal group at the end of the experiment (Table 3).
Oral administration of saline (control Group 3) for rats with ALI did not produce a significant change of the elevated level of CPK compared to the untreated model (Group 2), at the end of the experiment (Table 3).

Treatment of rats with ALI with simvastatin elevated significantly the mean level of serum (CPK) at the end of the experiment, compared to the untreated model group (Table 3). The percentages of elevation was 15%. Meanwhile treatment of rats with ALI with rosuvastatin showed an insignificant change of the mean (±SD) (CPK) level in serum at the end of the experiment (Table 3).

The mean serum (CPK) was significantly increased more with simvastatin therapy than with rosuvastatin at the end of the experiment.

Table (3): Effects of simvastatin and rosuvastatin on the mean serum levels of ALT (U/L), AST (U/L), creatinine (mg/dl) and creatinine phosphokinase (U/L) in acute lung injury at the end of the experiment.

<table>
<thead>
<tr>
<th>Serum level</th>
<th>ALT-7 days (U/L)</th>
<th>AST-7 days (U/L)</th>
<th>Creatinine-7 days (mg/dl)</th>
<th>CPK-7 days (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats (group 1)</td>
<td>33.00±2.53</td>
<td>34±7.46</td>
<td>0.20±0.034</td>
<td>81.83±8.35</td>
</tr>
<tr>
<td>LPS-treated rats (model)</td>
<td>40.50±3.45</td>
<td>40±6.03</td>
<td>0.22±0.10</td>
<td>85.00±5.06</td>
</tr>
<tr>
<td>ALI + saline oral (1ml/kg) (group 3)</td>
<td>41.50±4.66</td>
<td>39±6.5</td>
<td>0.21±0.05</td>
<td>82.00±7.05</td>
</tr>
<tr>
<td>ALI + simvastatin (15mg/kg/day) (group 4)</td>
<td>50.00±16.42</td>
<td>52.83±9.93</td>
<td>0.2600±0.11</td>
<td>100.00±6.36</td>
</tr>
<tr>
<td>ALI + rosuvastatin (15mg/kg/day) (group 5)</td>
<td>35.00±7.97</td>
<td>40.00±8.37</td>
<td>0.21±0.09</td>
<td>89.17±5.95</td>
</tr>
</tbody>
</table>

The data expressed as (mean ± S.D) (n=6).

**In-Vitro study:**

The effects of simvastatin and rosuvastatin treatment on ach induced contractile response of rat isolated tracheal rings.

Addition of ach to tracheal rings isolated from normal rats induced increasing contractile responses in a concentration dependent manner (Table 4 & Figs. 1,2).

Significant increase of the ach induced contractile responses were observed in tracheal rings isolated from the rats untreated that suffered from ALI compared to normal group (Table 4 & Figs. 1,3).

There was leftward shift of the ach induced contraction response curve Fig. (1).

The results of the present study showed that the increased ach contractile responses observed in the ALI model group were significantly reduced in tracheal rings isolated from rats that suffered from ALI and were treated with either simvastatin or rosuvastatin (Table 4 & Figs. 1,4,5) when compared to the untreated model group.

Both drugs nearly normalized the ach induced contractile responses of the isolated tracheal rings. The mean level of tracheal contractile responses were significantly reduced more with rosuvastatin than with simvastatin therapy (Table 4 & Fig. 1).

Table (4): Mean (±S.D) level of Ach induced contractile responses of tracheal rings isolated from rats that suffered from ALI either untreated or treated with simvastatin or rosuvastatin at the end of the experiment.

<table>
<thead>
<tr>
<th>Ach concentration (µg/10ml)</th>
<th>1 µg/10ml</th>
<th>2 µg/10ml</th>
<th>4 µg/10ml</th>
<th>8 µg/10ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (group 1)</td>
<td>0.37±0.07</td>
<td>0.63±0.04</td>
<td>0.91±0.06</td>
<td>1.09±0.04</td>
</tr>
<tr>
<td>ALI untreated (model) (group 2).</td>
<td>1.65±0.04</td>
<td>2.25±0.12</td>
<td>2.98±0.46</td>
<td>3.45±0.49</td>
</tr>
<tr>
<td>ALI + simvastatin (group 4)</td>
<td>0.63±0.03</td>
<td>0.87±0.03</td>
<td>1.20±0.05</td>
<td>1.40±0.08</td>
</tr>
<tr>
<td>ALI + Rosuvastatin (group 5)</td>
<td>0.50±0.05</td>
<td>0.7±0.1</td>
<td>1.00±0.05</td>
<td>1.19±0.07</td>
</tr>
</tbody>
</table>

The data expressed as (mean ± S.D) (n=6).

a: Significant as compared to normal (group 1) (p<0.05).

b: Significant as compared to untreated model (group 2) (p<0.05).

c: Significant as compared to simvastatin (group 4) (p<0.05).
Fig. (1): Dose response curve showing the effects of different doses of Ach on contraction of isolated tracheal ring of normal, LPS, simvastatin and rosuvastatin treated rats.

Fig. (2): The effects of increasing doses of Ach on isolated tracheal ring of the normal rats.

Fig. (3): The effects of increasing doses of Ach on isolated tracheal ring of the model of acute lung injury.
Discussion

In the current study induction of ALI (LPS group) produced significant increase in lung tissue TNF-α and IL-10 at 24, 72 hours post infection and at the end of the study. Treatment of ALI model with simvastatin resulted in significant decrease in lung tissue TNF-α, and IL-10 at 24, 72 hours post infection and at the end of the study.

Medeiros and his colleagues [21], studied the effects of simvastatin pretreatment on lung injury in rats with abdominal sepsis caused by Cecal Ligation and Puncture (CLP). TNF-α and IL-10 plasma levels was significantly lower in rats treated with simvastatin. Induced inflammation was greatly reduced by simvastatin pre-treatment.

In a randomized, double-blind, placebo-controlled clinical trial, LPS was inhaled via a nebulizer. Simvastatin reduced significantly TNF-α concentrations in BALF after LPS challenge. Simvastatin also decreased IL-10 but this did not
reach statistical significance. LPS inhalation induced a threefold up-regulation in nuclear nuclear factor βB (NF-κB) in monocyte-derived macrophages; pretreatment with simvastatin reduced this. LPS stimulates the induction of several inflammatory genes via the NF-κB pathway [22]. In addition, LPS stimulation of alveolar macrophage is known to induce NF-κB-dependent TNF-α and IL-1β secretion [23]. The data from the clinical trial suggested that simvastatin specifically reduced TNF-α and IL-1β, and it also reduced the secretion of two key macrophage-derived proteases matrix metalloproteinases (MMP-7/MMP-9) in vivo. Their data suggested that simvastatin modulated macrophage activation and thus identifying an important potential mechanism for the observed anti-inflammatory effect of simvastatin in vivo [24].

This results of the current work were in contrast with Pirat and his colleagues [25] who used a model of intestinal ischemia and reperfusion to induce ALI in mice. No significant differences were observed between their control group animals and simvastatin treated animals with respect to serum or BALF levels of IL-1, IL-6, and TNF-α at the end of reperfusion. The findings for these variables might be a reflection of the I/R model they used, which is associated with a generalized severe inflammatory response. This type of reaction could potentially overwhelm the inhibitory effects of simvastatin on chemokine expression.

In the current study rosuvastatin treatment resulted in a significant decrease in TNF α, and IL-1β, at 24, 72 hours and at the end of the experiment when compared to the untreated model.

These results were in agreement with Dolkart et al., [26]. A model of blunt chest injury in rats was employed. Lung contusion, is a leading risk factor for development of Acute Lung Injury (ALI). Administration of rosuvastatin decreased significantly the cytokine levels that were increased after the blunt chest trauma.

Neukamm and his colleagues [27] in a randomized, placebo-controlled, double-blind trial in patients with COPD showed that rosuvastatin therapy was associated with enhanced endothelium-dependent vascular function, improved pulmonary function and reduced systemic inflammation in patients with Chronic Obstructive Pulmonary Disease (COPD).

Zhu et al., [28] in a murine model of chronic asthma, showed that rosuvastatin reduced the number of total inflammatory cells, lymphocytes, macrophages, neutrophils, and eosinophils recruited into BALF, the level of TNF-α in BALF, along with the Histological Mucus Index (HMI) and Gamma-aminobutyric acid type A receptor (GABA-A) β2 expression. Changes occurred in a dose-dependent manner. Its ability to reduce the inflammatory response and mucus hypersecretion is mediated by regulating GABA-A receptor activity. The authors suggested that rosuvastatin reduced the recruitment of asthmatic inflammatory cells, possibly via the decrease of the expressions of TNF-α, IL-4 and IL-5.

Naito et al., [29] showed that exposure of the small intestine to Ischemia-Reperfusion (I-R) resulted in mucosal inflammation characterized by significant increases in thiobarbituric acid-reactive substances, tissue-associated myeloperoxidase activity, and the mucosal contents of rat Cytokine-induced Neutrophil Chemoattractant-1 (CINC-1) and TNF-α. These increases in inflammatory parameters were significantly inhibited by pretreatment with 10mg/kg rosuvastatin. Rosuvastatin-treated animals demonstrated preserved expression of eNOS protein compared to the I-R-treated group. This is likely to explain the reduction in tissue injury and neutrophil accumulation.

It was observed in the present work that rosuvastatin was more effective in treatment of ALI than simvastatin.

In agreement with the present result Sinori et al., [30] who showed that rosuvastatin, but not simvastatin, provided end organ protection in stroke prone rats by anti-inflammatory effects. Rosuvastatin treatment was shown to attenuate the transcription of monocyte chemoattractant protein-1, transforming growth factor-β1, IL-1β, and tumor necrosis factor-alpha in the kidney, and of P-selectin in brain vessels and increased the transcription of endothelial nitric oxide synthase mRNA in the aorta. Treatment with simvastatin (2 to 20mg/kg per day) did not exert any protective effects.

Also Melo et al., [31] considered the pleiotropic effects of 3 different statins in endotoxin-induced ALI in mice; atorvastatin, which is lipophilic and synthetic; pravastatin, which is hydrophilic and fermentation derived and simvastatin, which is lipophilic and fermentation derived were used. After histological, biochemical and functional analysis, they concluded that pravastatin was the best anti-inflammatory therapy, while atorvastatin was the best antioxidant therapy. Simvastatin showed the least pleiotropic activities.

The current study extended to test the safety profile of the used drugs. For this the following
parameters were measured in the serum (AST, ALT, CPK and creatinine). Simvastatin ALI treated group showed significant increase in AST, ALT, and creatinine phosphokinase level when compared to the untreated model group. Rosuvastatin ALI treated group, did not induce any significant increase in the previous parameters when compared to the untreated model group.

This could possibly because lipophilic statins are more widely distributed after administration and are therefore associated with a greater number of side effects, favoring the use of the more hydrophilic forms for study in critically ill populations with lung injury [32].

Liver injury was seen in a guinea pig model exposed to high doses of simvastatin. It was associated with a 10-fold elevation in serum aspartate and alanine aminotransferase activities [33].

The results of the present work were in contrast with Muller and his colleagues [34]. They showed that simvastatin had no impact on renal function or ALT levels in plasma. It did not alter blood pressure, urine output electrolyte levels or acid-base homeostasis in mechanically ventilated mice.

Although the increase in ALT, AST and CPK were observed with simvastatin treatment in the current study but it remains safe for use in ALI as the percentages of increase were 19%, 24.2%, 15%, respectively. While treatment should be discontinued only if serum transaminases persist 3 times, or serum CPK persist 5 times above the upper limit of normal [35].

Concerning the in-vitro part of the present study, the results showed that simvastatin and rosuvastatin significantly reduced Ach induced contractile responses of tracheal rings isolated from rats with ALI and treated by those two tested drugs compared to the untreated model. Rosuvastatin induced more significant reduction than simvastatin.

Cazzolla et al. [36] investigated the effect of LPS on the transmural contractile tension of human isolated bronchi. Their results provided mechanistic evidence for the enhanced bronchoconstriction induced by LPS in human isolated airways, the contribution of Rho/Ras pathways in this LPS response, and the protective role of simvastatin.

Sun et al. [37] tried to explore the association between anti-atherosclerosis effect of rosuvastatin and inhibition of the TNF-α-Rho kinase signaling pathway, which might be the internal mechanism of the anti-proliferation effect of rosuvastatin in smooth muscle cells. They indicated that rosuvastatin can inhibit the activation of TNF-α mediated Rho kinase pathway and reduce the TNF-α induced smooth muscle cell proliferation.

Zeki et al., [38] conducted a series of in vitro experiments using primary mouse tracheal epithelial cells. They showed that systemic treatment of mice with simvastatin attenuates allergic inflammation, decreases the production of IL-4 and IL-13, reduces goblet cell hyperplasia, and improves airway hyperreactivity. Collectively, these results suggested both an anti-inflammatory and epithelial anti remodeling effect of simvastatin in vivo. They hypothesized that simvastatin inhibits the expression of IL-13-induced cytokines and chemokines in primary mouse tracheal epithelial cells. Simvastatin also inhibited the basal gene expression of osteopontin independent of IL-13. Osteopontin is an integrin ligand and a cytokine with a variety of actions important in asthma pathogenesis.

Takeda et al., [39] found that simvastatin (0.1 - 1.0 µM) significantly inhibited airway smooth muscle cell proliferation and DNA synthesis in a concentration-dependent manner. This occurred due to prevention of geranylgeranylation of RhoA.

The current study concluded that simvastatin or rosuvastatin therapy could be beneficial in the management of ALI. Moreover rosuvastatin was more effective and more safe than simvastin. These results could offer a new opportunity in the management of ALI.

References
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