In Oleic Acid-Induced ARDS, Effects of Tocilizumab and Dexamethasone on the Downregulation of Proinflammatory Cytokines and Upregulation of Antioxidants in the lung.

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Research Article

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Abstract

Background

Acute respiratory shortage syndrome (ARDS) is a life-threatening disease formed by the induction of inflammatory cytokines and chemokines in the lungs. There is a dearth of drug treatments that can be used to prevent cytokine storms in ARDS treatment. This study aimed to investigate the efficacy of tocilizumab and dexamethasone on oxidative stress, antioxidant parameters, and cytokine storm in acute lung injury caused by oleic acid in rats.

Methods

Adult male rats, CN (healthy, n=6), OA (oleic acid, n=6), OA+TCZ-2 (Oleic acid and Tocilizumab 2mg/kg, n=6), OA + TCZ-4 (Oleic acid and Tocilizumab 4 mg/kg, n=6), and OA+DEX-10 (Oleic acid and Dexamethasone 10mg/kg, n=6) were divided into five groups. All animals were sacrificed for histopathological, immunohistochemical, biochemical, PCR analyses, and SEM imaging.

Results

The expression of TNF-α, IL-1β, IL-6, and IL-8 cytokines in rats with acute lung injury with oleic acid was downregulated in the TCZ and DEX groups compared to the OA group. (P<0.05). The MDA level in the lung tissue was statistically lower in the OA+TCZ-4 group compared to the OA group. It was determined that SOD, GSH, and CAT levels decreased in the OA group and increased in the TCZ and DEX groups (P<0.05). Histopathological findings such as thickening of the alveoli, hyperemia, and peribronchial cells were found to be similar to the control group in TCZ and DEX group rat lung tissue. With SEM imaging of the lung tissue, it was found that the alveolar lining layer became indistinct in the OA, OA+TCZ-2 and OA+TCZ-4 groups.

Conclusions

In the acute lung injury model caused by oleic acid, tocilizumab and dexamethasone are thought to be effective in preventing cytokine storm by downregulating the expression of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8. Against the downregulation of antioxidant parameters such as SOD and GSH in the lung tissue caused by oleic acid, tocilizumab and dexamethasone upregulated and showed a protective effect in cell damage.

Background

Acute lung injury (ALI), also known as acute respiratory distress syndrome (ARDS), is a life-threatening lung disease caused by a variety of direct and indirect causes. Pathological changes such as deterioration of pulmonary vascular permeability, pulmonary edema, hyaline membrane formation, micro-atelectasis of alveolar epithelial cells, microthrombosis, and microcirculation disorder are seen in ARDS [1, 2]. Oxidative stress and inflammatory response are two important factors in ARDS [3].

In ALI/ARDS, the inflammatory response is initiated by a complex network of cytokines and other pro-inflammatory molecules produced by various cell types in the lungs, including inflammatory cells such as recruitment of blood leukocytes, activation of tissue macrophages [3]. The early response proinflammatory cytokines are Tumor necrosis factor α (TNF-α) and IL-1β produced by macrophages and neutrophils (and other cell types) [4, 5]. TNF-α and IL-1β act locally on other cells, including monocytes/macrophages, endothelial cells, fibroblasts, and epithelial cells, and stimulate the production of other cytokines (such as IL-2, IL-4, IL-6, and IL-8) [3]. In ARDS patients, inflammatory cytokines such as IL-1β, TNF-α, IL-6, and IL-8 are elevated in bronchoalveolar lavage fluid and plasma [6, 7]. IL-6 is believed to play a crucial role in the development of the cytokine storm and contribute to the occurrence of ARDS and cause interstitial pneumonia in severe COVID-19 patients [8].
Reactive oxygen species (ROS) are produced in high amounts by damaged endothelium/epithelium as well as leukocytes, leading to aggravation of ARDS through lipid peroxidation, which can alter both the structure and function of pulmonary capillaries. [9] Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) are naturally present in the lung, but the intrapulmonary levels of these enzymes can be greatly elevated when the lung is faced with an oxidant burst (such as hyperoxia) that disrupts the redox balance. [10] SOD catalyzes superoxide anion radicals to hydrogen peroxide and oxygen, while CAT converts hydrogen peroxide to water and oxygen [11]. GSH scavenges various radicals and also acts as a direct antioxidant by participating in glutathione peroxidase reactions [12].

Tocilizumab (TCZ) is a recombinant humanized monoclonal antibody that also binds to its soluble forms [13]. The mechanism of TCZ blocks receptor complex signaling to inflammatory mediators responsible for B and T cell activation and inhibits cytokine storm [14]. The drug is used in the treatment of certain autoimmune disorders such as rheumatoid arthritis, juvenile idiopathic arthritis and Castleman disease [15]. In recent years, TCZ is widely preferred in the treatment of COVID-19 causing acute respiratory syndrome [16].

In the treatment of ALI/ARDS, glucocorticoids have been used for many years as they reduce inflammation and fibrosis through inhibition of various cytokines, including interleukin IL-1, IL-3, IL-5, IL-6, IL-8, and TNF-α [17, 18]. Dexamethasone, one of these steroids, reduces the production of inflammatory cytokines and pulmonary edema, alleviates alveolar epithelial and endothelial cell damage [19]. In the study, it was aimed to investigate the efficacy of tocilizumab and dexamethasone on oxidative stress, antioxidant parameters and cytokine storm in acute lung injury caused by oleic acid.

Materials And Methods

Animals

A total of 30 Wistar albino adult male rats aged 12-13 weeks weighing 200-220 g were purchased from Bolu Abant İzzet Baysal University Medical Experimental Research and Application Center. Rats were kept in at temperatures ranging between 19°C and 22°C, with a standard 12-h light–dark cycle. The experimental animal ARDS model was performed with oleic acid (OA).

Experimental procedures

Adult male rats were randomly selected and divided into five groups; CN (healthy, n = 6), OA (oleic acid, n = 6), OA+TCZ-2 (Oleic Acid and Tocilizumab 2mg/kg, n=6), OA + TCZ- 4 (Oleic Acid and Tocilizumab 4 mg/kg, n=6), and OA+DEX-10 (Oleic Acid and Dexamethasone 10mg/kg, n = 6). 50 µL of oleic acid was dissolved in 250 µL of 1% BSA and administered via tail vein to all groups except the healthy group. Intraperitoneally, dexamethasone (Deva llac, Dekort Ampul 8 mg/2 ml, Turkey) and Tocilizumab (Actemra, Roche, Germany) were administered twice with an interval of 12 hours using insulin injectors six hours after OA injection. Rats were euthanized by cervical dislocation. Systemic autopsy of rats was performed, and lung tissue was taken for biochemical and pathological analysis. Lung tissue -20°C was stored for biochemical analysis. It was taken in 10% formaldehyde solution for pathological analysis.

Histopathological analysis

The tissues were cut and moved to the cassettes. Routine pathology follow-up was done after the cassettes were cleaned under running water, and paraffin was blocked. Hematoxylin-eosin staining was conducted on 5 µm thick pieces of paraffin blocks cut in a microtome and mounted on adhesive slides filled with a coverslip. Sections were examined under a light microscope. Histopathological changes were scored semiquantitatively as (-, 0) absent, + (1): mild, ++ (2): moderate + + + (3): severe [20].
**Immunohistochemical analysis**

Immunohistochemical staining was performed according to the Mouse and Rabbit Specific HRP / DAB IHC Detection Kit-Micro polymer (ab236466) (Abcam, United Kingdom, UK) kit procedure. Proteinase K (ab64220) (Abcam, United Kingdom, UK) was used for antigen retrieval. Sections taken from paraffin blocks to adhesive were dropped 3% H₂O₂ peroxidase block solution. Then, the protein block solution was poured. In the sections were dropped 1:100 Anti-TNF-α monoclonal (Cat NO: ab 6671, Abcam, Boston ,USA), anti-IL-6 (Cat NO: ab6672, Boston, USA), and IL-8 (Cat NO: ab34100, Boston, USA), and left at room temperature for 1 hour. Subsequently, the Mouse Identification Reagent (Complementary) solution was added to the slides and Goat anti-rabbit HRP-conjugate was dropped. Slides stained by DAB (3,3'- diaminobenzidine tetrahydrochloride). After counter-staining with Mayer's hematoxylin, slides were closed by coverslips and evaluated under a light microscope (Leica DM 400B). The negative control slides were also stained according to the same procedure and PBS was used instead of the primer antibody. Immunohistochemical staining was scored semi quantitatively as low (+), moderate (++), and high (+++) expression.

**SEM analysis**

A tissue piece of 5x5x5 mm was taken from the peripheral ends of two rat lungs and washed in PBS. It was then fixed in 2.5% glutaraldehyde. It was kept in 1% OsO₄ and then the tissues were passed through a graded acetone series. All tissue fixation steps were performed under a fume hood. It was dried in a critical point dryer (Quorum Technologies, E3100) and then coated with gold palladium (Au-Pd) (Cressington, Sputter Coater 108 Auto). It was examined and visualized under a high vacuum at 5.00 kV, with an ETD detector, scanning electron microscope (FEI, Quanta FEG 250). SEM Analysis was performed in Kastamonu University Central Research Laboratory, Imaging Laboratory.

**Biochemical analyses**

**Preparation of tissue homogenates**

The tissues taken into the petri dish were pulverized with liquid nitrogen into a porcelain mortar. Then, it is weighed as 25 mg in sterile eppendorf tubes and homogenate buffers suitable for the parameter (LPO: 10% KCl, SOD: 50 mM KH₂PO₄, 10 mM EDTA, GSH: 50 mM Tris-HCl and CAT: 50 mM KH₂PO₄, pH 7) added. Tissues in the buffer were homogenized for 1 minute at a frequency of 35 hz using a tissue homogenizer (Qiagen Tissuelyser II, Germany) with a 5 mm steel ball. Subsequently, the homogenates were centrifuged in a refrigerated centrifuge (Hettich Rotina 320R, Germany) at +4°C at 4000 rpm for 30 minutes for LPO and GSH, 6000 rpm for 1 hour for SOD, and 8500 rpm for 1 hour for CAT. The resulting supernatants were then used in accordance with the measurement methods.

**Determining of lipid peroxidation levels**

For the determination of lung tissue LPO level, Ohkawa H, Ohishi N and Yagi K [21] described by a reaction method between thiobarbituric acid and malondialdehyde was used. In the calculation of tissue LPO level, absorbances read at 532 nm by spectrophotometer (Bio-Tek EPOCH, USA) were calculated using a standard graph created with 1,1,3,3-tetramethoxypropane, and the results were expressed as nmol MDA/g tissue.

**Determining of superoxide dismutase enzyme activity**

For the determination of lung tissue SOD activity, a method based on the detection of formazan dye of superoxide radicals formed by xanthine oxidase activity was used [22]. To determine the activity, absorbances read at 560 nm were calculated with the formulation specified in the method and expressed as U/mg tissue.

**Determining of catalase enzyme activity**
CAT activity in lung tissue was determined using a method that utilizes the conversion of H$_2$O$_2$ to water by an enzymatic reaction [23]. After the samples were read kinetically at 240 nm, they were calculated with the formula specified in the method and expressed as µmol/min/mg tissue.

**Determining of glutathione levels**

Glutathione (GSH) levels of lung tissues were determined according to the method described [24]. GSH levels of the tissues were determined at 412 nm and expressed as nmol/mg lung tissue.

**Molecular analysis of gene expressions**

**RNA isolation and cDNA synthesis**

The lung tissues were stabilized in RNA Stabilization Reagent (RNAlater, Qiagen), then homogenized with Tissue Lyser II (Qiagen) device. Total RNA was isolated from homogenized lung tissues following the RNeasy Mini Kit (Qiagen) instructions. cDNA was synthesized by reverse transcription of complementary DNA from isolated RNA samples according to the instructions of the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California, USA), as previously described [25]. The sequences of PCR primer pairs used for each gene are shown in Table 1.

**Relative analysis of gene expressions (Real-Time Quantitative PCR)**

Expression analysis of TNF-α, IL-1β and IL-6 as target genes was conducted with StepOne Plus Real-Time PCR System technology (Applied Biosystems) for each cDNA sample synthesized from rat lung RNA. The expression analysis of β-actin was used as an endogenous reference gene. Quantitative real-time PCR was conducted according to the One-Step TaqMan Gene Expression Assays Probe-based technology (Primer Design Ltd., Southampton, UK), as previously described. [26] The data obtained were expressed as a fold-change in expression using the 2$^{-\Delta\Delta \text{Ct}}$ method compared to the healthy group [27].

**Statistical analysis**

IBM SPSS Statistics 25.0 software was used for comparing the histopathology results. All values are presented as the mean±standard deviation. Biochemical analyzes and mRNA expression levels were statistically analyzed according to the One Way Anova test among study groups. Significant differences were determined after the data were analyzed by Tukey multiple range test. Histopathological and immunohistochemical scores were analyzed using The Kruskal Wallis and Mann-Whitney U tests. The $P$ values less than 0.05 were considered statistically significant.

**Results**

**Histopathological results**

Histopathological findings in the lungs in the control and experimental groups are summarized in Table 1. Thickening of the interalveolar septum in the lung was statistically more severe ($P$<0.05) in the OA group than in the other experimental groups. In addition, there was no statistically significant difference ($P$>0.05) in thickening of the interalveolar septum in the CN, OA+TCZ-4 and OA+DEX-10 groups (Figure 1(a)). Peribronchial cell infiltration was found to be statistically more severe in the OA group than in the experimental groups ($P$<0.05). The vessels in the lung interalveolar septum were statistically more severe in the OA group ($P$<0.05), and there was no statistically significant difference in the CN and experimental groups ($P$>0.05).

**Immunohistochemical results**

The immunohistochemical method was used to determine the expression of TNF-α, IL-6, and IL-8 cytokines in the lungs of control and experimental group rats. TNF-α expression was statistically significantly increased ($P$<0.05) in the OA group.
compared to the other groups (Figure 1(b)). In addition, it was determined that TNF-α expression was stained moderately in the TCZ and DEX groups. IL-6 expression was statistically significantly increased (P<0.05) in the OA group compared to the OA+TCZ-4 and OA+DEX-10 groups (Figure 1(b)). IL-6 expression was detected in the OA group and moderate staining in the OA+TCZ-2 group. IL-8 expression increased significantly (P<0.05) in the OA group compared to the other study groups. IL-8 stained moderately in the OA+TCZ-2 and OA+DEX-10 groups and negatively in the OA-TCZ-4 group and control group.

**SEM results**

All lung tissues were carefully examined. In SEM examination, a thin layer of alveolar lining was detected inside the saccus alveolar (Figure 2 (A) and (D)). In addition, alveolar macrophages and erythrocytes were prominent (Figure 2 (A) and (C)). Collagen fibrils and alveolar capillar vessels were observed in the interalveolar septum. Alveolar capillar vessels were found to be full in the oleic acid group (Figure 2 (B)). It was observed that the cell layer forming the alveolar lining layer became indistinct in oleic acid, OA+TCZ-2, and OA+TCZ-4 groups compared to CN and OA+DEX 10 groups (Figure 2). In the OA+TCZ 4 group, it was noted that the cells forming the alveolar lining layer completely deteriorated, the collagen fibrils under this layer were exposed, and the vascular structures deteriorated. (Figure 2(E)). In addition, collateral channels, defined as kohr openings, were observed as a rather large and emphysematous structure in the OA+TCZ 2 group (Figure 2D).

**Biochemical results**

In lung tissue, LPO level increased statistically in the OA group compared to the control group (P<0.05). In addition, LPO level was not statistically significant (P>0.05) in the OA+TCZ-2 and OA+DEX-10 groups, and in the CN and OA+TCZ-4 groups (Figure 3(a)). SOD activity was significantly lower in the OA and OA+TCZ-2 groups compared to the other groups (P<0.05). In addition, SOD activity was not statistically significant (P>0.05) in the OA+TCZ-4 and OA+DEX 10 groups (Figure 3(b)). GSH level increased significantly in the OA+TCZ-4 group compared to the OA group (P<0.05). In addition, GSH level, CN, OA+TCZ-2 and OA+DEX 10 gr groups were statistically (P<0.05) close (Figure 3(c)). CAT activity was significantly reduced in the OA group (P<0.05). There was no statistical difference (P>0.05) between CAT activity, OA+TCZ-4 group, and OA+DEX-10 group (Figure 3(d)).

**Molecular results**

To evaluate whether tocilizumab (2 ve 4 mg/kg) and dexamethasone (10 mg/kg) attenuated OA-induced ARDS, the expression levels of TNF-α, IL-1β, and IL-6 mRNA in the lung tissue of the rats were analyzed using real-time PCR (Figure 4 (a), (b), (c)). It was observed that OA in lung caused a significant increase in the expression of TNF-α, IL-1β and IL-6 mRNA levels p < 0.05. On the other hand, treatment with tocilizumab 4mg/kg and dexamethasone 10 mg/kg dose led to a significant decrease regarding the TNF-α, IL-1β, and IL-6 mRNA expression levels when compared to the OA-group (P< 0.05).

**Discussion**

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), occur due to various clinical conditions such as trauma, aspiration, sepsis, endotoxemia, and pneumonia [28]. The pathophysiology of ARDS is quite complex. [29] In ARDS, proinflammatory cytokines and interconnected inflammatory cascades are important to the inflammatory response [30]. As a result of this inflammatory response, alveolar-capillary permeability increases, microthrombus forms, and hypoxic pulmonary vasoconstriction is impaired, and endothelial and epithelial damage occurs with the ventilation-perfusion relationship, leading to alveolar edema, decreased lung compliance, and ultimately refractory hypoxemia [3]. In the treatment of the disease, glucocorticoids, surfactants, inhaled nitric oxide, antioxidants, protease inhibitors, and various other anti-inflammatory are used [31]. Oleic acid-induced lung injury, a well-defined laboratory model
of acute lung injury, is the most preferred for the evaluation of potential therapeutic agents [32, 33]. In the present study, it was aimed to investigate the effects of tocilizumab and dexamethasone on cytokine storm, oxidative stress, and antioxidants in rats with acute lung injury caused by oleic acid.

Some of the proinflammatory cytokines have prognostic significance in the pathogenesis of ARDS [6]. TNF-α and IL-1β are mostly produced from activated macrophages and act via specific cell membrane-bound receptors. These cytokines stimulate the expression of IL-6 and IL-8 in other cells, including monocytes/macrophages, endothelial cells, fibroblasts and epithelial cells [3]. The influx of neutrophils is triggered by increased expression and release of IL-8 [34]. Characterized by acute respiratory distress syndrome (ARDS), COVID-19, IL-6 is believed to play an important role in the development of the cytokine storm [35]. In the study, oleic acid increased the expression of TNF-α, IL-1β, IL-6, and IL-8 in lung tissue, similar to previous studies [36–38], and these findings show that oleic acid plays an important role in cytokine storm in lung tissue.

There has been increased interest in the use of anti-interleukin-6 (IL-6) agents as drugs in the treatment of patients with severe lung injury and elevated IL-6 levels, COVID-19/ARDS. The IL-6 inhibitor is widely available for use in COVID-19 patients as monoclonal antibodies targeting IL-6 (siltuximab) or IL-6R (tocilizumab and sarilumab). Tocilizumab (TCZ) is a neutralizing antibody against IL-6 and IL-6R and blocks both classical and signal transduction pathways [39]. In the study, it was determined that TNF-α, IL-1β, and IL-6, IL-8 expression in the lung tissue of rats using 4 mg/kg TCZ were significantly reduced (P < 0.05) compared to the oleic acid group by PCR and immunochemistry. IL-6 is positively correlated with the production of proinflammatory cytokines and chemokines [40]. The study showed that IL-6 is a cytokine with prognostic importance in ARDS patients and has a synergistic effect with other proinflammatory cytokines.

Glucocorticoids have long been used to treat ARDS [41]. Dexamethasone, a synthetic corticosteroid, acts as a broad-spectrum immunosuppressive, has greater activity and longer duration of action than cortisone [42]. The drug reduces the severity of ARDS and improves patient prognosis, with its potential to reduce inflammation in the lungs [43]. In a previous study [41], it was determined that serum TNF-α, IL-6 and VEGF levels of rats treated with oleic acid and dexamethasone were significantly lower than those of the oleic acid group. In the study, TNF-α, IL-1β, IL-6, and IL-8 expression were detected at significantly lower levels in the lung tissue of rats treated with 10 mg/kg dexamethasone by PCR and immunochemistry compared to the oleic acid group (P < 0.05). Dexamethasone is thought to be useful in the hyperinflammation or cytokine storm associated with COVID-19/ARDS [44].

Reactive oxygen species (ROS) such as superoxide anion radical (O), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical cause oxidative breakdown of polyunsaturated fatty acids (PUFA) of cell membranes, known as lipid peroxidation [45], and finally MDA is formed. In the study, similar to previous studies [20, 46], MDA level was found to be high in the lung tissue and oleic acid caused damage to the lung cells. It was also determined that the MDA level in the lung tissue was statistically significantly lower in the OA + TC-4 group compared to the OA + TCZ-2 and OA + DEX 0.1 groups (P < 0.05). Tocilizumab and dexamethasone reduced oxidative stress and free radical reactions in response to OA treatment.

Hydrogen peroxide has an important role in the pathogenesis of ALI/ARDS [47]. SOD, the enzyme catalyzes superoxide radicals in a dismutation reaction that produces hydrogen peroxide. In the study, SOD activity decreased in the lung tissue of rats treated with OA. In the study [48] conducted with Sodium escinate (SA), which has anti-inflammatory properties, they found a higher SOD level compared to animals given OA alone. In the study, it was determined that SOD activity was higher in the OA-TCZ-4 and OA + DEX 0.1 groups compared to the OA group. Catalase and glutathione (GSH) enable the conversion of hydrogen peroxide to water and oxygen [49]. GSH is an intracellular thiol found at high levels in all tissues and various body fluids, particularly lung tissue and BALF. [50] GSH is oxidized to GSSG via the enzyme glutathione peroxidase (GPx), while GSSG is reduced to GSH via glutathione reductase (GR). GSH acts as an antioxidant by repairing cellular damage [51] and helps relieve inflammation [52]. Similar to previous studies previous [20, 28, 53], it was determined that oleic acid reduced GSH and CAT activity in rat lung tissue (P < 0.05). In addition, it was noticed that GSH
and CAT activities increased statistically in Tocilizumab and dexamethasone groups (P < 0.05). Tocilizumab and
dexamethasone showed a protective effect against oxidative stress caused by OA.

Oleic acid (OA) induces acute diffuse lung injury similar to ALI and ARDS in humans [28]. Oleic acid causes focal bleeding and vascular congestion in lung tissue, diffuse interstitial, and alveolar edema, and interstitial and alveolar infiltration of leukocytes [33]. In the study performed, it was observed that alveolar thickening, hyperemia, and peribronchial cell infiltration in the lung tissue of rats treated with oleic acid were histopathologically severe, and histopathological findings in TCZ and DEX group rat lung tissue were similar to the control group. In addition, with SEM in the study, blood vessels in the lungs of OA group rats were full and the cell layer forming the alveolar lining layer became indistinct in the OA, OA + TCZ-2, and OA + TCZ-4 groups. In fact, in the OA + TCZ-4 group, it was noted that the cells forming the alveolar lining layer were completely disturbed, the collagen fibrils under this layer were exposed, and the vascular structures deteriorated. It is thought that more studies should be done on the imaging of tocilizumab with SEM on lung tissue.

**Conclusions**

Oleic acid was found to be a good model with its histopathological findings and cytokine storm in the lung tissue. In the model of acute lung injury caused by oleic acid, tocilizumab and dexamethasone are thought to be effective in preventing cytokine storm by downregulation the expression of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-8. Oleic acid reduces antioxidant parameters such as SOD and GSH in lung tissue, and tocilizumab and dexamethasone show a protective effect on cell damage by increasing these antioxidants.

**Abbreviations**

**OA**: Oleic acid  
**ARDS**: Acute respiratory distress syndrome  
**ALI**: Acute lung injury  
**CAT**: Catalase  
**COVID-19**: Coronavirus disease 2019  
**DAB**: 3,3’- diaminobenzidine tetrahydrochloride  
**DEX**: Dexamethasone  
**GR**: Glutathione reductase  
**GPx**: Glutathione peroxidase  
**GSH**: Glutathione  
**H₂O₂**: Hydrogen peroxide  
**H&E**: Haemotoxylin and Eosin  
**IL**: Interleukin  
**IL-1β**: Interleukin 1 beta  
**LPO**: Lipid peroxidation
MDA: Malondialdehyde
PUFA: Polyunsaturated fatty acids
ROS: Reactive oxygen species
SEM: Scanning electron microscopy
SOD: Superoxide dismutase
TCZ: Tocilizumab
TNF-α: Tumor necrosis factor α

Declarations

Ethical approval and consent to participate

Animal studies were approved by the local ethics committee of Kastamonu University (E.50144-6/31).

Consent for publication

Not applicable.

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Authors' contributions

All authors participated in the design, interpretation of the studies and analysis of the data, and review of the manuscript, HSE and FT; conducted the experiments, contributed to performing FT; histopathologically and immunochemistry analysis, BD; SEM imaging, HSE and MA; biochemical analysis, and IC; molecular analysis.

References


Table

Table 1. Primers used in real-time PCR experiments.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primer sequence</th>
<th>Accession number</th>
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| TNF-α     | F: 5′-CCAGGAGAAAGTCAGCCTCCT-3′  
           | R: 5′-TCATACCAGGGCTTGAGCTCA-3′  | X66539 |
| IL-1β     | F: 5′-CACCTCTCAAGCAGACGACACAG-3′  
           | R: 5′-GGGTTCATGGTAAGTCAAC-3′  | NM_031512 |
| IL-6      | F: 5′-TCCTACCCAATCTCAATGCTC-3′  
           | R: 5′-TTGGATGTCTTTGTCCTTGACC-3′  | NM_012589 |
| β-actin   | F: 5′-AGGCCGGCTTCGCGCGGCAGAC-3′  
           | R: 5′-CTCGGGAGGCCACACGCAGCT-3′  | NM_031144.3 |

Table 2. Immunohistochemical and histopathological results of the effect of tocilizumab and dexamethazone on oleic acid-induced acute lung.
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<td>1.75±0.95&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>1.5±1.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.034</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.00±1,15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.50±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25±0.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.50±0.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.75±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.75±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00±0.81&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.25±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.75±0.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.13</td>
</tr>
</tbody>
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<sup>a,b</sup>: P < 0.05, All values are mean ± Sd.

**Figures**
Figure 1

(a) Peribronchiole hyperplasia in lymphoid tissue and thickening of the interalveolar septum (black arrows) and hyperemia (red arrows) of the interalveolar septum such as pathological change were detected of lung by H&E staining. Bar: 200 and 500 µm. (b). The expressions of TNF-α, IL-6, and IL-8 were measured by immunohistochemistry. Bar: 50 and 200 µm.
Figure 2

Scanning electron microscopic images of the rat lungs. (A) Cross-section area of the control group (Bar: 500 µm). (a) alveolar surface of the control group (Bar: 30 µm). (B) Cross-section area of the OA group (Bar: 500 µm). (b) alveolar surface of the OA group (Bar: 50 µm). (C) Cross-section area of the OA+DEX 10 group (Bar: 500 µm). (c') alveolar surface of the OA+DEX 10 group (Bar: 30 µm). (D) Cross-section area of the OA+TCZ 2 group (Bar: 500 µm). (d) alveolar surface of the OA+TCZ 2 group (Bar: 100 µm). (E) Cross-section area of the OA+TCZ 4 group (Bar: 500 µm). (e) alveolar surface of the OA+TCZ 4 group (Bar: 50 µm). #: kohr pore, *: erythrocyte, Arrow: interalveolar septum, Double arrow: alveolar capillar vessel, p: pleura, s: alveolar sac, m: alveolar macrophage, all: alveolar lining layer, cf: collagen fibrils.
Figure 3

(a) In lung tissue, the LPO level increased significantly (P < 0.05) in the OA group and was significantly lower in the DEX and TCZ groups (P < 0.05). (b) It significantly decreased SOD activity in OA and OA+TCZ-2 groups (P < 0.05) and increased SOD activity in OA+TCZ-4 and OA+DEX-10 groups compared to OA group (P < 0.05). (c) In the OA+TCZ-4 group, GSH level was significantly increased compared to the OA and other experimental groups (P < 0.05). (d) CAT activity was significantly decreased in the OA group (P < 0.05) and significantly increased in all treatment groups compared to the OA group (P < 0.05). Different letters (a,b,c,d) in each graph indicate statistical differences between control and experimental groups according to Tukey's multiple range test (P < 0.05).
(a), (b), (c). Effect of tocilizumab and dexamethasone on OA-induced of lungs tissue changes in relative mRNA expression TNF-α, IL-1β, and IL-6. Each bar with vertical line represents the mean fold change ± S.E.M of 6 rats compared to control. OA significantly increased TNF-α, IL-1β and IL-6 levels in lungs tissue compared with control group (P < 0.05). All treatments markedly decreased TNF-α, IL-1β and IL-6 level in lungs tissue compared with OA group (P < 0.05). Different letters (a, b, c, d) indicate statistical differences between groups according to Duncan's multiple range test (P < 0.05) in each graphics. There is no statistically significant difference between the groups which have the same letters (P > 0.05).