Protective Effects of Rutin on Acute Lung Injury Induced by Oleic Acid in Rats

Mustafa Sinan AKTAŞ1 Fatih Mehmet KANDEMİR2 Mustafa ÖZKARACA3 Başak HANEDAN1 Akın KIRBAŞ1

[1] This study was presented in 5th Animal Health and Veterinary Medicine Congress (September 26-27, 2016) in Valencia, Spain

1 Atatürk University, Faculty of Veterinary Medicine, Department of Internal Medicine, TR-25240 Erzurum - TURKEY
2 Atatürk University, Faculty of Veterinary Medicine, Department of Biochemistry, TR-25240 Erzurum - TURKEY
3 Atatürk University, Faculty of Veterinary Medicine, Department of Pathology, TR-25240 Erzurum - TURKEY

Abstract

The purpose of this study was to explore the protective effects of different doses of rutin with the antioxidant and anti-inflammatory properties on acute lung injury (ALI) induced by oleic acid (OA) in rats. Thirty-five Sprague-Dawley male rats were randomly separated into five groups comprising control, rutin 150 mg, OA, rutin 75 mg + OA and rutin 150 mg + OA. In the rutin 75 mg + OA and rutin 150 mg + OA groups, the lung malondialdehyde level (MDA) was significantly lower than that of the OA group. In the rutin 75 mg + OA and rutin 150 mg + OA groups, the lung GPx (glutathione peroxidase), CAT (catalase) and SOD (superoxide dismutase) activities and GSH (glutathione) levels were significantly higher than those of the OA group, and significantly lower than those of the control group. iNOS expressions in the interstitial parts of the lungs were significantly lower than those of the OA group. It was concluded that on the ALI induced by OA, rutin had protective effects through the antioxidant and anti-inflammatory properties and that the treatment of rutin as a supportive treatment in ALI was found to be practically useful.

Keywords: Acute lung injury, Oleic acid, Oxidative stress, Rutin

INTRODUCTION

Acute lung injury (ALI) is a disease characterized by edema due to intra or extra pulmonary risk factors, hypoxemia resistant to oxygen treatment, alveolar hemorrhage, development of hyaline membrane, increase in the alveolar wall thickness and histopathologic changes containing pulmonary inflammation [1], and clinically characterized by pulmonary edema and respiratory distress with an acute onset [2]. Sepsis, pneumonia, shock, aspiration, pancreatitis, blood transfusion, severe trauma and the inhalation of toxic gases are all factors creating...
Protective Effects of Rutin on...

The rats were randomly divided into five groups and each group consisted of seven rats. The rats in Group I were given intravenously (i.v.) sterile saline once (control group). The rats in Group II were given rutin (rutin hydrate, Sigma Chemical Company, USA) orally in the doses of 150 mg/kg/day for 7 days. The rats in Group III were given i.v. 50 μl OA (Cis-9-octadecenoic acid, Sigma Aldrich, Germany) dissolved in 250 μl 1% BSA (Bovine Serum Albumin, Sigma Aldrich, Germany) through their tail veins once. The rats in Group IV were given rutin orally in the doses of 75 mg/kg/day for 7 days and on the 7th day the rats were given 50 μl OA i.v. through the tail veins once. The rats in Group V were given rutin orally in the doses of 150 mg/kg/day for 7 days and on the 7th day the rats were given 50 μl OA i.v. through the tail veins once. The rats in all of the groups were sacrificed with decapitation under sevoflurane anesthesia (Sevorane liquid 100%, Abbott Laboratory, Istanbul, Turkey) 24 h after the last application.

Analysis of Oxidants and Antioxidants

The homogenization of lung tissues was performed in a Teflon-glass homogenizer with the use of a buffer of 1.15% KCl in order to obtain a 1:10 (w/v) homogenate. The malondialdehyde (MDA) levels in the lung homogenate were measured by the thiobarbituric acid reaction according to the method of Placer et al. The lung CAT activity was measured by the decomposition of hydrogen peroxide at 240 nm according to the method of Aebi. The measurement of protein concentration in the supernatant was also performed according to the method of Lowry et al. The measurement of lung SOD activity was performed by superoxide radical production via xanthine and xanthine oxidase, following the reaction of nitro blue tetrazolium and the formation of formazan dye. The measurement of GSH content according to the method of Sedlak and Lindsay and the measurement of GSH activity was performed according to the method of Matkovics et al.

Histopathological Examination

Rats were killed by decapitation. The lungs were immediately removed, fixed in 10% neutral formalin solution for 24-48 h, then processed to obtain paraffin blocks. Paraffin-embedded blocks were routinely processed. 5-μm thick sections were stained with hematoxylin-eosin, and examined under a microscope under 20X magnification. Slides in the sections were graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe).

Immunohistochemical Examination

After deparaffinization, the slides were immersed
sections were graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe).

**Statistical Analysis**

The biochemical, histopathologic and immunohistochemical parameters were analyzed with one-way ANOVA using SPSS package program (version 20.0; SPSS, Chicago, IL). The Duncan test was used in the comparison of the groups. All data were presented in mean (±) standard error of means (SEM). Differences in histopathologic and immunohistochemical measured parameters among the five groups were analyzed with a nonparametric test (Kruskal-Wallis). Dual comparisons between the groups exhibiting significant values were evaluated with a Mann-Whitney U-test (P<0.05).

**RESULTS**

The lung tissue MDA levels were significantly higher in the OA group compared to the control and rutin 150 mg groups. The lung tissue MDA levels significantly decreased in the rutin 75 mg + OA and rutin 150 mg + OA groups compared to the OA group. But there were no significant changes in the lung tissue MDA levels between the rutin 75 mg + OA and rutin 150 mg groups. The lung tissue MDA levels significantly increased in the rutin 150 mg + OA group compared to the control group. There were no significant changes in the lung tissue MDA levels between the rutin 75 mg + OA group and the rutin 150 mg + OA group (Table 1).

The lung tissue SOD activity significantly decreased in the OA group compared to the control and rutin 150 mg groups. The lung tissue SOD activity significantly increased in the rutin 150 mg + OA group compared to the control group. The lung tissue SOD activity was significantly higher in the rutin 150 mg + OA group compared to the rutin 75 mg + OA group (Table 1).

The lung tissue CAT activity significantly decreased in the OA group compared to the control and rutin 150 mg groups. The lung tissue CAT activity significantly increased in the rutin 150 mg + OA group compared to the control group. There were no significant changes in the lung tissue CAT activity between the rutin 150 mg + OA group and the control group. Furthermore, there were no differences in the lung tissue CAT activity between the rutin 150 mg + OA group and the rutin 75 mg + OA group (Table 1).

The lung tissue GPx activity significantly decreased in the OA group compared to the control and rutin 150 mg groups. The lung tissue GPx activity significantly increased in the rutin 150 mg + OA group compared to the OA group. But there were no significant changes in the lung tissue GPx activities between the rutin 150 mg + OA group and the control group. The lung tissue GPx activities were significantly higher in the rutin 150 mg + OA group compared to the rutin 75 mg + OA group (Table 1).

**Histopathologic Evaluation**

It was observed that the lung structures in the control and rutin 150 mg groups were normal (Fig. 1A-B). Severe inflammatory cell infiltrations and hemorrhagia were observed in the interstitial areas of the OA group (Fig.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I (Control)</td>
</tr>
<tr>
<td>MDA (nmol/g tissue)</td>
<td>51.26±1.02a</td>
</tr>
<tr>
<td>GPx (U/g protein)</td>
<td>5.90±0.22a</td>
</tr>
<tr>
<td>GSH (nmol/g tissue)</td>
<td>34.08±1.02a</td>
</tr>
<tr>
<td>CAT (katal/g protein)</td>
<td>45.74±1.40b</td>
</tr>
<tr>
<td>SOD (U/g protein)</td>
<td>26.93±0.33a</td>
</tr>
</tbody>
</table>

MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase, GPx: glutathione peroxidase, GSH: glutathione. a,b,c,d Means in rows with different superscripts differ significantly at P<0.01. All the values are expressed as the mean±SEM of seven rats in each group.
Protective Effects of Rutin on...

1C). The severity of the inflammatory cell infiltrations and hemorrhage in ALI induced by OA were significantly decreased in the rutin 75 mg + OA and rutin 150 mg + OA groups (Fig. 1D-E, Table 2).

**Immunohistochemical Evaluation**

The lung iNOS, which is used as an inflammatory marker, were expressed in low levels in the lungs of the rats in the control and rutin 150 mg groups (Fig. 2A-B, Table 3). The lung iNOS was severely expressed in the interstitial areas in the OA group, and the lung iNOS expression in the interstitial areas was significantly decreased in the rutin 75 mg + OA group (Group IV) (D), Moderate inflammatory cell infiltrations in the interstitial areas (*) of the rutin 150 mg + OA group (Group V) (E), (H-E)

**Table 2. Evaluation of the inflammatory cell infiltrations and hemorrhage in the lung tissue samples of the groups under a light microscope with x20 magnification: 0 (none), 1 (light), 2 (moderate), 3 (severe)**

<table>
<thead>
<tr>
<th>Histopathologic Findings</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I (Control)</td>
</tr>
<tr>
<td>Inflammatory cell infiltrations and hemorrhage</td>
<td>0.14±0.14a</td>
</tr>
</tbody>
</table>

All data were presented in mean (±) standard error of means (SEM) and P<0.05 versus other groups.
DISCUSSION

Because the morphologic, cellular and functional changes caused by the intravenous treatment of OA are similar to those of ALI, OA treatment in experimental studies in order to research the therapeutic effects of different agents on ALI is quite a common method [24]. There are many studies where OA is used to form ALI in many species such as rat [25], mouse [17], dog [26] and rabbit [27]. Nonenzymatic lipid peroxidation is an important point in the oxidative stress related cellular damage caused by free radicals. MDA, which is the last product of lipid peroxidation, is a good indicator of cellular damage caused by free radicals and oxidative stress [28]. Koksel et al. [25] have indicated the increased MDA levels and the development

<table>
<thead>
<tr>
<th>Immunostaining</th>
<th>Groups</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>iNOS</td>
<td>Group I (Control)</td>
<td>1.00±0.21a</td>
</tr>
<tr>
<td></td>
<td>Group II (Rutin 150 mg)</td>
<td>1.14±0.14a</td>
</tr>
<tr>
<td></td>
<td>Group III (OA)</td>
<td>2.85±0.14b</td>
</tr>
<tr>
<td></td>
<td>Group IV (75 mg rutin + OA)</td>
<td>2.14±0.14c</td>
</tr>
<tr>
<td></td>
<td>Group V (150 mg rutin + OA)</td>
<td>1.42±0.20d</td>
</tr>
</tbody>
</table>

All data were presented in mean ± standard error of means (SEM) *P<0.05 versus other groups.
of oxidative stress in rats with ALI induced by OA. The same results have been determined in various other studies [6,29]. In the present study in conformity with the results of Koksel et al. [25] it was determined that the MDA level in the OA group was significantly higher than that of the control group. The MDA increase in the rat brains and kidneys caused by ischemia-reperfusion has been reported to be lessened by rutin [13,30]. Furthermore, it was determined that the increase in MDA level caused by β-amyloid 42 in BV-2 cells reduced by rutin [31]. It was found in this study that although the lung MDA level of the rutin 75 mg + OA and rutin 150 mg + OA groups significantly decreased compared to that of the OA group, it still was very high compared to that of the control group. Similarly, Yeh et al. [32] have determined that rutin has an inhibiting effect on the lipid peroxidation of rats with ALI induced by LPS.

Under normal physiological circumstances, cellular defense against oxidative damage is provided through various mechanisms and antioxidant molecules such as SOD, CAT, GSH, and GPx [33]. It is indicated that the antioxidant enzymes are consumed during ALI [34]. This result was supported in the presented study where the GSH level and the GPx, CAT and SOD activities of the lung tissue in the OA group were significantly lower compared to those in the control and rutin 150 mg groups. It is indicated that rutin is an antilipoperoxidant agent [35] and a strong free radical scavenging [36]. Rutin has been found to increase the levels of SOD and CAT in rats with cerebral ischemia-reperfusion [37]. Furthermore, rutin treatment has been indicated to increase the SOD, CAT and GPx activities in neurotoxicity [38], liver damage [39], renal damage [40] and testicular damage [41]. Similarly, Yeh et al. [32] have determined that rutin increases the SOD, CAT and GPx activities in rats with ALI induced by LPS. Also, Martinez et al. [42] have revealed that quercetin increases the SOD, CAT and GPx activities in the lung tissue, especially in the interstitial areas, of the rats in the control and rutin 150 mg groups and there was a severe iNOS expression in the interstitial areas in the OA group treated with OA alone. In accord with the results in the presented study, Yi-Chun et al. [44] have found that rutin shows the protective effect by inhibiting neutrophil infiltration and the expression of iNOS and vascular cell adhesion molecule (VCAM)-1 in ALI model induced by LPS in mice. The studies have revealed that rutin has the protective effect via anti-inflammatory properties in ALI model [45,46]. Also, Kandemir et al. [47] have reported that iNOS is expressed in the glomeruli and mesangial cells in rats with renal damage induced by gentamicin, and that the treatment of rutin decreases the iNOS expression and thus decreases inflammation.

As a result, it was concluded that rutin has a protective effect through its antioxidant and anti-inflammatory properties in the ALI induced by OA and that this protective effect is higher in the 150 mg dosage than the 75 mg dosage and that it would be beneficial to use rutin in the supportive treatment of ALI patients.

REFERENCES

of nitric oxide and proinflammatory cytokines. Neurotox, cytotoxicity, attenuates oxidative stress, and decreases the production of lipid peroxidation (as malondialdehyde) in biochemical systems. Rutin inhibits β-amyloid aggregation and β-amyloid plaque formation.


