The effect of curcumin on lung injuries in a rat model induced by aspirating gastrointestinal decontamination agents

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Abstract
Background: Aspiration is one of the most feared complications of gastrointestinal decontamination procedures with nonabsorbed polyethylene glycol (PEG) solution and activated charcoal (AC). We aimed to investigate the protective effects of curcumin (CUR) on lung injury in rats induced by aspiration of these agents.

Methods: Experimental rats were divided randomly into 6 groups (n = 7): a saline-aspirated control (group I), sterile saline aspirated with CUR treatment (group II), PEG aspirated (group III), PEG aspirated with CUR treatment (group IV), AC aspirated (group V), and AC aspirated with CUR treatment (group VI). After aspiration, treatment groups II, IV, and VI were given 150 mg/kg CUR intraperitoneally once a day for 7 days. After 7 days, the rats were humanely killed, and both the lungs and serum specimens from all groups were evaluated histopathologically, immunohistochemically, and biochemically.

Results: Aspiration of gastrointestinal decontamination agents produced histopathologic changes, elevated levels of malondialdehyde and surfactant protein D, reduced levels of antioxidant enzymes, and increased expression of inflammatory cytokines interleukin-1β and tumor necrosis factor α. Curcumin treatments effectively attenuated the rats’ pulmonary inflammation responses (as shown by reduced alveolar damage), decreased serum malondialdehyde and surfactant protein D levels, and inhibited the expressions of tumor necrosis factor α and interleukin-1β.

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Gastrointestinal decontamination with nonabsorbed polyethylene glycol (PEG) solution and activated charcoal (AC) are modalities used widely by emergency departments in treating patients who ingested toxic agents [1]. These gastrointestinal decontamination agents attempt to interrupt the enterohepatic and enteroenteric circulation of potentially toxic ingestions of sustained-release or enteric-coated drugs [1,2]. Whole bowel irrigation with a PEG solution arguably is the only option for gastrointestinal decontamination in patients presenting more than 2 hours after poisoning, when the amount of poison adsorbed by AC is insignificant [2]. Although PEG and AC are known to be safe and reliable, some complications may occur during or after the use of these agents. One of the most serious complications is aspiration of PEG or AC [3,4]. Therefore, administration of these agents is contraindicated in patients whose airway reflexes are already impaired because of aspiration, or if cerebrovascular accidents, head trauma, seizure, and ingestions are likely to produce central nervous system depression [2].

The pathogenesis and molecular basis of lung injury induced by aspiration have been investigated extensively in recent experimental studies [5,6]. Numerous inflammatory mediators have been reported in lung injury induced by aspirating contents from the gastrointestinal tract. Among these mediators are ICAM-1 (Intercellular Adhesion Molecule-1)/lymphocyte function–associated antigen-1, neutrophil adhesion receptor CD18, interleukins (IL-1β, IL-6, IL-8, and IL-10), macrophage inflammatory proteins-2 and keratinocyte-derived cytokine, monocyte chemoattractant protein-1, tumor necrosis factor (TNF)-α, and cytokine-induced neutrophil chemoattractant-1 [5]. However, there is limited knowledge about the histopathologic events caused by aspiration of AC and PEG solutions. Previous studies have demonstrated that aspiration of gastrointestinal decontamination agents brought about increased microvascular permeability, pulmonary edema, and acute respiratory distress syndrome [7,8]. It is important to clarify the histopathologic mechanisms of acute lung injury caused by aspiration of AC and PEG solutions to develop an effective therapeutic strategy. However, biochemical and histologic changes in the lung tissue if AC and PEG solutions are aspirated are still unclear.

Curcumin (CUR), an active component of the turmeric root (Curcuma longa), is known to be an anti-inflammatory, antioxidant, anticancer, and antibacterial agent [9]. Curcumin inhibits the production of TNF-α and IL-1 and reduces the activation of nuclear factor κB and activator protein-1 [10]. Recently, CUR was shown to attenuate acute lung injury, probably through improving oxidative stress levels and inhibiting expression of inflammatory cytokines [10-12]. To date, however, no further histopathologic and biochemical results on lung injury caused by gastrointestinal decontamination agents in rats by CUR treatment have been reported. This study investigates further the potential preventive effect of CUR on lung injury resulting from aspiration of gastrointestinal decontamination agents.

**1. Materials and methods**

Permission for the study was obtained from the Local Ethics Committee of Animal Testing at Ondokuz Mayis University, Samsun, Turkey; the study was performed at the Ondokuz Mayis University Research and Application Centre of Laboratory Animals.

**1.1. Experimental animals and study groups**

Forty-two Sprague-Dawley female rats weighing approximately 250 to 300 g were used in our study. The temperature of the environment where the animals’ were housed was 20°C to 22°C. The animals were kept in individual cages in a lighted environment for 12 hours and in a dark environment for 12 hours daily and fed with water and food ad libitum. The rats were checked on a daily basis.

The rats were divided randomly into 6 groups (n = 7):

- The animals in group I were the control group. These animals were aspirated intratracheally with 1 mL/kg of 0.9% sterile saline.
- The animals in group II were also aspirated intratracheally with 1 mL/kg of 0.9% saline; after aspiration, these animals were administered 150 mg/kg of CUR (Sigma-Aldrich, St Louis, MO) intraperitoneally (i.p.) once a day for 7 days.
- The animals in group III were aspirated intratracheally with 1 mL/kg of PEG (Paddock Laboratories, Inc, USA).
- The animals in group IV were aspirated intratracheally with 1 mL/kg of PEG; after aspiration, they were administered 150 mg/kg of CUR i.p. once a day for 7 days.
- The animals in group V were aspirated intratracheally with 1 mL/kg of AC (Char-Flo-Aqua Suspension, 206 mg/240 mL; SSM, Chemicalskey, England).
- The animals in group VI were aspirated intratracheally with 1 mL/kg of AC; after aspiration, they were administered 150 mg/kg of CUR i.p. once a day for 7 days.

**1.2. Surgical procedure**

Before the rats were given intratracheal solutions, they were anesthetized using ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg). The animals were positioned supine, and xylazine (10 mg/kg). The animals were positioned supine, and...
an anterior neck incision provided tracheal exposure. The materials specified in the description of the groups were injected into the trachea using a 20-gaude needle on a 1-mL tuberculin syringe. The skin incision then was closed using 6-0 Ethilon suture.

Starting the first postoperative day, animals in groups II, IV, and VI were injected i.p. with CUR daily for 7 days. After the last CUR injections, all the rats were humanely killed by an i.p. injection of a sufficient dose of ketamine hydrochloride, and both lungs were removed. Portions of the right lung (the anterior lobe, the median lobe, the posterior lobe, and the postcaeval lobe) and the left lung (the upper left lobe and the lower left lobe) were evaluated. Each lung lobe section was flattened out in preparation for the histopathologic, immunohistochemical, and biochemical procedures. Histopathologic and immunohistochemical analyses were performed in at least 8 areas of each lung section. The final score in each category for each individual animal was the average of the scores from examining all sections of its lungs.

1.3. Biochemical procedure

1.3.1. Collection of samples

The animals were humanely killed at the end of day 7. The lungs of the animals were studied grossly, and the samples were taken from all lobes of the right and left lungs for both histopathologic and immunohistochemical examination. The lung tissue samples were kept in 10% formaldehyde until the necessary examinations were performed.

Blood samples from the animals that were killed were placed into noncoagulant tubes for biochemical studies. The blood samples in the tubes were centrifuged at 1000 × g for 10 minutes within 2 hours after acquisition; the resulting serum samples were kept at −80 °C until they were studied.

1.3.2. Rat pulmonary surfactant-associated protein D

Surfactant-associated protein D (SP-D) derived from the serum samples was studied using an enzyme-linked immunosorbent assay (ELISA) following the manufacturer’s instructions (CSB-E12632r, Rat pulmonary SP-D ELISA kit; Cusabio, China). The color generated was read on a microplate reader at 450 nm, and the results were presented in nanograms per milliliter.

1.3.3. Glutathione peroxidase

The activity of glutathione peroxidase (GSH-Px) in the serum was studied using ELISA following the manufacturer’s instructions (ELISA kit; Cayman Chemicals Company). One unit of SOD was defined to be the amount of enzyme ensuring dismutation of 50% of the superoxide radicals. The results were presented in units per milliliter.

1.3.4. Total superoxide dismutase

The total superoxide dismutase (SOD) activity (Cu/Zn, Mn, and Fe SOD) is observed in our study. Total SOD activity in the serum was studied in accordance with the manufacturer’s company’s instructions (ELISA kit; Cayman Chemicals Company). One unit of SOD was defined to be the amount of enzyme ensuring dismutation of 50% of the superoxide radicals. The results were presented in units per milliliter.

1.3.5. Malondialdehyde

Malondialdehyde (MDA) was studied using the method described by Draper et al [12]. Malondialdehyde values were calculated using the extinction coefficient of the complex of thiobarbituric acid–MDA (1.56 × 10^5 cm⁻¹ [mol/L]⁻¹). The results were presented in micromoles per deciliter.

1.4. Histopathologic examination

Specimens taken from each lobe of both lungs were fixed immediately in 10% neutral-buffered formalin and embedded in paraffin wax. Next, the specimens were sectioned (5 μm), and microslides were stained with hematoxylin and eosin for histopathologic assessment under a light microscope by a pathologist blind to the aspirated solution.

Histopathologic examination looked for the peribronchial inflammatory cell infiltration (PICI), alveolar septal infiltration (ASI), alveolar edema (AED), alveolar exudate (AEX), granuloma (GRA), and necrosis (NEC) formation. All histopathologic findings were evaluated using a scoring system developed by Takl et al [13] (Table 1). All slides were scored from 0 to 3 according to the degree of histopathologic findings.

1.5. Immunohistochemical examination

Sections perpendicular to the lobar bronchioles were taken from each lobe of both lungs and fixed immediately in 10% neutral-buffered formalin. The fixed tissues were dehydrated in ethanol, cleared in xylene, and embedded in paraffin blocks. Five-micrometer sections were cut and mounted on 3-aminopropyltriethoxysilane–coated slides (Sigma, St Louis, MO). Tissue sections were deparaffinized and rehydrated with distilled water. The microslides were stained using the streptavidin-biotin-peroxidase complex technique (Histostain Plus Kit; Zymed, USA). Endogenous peroxidase was blocked with 3% H₂O₂ for 5 minutes. Slides were then placed in antigen retrieval solution (sodium citrate, pH 6) and heated by microwave oven to room temperature. Next, the tissues were incubated with protein blocking solution for 10 minutes at room temperature. Immediately afterward, the tissues were incubated with the primary antibody for 30 minutes at room temperature.

Anti–TNF-α antibody (1:50; Endogen, IL, USA) and anti–IL-1β (1:100; Endogen, IL, USA) were used as the primary antibodies. For negative control, the primary antibody was evaluated by substituting phosphate-buffered saline for the primary antibody.

All slides were incubated with the secondary antibody, and the horseradish peroxidase–streptavidin solution, respectively, for 30 minutes at room temperature. The slides were stained
with amino ethylcarbazole chromogen and counterstained with Gills hematoxylin. After each step was completed, the slides were washed twice with phosphate-buffered saline solution for 5 minutes, except the protein-blocking step.

The number of TNF-α– and IL-1β–positive cells was assessed using a microscopy image analysis system (Bs200Pro Image analysis system, BAB software, Ankara, Turkey). The number of TNF-α– and IL-1β–positive cells was scored by counting 1000 cells in randomly selected ×10 high-power magnification fields per lung sample. Every stained cell was considered positive regardless of the intensity of the staining. The number of immunopositive cells was recorded as follows: weak, less than 5% (−/+); mild, 6% to 25% (+); moderate, 26% to 50% (++), and strong, more than 50% (+++).

1.6. Statistical methods

All biochemical findings are presented as means ± SD, and the histopathologic scores are presented as median. All findings were developed using SPSS statistical software (SPSS for Windows, version 15.0; SPSS, Chicago, IL). Nonparametric values were analyzed with the Mann-Whitney U test. The degrees of histopathologic scores were analyzed using the analysis of variance followed by the Bonferroni post hoc test for detection of significant differences between groups. A P value less than .05 was regarded as statistically significant.

2. Results

2.1. Biochemical findings

The measurements of serum surfactant protein D, MDA, SOD, and GSH-Px levels and the statistical significance of these measurements are shown in Fig. 1. Aspiration of gastrointestinal decontamination agents (AC and PEG) increased the levels of serum surfactant...
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Table 2  Histopathologic scores of pulmonary tissue for each group (n = 7)

<table>
<thead>
<tr>
<th>Group</th>
<th>PICI</th>
<th>ASI</th>
<th>AED</th>
<th>AEX</th>
<th>AHI</th>
<th>IF</th>
<th>GRA</th>
<th>NEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>2.14 ± 0.34</td>
<td>1.85 ± 0.34</td>
<td>0.85 ± 0.14</td>
<td>1.71 ± 0.18</td>
<td>1.14 ± 0.14</td>
<td>0.71 ± 0.18</td>
<td>0.85 ± 0.14</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>IV</td>
<td>0.57 ± 0.20 **</td>
<td>0.42 ± 0.42 **</td>
<td>0.71 ± 0.18</td>
<td>0.14 ± 0.14 ***</td>
<td>0.71 ± 0.18</td>
<td>0.28 ± 0.18</td>
<td>0.42 ± 0.42 **</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>V</td>
<td>2.00 ± 0.37</td>
<td>2.14 ± 0.34</td>
<td>1.57 ± 0.20</td>
<td>0.85 ± 0.14</td>
<td>2.00 ± 0.21</td>
<td>0.85 ± 0.14</td>
<td>0.57 ± 0.20</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>VI</td>
<td>0.71 ± 0.18</td>
<td>0 ± 0</td>
<td>0.42 ± 0.20</td>
<td>0.14 ± 0.14 **</td>
<td>0.28 ± 0.18</td>
<td>0.14 ± 0.14 **</td>
<td>0.14 ± 0.14 **</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. Group III, PEG-aspirated group; group IV, PEG-aspirated group treated with CUR; group V, AC-aspirated group; group VI, AC-aspirated group treated with CUR.

* P < .05 compared with untreated groups.
** P < .01 compared with untreated groups.
*** P < .001 compared with untreated groups.

2.2. Histopathologic findings

The histopathologic scores of PICI, ASI, AED, AEX, AHI, IF, GRA, and NEC in each study group and their statistical significances are shown in Table 2. In this study, the saline groups (groups I and II) showed no obvious changes (Table 2). No IF, GRA, or NEC was observed in these 2 groups. In addition, no NEC was detected in the PEG-aspiration groups (groups III and IV).

According to the scoring system of Takil et al [13] (Table 1), the alveolar damage in the gastrointestinal decontamination-aspiration groups (groups III and V) was found to have increased, compared with the control saline-aspiration group (group I) (Table 2). Treatment with CUR greatly reduced the morphologic alterations associated with aspiration of gastrointestinal decontamination agents and protecting the alveolar structure (Table 2).

Table 3  Semiquantitative comparison of the positive immunostaining for iNOS, IL-1β, and TNF-α in lung tissues for each group (n = 7)

<table>
<thead>
<tr>
<th>Group</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>+</td>
<td>±</td>
<td>+++</td>
<td>±</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

Group I, saline-aspirated group; group II, saline-aspirated group treated with CUR; group III, PEG-aspirated group; group IV, PEG-aspirated group treated with CUR; group V; AC-aspirated group; group VI, AC-aspirated group treated with CUR.

2.3. Immunohistochemical findings

The immunopositive cell numbers of TNF-α and IL-1β were semiquantitatively higher in groups III and V than in control group I (Table 3; Fig. 2). The immunopositive cell numbers of TNF-α and IL-1β decreased significantly in all treatment groups (Table 3).

The IL-1β immunostaining of lung specimens was significantly higher in groups III and V (median, 2+ and 3+, respectively) than in the control group I (median, 1+) (P < .05 and P < .01, respectively) (Table 3; Fig. 2). After CUR treatment, the IL-1β immunostaining of lung specimens decreased significantly in group II (median, ±), group IV (median, 1+), and group VI (median, 1+) in contrast to the control group I (median, 1+), group III (median, 2+), and group V (median, 3+) (P > .05, P < .05, and P < .01, respectively) (Table 3).

The TNF-α immunostaining of lung specimens was significantly higher in groups III and V (medians, 3+ and 3+, respectively) than in the control group I (median, 1+) (P < .01 and P < .01, respectively) (Table 3). After CUR treatment, the TNF-α immunostaining of lung specimens decreased significantly in group II (median, ±), group IV (median, ±), and group VI (median, 1+) in contrast to the control group I (median, 1+), group III (median, 3+), and group V (median, 3+) (P > .05, P < .001, and P < .01, respectively) (Table 3).

3. Discussion

We have demonstrated in this study that CUR, an anti-inflammatory, antioxidant, and antibacterial agent, prevents lung damage caused by aspiration of AC and PEG solutions. Curcumin attenuated lung parenchymal destruction and inflammatory infiltration induced by aspirating AC and PEG solutions by decreasing the serum MDA and surfactant protein D levels, increasing the antioxidant enzyme levels, and inhibiting the expressions of TNF-α and IL-1β in lung tissue. Our findings indicate that CUR can prevent lung injury induced by gastrointestinal decontamination agents.
Administration of AC and PEG solutions is a gastrointestinal decontamination procedure commonly used in treating these poisoning patients. The most common complication associated with gastrointestinal decontamination is aspiration that results from a combination of the patient’s depressed mental status, loss of protective airway

Fig. 2 Immunohistochemical expressions of IL-1β and TNF-α in all study groups (n = 7). IL-1β(A1), group V, strong immunopositive reaction of IL-1β and immunopositive cells seen in interalveolar septal tissue (arrowhead). B1, Strong immunopositive reaction of IL-1β in interalveolar septal tissue and alveolar type II cells (arrowhead) in group III. C1, Weak immunopositive reaction of IL-1β (arrowhead) and moderate inflammatory reaction in interalveolar tissue in group I. TNF-α (A2), strong immunopositive reaction of TNF-α and immunopositive cells seen in interalveolar septal tissue (arrowhead) in group V. B2, Strong immunopositive reaction of TNF-α in interalveolar septal tissue (arrowhead) and moderate inflammatory reaction in group III. C2, Weak immunopositive reaction of TNF-α (arrowhead) and moderate inflammatory reaction in interalveolar tissue in group I. Immunoperoxidase technique, Harris hematoxylin counter staining, amino ethylcarbazole as a chromogen, ×20.
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Curcumin is a biologically active phytochemical substance derived from the rhizomes of Curcuma longa, commonly known as turmeric. It has been used in traditional medicine for centuries for its anti-inflammatory, antimicrobial, and antioxidant properties. Several studies have shown its potential therapeutic benefits in various conditions, including inflammatory diseases, neurodegenerative disorders, and cancer.

4. Conclusion

Although previous experimental studies have used several antioxidant and anti-inflammatory drugs to treat lung injury caused by aspiration, there have been no studies evaluating the role of CUR in treating lung injury caused by aspiration of gastrointestinal decontamination agents. To the best of our knowledge, our findings provide the first study evaluating the effect of CUR in the pathogenic process of lung injury induced by aspiration of gastrointestinal decontamination agents. Our results show that CUR has a preventive effect on lung damage, and further studies are required to validate our findings.

References


