Int Surg 2016;**101:**304–312
DOI: 10.9738/INTSURG-D-15-00256.1

Protective Effect of Curcumin on Carbapenem-Resistant *Escherichia coli*–Induced Lung Injury in Rats

Cagla Bali[1], Nejat Altintas[2], Ozlem Ozmete[1], Ibrahim Gelincik[3], Hakan Yabanoglu[4], Neslihan Tekin[5], Bulent Altinsoy[6], Bunyamin Cuneyt Turan[7], Anıs Aribogan[1]

[1]*Department of Anesthesiology and Reanimation and* [4]*Department of General Surgery, Baskent University School of Medicine Adana Teaching and Research Center, Adana, Turkey*

[2]*Department of Pulmonary Medicine,* [3]*Department of Pathology, and* [7]*Department of Anesthesiology and Reanimation, Namık Kemal University School of Medicine, Tekirdag, Turkey*

[5]*Department of Chemistry, Biochemistry Division, Aksaray University Faculty of Science and Letters, Aksaray, Turkey*

[6]*Department of Pulmonary Medicine, Bulent Ecevit University School of Medicine, Zonguldak, Turkey*

Curcumin has remarkable anti-inflammatory and antioxidant properties. The aim of this study was to investigate the protective effects of curcumin on a rat model of carbapenem-resistant *Escherichia coli*–induced acute lung injury (ALI). Thirty-two rats were randomly allocated to 4 groups to induce an ALI: negative control group (rats not infected with *E coli* with no antibiotic treatment), positive control group (rats infected with *E coli* with no antibiotic treatment), imipenem group (rats infected with *E coli* that received intraperitoneal injection of imipenem), and the imipenem+curcumin group (rats infected with *E coli* that received intraperitoneal injection of imipenem and were fed on curcumin).The rats were killed, and lung tissues samples were harvested for biochemical analyses and histopathologic examination. Total antioxidant status (TAS), total oxidant status (TOS), tumor necrosis factor α (TNFα), and interleukin-6 (IL6) were measured. TOS increased in the positive control group ($P < 0.001$) and decreased in the imipenem and imipenem+curcumin groups ($P < 0.001$ and $P < 0.001$, respectively). TAS decreased in the positive control group ($P = 0.005$). Imipenem treatment did not increase TAS, but

Corresponding author: Cagla Bali, MD, Anesthesiology and Reanimation Department, Baskent University School of Medicine, Baskent Universitesi Tip Fakultesi Adana Uygulama ve Arastirma Merkezi Dadaloglu Mh 39, Sk. No.6 01250, 01250 Adana, Turkey. Tel.: +90 322 3272727, ext.: 1472; Fax: +90 322 3271273; E-mail: caglaetike@hotmail.com

the imipenem+curcumin group increased TAS ($P = 0.014$). TNFα and IL6 increased in the positive control group compared with the negative control group ($P < 0.001$ and $P = 0.010$, respectively). Imipenem decreased TNFα ($P < 0.001$), but did not decrease IL6 ($P = 0.418$). Imipenem+curcumin decreased TNFα ($P < 0.001$); this decrease was more pronounced compared with the imipenem group ($P = 0.008$). IL6 decreased in the curcumin group compared with the positive control group ($P = 0.011$). Curcumin combined with imipenem can be an alternative therapeutic agent to overcome the resistance of *E coli* strains.

Key words: Curcumin – Carbapenem-resistant *E coli* – Acute lung injury

Acute lung injury (ALI), characterized by excess production of inflammatory factors, pulmonary edema, and severe hypoxemia in the lung, is a common clinical problem associated with significant morbidity and mortality.[1] The primary source of ALI is sepsis, wherein Gram-negative bacteria are a foremost cause.[2] The intraperitoneal injection of the outer cell wall of virtually all Gram-negative bacteria imitates human Gram-negative ALI, which is a commonly accepted model for ALI.[3] The outer cell wall, binding to its Toll-like receptor, activates various proinflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin (IL)1β.[4] Consequently, a robust inflammatory response develops that results in increased endothelial and alveolar permeability, decreased alveolar fluid clearance, and disrupted delicate alveolar structures; this critically impairs lung function.[2] Despite considerable developments in the understanding of the pathophysiology of ALI, recently available treatment options have failed to reduce ALI-related mortality. Accordingly, new treatment choices are needed to achieve effective treatment of ALI.

Escherichia coli is a commensal bacterium in the Enterobacteriaceae family. It causes infections in humans and animals, leading to community-acquired and nosocomial-acquired infections in humans.[5]

The increased prevalence of extended-spectrum β-lactamase (ESBL) produces carbapenem, a preferred drug in the treatment of multidrug-resistant *E coli*. However, in health care settings, the emergence of carbapenem resistance has become a serious mortality factor in the last decade.[6]

There is a need to add therapies to potentiate the effects of antibiotics. First, antibiotics can decrease the bacterial load; however, they cannot preserve organs from the overstimulated inflammation stage. Numerous approaches have been attempted to keep the exaggerated immune response under control, such as the use of various corticosteroids, the use of anti-TNF antibodies, TNF blockers, cyclooxygenase (COX)2 inhibitors, and leukotriene synthetase inhibitors.[7] However, clinical trials showed that these drugs failed and did not protect patients with acute infections.[7] Therefore, a new anti-inflammatory agent, which suppresses exaggerated immune system while preserving its safeguarding activity, is needed. Second, the outburst of drug-resistant microbial strains requires studies of the synergistic effects of antibiotics in combination with the plant's derivatives to develop an antimicrobial cocktail with a wider spectrum of activity and a reduction of adverse side effects of antimicrobial agents.

Curcumin, an orange-yellow curry pigment from turmeric (*Curcuma longa*) has been used as a dietary spice for many years in Asian countries. Curcumin is available in several forms, including tablets, ointments, drinks, soaps, and cosmetics.[8] It is an effective anti-inflammatory, anticancer, and antioxidant agent under investigation for cancer prevention and anti-inflammation.[9,10] Curcumin is more efficient than standard antioxidant compounds as an antioxidative agent.[11] Furthermore, curcumin has antimicrobial activity; in a recent research, synergistic antimicrobial activity of curcumin with Augmentin against *Klebsiella pneumoniae* was reported.[12] Therefore, the addition of curcumin to imipenem may be considered a good option in potentiating the antimicrobial activity of imipenem and in decreasing the detrimental action of the overstimulated immune response.

This study investigated whether an herbal compound, curcumin in combination with imipenem, has a protective effect on ALI. It also assessed if it can be an alternative therapeutic agent to overcome the imipenem resistance of *E coli* strains.

Material and Methods

Bacterial strain

Imipenem-resistant *E coli* isolated from urine in the Namik Kemal University laboratory was used. A 5% sheep blood agar and an eosin methylene blue (EMB) agar were used for culturing bacteria. The plates were incubated at 37° C for 18 to 24 hours. Colony morphology (EMB agar metallic green reflection) and conventional biochemical methods [lactose fermentation, urease activity, citrate utilization, triple sugar iron (TSI) agar for carbonhydrate fermentation detection, and indole production test] were used for the bacteria identification. The carbapenem sensitivity of bacteria was evaluated with the Kirby-Bauer disk diffusion method in Mueller-Hinton agar in accordance with the Clinical and Laboratory Standards Institute.[13]

Animals

Hence, there are no age and sex differences in curcumin pharmacokinetics.[14] Pathogen-free Wistar albino rats of either sex (10 weeks old, 280–320 g) were obtained from the Namik Kemal University Laboratory Animals Research Center. They were housed in separate cages under a standard temperature (22 ± 2°C) and humidity-controlled (60 ± 5%) room with an alternating cycle of 12-hour light and dark. The rats were acclimatized for 5 days before the experiment, and they were fed with pelleted rat food and water *ad libitum*. The study was approved by Baskent University Institution and Review Board (Project DA 15/05) and conducted in accordance with *The Guide for the Care and Use of Laboratory Animals*.[15]

Experimental design

Thirty-two rats were randomly allocated in 4 experimental groups with 8 animals each. They were grouped according to their experimental treatment: negative control group, positive control group, imipenem group, and imipenem+curcumin group.

Induction of ALI

ALI in the rats was induced with imipenem-resistant *E coli* following the method of Oliveira-Junior *et al*.[16] A single isolated colony of *E coli* was transferred to nutrient broth (Mueller-Hinton) and was incubated at 37°C for 12 hours. The solution was prepared to acquire an *E coli* concentration of approximately 1.5×10^8 colony-forming units (CFU)/mL as determined by the 0.5 McFarland standard. The solution (0.1 mL) was injected intraperitoneally (i.p.) into the rats.

Negative control group

The rats were not infected with *E coli* and were given no antibiotic treatment; they received only normal saline i.p.

Positive control group

The rats were infected with *E coli* and were given no antibiotic treatment; they received only normal saline i.p.

Imipenem group

The rats received an i.p. injection of imipenem at a dose of 7 mg/kg for 5 days (Tienam Flacon, Merck Sharp Dohme, Istanbul, Turkey), 24 hours after the establishment of infection with *E coli*.

Imipenem+curcumin group

The rats were fed on curcumin (Curcumin, Sigma Aldrich Company, St Louis, Missouri) (150 mg/kg) for 5 days; they also received an i.p. injection of imipenem at a dose of 7 mg/kg for 5 days, 24 hours after the establishment of infection with *E coli*.

Experimental rat model studies were searched in the *Index Medicus* database to find the most effective curcumin dose for protecting against tissue damage[17] and an effective *E coli* dose for inducing ALI.[18]

All rats in the 4 groups were euthanized by cervical dislocation under deep anesthesia (ketamine/xylazine) on the sixth day of imipenem administration. At the end of the experiment, the lung tissues samples were quickly harvested and washed with cold saline. The right section of the lung was placed into a glass bottle, labeled, and stored at –80 °C until assayed for biochemical analyses; the left part of the lung was placed in Bouin's solution for routine histopathologic and immunohistochemical examination by light microscopy.

Histopathologic assessment

The lung specimens were immersed in Bouin's solution, dehydrated in alcohol, and embedded in paraffin. Slides with 5-μm sections were obtained and were stained with hematoxylin and eosin

(H&E) using standard procedures. The lung tissues were examined and evaluated with standard light microscopy by a pathologist, who was blinded to the groups.

Biochemical procedures

The frozen lung tissue samples were cut into small pieces and were homogenized in 2 mL TrisHCl buffer (pH 7.4) for 3 minutes at 16,000 rpm by a homogenizer (yellow line DI25 digital, IKA, Burladingen, Germany). To clear the debris, the homogenates were centrifuged at 5000*g* for 60 minutes. The resulting upper supernatant fluid was used for analysis.

Measurement of total antioxidant status

The total antioxidant status (TAS) levels were verified using an automated colorimetric measurement method developed by Erel.[19] This method is based on bleaching the characteristic color of the stable radical cation with antioxidants. The assay has excellent precision values that are lower than 3%. The results were expressed as millimoles (mmol) of Trolox equivalent per liter.

Measurement of total oxidant status

The total oxidant status (TOS) was determined using an automated measurement method developed by Erel.[20] The oxidants present in the sample oxidize the ferrous ion–*O*-dianisidine complex to ferric ion. The ferric ion and xylenol orange produced a colored complex in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide (H_2O_2); the results were expressed in terms of micromoles of H_2O_2 equivalent per liter.

Measurement of oxidative stress index

The percentage ratio of TOS to TAS yields the oxidative stress index (OSI), an indicator of the degree of oxidative stress[21] calculated as OSI (arbitrary units) = TOS (mmol H_2O_2 Eq/L)/TAS (mmol Trolox Eq/L). The OSI value for the lung samples was also calculated as OSI (arbitrary units) = TOS (mmol H_2O_2 Eq/g protein)/TAS (mmol Trolox Eq/g protein).

Measurement of cytokines: TNFα and IL6

The TNFα and IL6 concentrations were measured in a serum by commercial enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, Vienna, Austria) according to the manufacturer's instructions.

Statistics

Similar study for sample size calculation was not achieved in the literature. Therefore, sample size is calculated from the web site http://www.danielsoper.com. It was estimated that 8 rats were needed for each group to detect a statistical power (1 – β value) of 80%, allowing for a type I (α) error of 0.05. The normality of the distribution was confirmed using the Shapiro-Wilk test. According to the results obtained from the normality test, a one-way analysis of variance (ANOVA) and Kruskal-Wallis H test were appropriate for use in the statistical analysis. Multiple comparisons were carried out by Tukey's honest significant difference (HSD) test after the ANOVA test and Mann-Whitney U test with the Bonferroni correction; the cutoff level of the α error was reduced to 0.005/(number of tests) after the Kruskal-Wallis H test. The results were expressed as mean ± SD and as a median with minimum and maximum values where appropriate. All statistical analyses were performed with SPSS software (version 21.0, IBM Corporation, Armonk, New York; serial number = 10229569). A 2-sided probability value of less than 0.05 was considered statistically significant.

Results

Histopathologic change

The lung structure of the negative control group was similar to a healthy lung structure. The lung tissues presented open alveoli, interalveolar spaces with customary terminal bronchi, a normal view of the bronchiolar epithelium, thin interalveolar septa, and a lack of inflammatory cells and fibrosis (Fig. 1). However, markedly increased histopathologic abnormalities were seen in the lung tissue of the *E coli*–injected (positive control) group, including broad alveolar injury with intraalveolar septal thickening, large fibrous areas, and collapsed alveolar spaces; inflammatory cells were also seen in the interstitium around small airways and mucosal epithelium. Significant pulmonary edema and alveolar exudate were also observed in this group (Fig. 2). The imipenem protected the lung against *E coli*; howev-

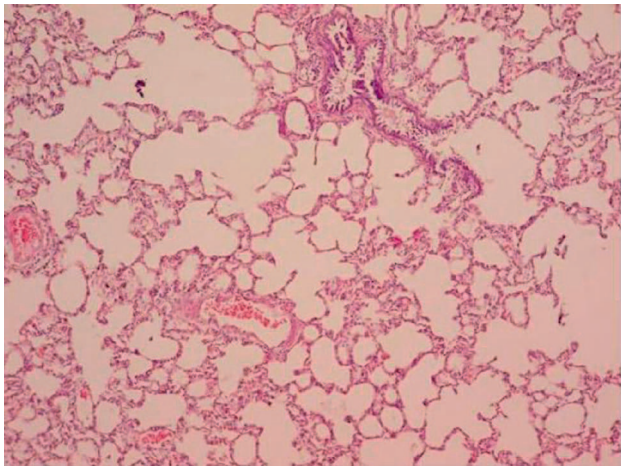

Fig. 1 Negative control group animals (treated with saline alone) showing normal lung tissue morphologies (H&E, ×200).

er, there was still alveolar injury, pulmonary edema, and inflammatory cell infiltration in the imipenem alone–treated group (Fig. 3). On the contrary, curcumin (imipenem+curcumin group) provided protection against *E coli*–induced lung tissue damage. The interstitium of the lungs seemed thinner, and the number of inflammatory cells were obviously reduced. Additionally, pulmonary edema, alveolar exudate, and alveolar airways pathology were significantly less observed in the curcumin-treated rats (Fig. 4). All histopathologic changes were similar and uniform in each group.

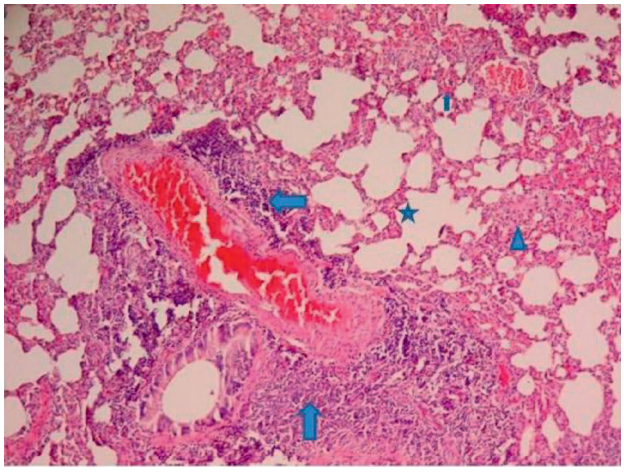

Fig. 2 Positive control group animals (*E coli*–infected, treated with saline alone) showing alveolar congestion (thin arrows), perivascular and peribronchial infiltration (thick arrows), moderate parenchymal fibrosis (arrowhead), and thickening of the alveolar walls (asterisks) (H&E, ×200).

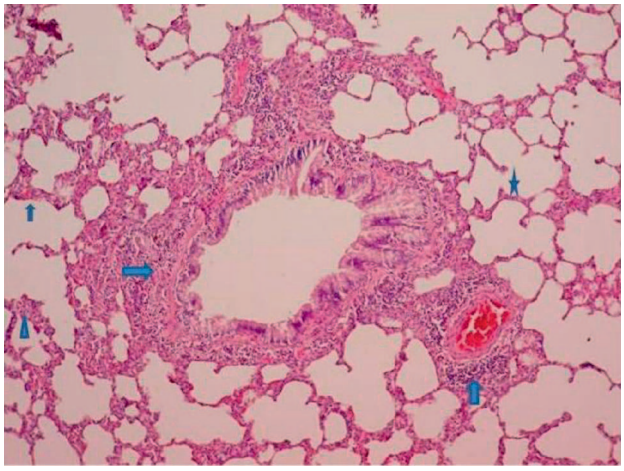

Fig. 3 Imipenem group (infected with *E coli*, received only imipenem treatment) showing slight alveolar congestion (thin arrows), mild perivascular and peribronchial infiltration (thick arrows), mild parenchymal fibrosis (arrowhead), and slight thickening of the alveolar walls (asterisks) (H&E, ×200).

Changes in oxidative stress parameters

The levels of TOS, TAS, and OSI are given in Table 1. The *E coli* inoculation resulted in an increase in the oxidative stress parameters of TOS and OSI ($P < 0.001$ and $P < 0.001$, respectively) and a decrease in the antioxidant parameter of TAS ($P = 0.005$). Imipenem treatment significantly decreased TOS and OSI ($P < 0.001$ and $P = 0.001$, respectively) and increased TAS; however, this increase did not reach statistical significance compared with the positive control group ($P = 0.899$). The imipenem+curcumin

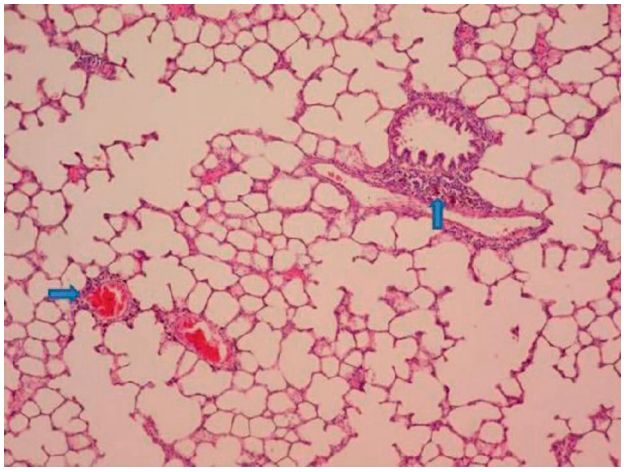

Fig. 4 Imipenem+curcumin group (infected with *E coli*, received imipenem+curcumin treatment) showing only mild perivascular and peribronchial infiltration (arrows) (H&E, ×200).

Table 1 Comparison of the tissue oxidative stress parameters among study groups

Parameters	Negative control	Positive control	Imipenem group	Imipenem+ curcumin group	*P*
TOS (μmol H_2O_2 Eq/L)	4.87 ± 2.16[a,d]	6.01 ± 3.21[a,b,c]	8.93 ± 2.38[b,d]	7.23 ± 1.78[c]	<0.001
TAS (mmol Trolox Eq/L)	2.83 ± 0.17[a]	2.28 ± 0.57[a,b]	2.41 ± 0.36[c]	2.90 ± 0.25[b,c]	0.005
OSI (arbitrary unit)	1.74 ± 0.84[a,d]	6.40 ± 0.92[a,b,c]	3.78 ± 1.22[b,d]	2.53 ± 0.72[c]	<0.001

Where the *P* value is significant, values within a row with the same superscript letter are significantly different. Data are presented as mean and SD.

combination significantly decreased TOS and OSI ($P < 0.001$ and $P < 0.001$, respectively) and increased TAS ($P = 0.014$).

Changes in cytokines (IL6 and TNF α)

E coli inoculation significantly increased IL6 levels compared with the negative control group ($P = 0.010$), and imipenem did not significantly decrease the IL6 level ($P = 0.418$). However, the imipenem+curcumin combination significantly decreased the IL6 level compared with the *E coli*–inoculated group ($P = 0.011$), as shown in Fig. 5.

TNFα levels were significantly increased in *E coli*–inoculated rats compared with the negative control group ($P < 0.001$). Imipenem and the imipenem+curcumin combination significantly decreased TNFα ($P < 0.001$ and $P < 0.001$, respectively), but this decrease was more pronounced in the imipenem+curcumin group compared with the imipenem group ($P = 0.008$), as shown in Fig. 6.

Discussion

Acute respiratory failure is a major source of morbidity and mortality in critically ill patients, with an estimated incidence of 10 to 14 per 100,000 people and a mortality rate of 36% to 52%, respectively.[21] *E coli* may result in uncontrollable inflammation, microvascular leakage, and edema of the lungs in rats. Furthermore, carbapenem-resistant *E coli* is spread in health care settings, and treatment is a major challenge.[22] This study showed that adding curcumin to imipenem as a treatment substantially alleviated inflammation and injury in the lung tissue of carbapenem-resistant *E coli*–induced rats. Curcumin in combination with antibiotics could potentiate the effects of the antibiotics.

E coli replicates at the site of infection and activates several inflammatory cytokines and expressions of many cell surface leukocyte and endothelial adhesion molecules, which result in neutrophil recruitment into the airways.[23] The increased recruitment and subsequent lysis of

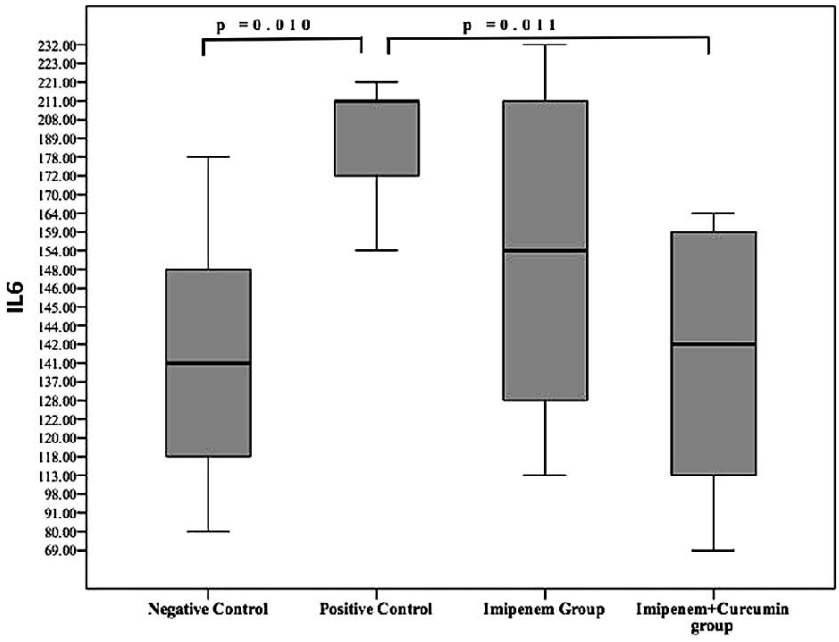

Fig. 5 Serum IL6 levels among the groups. Serum IL6 levels were not significantly decreased with imipenem treatment ($P = 0.418$); however, curcumin combination therapy significantly decreased IL6 levels ($P = 0.011$). The box encompasses the 25% to 75% quartiles, and the median is represented by the horizontal line within the box. The whiskers extend to the highest and lowest values within the higher and lower limits, respectively.

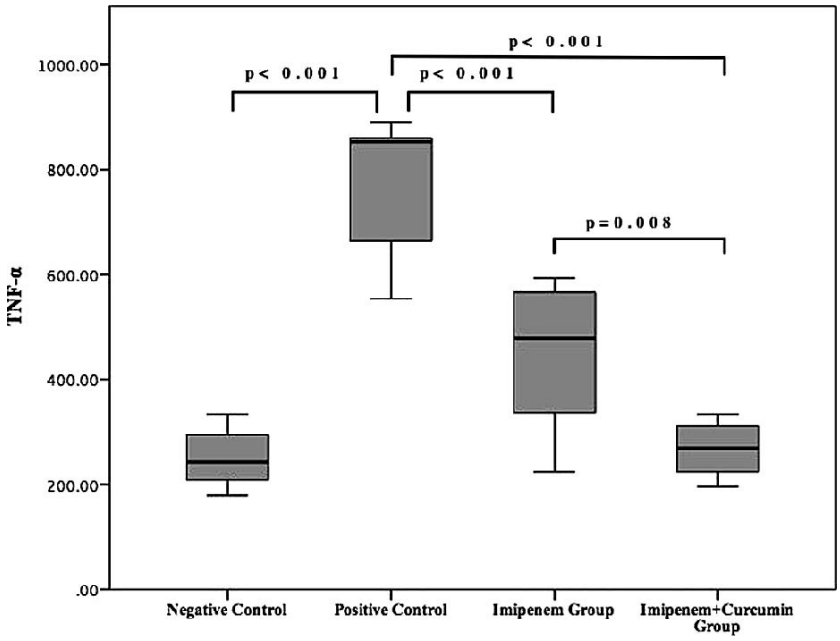

Fig. 6 Serum TNFα levels among the groups. Both imipenem therapy and curcumin combination therapy significantly decreased TNFα ($P < 0.001$ and $P < 0.001$, respectively); however, this decrease was more pronounced in the curcumin group compared with the imipenem group ($P = 0.008$). The box encompasses the 25% to 75% quartiles, and the median is represented by the horizontal line within the box. The whiskers extend to the highest and lowest values within the higher and lower limits, respectively.

neutrophils in the lung can lead to the overproduction of reactive oxygen species (ROS) and oxidative stress.[24] There is evidence that ROSs play a significant role in mediating structural cell apoptosis, lipid peroxidation, and in upregulating proinflammatory cytokine synthesis, such as TNFα and IL6,[25] which, in turn, increases pulmonary endothelial permeability, and subsequently extravasation, and the cytotoxicity of inflammatory cells and tissue injury.[26] In this study, there was increased recruitment of neutrophils to the lung tissue of *E coli*–injected rats, which was confirmed by the histopathologic examination of lung tissue. Antioxidant therapy has been successful in the protection and the treatment of lung injury in some animal studies.[7,27] Pignatelli *et al* showed that curcumin decreased neutrophil infiltration in the tissue spaces.[28] In this study, the histopathologic examination of the lung tissue of rats, which were treated with imipenem alone, showed that imipenem had very little effect on neutrophil infiltration; the alveoli were filled with neutrophils similar to the group injected with *E coli* and not treated at all. However, imipenem combined with curcumin completely reduced markers of inflammation, and the alveoli were lacking neutrophils.

TNFα is a critical cytokine that regulates cell proliferation, survival, differentiation, and apoptosis in the inflammatory and fibrotic responses in the lung following toxicant exposure. It is released mainly from macrophages, but it is also released from neutrophils, $CD4^+$ and $CD8^+$ T cells, B cells, natural killer cells, endothelial cells, smooth muscle cells, osteoclasts, and fibroblasts.[26] TNFα increases pulmonary vascular and alveolar permeability by consuming intracellular glutathione. This event increases vulnerability to oxidative stress, supports the increased expression of adhesion molecules (intercellular adhesion molecule1 and vascular cell adhesion molecule1), and facilitates leukocyte rolling and adhesion.[29,30] Murine lungs overexpressing TNFα expose airspace expansion, loss of small airspaces, increased collagen, and thickened pleural septa on microcomputed tomography. Increased elastance and lung volumes were also found during spirometry.[31] IL6 is released by T cells and macrophages and is usually induced with the other cytokines TNFα and IL1 in many situations, such as infection and trauma; all tissue damage leads to inflammation and also plays a major role in the induction of the acute phase reaction.[32] Reports show that the infection of airways by pathogens, such as *E coli*, triggers the secretion of TNFα and IL6. In this study, TNFα and IL6 increased significantly and correlated with inflammatory cell infiltration in *E coli*–injected rats.

In contrast, curcumin in combination with imipenem led to a decrease in TNFα and IL6 levels and inflammatory cell infiltration in the lungs of rats infected with *E coli* without any adverse effects. Accordingly, curcumin combined with imipenem

can be used to decrease ALI by suppressing the cytokine.

In organisms, the levels of antioxidants and free radicals should at least be in balance to prevent oxidative stress.[33] To explore the protective mechanisms of curcumin, the changes in the oxidative stress status of the lungs were assessed. In the present study, the ratio of TOS to TAS, so-called OSI, shows the oxidant status increased in rats given *E coli*. It was demonstrated that imipenem administration decreased the level of OSI. However, the treatment of rats with a combination of imipenem with curcumin resulted in more decrease compared with the imipenem alone group, thereby reducing lung injury. These results show that curcumin can be considered an adjunct treatment to the antibiotic imipenem, which clears bacteria from the lungs, with the tissue protection from injury caused by an excessive immune response. Curcumin also has antimicrobial effects other than its anti-inflammatory effect. Curcumin is considered a potential candidate against *Helicobacter pylori*, has an excellent protective effect against *Vibrio vulnificus* infection,[34] and has reduced the levels of various virulence factors of *Pseudomonas aeruginosa*.[35] In a recent study, Mun *et al* showed that curcumin has a synergistic antibacterial effect with many antibiotics against methicillin-resistant *Staphylococcus aureus*.[17] It is also reported that curcumin has a synergistic effect with the other adjuncts to multidrug-resistant *Acinetobacter baumannii* and *Staphylococcus epidermidis*.[36,37] Accordingly, in the present study, curcumin in combination with imipenem decreased inflammatory cell infiltration in imipenem-resistant *E coli*–injected rats. However, absence of a curcumin group alone is a limitation for this study. The investigators first thought curcumin as an anti-inflamatory adjunct agent and they planned to use curcumin beside the common used antibiotic "imipenem." New experimental studies can be planned for curcumin as a sole agent for its other unknown properties.

Conclusion

In conclusion, curcumin attenuates imipenem-resistant *E coli*–induced ALI. It is believed that the antioxidant and anti-inflammatory properties of curcumin decreased the production of TNFα, IL6, OSI, and neutrophil infiltration because the immune system plays a major role in the development of ALI and mortality. Furthermore, curcumin in combination with imipenem has the potential quality of an alternative therapeutic agent to overcome the resistance of *E coli* strains.

Acknowledgments

This study was supported by the Baskent University Research Fund (Project DA 15/05).

References

1. Martínez O, Nin N, Esteban A. Prone position for the treatment of acute respiratory distress syndrome: a review of current literature. *Arch Bronconeumol* 2009;**45**(6):291–296
2. Matute-Bello G, Frevert CW, Martin TR. Animal models of acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2008; **295**(3):L379–L399
3. Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 2000;**342**(18):1334–1349
4. Beutler B, Rietschel ET. Innate immune sensing and its roots: the story of endotoxin. *Nat Rev Immunol* 2003;**3**(2):169–176
5. Oteo J, Pérez-Vázquez M, Campos J. Extended-spectrum [beta]-lactamase producing *Escherichia coli*: changing epidemiology and clinical impact. *Curr Opin Infect Dis* 2010;**23**(4): 320–326
6. Xu Y, Gu B, Huang M, Liu H, Xu T, Xia W *et al*. Epidemiology of carbapenem resistant Enterobacteriaceae (CRE) during 2000-2012 in Asia. *J Thoracic Dis* 2015;**7**(3):376–385
7. Tasaka S, Hasegawa N, Ishizaka A. Pharmacology of acute lung injury. *Pulm Pharmacol Ther* 2002;**15**(2):83–95
8. Gupta SC, Patchva S, Koh W, Aggarwal BB. Discovery of curcumin, a component of golden spice, and its miraculous biological activities. *Clin Exp Pharmacol Physiol* 2012;**39**(3):283–299
9. Strimpakos AS, Sharma RA. Curcumin: preventive and therapeutic properties in laboratory studies and clinical trials. *Antioxid Redox Signal* 2008;**10**(3):511–545
10. Punithavathi D, Venkatesan N, Babu M. Curcumin inhibition of bleomycin-induced pulmonary fibrosis in rats. *Br J Pharmacol* 2000;**131**(2):169–172
11. Ak T, Gülçin I. Antioxidant and radical scavenging properties of curcumin. *Chem Biol Interact* 2008;**174**(1):27–37
12. Bansal S, Chhibber S. Curcumin alone and in combination with augmentin protects against pulmonary inflammation and acute lung injury generated during Klebsiella pneumoniae B5055-induced lung infection in BALB/c mice. *J Med Microbiol* 2010;**59**(Pt 4):429–437
13. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial testing; 22nd informational supplement. Available at: http://antimicrobianos.com.ar/ATB/wp-content/uploads/2012/11/M100S22E.pdf. Accessed June 24, 2015

14. Kocher A, Schiborr C, Behnam D, Frank J. The oral bioavailability of curcuminoids in healthy humans is markedly enhanced by micellar solubilisation but not further improved by simultaneous ingestion of sesamin, ferulic acid, naringenin and xanthohumol. *J Funct Foods* 2015;**14**:183–191
15. National Academies Press. *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academies Press, 1996
16. Oliveira-Junior IS, Brunialti MKC, Koh IHJ, Junqueira VBC, Salomão R. Effect of pentoxifylline on lung inflammation and gas exchange in a sepsis-induced acute lung injury model. *Braz J Med Biol Res* 2006;**39**(11):1455–1463
17. Mun SH, Joung DK, Kim YS, Kang OH, Kim SB, Seo YS *et al*. Synergistic antibacterial effect of curcumin against methicillin-resistant *Staphylococcus aureus*. *Phytomedicine* 2013;**20**(8–9):714–718
18. Wu Q, Li H, Qiu J, Feng H. Betulin protects mice from bacterial pneumonia and acute lung injury. *Microb Pathog* 2014;**75**:21–28
19. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem* 2004;**37**(4):277–285
20. Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005;**38**(12):1103–1111
21. Reynolds HN, McCunn M, Borg U, Habashi N, Cottingham C, Bar-Lavi Y. Acute respiratory distress syndrome: estimated incidence and mortality rate in a 5 million-person population base. *Crit Care* 1998;**2**(1):29–34
22. Yamamoto M, Pop-Vicas AE. Treatment for infections with carbapenem-resistant Enterobacteriaceae: what options do we still have? *Crit Care* 2014;**18**(3):229
23. Godaly G, Bergsten G, Hang L, Fischer H, Frendéus B, Lundstedt AC *et al*. Neutrophil recruitment, chemokine receptors, and resistance to mucosal infection. *J Leukoc Biol* 2001;**69**(6):899–906
24. Diep BA, Chan L, Tattevin P, Kajikawa O, Martin TR, Basuino L *et al*. Polymorphonuclear leukocytes mediate *Staphylococcus aureus* Panton-Valentine leukocidin-induced lung inflammation and injury. *Proc Natl Acad Sci U S A*. 2010;**107**(12):5587–5592
25. Rossi RE, Parisi I, Despott EJ, Burroughs AK, O'Beirne J, Conte D *et al*. Anti-tumour necrosis factor agent and liver injury: literature review, recommendations for management. *World J Gastroenterol* 2014;**20**(46):17352–17359
26. Khasnis AA, Calabrese LH. Tumor necrosis factor inhibitors and lung disease: a paradox of efficacy and risk. *Semin Arthritis Rheum* 2010;**40**(2):147–163
27. Bhatia M, Moochhala S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *J Pathol* 2004;**202**(2):145–156
28. Ohene-Abuakwa Y, Pignatelli M. Adhesion molecules in cancer biology. *Adv Exp Med Biol* 2000;**465**:115–126.
29. Mazzon E, Cuzzocrea S. Role of TNF-alpha in lung tight junction alteration in mouse model of acute lung inflammation. *Respir Res* 2007;**8**(75):1–19
30. Ishii Y, Partridge CA, Del Vecchio PJ, Malik AB. Tumor necrosis factor-alpha-mediated decrease in glutathione increases the sensitivity of pulmonary vascular endothelial cells to H_2O_2. *J Clin Invest* 1992;**89**(3):794–802
31. Lundblad LKA, Thompson-Figueroa J, Leclair T, Sullivan MJ, Poynter ME, Irvin CG *et al*. Tumor necrosis factor-alpha overexpression in lung disease: a single cause behind a complex phenotype. *Am J Respir Crit Care Med* 2005;**171**(12):1363–1370
32. Xing Z, Gauldie J, Cox G, Baumann H, Jordana M, Lei XF *et al*. IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. *J Clin Invest* 1998;**101**(2):311–320
33. Rahman K. Studies on free radicals, antioxidants, and co-factors. *Clin Interv Aging* 2007;**2**(2):219–236
34. Na HS, Cha MH, Oh DR, Cho CW, Rhee JH, Kim YR. Protective mechanism of curcumin against *Vibrio vulnificus* infection. *FEMS Immunol Med Microbiol* 2011;**63**(3):355–362
35. Rudrappa T, Bais HP. Curcumin, a known phenolic from *Curcuma longa*, attenuates the virulence of *Pseudomonas aeruginosa* PAO1 in whole plant and animal pathogenicity models. *J Agric Food Chem* 2008;**56**(6):1955–1962
36. Betts JW, Wareham DW. In vitro activity of curcumin in combination with epigallocatechin gallate (EGCG) versus multidrug-resistant *Acinetobacter baumannii*. *BMC Microbiol* 2014;**14**(172):1–5
37. Sharma G, Raturi K, Dang S, Gupta S, Gabrani R. Combinatorial antimicrobial effect of curcumin with selected phytochemicals on *Staphylococcus epidermidis*. *J Asian Nat Prod Res* 2014;**16**(5):535–541