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Protective Effects of Probiotics Against Systemic Inflammation in Mice Model with Chronic Obstructive Pulmonary Disease Induced by Cigarette-smoke

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Abstract

Background: Systemic inflammation is one of hallmarks in chronic obstructive pulmonary disease (COPD), contributing to high morbidity and mortality due to elevated levels of interleukin-6 (IL-6) and reduced level of interleukin-10 (IL-10). Probiotics have the potential to reduce systemic inflammation through the gut-lung axis. This study aims to assess the effect of probiotics compared with an inhaled bronchodilator on serum IL-6 and IL-10 levels in mice model of COPD.

Methods: This was an in vivo experimental study with a post-test only control group design. Thirty C57BL/6 mice were randomized into five groups; NC (healthy mice), PC (COPD induced mice); T1 (COPD mice treated with a bronchodilator), T2 (COPD mice treated with probiotics) and T3 (COPD mice treated with both a bronchodilator and probiotics). COPD was induced for 12 weeks, followed by a 6-week treatment period. After completing the treatment, serum IL-6 and IL-10 levels were measured using the enzyme-linked immunosorbent assay (ELISA).

Results: The IL-6 levels in T2 group were reduced to levels comparable to the negative control group (13.5 vs 12.0 pg/ml respectively, p=0.84). The IL-10 levels were higher in T2 group compared to T1 group, however; this difference was not statistically significant (181.4 vs 155.0 respectively, p>0.05).

Conclusion: In mice model of COPD, probiotics have been shown to lower IL-6 levels and, to a lesser extent, increased IL-10. As a result, probiotics may have a protective effect against systemic inflammation.

Keywords: COPD, Inflammation, Interleukin-6, Interleukin-10, Probiotics.

Introduction

Chronic obstructive pulmonary disease (COPD) continues to be a major global health issue, impacting millions of people worldwide (1). Recent epidemiological data show that the prevalence of COPD is rising, especially in low- and middle-income countries, primarily due to the increasing use of tobacco and exposure to air pollutants (2). The burden of COPD is profound, characterized by substantial morbidity and

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mortality (3,4). Since 2020, COPD has been recorded as the third leading cause of death worldwide, following cardiovascular and cerebrovascular diseases (5).

Chronic obstructive pulmonary disease (COPD) is characterized by systemic inflammation (6–8). This inflammation is due to elevated levels of pro-inflammatory cytokines (e.g., Interleukin 6) and reduced levels of anti-inflammatory cytokines (e.g., Interleukin 10) (9). Such systemic inflammation contributes to the development of comorbidities, including cardiovascular diseases, osteoporosis, metabolic dysfunction (10), and muscle-wasting (11), thereby worsening the overall burden of the disease. These comorbidities not only complicate the treatment of COPD but also increase mortality in affected individuals. Therefore, it is essential to implement comprehensive treatments to address systemic inflammation and enhance the quality of life for patients with COPD.

Recent studies suggest that probiotics could regulate inflammation (12) in pulmonary tissue through the gut-lung axis, two-way communication pathway between the intestine and pulmonary tissues (13,14). Probiotic-derived metabolites, specifically short-chain fatty acids (SCFAs), have been demonstrated to have a beneficial effect in mitigating damage in the alveoli and respiratory tract (15,16). However, the degree to which probiotics can reduce inflammation at the systemic level remains uncertain. The objective of this study is to examine the impact of probiotics on regulating systemic inflammation in mice model of COPD, characterized by pro-inflammatory cytokines (IL-6) and anti-inflammatory (IL-10) in COPD model mice.

Materials and Methods

Experimental design

This study was conducted *in vivo*, utilizing an experimental design with a post-test only control group. The research took place at the Faculty of Medicine, Universitas Sumatera Utara in Medan, Indonesia, and Institut Biosains, Brawijaya University in Malang, Indonesia.

A total of 30 male C57BL/6 mice (*Mus musculus*), aged 2-3 months were involved in this study. All mice were randomized into five groups as illustrated in Table 1. At the end of the 12th week, all mice were euthanized using an intraperitoneal ketamine injection. Blood specimens were collected intracardially, and ELISA examinations were performed to determine serum IL-6 and IL-10 levels.

Table 1. Description of animal groups.

Groups	Description	Number of mice	COPD-induced	Treatment	
				Standard drugs	Probiotics
NC	Negative control	6	No	No	No
PC	Positive control	6	Yes	No	No
T1	Treatment 1	6	Yes	Yes	No
T2	Treatment 2	6	Yes	No	Yes
T3	Treatment 3	6	Yes	Yes	Yes

Induction of COPD

The COPD induction was carried out using the whole-body exposure method to cigarette smoke. Five mice were placed in a container measuring 69x47x38cm which was supplied with cigarette smoke through a smoking pump (17). The exposure to cigarette smoke was carried out for 12 weeks, twice a day, with 12 cigarettes per session, each session lasting 45 minutes. Each cigarette contains 1 mg of nicotine and 15 mg of tar (18,19). To ensure the development of the COPD model, histopathological examination of pulmonary

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tissue was performed with Haematoxylin-Eosin staining at 400x magnification.

Probiotics and standard medication

The probiotics used in this study were commercially available L-Bio products, each gram of which contains 10^8 colony-forming units (CFU) strains of *Bifidobacterium sp and Lactobacillus sp* (12). The probiotics were administered twice daily at a dosage of $1x10^9$ CFU each time (20). An oral gavage was used to administer a solution containing 26 mg of probiotic powder dissolved in 0.2 ml of water for a duration of six weeks.

Bronchodilators are the standard medications prescribed for the treatment of COPD, and so the study employed them. Salbutamol, at a dose of 1.25 mg, was delivered via inhalation using a nebulizer apparatus. The aerosol produced was introduced into a sealed container with dimensions of 20x20x15cm, housing a total of 3 mice. The administration of standard drug was carried out twice a day for six weeks.

Results

Confirmation of COPD model

The verification of successful induction of COPD procedure in mice, a histopathological examination of lung tissue was carried out in the NC and PC groups. The NC group showed normal small airway features with well-organized alveoli tissue and an intact interalveolar septum. In contrast, mice in the PC group exhibited emphysematous changes such as interalveolar septal destruction, air sac dilation, fibrosis in the small airways, and inflammatory cell infiltration (Fig. 1). These findings confirmed the expected COPD model in mice.

Effect of probiotics on serum IL-6 levels

The study found that there was a significant difference in serum IL-6 levels (a pro-inflammatory cytokine) among the five groups of mice.

Table 2. The effect of probiotics on serum IL-6 levels

Groups	IL-6 levels (pg/mL)	*p-value*	*Post-Hoc*			
	Mean ± SD		PC	T1	T2	T3
NC	13.06 ± 2.24	0.035[a]	0.01[b]	0.04[b]	0.84	0.57
PC	19.45 ± 6.71			0.51	0.01[b]	0.03[b]
T1	17.92 ± 4.94				0.06[b]	0.13
T2	13.51 ± 0.43					0.71
T3	14.37 ± 2.15					

[a] $p< 0.05$ statistically significant with *One-Way Anova*. [b] $p< 0.05$ statistically significant with Post-Hoc least significant difference (LSD). NC: negative control, PC: positive control, T: treatment, SD: standard deviation.

The highest serum IL-6 levels were found in the PC group, indicating that COPD caused inflammation not only in the lungs but also at the systemic level. The administration of probiotics was successful in lowering serum IL-6 levels, leveling them close to those of the negative control group, thus demonstrating the protective role of probiotics against systemic inflammation in COPD. The combination of probiotics and bronchodilators (T3) did not yield better results compared to probiotics alone, as shown in Table 2.

Effect of probiotics on serum IL-10 levels

The study found that there was no statistically significant difference in IL-10 levels ($p>0.05$) (Table 3). However, the highest levels of IL-10 were observed in the T2 group, indicating that probiotics might have the potential to increase serum IL-10 levels.

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Table 3. The effect of probiotics on serum IL-10 levels

Groups	Serum IL-10 (pg/mL) Mean ± SD	*p-value*
NC	155.3 ± 30.94	
PC	155.0 ± 50.59	
T1	141.6 ± 25.75	0.38[c]
T2	181.4 ± 58.15	
T3	137.3 ± 23.49	

[c] *One-Way Anova* test. NC: negative control, PC: positive control, T: treatment, SD: standard deviation.

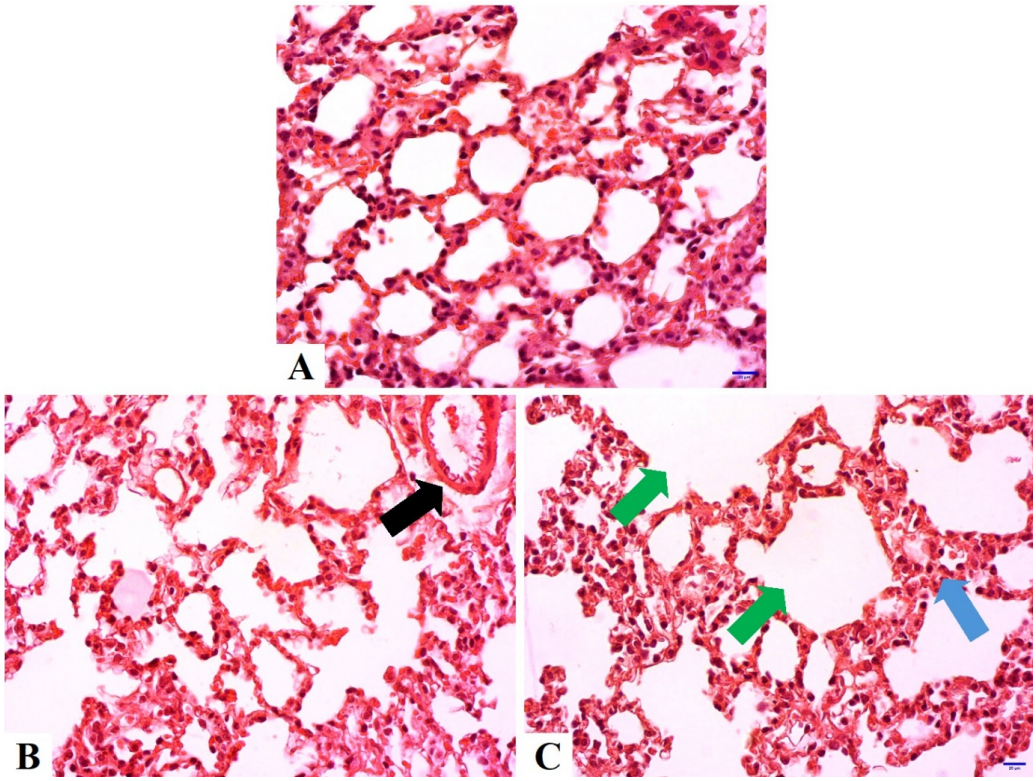

Fig. 1. Histopathological feature of pulmonary tissue. (A) Normal pulmonary tissue in NC mice, (B) Fibrosis of small airway in PC mice (black arrow), (C) Alveolar dilatation and destruction of interalveolar septa (green arrow), inflammatory cell infiltration (blue arrow).

Discussion

This present study demonstrates that administering oral probiotics for six weeks effectively lowers serum IL-6 levels in a COPD mice model although their effect on elevating serum IL-10 levels was not statistically significant.

As a pro-inflammatory cytokine, IL-6 promotes T-helper17 (Th17) cell differentiation and the production of other pro-inflammatory cytokines, thereby amplifying the inflammatory cascade. High serum IL-6 levels correlate with a higher frequency of exacerbations, muscle wasting, insulin resistance, and cardiovascular disease, all of which lead to increased mortality in COPD patients (21–23). In contrast, IL-10 plays an important role as an anti-inflammatory cytokine that helps limit the inflammatory response by inhibiting the production of pro-inflammatory cytokines, including IL-6 (11,24,25). Unfortunately, IL-10 levels of COPD patients are often insufficient to counteract excessive pro-

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inflammatory stimulation. Studies have shown that lower levels of IL-10 are associated with increased inflammation (25) and poorer clinical outcomes in COPD patients (26,27).

Previous study by Carvalho et al., found that administration of probiotics containing *Lactobacillus rhamnosus* for 9 weeks was effectively in lowered IL-6 levels and increasing IL-10 levels in pulmonary tissue from bronchoalveolar lavage fluid (BALF) specimens (28). Vasconcelos et al., discovered the same results, demonstrating the efficacy of 9-week probiotics administration in increasing IL-10 levels of BALF specimens in a mice model of Asthma-COPD overlap syndrome (29). A key distinction lies in the fact that the studies conducted by Carvalho et al., and Vasconcelos et al., employed COPD mice model for prevention, in which the mice were administered probiotics prior to the induction of COPD (28,29). In contrast, this present study employed mice model of COPD for treatment, where mice were initially induced to develop COPD and subsequently administered probiotics.

The different outcomes of this study compared to previous ones yield important insights. The administration of probiotics effectively reduces IL-6 levels in both pulmonary tissue and the systemic level. However, their impact on IL-10 was only observed in pulmonary tissue, not at the systemic level. It is worth noting that the effect of probiotics on IL-10 levels is only evident after a minimum duration of 9 weeks (28,29).

Probiotics help metabolize indigestible fiber and produce metabolite products, specifically SCFAs, mainly acetic acid, propionate and butyrate. SCFAs have been shown to inhibit the activation of the nuclear factor – kappa beta (NF-κB) (30) signaling pathway which acts as transcription factor for IL-6 synthesis. Acetate and butyrate in macrophages also inhibit the secretion of tumor necrosis factor (TNFα) and IL-6 through the activation of free fatty acid (FFA)3 receptors (31,32). Furthermore, butyric acid also stimulates the differentiation of T-naïve cells into T-regulatory cells, then releases mediators such as IL-10, which will suppress the production of IL-6 and TNF-α from macrophages (33). In addition, previous studies have demonstrated that probiotic strains, specifically *Lactobacillus* and *Bifidobacterium*, can increase the production of IL-10 by interacting with the gut-associated lymphoid tissue (GALT) and activating regulatory T cells (34,35).

Regrettably, the probiotics used in the current study were a combination of two bacterial strains, *Lactobacillus* and *Bifidobacterium*. Therefore, it is not possible to conclusively determine which bacterial strain provides the most advantage. To achieve the strongest evidence, it is imperative to carry out further pre-clinical studies on experimental animals before advancing to the clinical trial stage.

In conclusion, our study demonstrates the significant impact of probiotics on reducing pro-inflammatory cytokine IL-6 levels in mice model of COPD. Probiotics have the potential to be used as additional therapy to improve the quality of life for COPD patients due to their protective effect against systemic inflammation.

Ethical Approval

The methodologies used in this study have obtained ethical clearance from the Health Research Ethical Committee of the Universitas Sumatera Utara, Medan, Indonesia, under decree number 324/KEPK/USU/2023.

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Conflict of Interest
The authors declare that there is no conflict of interest.

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