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Short Communication

Probiotics-derived butyric acid may suppress systemic inflammation in a murine model of chronic obstructive pulmonary disease (COPD)

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Abstract

Systemic inflammation in chronic obstructive pulmonary disease (COPD) contributes to multimorbidity and a diminished quality of life. Probiotics, through the gut-lung axis, have shown potential to mitigate systemic inflammation; however, their specific role in COPD-related inflammation remains unclear. The aim of this study was to evaluate the efficacy of probiotics in reducing serum interleukin-6 (IL-6) levels by enhancing butyric acid production in a murine model of COPD. An in vivo experimental study with a post-test-only control group design was conducted using 30 C57BL/6 mice randomized into five groups: non-COPD healthy control, untreated COPD, COPD treated with bronchodilator, COPD treated with probiotics, and COPD treated with a combination of bronchodilator and probiotics. COPD was induced by six weeks of cigarette smoke exposure, followed by six weeks of treatment while continuing the smoke exposure. Caecal butyric acid and serum IL-6 levels were measured using enzyme-linked immunosorbent assay (ELISA) and gas chromatography, respectively. Caecal butyric acid levels were lowest in untreated COPD mice (1.2±0.28 mmol/L) and significantly increased with probiotic administration (6.6±4.43 mmol/L, p=0.010), exceeding levels observed in healthy controls (3.9±2.05 mmol/L). Serum IL-6 levels were highest in COPD-induced mice (19.4±6.71 pg/mL) and significantly reduced with administration of probiotics (13.5±0.43 pg/mL, p=0.035), approaching levels of healthy controls (13.0±2.24 pg/mL, p=0.847). A negative correlation was observed between butyric acid and serum IL-6 levels (r=-0.420; p=0.021), suggesting that higher butyric acid levels were associated with reduced systemic inflammation. These findings demonstrated that probiotics, via their metabolite butyric acid, effectively reduced systemic inflammation in a COPD mouse model, highlighting their potential as a therapeutic approach for managing COPD-related inflammation.

Keywords: Chronic obstructive pulmonary disease, probiotics, gut-lung axis, butyric acid, interleukin-6

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by a persistent airflow limitation due to irreversible damage to the airways and alveolar tissue, leading to chronic

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respiratory symptoms such as shortness of breath, wheezing, chronic cough, and reduced activity tolerance [1]. This condition not only affects the lungs but also triggers systemic inflammation, which contributes to various comorbidities and results in a diminished quality of life [2-4]. Unfortunately, the current standard treatment focuses more on reducing respiratory symptoms rather than lowering the inflammation at the systemic level. Systemic inflammation in COPD is associated with elevated levels of circulating inflammatory mediators [5], particularly interleukin-6 (IL-6) [6,7], which is correlated with disease severity and overall mortality [8], thus highlighting the importance of effective anti-inflammatory treatments.

Recent studies have emphasized the gut-lung axis, illustrating the role of gut microbiota in modulating systemic inflammation [9,10]. The gut microbiota, which is mainly dominated by *Actinobacteria*, *Firmicutes*, and *Bacteroidetes*, produces metabolites such as short-chain fatty acids (SCFAs), bile acid derivatives and fermented gas. Of all metabolites, SCFAs, particularly butyric acid, are the most widely studied for their anti-inflammatory properties [11] and is also assumed to potentially reduce systemic inflammation in COPD.

Patients with COPD often experience gut microbiota dysbiosis due to chronic inflammation, which leads to reduced levels of SCFAs [12]. While probiotics containing non-pathogenic microorganisms are assumed to help restore microbial balance [13], their potential effectiveness in reducing systemic inflammation remains uncertain. The aim of this study was to assess the effect of probiotics on reducing serum IL-6 levels by promoting butyric acid production in a COPD mice model.

Methods

Study design and setting

An in-vivo study was conducted in 2023 employing a post-test-only control group design. All procedures on mice (COPD induction, administration of bronchodilators and probiotics, and termination), as well as enzyme-linked immunosorbent assay (ELISA) examinations, were carried out at the Institute of Bioscience, Universitas Brawijaya, Malang, Indonesia. Histopathology analysis was conducted at the Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Sampling strategy and inclusion criteria

The C57BL/6 mice aged 2 to 3 months, with a weight range of 18 to 22 grams, were used in this study. The sample size was determined using the Federer formula, yielding five groups consisting of six mice in each group. All subjects were male mice, deliberately selected to eliminate the effects of estrogen and progesterone. Mice that did not survive until the end of the study were classified as dropouts and excluded.

Randomization and study groups

Thirty C57BL/6 mice were acclimatized for one week, during which they were provided with standard meal and water *ad libitum*. Subsequently, all mice were randomized and assigned to five groups. All mice, except for negative control/healthy mice (Group A), underwent a 12-week exposure to cigarette smoke to generate COPD models. The mice were then treated with either inhaled bronchodilator only (C), oral probiotics only (D), or a combination of both inhaled bronchodilator and oral probiotics (E) for six weeks, except for the positive control (B), which was left untreated.

Induction of COPD model

COPD was induced in mice using a cigarette smoke exposure method in which each cigarette contained 1 mg of nicotine and 15 mg of tar [14]. The mice were exposed to cigarette smoke through a whole-body system, in which they were placed inside a smoking chamber measuring 69×47×38 cm [15]. During each 45-minute session, the mice inhaled smoke from 12 cigarettes, every morning and night, five days a week [16]. This exposure continued for six weeks, after which the mice received treatment while still being exposed to cigarette smoke for an additional six weeks, making the total exposure period 12 weeks, as illustrated in **Figure 1**.

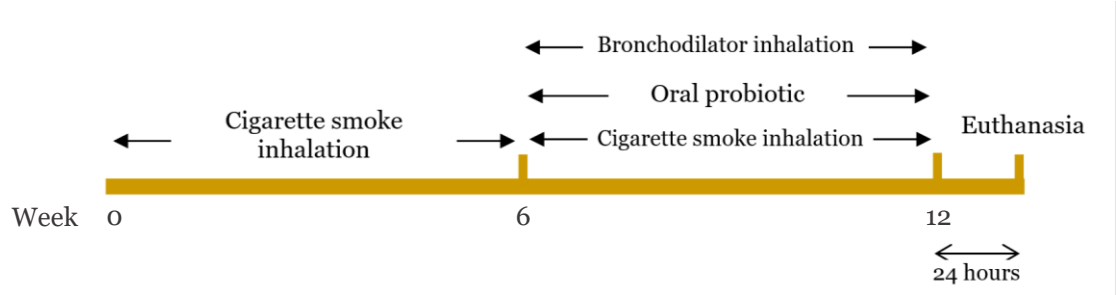

Figure 1. Timeline of chronic obstructive pulmonary disease (COPD) induction and treatment.

Probiotics and bronchodilator treatment

The probiotics used in the study were commercial preparation, L-Bio™ (PT Lapi Indonesia, Jakarta, Indonesia), containing 10^8 colony-forming unit (CFU) per gram of *Lactobacillus* sp. and *Bifidobacterium* sp. These probiotics were administered at a dosage of 26 mg twice daily, one hour after each cigarette smoke exposure. The probiotics were diluted in 0.2 mL of normal saline and delivered via oral by utilizing a gavage needle, instead of a syringe needle, to probe the animal's oesophagus and stomach [17].

Salbutamol 1.25 mg solution was administered via inhalation twice daily, two hours after cigarette smoke exposure. The bronchodilator nebulization procedure involves inserting the nebulizer mouthpiece, coupled to the nebulizer machine, into the smoking chamber. Both, the probiotics and bronchodilator were administered for six weeks after the initial six-week period of COPD induction.

Data collection

At the end of the 12th week, all mice were purposely euthanized using an intraperitoneal injection of ketamine to obtain three specimens: caecum digesta, blood and pulmonary tissue. Caecum digesta were collected to measure the amount of butyric acid by gas chromatography analysis (GC-2010 Plus, Shimadzu, Kyoto, Japan). Blood specimens were collected intracardially, and 100 mL serum was processed to measure the serum IL-6 levels using 555240 BD Bioscience ELISA kit (Bioscience Pharmingen, San Diego, USA) [18]. Pulmonary tissues were obtained from the superior lobe of the right lung, stained with hematoxylin-eosin (HE), and observed at 400× magnification.

To ensure that the COPD-induced mice groups (Groups B, C, D, and E) accurately represented the COPD model, a qualitative analysis of the histological features of the small airways and alveolar tissue was performed by an independent pathologist. The COPD model was validated through histological analysis of the small airways and pulmonary parenchyma, showing pathognomonic characteristic emphysematous changes in the pulmonary tissues and features of chronic bronchitis in the small airways [14].

Statistical analysis

To assess the effect of probiotics on butyric acid and IL-6 levels, a one-way analysis of variance (ANOVA) with least significant difference (LSD) post-hoc test was performed. Correlation between butyric acid and IL-6 levels was assessed using Spearman correlation. Data analyses were conducted using SPSS software version 24 (IBM, New York, USA), in which $p<0.05$ was considered significant.

Results

Validation of the COPD model

Mice in the unexposed cigarette group (Group A) showed normal regular epithelial structure surrounding the bronchioles and intact air sacs in the alveolar tissue (**Figure 1A**). In contrast, mice in Group B showed abnormalities in the small airways and alveolar tissue. These abnormalities included bronchial wall fibrosis, epithelial traction on the bronchial lumen, goblet cell hyperplasia, and mucus accumulation within the lumina (**Figure 1B**). Additionally, the

pulmonary parenchyma showed characteristic emphysematous changes, such as the enlargement of distal air sacs in the terminal bronchioles and the loss of alveolar septa (**Figure 1C**).

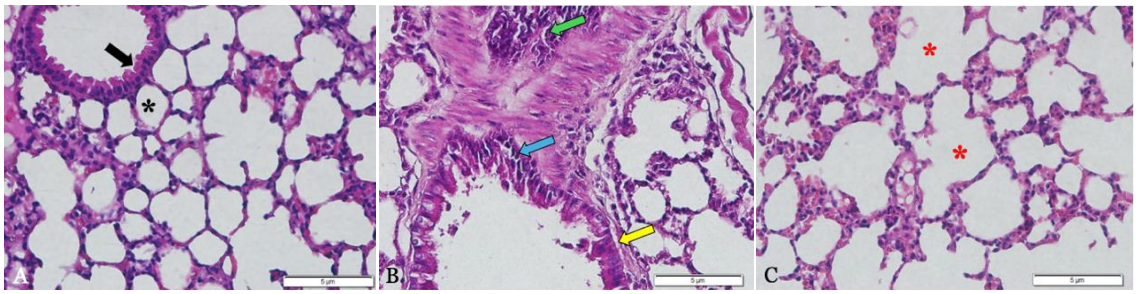

Figure 2. Histological analysis of pulmonary tissue. (A) Normal pulmonary tissue in cigarette-unexposed negative control group. (B) Small airway abnormalities and (C) alveolar abnormalities in cigarette-exposed groups (positive control group). Black arrow: normal bronchioles epithelium, blue arrow: fibrosis and traction of the small airway wall, yellow arrow: goblet cell hyperplasia, green arrow: inflammatory cell infiltration, black asterisk: normal air sacs, red asterisk: emphysematous alveolar and destruction of alveolar septa.

Effect of probiotics on butyric acid levels

Our data indicated significant differences in butyric acid levels across the groups (p=0.010) (**Table 1**). The positive control group had the lowest levels (1.2±0.28 mmol/L), while the group receiving probiotics only exhibited the highest levels (6.6±4.43 mmol/L) (**Table 1**). In contrast, there was no significant increase in butyric acid levels in the inhaled bronchodilator only group compared to the positive control group (1.5±0.79 vs 1.2±0.28 mmol/L, p=0.844). However, the group receiving probiotics had significantly higher levels of butyric acid compared to the other groups, with a p-value of 0.039 (**Table 1**).

Table 1. Effect of probiotics on butyric acid levels among groups (n=30)

Groups	n	Butyric acid (mmol/L)	p-value	LSD post-hoc			
		Mean±SD		B	C	D	E
Negative control (A)	6	3.9±2.05	0.010[a]	0.084	0.122	0.085	0.707
Positive control (B)	6	1.2±0.28			0.843	0.001[b]	0.168
Bronchodilator (C)	6	1.5±0.79				0.002[b]	0.234
Probiotics (D)	6	6.6±4.43					0.039[b]
Combination (E)	6	3.3±2.95					

LSD: least significant difference
[a]Analyzed with one-way Anova
[b]Analyzed with LSD post-hoc

Effect of probiotics on serum IL-6

The highest mean levels of serum IL-6 were observed in the positive control group. Although bronchodilator group had lower IL-6 levels compared to the positive control group, the difference was not statistically significant (17.92 vs 19.45 pg/mL, p=0.514). However, the group receiving probiotics had significant reduced IL-6 levels compared to the positive control group (13.51 vs 19.45 pg/mL, p=0.016), bringing the levels close to those of the negative control group (13.51 vs 13.06 pg/mL, p=0.848) (**Table 2**).

Table 2. Effect of probiotics on serum interleukin-6 (IL-6) among groups (n=30)

Groups	n	IL-6 (pg/mL)	p-value	LSD post-hoc			
		Mean±SD		B	C	D	E
Negative control (A)	6	13.0±2.24	0.035[a]	0.010[b]	0.045[b]	0.848	0.57
Positive control (B)	6	19.4±6.71			0.514	0.016[b]	0.037[b]
Bronchodilator (C)	6	17.9±4.94				0.066	0.135
Probiotics (D)	6	13.5±0.43					0.710[b]
Combination (E)	6	14.3±2.15					

LSD: least significant difference
[a]Analyzed with one-way Anova
[b]Analyzed with LSD post-hoc

Correlation of butyric acid and serum IL-6

This study demonstrated statistically significant negative correlation between butyric acid levels and serum IL-6 levels, which means that the higher the butyric acid level in the colon, the lower the serum IL-6 level (r=-0.420; p=0.021). This finding suggested that butyric acid might play an important role as a pathway that mediates the action of probiotics in modulating systemic inflammation in COPD. Detailed scatter plot diagram of the correlation between butyric acid levels and serum IL-6 in mice model of COPD is presented in **Figure 2**.

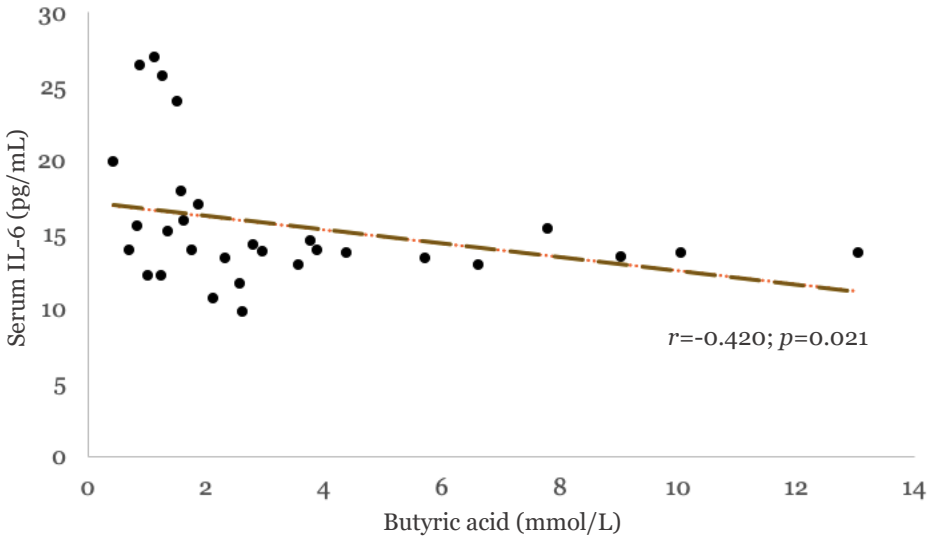

Figure 2. Correlation of butyric acid and serum interleukin-6 (IL-6) in mice model of chronic obstructive pulmonary disease (COPD) (r=-0.420; p=0.021; R^2=0.176; analyzed with Spearman correlation).

Discussion

Systemic inflammation is a crucial problem in COPD which results in damage to other organs and leads to high morbidity and mortality [19,20]. Previous studies have highlighted that serum IL-6 can be used as a marker of systemic inflammation which is closely correlated with the frequency of exacerbations and disease severity in COPD [21,22]. It is now well-recognized that high IL-6 levels are correlated with cardiovascular complications [23], decreased functional capacity [23], sarcopenia [24], osteoporosis [24], and depression [25]. Another study has highlighted the importance of IL-6-targeted therapies to improve quality of life in people with chronic diseases [6] including COPD. Unfortunately, the current standard treatment with inhaled bronchodilator focuses more on reducing respiratory symptoms rather than on targeting systemic inflammation [26]. Thus, a supplementary treatment that might intervene in the systemic inflammatory pathway is needed to enhance the quality of life of COPD patients.

Previous studies have demonstrated the role of probiotics in lowering local inflammation in pulmonary tissue, by the reduction of IL-1β, IL-6, TNFα [27], as well as expression of matrix metalloproteinase (MMP)-9 and MMP-12 in bronchoalveolar lavage fluid specimen [18,28], but their effects at the systemic level are still unknown. The present study demonstrated that the anti-inflammatory effect of probiotics not only occurs locally in pulmonary tissue but also at the systemic level, as evidenced by a decrease in serum IL-6 levels in COPD mice model administered with probiotics, bringing them close to the levels observed in normal mice (**Table 2**).

The effect of probiotics in lowering systemic inflammation has been widely reported through the concept of the gut-lung axis [9,29,30], which reflects the bidirectional relationship between the gut and the lungs [31], but its precise mechanism by which probiotics may affect the gut-lung axis remains unidentified. In order to demonstrate the pathway, our study measured the levels of certain metabolites produced by the gut microbiota, particularly SCFAs [32], with a focus on butyric acid. The present study demonstrated a substantial correlation between the administration of probiotics and the concentration of butyric acid in the colon. COPD mice model receiving probiotics demonstrated the most elevated concentrations of butyric acid. In contrast,

untreated COPD mice model exhibited the most depleted levels of butyric acid due to gut microbiota dysbiosis caused by systemic inflammation (**Table 1**).

More importantly, this study demonstrated a negative correlation between butyric acid levels and serum IL-6 levels, implying that an increase in butyric acid levels in the colon would result in a decrease in serum IL-6 levels (**Figure 2**). A previous study demonstrated promising anti-inflammatory properties of butyric acid by inhibiting nuclear factor-kappa B (NF-κB), which serves as a transcription factor of pro-inflammatory cytokines, including IL-6 [33]. It also promotes the differentiation of regulatory T cells (Tregs) from T naive cells, resulting in the production of IL-10 that inhibits the synthesis of IL-6 and TNFα by macrophages [34,35]. Butyric acid could also regulate the intracellular signaling system responsible for the polarization of M0 macrophages which prevents the macrophages from adopting the pro-inflammatory M1 phenotype and instead promotes the anti-inflammatory M2 phenotype [36]. Furthermore, butyric acid could enhance the integrity of the intestinal mucosa, thereby preventing the translocation of pathogenic bacterial toxins into the bloodstream [37].

This study indicated the importance of increasing levels of butyric acid in the colon to reduce systemic inflammation in COPD patients, one possible strategy is through the administration of probiotics. Butyric acid-producing microbes are mostly included in the phylum Firmicutes [38], which directly ferment monosaccharides, producing acetyl coenzyme A and leading to the formation of butyrate. In addition, the cross-interaction between *Bifidobacterium* and *Lactobacillus* will further increase the production of butyric acid, as the two bacteria produce acetic and lactic acid, which will then be utilized as a substrate by *Eubacterium* to be converted into butyric acid [11,38]. Administering probiotics containing all the bacteria has the potential to serve as an additional therapy for the reduction of inflammation associated with COPD [39-41], hence enhancing the quality of life for patients. However, the probiotics utilized in the present study consisted of a mixture of two bacterial strains, *Lactobacillus* and *Bifidobacterium*. Consequently, this study could not ascertain which specific bacterial strain had the most benefit. In order to follow the principles of evidence-based medicine, it is therefore necessary to conduct additional pre-clinical investigations on experimental animals before progressing to the clinical trial phase.

Conclusion

The administration of probiotics for six weeks in a COPD model of mice increased the butyric acid levels, accompanied by a significant reduction in blood IL-6 levels. The butyric acid had significant negative correlation with serum IL-6, confirming the important role of probiotics-derived butyric acid in lowering systemic inflammation in COPD. Further studies are needed to explore the underlying mechanisms by which probiotics-derived butyric acid reduces systemic inflammation in COPD and to evaluate its therapeutic potential in clinical settings.

Ethics approval

The protocol of this study was approved by the Health Research Ethical Committee of the Universitas Sumatera Utara, Medan, Indonesia, under decree number 324/KEPK/USU/2023.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

This study used an artificial intelligence (AI) tool for manuscript writing support. The AI tool was employed for language refinement (improving grammar, sentence structure, and readability of the manuscript. We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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