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Original article

Effects of phycocyanin on pulmonary and gut microbiota in a radiation-induced pulmonary fibrosis model

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ABSTRACT

Objective: Radiation pneumonia and fibrosis are major clinical complications of radiotherapy for thoracic tumor patients, and may significantly reduce survival and quality of life. At present, no safe and effective radiation protection measures have been approved for clinical use. Phycocyanin, a protein responsible for photosynthesis from *Spirulina*, has been shown to have a variety of biological activities and to be beneficial for a variety of diseases, including pulmonary fibrosis. However, the preventive and protective effects of phycocyanin on radiation-induced pulmonary fibrosis have not been studied.

Design: X-ray single dose irradiation was used on the chest of mice to prepare a mouse model of pulmonary fibrosis, from which the effect of phycocyanin on pulmonary histopathologic change, pulmonary fibrosis, the microbiota in lung and gut, LPS, TNF-α, and IL-6 at different time after irradiation were evaluated.

Results: Phycocyanin alleviated the radiation-induced lung injury and reduced the level of inflammatory factors. Thorax irradiation led to the disorder in microbiota of the lung and gut. The variation trend of the diversity of the two tissues was opposite, but that of the microbiota composition was similar. The phycocyanin intervention regulated the composition of the lung and gut microbiota, transformed them into normal state, and reduced the level of LPS, which significantly reduced the abundance of inflammation-related bacteria, and increased the abundance of probiotics that produce short-chain fatty acids.

Conclusion: Phycocyanin could regulate the radiation-induced disorder in lung and gut microbiota of mice, and reduce the radiation-induced lung inflammation and fibrosis.

1. Introduction

Radiation therapy for esophageal cancer, lung cancer, or other thoracic spine cancer often causes acute radiation pneumonia or chronic radiation pneumonia. The radiation-induced lung injury (RILI) can even further lead to lung fibrosis. The incidence of RILI in patients receiving thoracic radiotherapy and lung cancer patients receiving high-dose radiotherapy can reach 30 %–40 %, respectively [1,2] Patients with RILI often present with cough, fever and chest pain. The consequences of RILI can even lead to respiratory failure and even death of patients. As it involves a variety of cells, the mechanism of RILI causing pulmonary fibrosis is more complicated, including complex intermolecular interactions. Radiation can cause apoptosis of epithelial cells, as well as a large amount of oxidative damage and the release of fibrotic cytokines. These processes eventually lead to excessive deposition of extracellular matrix ECM and the formation of lung fibrosis. In recent years, with the in-depth research on lung diseases, more and more studies have proved that there is an important crosstalk between gut microbes and the lungs, which plays an important role in maintaining the health of the host. Studies have shown that gut microbes can affect lung immunity, and many lung diseases are often accompanied by changes in gut microbes [3–5]. However, reports on the changes of lung microbiota in the lung and gut after RILI. In addition, facing the global pandemic of Covid-19, it is urgently needed to find active substances that can slow down the pneumonia and enteritis. Covid-19 can causes serious inflammation of human lungs, causing difficulty in breathing and pulmonary fibrosis,

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and eventually lung failure. ACE2 in lungs and gastrointestinal tract may be the transmission route of Covid-19.

Phycocyanin (PC) is a light-harvesting pigment protein isolated from cyanobacteria and can participate in the photosynthesis of algae. In the past 20 years, the role of PC in promoting health and treating diseases has been widely studied and reported. Previous studies have shown that PC has multiple activities such as antioxidant and anti-inflammatory [6,7]. It has been reported that PC can alleviate paraquat-induced lung injury in rats [8]. Research found that in the bleomycin-induced mouse lung fibrosis model, PC could regulate TLR2-MyD88-NF-κB signaling pathways alleviate pulmonary fibrosis [9], and in addition, could significantly reduce bacteria that associated with inflammation, thereby alleviating pulmonary fibrosis [10–12].

Thus, we hypothesized that, before or after RILI, PC intervention could change the colonizing pattern of the gut microbiota and affect the composition of lung microbiota, thereby alleviating the lung injury. Therefore, we induced pulmonary fibrosis in mice by X-ray single-dose irradiation on thorax and studied the effect of PC on radiation induced pulmonary fibrosis by evaluating the changes in lung histopathology, inflammatory factor levels, and microbiota in the lung and gut.

2. Materials and methods

2.1. Ethics statement

Our experimental protocol was formulated in accordance with the "Laboratory Animal Care Guidelines". The animal experiment ethics committee of Lanzhou University approved the experimental protocol.

2.2. Materials and animals

PC was extracted from *Spirulina*. King Dnarmse Spirulina Company (Fuqing, China) provided a medical grade PC for this experiment. Male C57BL/6 mice were purchased from Lanzhou Medical College in China. All animals were raised at 22 ± 2 °C and were given standard food and water.

2.3. Experimental design

4 to 6-week-old and male C57BL/6 mice were randomly divided into 4 groups in body weight, 15 mice in each group, i.e., the normal control group (C), radiotherapy only (RT), PC pre-administration plus radiotherapy group (PC + RT group), and radiotherapy plus PC administration group (RT + PC group). RT group received chest RT alone. PC + RT group received RT after 30 days with PC (50 mg/kg/d), no further administration after irradiation, only the same volume of double distilled water was given. RT + PC group was treated with PC (50 mg/kg/d) after RT. Animals in groups C and RT were given equal volume of distilled water. At the time points of 1, 3, and 5 months after RT, five animals in each group were given general anesthesia, blood samples, intestine, and lung tissues were collected. The RT conditions were as follows. All mice (C group, RT group, PC + RT group and RT + PC group) were under general anesthesia. Part of the mice (RT group, PC + RT group and RT + PC group) were irradiated to the entire chest by the X-ray therapy instrument (20 Gy, 2 Gy/min).

2.4. Sample collection

One month after the mice were irradiated, the feces from the cecum of the animals were collected and stored in liquid nitrogen at −80 °C for detection of bacterial 16S ribosomal RNA (rRNA) V3 and V4. At 1, 3 and 5 months after the mice were irradiated, 5 mice from each experimental group were selected and sacrificed. Animal serum, intestinal tissue and lung tissue were collected and stored in a refrigerator at −80 °C. The lung tissue was stained with 4 % hematoxylin-eosin (HE) and MASSON.

2.5. Histopathological analysis

The tissue sections were stained with HE and Masson and three C57BL/6 mice were used in each group. A Pannoramic MIDI scanner was used to take pictures of the stained sections. Lung injury scores were assessed according to previous methods. In brief, a score of 0, 1, 2, 3 and 4 represents no damage, mild damage, moderate damage, severe damage and very severe histologic changes, respectively [55]. In addition, fibrosis was quantified using the modified Ashcroft scoring [13].

2.6. Lung and intestinal microbiota analysis

Wash the lungs of the mice with 0.5 mL of phosphate buffer. The lavage fluid is then collected and used for genetic testing [12,14]. The extraction of total DNA and the evaluation of bacterial diversity used PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, USA, California) and Illumina Hiseq platform.

2.7. Enzyme-linked immunosorbent assay (ELISA)

ELISA kits (Shanghai Enzyme Link Biotechnology Co., Ltd., China) were used to detect the contents of interleukin (IL)-6, lipopolysaccharide (LPS) and tumor necrosis factor alpha (TNF-α) in the samples.

2.8. Statistical analysis

Flora analysis used Wilcox rank sum test to determine the differences among groups. We use SPSS 19.0 software to analyze the results. If $P < 0.05$, the result was considered statistically significant.

3. Results

3.1. PC intervention reduced lung tissue damage and fibrosis caused by thoracic irradiation

During the experiment, we found that after irradiation at the chest cavity, the fur around the mouse's chest was shed and turned pale with time. One month after irradiation, the fur was basically the same as that of the normal control group. However, after 3 and 5 months after irradiation, the fur was obviously whitened. From the appearance of fur, the whitening phenomenon of the PC intervention group was significantly less than that of the irradiation group (Fig. 1).

HE staining and Masson staining were used to analyze lung injury and fibrosis, and the results were compared 1 month after thoracic irradiation with that of group C, showing that the mouse lung tissue had obvious lymphocyte and neutrophil infiltration (green arrow in Fig. 2), alveolar tissue edema, and a small amount of blue collagen fibers were deposited. Three to 5 months after thoracic irradiation, with the prolongation of time, the lung tissue damage in mice increased, inflammatory cell infiltration increased, and eosinophilic mucus secretion appeared in the bronchial cavity (yellow arrow in Fig. 2). There is epithelial cell shedding in the tracheal cavity (blue arrow in Fig. 2), edema around the blood vessels, and loose connective tissue (red arrow Fig. 2). The alveolar wall thickens, collagen fibers proliferate, and fibroblasts increase (black arrows Fig. 2). Both prophylactic and therapeutic administration of PC reduced the infiltration of inflammatory cells and reduced the deposition of collagen fibers. This shows that PC intervention can alleviate lung injury caused by chest irradiation.

3.2. PC intervention reduces the increase of inflammatory factors and LPS induced by thoracic irradiation

Using the ELISA method, we detected the content of TNF-α, LPS and IL-6 in lung tissue, intestine, and serum. The results show that, the content of TNF-α and IL-6 in lung tissue or serum increased significantly at the three time points (1, 3, and 5 months) after thoracic irradiation

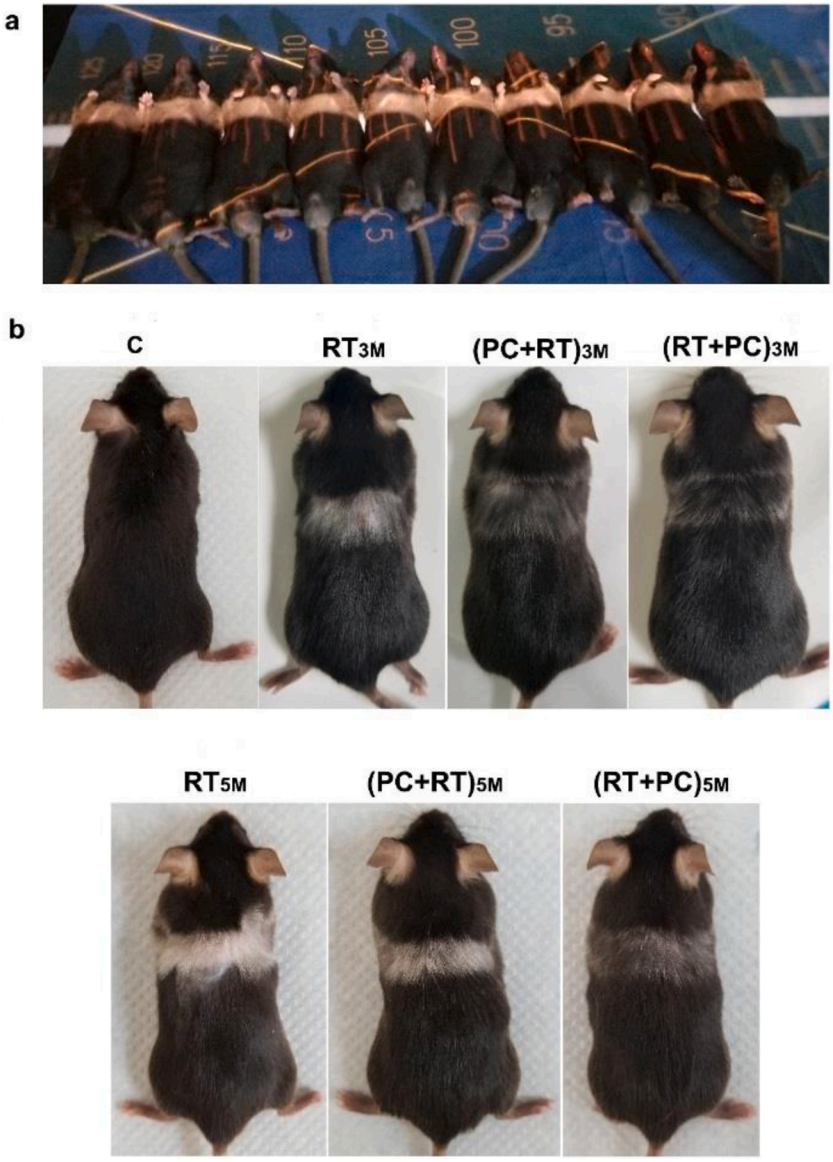

Fig. 1. Thoracic irradiation conditions and appearance changes of mice at different times.
a: irradiation conditions; b: changes in the appearance of mice. (C: normal control group; RT3M: 3 months after thoracic irradiation alone; RT5M: 5 months after thoracic irradiation alone; (PC + RT) 3M: PC pre-administered for one month and 3 months after chest irradiation Group; (PC + RT) 5M: PC pre-administered for one month and 5 months after thoracic irradiation; (RT + PC) 3M: thoracic irradiation and 3 months after PC administration; (RT + PC) 5M: 5 months after chest irradiation and PC intervention).

compared with the normal control group, while the PC medication group the contents of TNF-α and IL-6 were decreased. Although compared with lung tissue and serum, the contents of IL-6 and TNF-α in intestinal tissue did not change significantly, but at the 5-month time point, the content of IL-6 and TNF-αin intestinal tissue was significantly higher than that of normal control group (Fig. 3 a–b). Thoracic irradiation significantly increased the content of LPS in lung tissue, serum, and feces, and PC intervention also reduced its content to varying degrees (Fig. 3 c).

3.3. PC intervention regulates lung and intestinal flora

We measured the lung and intestinal flora using 16srRNA technology. The results of α diversity analysis show that the diversity index of lung flora increased after irradiation compared to the normal control group. In addition, although the diversity of intestinal flora decreased but not as significantly as the lung flora did, the trend was opposite. Both pre-administration and therapeutic administration have reduced the change of the flora diversity to a certain extent (Fig. 4), which showed that PC had a certain ability to regulate the disturbance of flora caused by thoracic irradiation.

After principal component analysis of each group, we found that no matter how the lung flora or intestinal flora varied, the difference in flora between groups C and RT was remarkable, and the bacterial structure after PC intervention was similar to that of group C (Fig. 5). In terms of the regulation of lung flora, the flora was more similar to that of group C. For the regulation of intestinal flora, the flora structure of PC + RT group was closer to that of group C (Figs. 4–6).

After analyzing the flora composition of each group, we found that at portal level, the main bacteria of normal lung flora are Fimicutes, Bacteroidetes, and Proteobacteria. Meanwhile, the intestinal flora included mainly Bacteroidetes and Fimicutes. Before irradiation, the content of Bacteroidetes in the lung tissue was only one-fourth of that of Fimicutes. After irradiation, the proportion of Bacteroidetes doubled that of the original, while the content of Fimuteutes decreased by about 15 %. Although the change of intestinal flora was not as great as that of abdominal flora, the variation trends of Bacteroidetes and Fimutes were the same to that of the lung flora for showing also the increase of Bacteroidetes and the decrease of Fimuteutes. The PC intervention stabilized the normal flora to a certain extent. At the same time, in the PC-administered group, the content of Actinobacteria increased in both lung and intestinal flora (Fig. 6).

Additional analysis of the flora composition at genus level showed that the abundances of *Alisipes*, *Lachnoclostridium* and *Bacteroides* in the

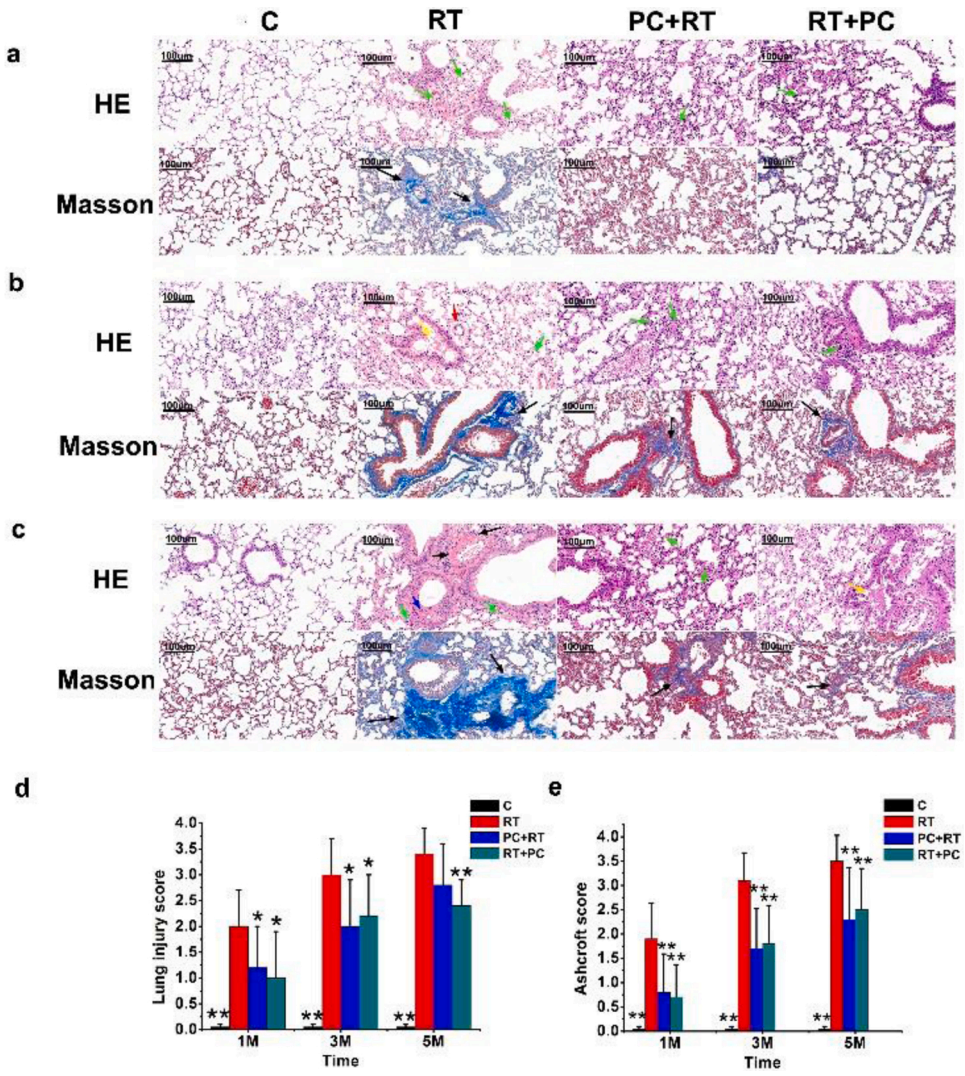

Fig. 2. Effect of PC on lung tissue injury and fibrosis induced by thoracic irradiation.
a: 1 month after chest cavity irradiation; b: 3 months after chest cavity irradiation; c: 5 months after chest cavity irradiation; d: lung injury score result; e: lung fibrosis score result. (HE: Hematoxylin and eosin staining; Masson: Masson staining; C: normal control group; RT: thoracic irradiation group only; PC + RT: thoracic irradiation group one month after PC pre-administration; RT + PC: PC administration group after thoracic irradiation; scale bar length is 100 μm, the difference is statistically significant: * $P < 0.05$, ** $P < 0.01$, compared with RT group).

lung flora increased after irradiation, while those of *Lactococcus*, *Dubosiella*, *Lactobacillus*, *Turicibacter*, *Candidatus-Saccharimonas*, *Romboutsia,* and *Bifidobacterium* decreased. In the intestinal flora after irradiation, the contents of *Alisipes*, *Mucispirillum*, *Helicobacter*, *Turibacter*, *Parabacteroides*, *Lachnoclostridium*, and *Intestinimonas* increased, while those of *Alloprevotella*, *Muribaculum*, *Anaerotruncus*, *Enterococcus*, *Bacteroides*, *Ruminiclostridium*, *Lactococcus*, and *Lactobacillus* decreased. After irradiation, five genera of *Alisipes*, *Lactococcus*, *Lactobacillus*, *Lachnoclostridium*, and *Bifidobacterium* showed the same trend in the lungs and intestines. However, PC administration alleviated the changes of genera such as *Alisipes*, *Lachnoclostridium*, *Lactococcus*, *Lactobacillus*, *Mucispirillum*, *Helicobacter*, *Turibacter*, *Parabacteroides*, *Intestinimonas*, *Bifidobacterium* etc. Although the *Faecalibacterium* in the lungs and intestines did not change significantly after irradiation, PC intervention increased the relative abundance of *Faecalibacterium* in the lungs and intestines (Fig. 7).

The Spearman method was used to analyze the correlation between the levels of factors (TNF-α, LPS and IL-6) in the sample and the abundance of different bacterial species. The results show that *Lachnoclostridium* was positively correlated with the above indicators in the lung flora; *Lactococcus*, *Dubosiella*, *Lactobacillus*, *Turicibacter*, *Candidatus-Saccharimonas*, *Romboutsia*, and *Bifidobacterium* were negatively correlated with the above indicators, and the correlation was statistically significant (Fig. 8a). In the intestinal flora, *Alistopes*, *Parabacteroides*, *Parasutterella*, *Lachnoclostridium*, and *Intestinimonas* were positively correlated with the above indicators; while *Bifidobacterium*, *Lactococcus,* and *Lactobacillus* were negatively correlated with the above indicators (Fig. 8b).

4. Discussion

In this paper, we studied the preventive and protective effects of PC on radiation-induced lung injury, and analyzed its regulatory effects on radiation-induced disturbances of lung and intestinal flora. The results show that the whitening phenomenon of the PC intervention group was significantly less than that of the irradiation group and both PC pre-administration and therapeutic administration reduced the levels of inflammatory factors and LPS in lung tissue, serum, and intestinal tract. In addition, chest irradiation can lead to disorders of the lung and intestinal flora, while PC intervention significantly alleviated the disorder of the flora. This result is very similar to our previous report on the effects of phycocyanin on bleomycin-induced pulmonary fibrosis and the intestinal microbiota in C57BL/6 mice [10].

Radiation-induced pulmonary fibrosis is a common and serious side effect of radiotherapy, which is usually inevitable and seriously affects the quality of life and survival rate of patients [15]. According to the different irradiation time, radiation pulmonary fibrosis includes early inflammation and late fibrosis [16]. In the experiment, we used HE and Masson staining methods to analyze the degree of lung tissue damage and fibrosis. After irradiating the chest cavity with a dose of 20 Gy, we

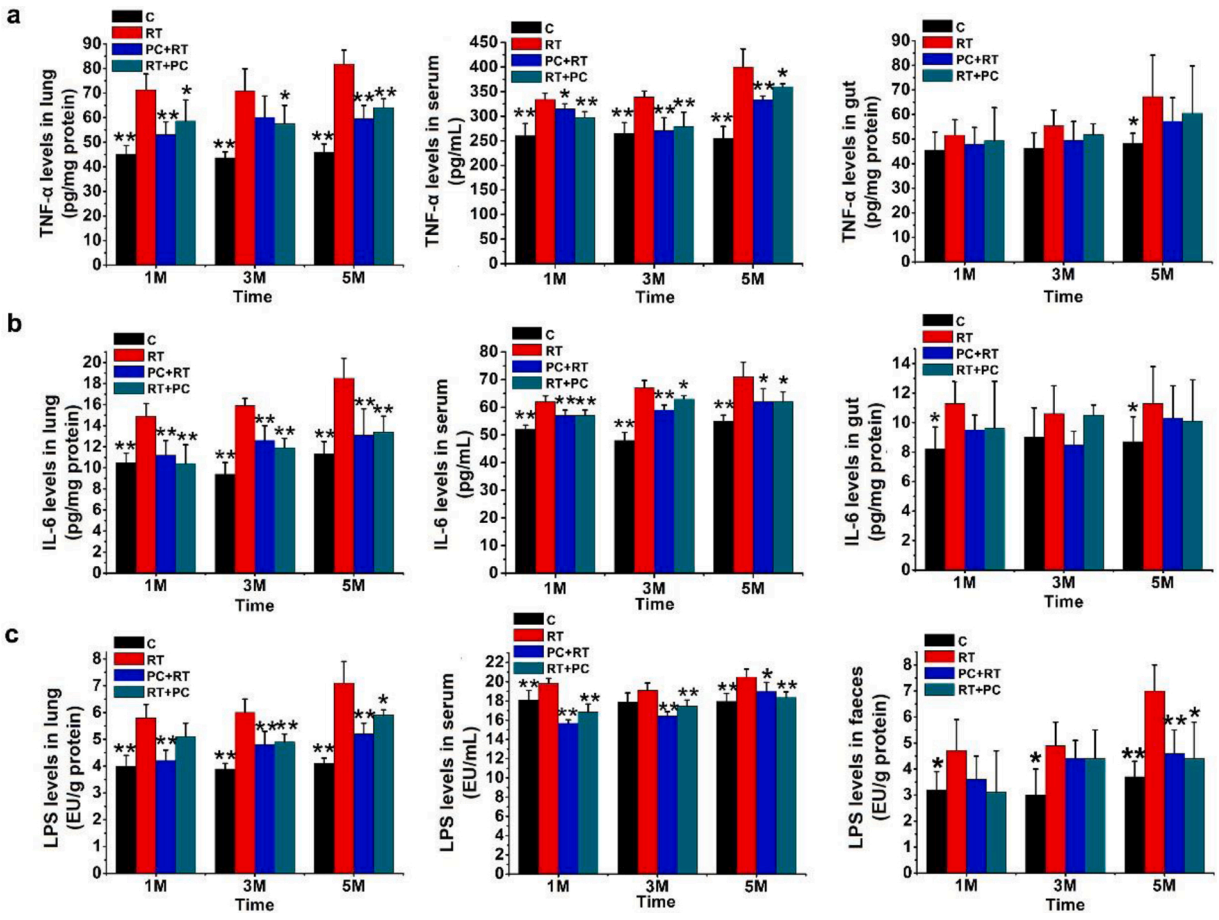

Fig. 3. The effect of PC on the levels of LPS and inflammatory factors after thoracic irradiation.
a: tumor necrosis factor alpha (TNF-α) content in lung tissue, serum and intestinal tissue; b: interleukin 6 (IL-6) content in lung tissue, serum and intestinal tissue; c: lipopolysaccharide (LPS)) Content in lung tissue, serum and feces.
(C: normal control group; RT: thoracic irradiation alone group; PC + RT: thoracic irradiation group one month after PC pre-administration; RT + PC: PC administration group after thoracic irradiation; data is average ± standard. Poor ($n = 5$) means that the difference is statistically significant: * $P < 0.05$, ** $P < 0.01$, compared with RT group).

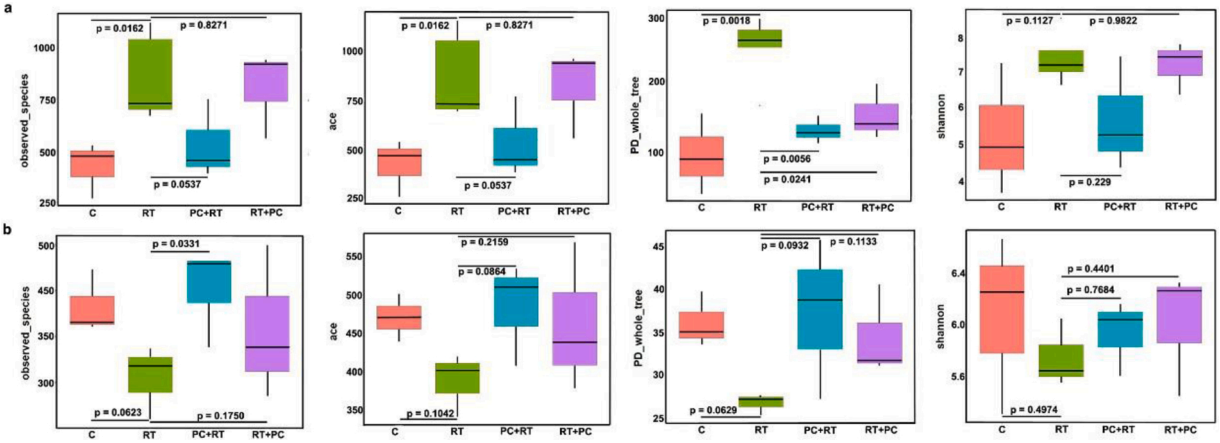

Fig. 4. The effect of PC on alpha diversity of lung and intestinal flora after thoracic irradiation.
a: diversity of lung flora; b: diversity of intestinal flora.
(C: normal control group; RT: thoracic irradiation alone group; PC + RT: thoracic irradiation group one month after PC pre-administration; RT + PC: PC administration group after thoracic irradiation; data is average ± standard Poor ($n = 5$); Observed_species: with the increase of sequencing depth, the number of OTUs actually observed; PD-whole-tree: community pedigree diversity; Shannon index: the diversity of the flora; ACE index: the abundance of the flora.).

found that the structure of the alveoli showed varying degrees of damage and pulmonary fibrosis. Both pre-administration and therapeutic administration of PC reduced inflammation damage and collagen fiber deposition, which indicates that PC can relieve fibrosis to a certain extent.

A large number of early inflammatory factors can directly damage

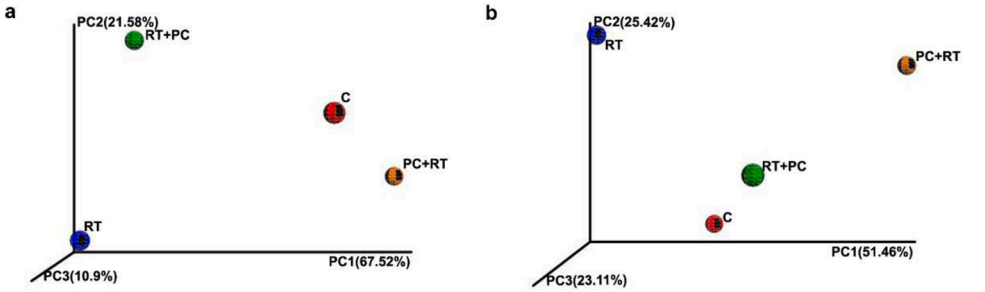

Fig. 5. The effect of PC on β diversity of lung and intestinal flora after chest irradiation revealed by PCA three-dimensional analysis.
a: β diversity of lung flora; b: β diversity of intestinal flora.
(C: normal control group; RT: thoracic irradiation alone group; PC + RT: thoracic irradiation group one month after PC pre-administration; RT + PC: PC administration group after thoracic irradiation).

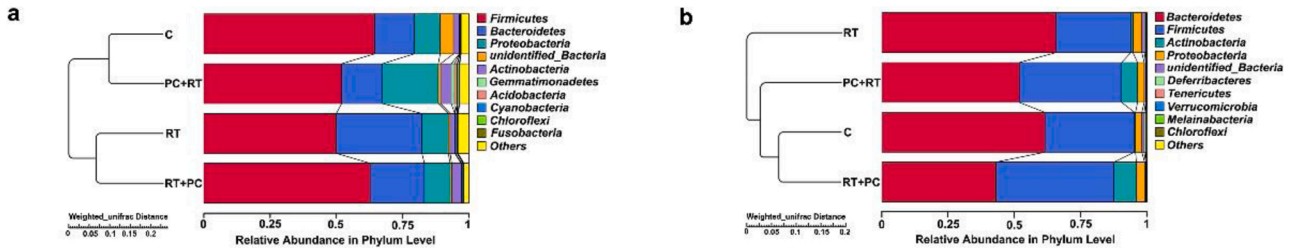

Fig. 6. The effect of PC on β diversity of lung flora and intestinal flora after chest irradiation (UPGMA cluster analysis).
a: β diversity of lung flora; b: β diversity of intestinal flora.
(C: normal control group; RT: thoracic irradiation group only; PC + RT: thoracic irradiation group one month after PC pre-administration; RT + PC: PC administration group after thoracic irradiation).

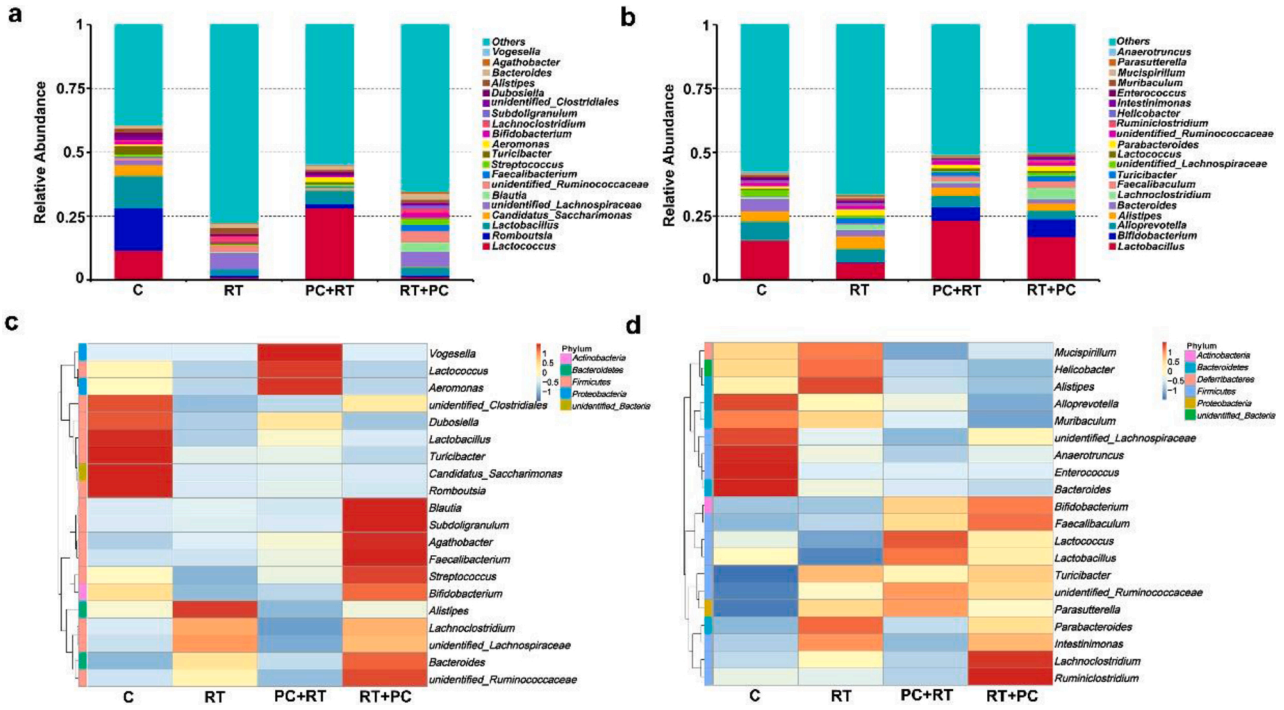

Fig. 7. The effect of PC on the abundance of lung and intestinal flora in each group after thoracic irradiation.
a: lung flora abundance-histogram; b: intestinal flora abundance-histogram; c: lung flora abundance-heat diagram; d: intestinal flora abundance-heat diagram.
(C: Normal control group; RT: thoracic irradiation group only; PC + RT: thoracic irradiation group one month after PC pre-administration; RT + PC: PC administration group after thoracic irradiation).

lung cells and further increase the permeability of pulmonary vascular endothelial cells and alveolar epithelium, leading to pulmonary edema and thrombosis. These processes eventually cause pulmonary fibrosis. The release of pro-inflammatory cytokines is related to various signaling pathways, such as the activation of NF-κB. If the expression of inflammatory cytokines can be reduced, the progression of pulmonary fibrosis can be reduced. Studies have shown that the progression of pulmonary fibrosis is related to the ongoing inflammation, mainly related to increased interleukins and tumor necrosis factor [17,18].

In this study, PC intervention significantly reduced the levels of TNF-α, LPS and IL-6, in the lung tissue, intestine, and blood of a mouse model with lung injury induced by chest irradiation. This indicates that the

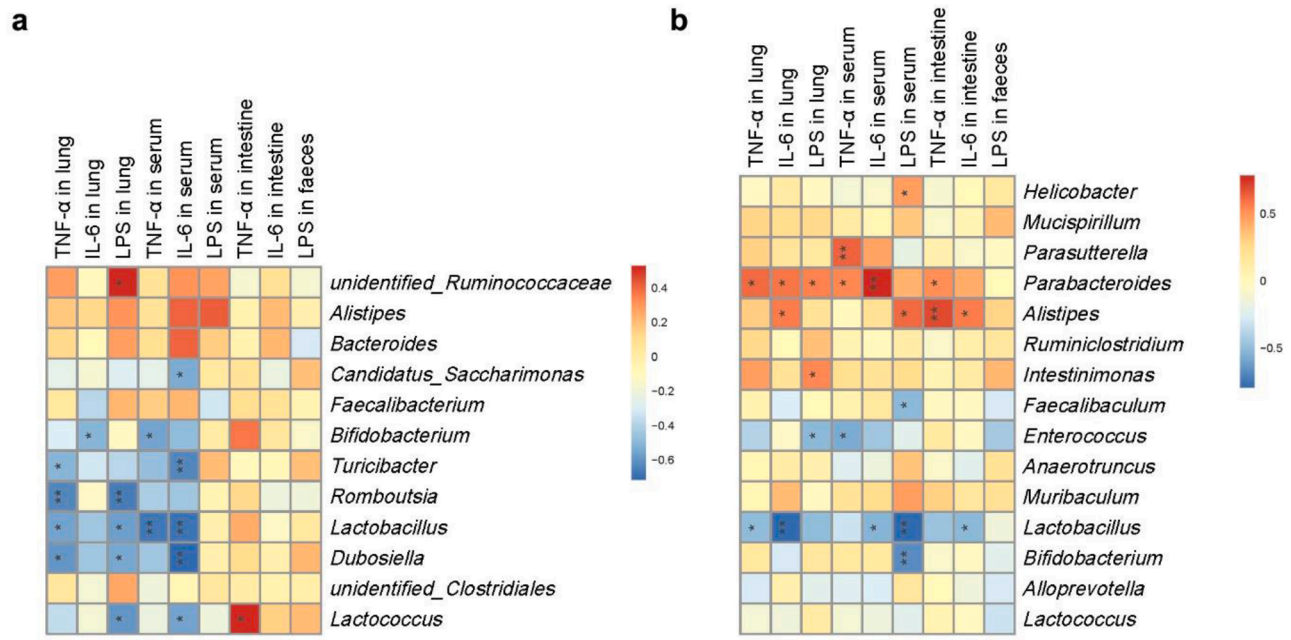

Fig. 8. Correlation analysis of flora and TNF-α, IL-6 and LPS.
a: Correlation between lung flora and biochemical indicators; b: Correlation between intestinal flora and biochemical indicators.
(The difference is statistically significant: *P < 0.05, **P < 0.01).

anti-pulmonary fibrosis effect of PC may be related to the reduction of inflammation and lipopolysaccharide levels. It has been shown that in allergic inflammation, PC can significantly inhibit the release of TNF-α, IL-6, IL-1β, and neutrophil infiltration. PC can reduce the increase of TNF-α, IL-1β, and IL-6 caused by LPS-induced microglia [19]. In addition, PC can also have an important protective effect on inflammatory bowel diseases and macrophage activation [20]. These results indicate that PC can reduce the symptoms of various diseases by inhibiting the inflammatory response, including pulmonary fibrosis induced by chest irradiation. These results indicate that PC can inhibit related diseases by inhibiting inflammation. The anti-pulmonary fibrosis effect of PC may also be related to the reduction of inflammatory factors and LPS levels. In allergic inflammation, PC can significantly inhibit the release of inflammatory factors and the infiltration of neutrophils [19,20]. These results indicate that PC can reduce lung tissue damage caused by chest irradiation by inhibiting inflammation.

By analyzing the correlation between the flora and the physiological indicators of pulmonary fibrosis, we determined the importance of PC in regulating the flora. First, a large number of studies have shown that a variety of flora is involved in inflammation [21–23]. For example, *Helicobacter* can induce various cytokines and chemokines, such as TNF-α and IL-6 [24]. *Helicobacter* can produce ammonia and other harmful substances, causing damage to the intestinal wall. Some substances such as phospholipase and cytotoxin produced by *Helicobacter* can destroy the tight junctions of epithelial cells and cause apoptosis [25]. *Helicobacter* can cause inflammatory bowel disease in immuno-deficient mice [26], and is associated with a variety of enteritis diseases [27]. *Alistairs* were shown capable of inducing inflammation, and their abundance was positively correlated with blood levels of IL-6 and LPS [28,29]. Other bacteria related to inflammation are *Parabacteroides* and *Bacteroides*. Studies have shown that they were related to chronic inflammatory bowel disease and are more abundant in chronic inflammatory bowel disease [30,31]. Moreover, *Intestinimonas* could increase in abundance in animal models of inflammation [14]. *Mucispirillum* showed high abundance in the intestinal tract with oxidative stress and inflammation, which was also a sign of serious indigestion [32,33]. After thoracic irradiation, *Helicobacter*, *Alisipes*, *Parabacteroides*, *Bacteroides*, *Intestinimonas,* and *Mucispirillum* in the intestine proliferated in large quantities. PC intervention significantly reduced the relative abundance of these strains and reduced the inflammatory damage of the bacteria to the tissue.

Secondly, a large number of studies show that many bacteria are involved in the production of short-chain fatty acids (SCFAs), such as *Faecalibacterium*, *Ruminiclostridium*, *Muribaculum*, *Anaerotruncus*, *Romboutsia*, *Turicibacter*, and *Clostridiales* [34–38]. They are beneficial bacteria, so they can ferment and produce SCFA in the intestine. SCFAs can reduce the level of intestinal inflammation, thereby protecting the intestinal barrier [39]. SCFAs can inhibit histone deacetylase (HDAC) and G protein coupled receptor (GPCR), while GPCRs are involved in lung diseases [40]. Compared with the irradiated group, the therapeutic administration of PC significantly increased the relative abundance of *Faecalibacterium*, *Lachnoclostridium*, *Ruminiclostridium*, and *Clostridiales*, increased the synthesis of SCFAs, and enhanced its inhibitory effect on HDACs and GPCRs.

In addition, many microorganisms are probiotics, such as *Lactobacillus*, *Lactococcus*, and *Bifidobacteriu* [41]. Probiotics can improve the immunity of human mucosa by increasing immunoglobulins, and play an important role in human health [42]. Probiotics can regulate the balance of intestinal flora in the body by inhibiting the increase of pathogenic bacteria, which can help treat diseases [43]. For example, *Lactobacillus* interacts with many pathogens by secreting salivary hormone B (an antibiotic) [44]. Other studies have found that the fermentation of lactic acid bacteria can not only enhance the physiological activity of intestinal peristalsis, but also improve immunity and avoid hyperglycemia [45,46]. *Bifidobacterium* is also a beneficial bacteria in the intestinal tract, and it has immuno-modulation and hypoglycemic effects [47]. In this study, the relative abundance of *Lactobacillus*, *Lactococcus,* and *Bifidobacterium* increased significantly after PC intervention compared with the irradiated group alone, improving the immunity and balance ability of the flora.

Dysfunctional flora promotes the production of lipopolysaccharide (LPS). By binding to Toll-like receptor 4 (TLR4), LPS induces chronic low-grade inflammation [48]. LPS could not only directly damage tissues, but also cause systemic inflammatory cascades through blood circulation, leading to the release of inflammatory cytokines [49]. In this study, LPS levels were increased in lung tissue, blood, and feces after

irradiation, while PC intervention reduced its levels in various tissues. In the correlation analysis, we also found that a variety of bacteria was significantly associated with LPS.

When analyzing the changes in the composition of the lung flora and intestinal flora, we found that there were many similarities between the changes in the lung flora and intestinal flora regardless of whether they were analyzed on the portal level or the genus level. For example, the abundance of *Fimicutes* was decreased after irradiation, and those of *Bacteroidetes* and *Actinobacteria* were increased after PC intervention. A previous study found that *Fimutes* abundance was inversely related to the levels of various inflammatory factors, while *Bacteroidetes* was just the opposite [50]. This shows that the inflammation level of lung tissue is closely related to the flora of the lungs and even the intestine. Specific to the genus level, irradiation simultaneously reduced the abundance of *Lactobacillus*, *Lactococcus,* and *Bifidobacterium* in the lungs and intestines. After PC intervention, the abundance of the three bacteria in the lungs and intestinal flora were also increased. In addition, the experimental results of the PC + RT group and the RT + PC group show that both preventive and therapeutic administration of PC can alleviate pulmonary fibrosis, but there are some differences in regulating the composition of the flora. This may be due to the PC intervention promoted the colonization of beneficial bacteria after the mice in the PC + RT group took PC continuously for one month before irradiation, which may increased the tissue tolerance to irradiation. On the other hand, mice in the RT + PC group were irradiated before taking PC. Since irradiation itself can affect the composition of the mouse flora of the RT PC group, and PC intervenes on the basis that the flora had been affected by radiation, the degree of repair was different from the effect of PC preventive administration.

In 2020, Covid-19 has become an infectious disease worldwide. Lymphopenia and inflammatory cytokine storms are typical syndrome observed in Covid-19 patients and are believed to be related to the severity of the disease. Covid-19 can also cause very severe inflammation of the lungs, causing difficulty in breathing and pulmonary fibrosis, and eventually the lung failure [51,52]. The Covid-19 virus attacks our body through angiotensin converting enzyme 2 (ACE2). ACE2 exists not only in our lungs, but also in our gastrointestinal tract. This may be the cause of gastrointestinal symptoms such as diarrhea, nausea, and vomiting in a large number of the patients. The gastrointestinal tract may be the potential transmission route and target organ of Covid-19 [53,54].

Therefore, there is an urgent need to find a natural active substance that can not only slow down the fibrosis caused by lung inflammation, but also adjust the structure of intestinal flora and maintain intestinal health. The findings of this study may provide a reference in this regard for the treatment of Covid-19 patients.

5. Conclusions

In summary, this study established a model of pulmonary fibrosis in thoracic irradiated mice, and evaluated the preventive and protective effects of PC. Wee believe that there was a communication mechanism between the intestine and lungs.

HE and Masson staining results showed that PC significantly attenuated pulmonary fibrosis caused by radiation, reduced levels of IL-6, TNF-α, and LPS in the lung, serum, and intestine, which indicate that PC significantly reduced the levels of proinflammatory cytokines and lipopolysaccharide. PC intervention can obviously remedy the microflora disorder induced by irradiation. The correlation between the biochemical indexes related to pulmonary fibrosis and the flora reveals that lung injury is closely related to the composition of the flora, and that there is communication between the intestine and the lungs. We infer that PC may play a protective role against radiation-induced lung injury by regulating the intestinal and lung flora, thus reducing LPS content and inflammatory response (Fig. 9). However, the mechanism of the interaction with the intestinal flora needs to be further explored.

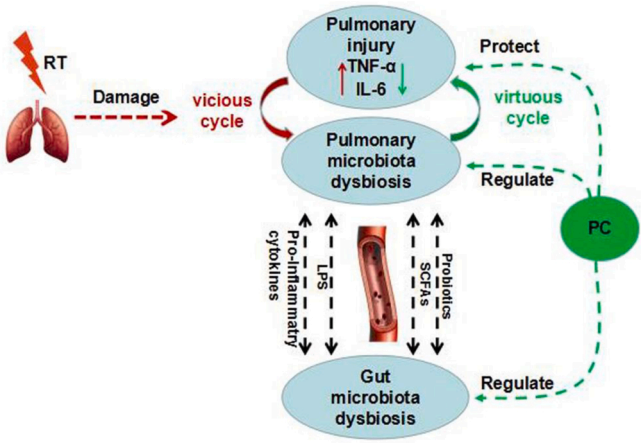

Fig. 9. The effect of PC on pulmonary fibrosis caused by radiation.

Declaration of Competing Interest

No competing interests.

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