Original Article Probiotic Saccharomyces boulardii attenuates cardiopulmonary bypass-induced acute lung injury by inhibiting ferroptosis

Jian Li^{1,2*}, Peng-Fei Gao^{1,2*}, Yun-Xin Xu¹, Hao Gu³, Qing-Xiu Wang^{1,4}

¹Shanghai East Clinical Medical School, Nanjing Medical University, Shanghai 200000, China; ²Department of Anesthesiology, The Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University, Huai'an 223001, Jiangsu, China; ³Department of Pediatrics, The Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University, Huai'an 223001, Jiangsu, China; ⁴Department of Anesthesiology, East Hospital Affiliated to Tongji University, Shanghai 200120, China. ^{*}Equal contributors.

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Abstract: Objective: Acute lung injury (ALI) is one of the most common and fatal complications of cardiopulmonary bypass (CPB). Probiotics treatment has been shown to reduce lung injury in different experimental models. However, the effect of probiotics on CPB-induced ALI is still poorly understood. This study aimed to investigate whether probiotic Saccharomyces boulardii CNCM I-745 treatment protects against lung injury in a rat model of CPB. Methods: Rats were orally gavaged with Saccharomyces boulardii CNCM I-745 once a day for 5 days before being subjected to CPB. Rats were euthanized post-CPB, and samples of lung tissue were processed for later investigation. The levels of inflammatory cytokines were measured by ELISA. The expression levels of ferroptosis markers in lungs were assessed by western blot. The microbes in feces and proximal colon of rats were analyzed by using 16S rDNA amplicon sequencing method. The ratio and maturity of conventional dendritic cells (cDCs) were determined by flow-cytometry. Results: Saccharomyces boulardii CNCM I-745 treatment improved lung function, attenuated pathologic lung changes and decelerated the exacerbation of inflammatory cytokine level after experimental CPB. Saccharomyces boulardii CNCM I-745 treatment also inhibited CPB-induced ferroptosis, as evidenced by the changes of main markers of ferroptosis, namely, the increase of Glutathione peroxidase 4 (GPX4) and the decrease of Acyl-CoA synthetase long chain family member 4 (ACSL4). In addition, after Saccharomyces boulardii CNCM I-745 treatment, the ratio and maturity of conventional dendritic cells (cDCs) in the guts of rats with CPB were significantly up-regulated. Conclusion: Our findings suggest that probiotic Saccharomyces boulardii CNCM I-745 reduces CPB-induced lung injury through suppression of the ferroptosis in lung and up-regulation of the ratio and maturity of cDCs in gut.

Keywords: Probiotics, cardiopulmonary bypass (CBP), lung injury, ferroptosis, conventional dendritic cells, guts

Introduction

Acute lung injury (ALI) is one of the most fatal complications of cardiopulmonary bypass (CPB) [1, 2]. ALI not only can significantly prolong the use of ventilator and stay in intensive care unit, but also is the main cause of increased mortality of CPB. Although the treatment of ALI has been improved continuously, the mortality of patients still reaches approximately 30% [3, 4]. Unfortunately, the mechanisms underlying CPB-induced ALI are complex and remain largely unclear. Thus, identification of novel strategies for ameliorating CPB-induced ALI will be beneficial for a large group of such patients. Ferroptosis, a novel iron-dependent form of regulated cell death, has been demonstrated to participate in the occurrence and development of various pathological conditions and diseases [5, 6]. Recently, ferroptosis was also shown to play a critical role in the pathogenesis of many lung diseases, including lung cancer, pulmonary fibrosis as well as ALI [5, 6]. However, the involvement of ferroptosis in CPBinduced lung injury remains unknown.

Probiotics treatment was demonstrated to be effective in a growing number of diseases, including lung diseases [7-9]. Probiotics have been suggested to inhibit growth of pathogens, improve intestinal integrity, reduce the production of cytokines and maintain immunomodulation activity [9-11]. Previous studies demonstrated that probiotics improve viral respiratory diseases by affecting the gut-lung microbiota axis [12, 13]. In addition, probiotics treatment was shown to reduce lung injury in different experimental models, such as aortic clamping, experimental sepsis and allergic asthma [14-16]. Furthermore, it was demonstrated that administration of probiotic prevented the ferroptotic symptoms induced by perfluorobutane sulfonate [17], indicating a protective effect of ferroptosis on probiotics.

In this study, we evaluated if probiotic Saccharomyces boulardii treatment would protect against lung injury in a rat model of CPB. Our results demonstrated that probiotic Saccharomyces boulardii CNCM I-745 reduced CPBinduced lung injury through suppression of the ferroptosis in lung and up-regulation of the ratio and maturity of conventional dendritic cells (cDCs) in gut. Our findings indicate that Saccharomyces boulardii CNCM I-745 treatment may be a promising strategy of intervention to improve clinical lung injury following CPB.

Materials and methods

Animal experiments

Animal experiments were approved by Ethics Committee of the Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University (approval No. TJBB03121201). A total of 30 male Sprague-Dawley rats (6-8 months old, weighing 350-500 g) were randomized into 5 groups (6 rats per group): sham group, CPB group, CPB + antibiotics (ANT) group, CPB + Saccharomyces boulardii CNCM I-745 (PRO) group and CPB + deferoxamine mesylate (DFO) group. The rats in the sham group underwent only canulation. Rats in the CPB group were subjected to the CPB treatment. Rats in the ANT group received intragastric administration of vancomycin (50 mg/kg, CV11721, Coolaber, China), neomycin (100 mg/kg, CN7741, Coolaber, China) and ampicillin (100 mg/kg, CA-2041, Coolaber, China) once a day for 3 days before CPB. The concentration and pretreatment time of antibiotics were determined according to a previous study [18]. Rats in the PRO group received intragastric administration

of Saccharomyces boulardii CNCM I-745 (5× 10⁷ CFU/D, Biocodex, France) once a day for 5 days before CPB. The concentration and pretreatment time of Saccharomyces boulardii CNCM I-745 were converted according to the different dosages of humans and animals following the manufacture instructions. Rats in the DFO group received intraperitoneal injection of DFO (HY-B0988, Coolaber, China) at a dose of 200 mg/kg 30 min before CPB. The concentration and pretreatment time of DFO were determined according to a previous study [19]. Rat model of CPB was established as described in our previous study [20]. Before operations, rats received intraperitoneal injection of 1% pentobarbital at a dose of 50 mg/kg. After full anesthesia, relevant surgical operations were carried out. After the operations, the rats were euthanized by intraperitoneal injection of 1% pentobarbital at a dose of 100 mg/kg.

Measurement of respiratory index (RI) and oxygenation index (OI)

GEM Premier 3000 Blood Gas Analyzer (Instrumentation Laboratory, MA) was used to calculate the RI and OI. The following equation were used for calculation: RI = $P(A-a)O_2/PaO_2$, OI = PaO_2/FiO_2 , $P(A-a)O_2 = PAO_2 - PaO_2$. $P(A-a)O_2$ represented alveolar-arterial oxygen tension gradient, PaO_2 represented arterial oxygen tension, PAO_2 represented alveolar oxygen tension, and FiO_2 represented inspired oxygen fraction.

Histopathological evaluation by H&E staining

Lung tissue samples in each group were collected and fixed using 10% buffered formalin, then the tissue samples were embedded in paraffin for histological analyses as described in our previous study [20]. The lung histological injury was evaluated by two independent pathologists by counting the total scores of the following 4 pathological parameters: (A) hemorrhage, (B) alveolar congestion, (C) alveolar wall thickness and (D) leukocyte infiltration. The histologic findings were evaluated by using the modified semiguantitative scores reported by Taki et al. [21]. A scoring system of 0-4 was used for the evaluation of lung injury: 0, normal lung (no injury); 1, mild injury (<25%); 2, moderate injury (25%-50%); 3, severe injury (50%-75%); 4, very severe injury (>75%).

Measurement of serum proinflammatory cytokines

Serum proinflammatory cytokines levels (TNF- α , IL-1 β , IL-22 and IL-6) of rats were determined using the specific ELISA kits for TNF- α (JL13202, Jianglaibio, Shanghai, China), IL-1 β (JL20884, Jianglaibio), IL-22 (JL20886, Jianglaibio) and IL-6 (JL20896, Jianglaibio), respectively, following the manufacture's instruction.

Western blot analysis

Western blot was performed according to our previous study [20]. Briefly, 20 µg of total protein per sample was subjected to SDS-PAGE and transferred to PVDF membranes for immunoblotting. The membranes were blocked with 5% nonfat milk for 1 h at room temperature, and then were incubated with the following primary antibodies overnight at 4°C. The primary antibodies were all from Proteintech, Wuhan, China, including rabbit anti-ALCS4 polyclonal antibody (22401-1-AP, 1:2000), rabbit anti-GAP4 polyclonal antibody (14432-1-AP, 1:1000), rabbit anti-GAPDH polyclonal antibody (10494-1-AP, 1:5000). The membranes were then incubated with goat anti-rabbit HRP conjugate secondary antibody (7074, 1:5000; CST) for 1 h at room temperature. Enhanced chemiluminescence was used to measure the protein expressions.

Phenotyping and cell sorting by flow cytometry

Relative percentage of cDCs was measured by flow cytometry (BD FACSArrayo, SanDiego, CA). The monoclonal antibodies used to stain cells were as follows: DAPI-labeled anti-CD45 (202-225, Biolegend, San Diego, CA), FITC-labeled anti-CD103 (205505, Biolegend), PE-labeled anti-CD86 (200307, Biolegend) and APC-labeled anti-MHC-II (110211, Biolegend). The percentage of CD45⁺ CD103⁺ double positive cells reflects the number of lung cDCs. The percentage of MHC II⁺ CD86⁺ double positive cells reflects the maturity of lung cDCs.

DNA extraction and sequencing

The E.Z.N.A.[®]Stool DNA Kit (D4015-00, Omega, Inc., USA) was used to extract Genomic DNA from feces and proximal colon of rats in each group. Sequencing was performed as described by a previous study [22]. Briefly, the PCR for prokaryotic 16S fragments was performed in 35 cycles of 98°C for 10 s, 54°C for 30 s and 72°C for 45 s, and a final extension at 72°C for 10 min. The PCR products were confirmed by electrophoresis on 2% agarose. AMPure XT beads (Beckman Coulter Genomics, USA) and Qubit (Invitrogen, USA) were used to purify and quantify the PCR products, respectively. The amplicon library was sized by Agilent 2100 Bioanalyzer (Agilent, USA) and quantified by the Library Quantification Kit (Kapa Biosciences, USA). HiSeq platform with PE150 was used to sequence the libraries.

Statistical analysis

Data were presented as mean \pm standard error of the mean (SEM). SPSS 17.0 software (IBM, New York, NY) was used for statistical analysis. One-way ANOVA or Dunnett's T3 test was used for comparisons among multiple groups. The measurement data at three time points were tested for normality, and one-way ANOVA and t-test were used to assess the differences between and within groups. P<0.05 was considered statistically significant.

Results

Saccharomyces boulardii CNCM I-745 improved lung function after CPB

As shown in **Figure 1**, RI and OI were measured at three time points: before CPB (T1), at the reopening of the left hilus pulmonis (T2), and the end of the CPB (T3). Compared with at T1, the RI (**Table 1**) was obviously increased, and the OI (**Table 2**) was obviously decreased in the CBP group, the ANT group and the PRO group at T2, indicating successful induction of CPB. At T3, the OI (**Table 2**) was obviously increased, and the RI (**Table 1**) was obviously decreased in the PRO group as compared with the CPB group, indicating that Saccharomyces boulardii CNCM I-745 improved lung function after CPB.

Saccharomyces boulardii CNCM I-745 attenuated pathologic changes in the lung after CPB

As depicted in **Figure 2A**, results of H&E staining showed an intact lung tissue structure in sham group. CPB induced severe inflammatory changes in lungs, including interstitial edema, alveolar structural destruction and an increase of inflammatory cells. The pathologic changes were obviously decreased after probiotics treatment. Antibiotics treatment increased pa-



Figure 1. Saccharomyces boulardii CNCM I-745 treatment improved lung function after CPB in rats. A. Comparisons of OI of among groups at different time points. B. Comparisons of RI of among groups at different time points. *P<0.05 vs. T1; #P<0.05 vs. CPB group. CPB: cardiopulmonary bypass; OI: oxygenation index; RI: respiratory index.

Table 1. The values of RI in each	n group at different time	points
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Groups	T1	T2	ТЗ	F	Р
Sham	385.67 ± 9.79	380.67 ± 12.42	376.00 ± 14.06	0.396	1.000
CPB	381.83 ± 17.43	206.67 ± 15.07 ^{#,a}	231.50 ± 8.46 ^{#,a,b}	254.286	<0.001
ANT	312.67 ± 13.00	186.00 ± 10.39 ^{#,*,&,a}	209.33 ± 9.87 ^{#,*,&,a,b}	256.748	<0.001
PRO	401.00 ± 21.00	257.33 ± 12.34 ^{*,a}	292.00 ± 13.90 ^{#,*,a,b}	120.688	<0.001
F	29.687	217.069	181.347		
Р	<0.001	<0.001	<0.001		

°P<0.05 vs. T1; °P<0.05 vs. T2; "P<0.05 vs. Sham; *P<0.05 vs. CBP; °P<0.05 vs. PR0.

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Groups	T1	T2	Т3	F	Р	
Sham	0.61 ± 0.10	0.70 ± 0.14	0.65 ± 0.21	3.287	0.400	
СРВ	0.77 ± 0.16	1.25 ± 0.17#,a	2.87 ± 0.46 ^{#,a,b}	128.256	<0.001	
ANT	1.09 ± 0.11	2.37 ± 0.84 ^{#,*,&,a}	5.05 ± 1.34 ^{#,*,&,a,b}	39.846	<0.001	
PRO	0.51 ± 0.19	0.91 ± 0.16 ^{*,a}	1.60 ± 0.23 ^{#,*,a,b}	54.289	<0.001	
F	10.671	15.684	35.443			
Р	< 0.001	<0.001	< 0.001			

Table 2. The values of OI in each group at different time points

°P<0.05 vs. T1; °P<0.05 vs. T2; *P<0.05 vs. Sham; *P<0.05 vs. CBP; °P<0.05 vs. PRO.

thologic changes in lungs. Evaluation of lung injury showed that the score of the PRO group was significantly lower than that of the CPB group, and the score of the ANT group was higher than that of the PRO group (**Figure 2B**). These data suggested that *Saccharomyces boulardii* CNCM I-745 attenuated the severity of lung injury after CPB.

Saccharomyces boulardii CNCM I-745 decelerated exacerbation of inflammatory cytokine level after CPB

The anti-inflammatory effect of Saccharomyces boulardii CNCM I-745 pretreatment was then

investigated by examining the levels of inflammation cytokines, including IL-1 β , TNF- α , IL-22 and IL-6. As shown in **Figure 3**, at both T2 and T3, serum concentrations of proinflammatory cytokine TNF- α (**Table 3**), IL-1 β (**Table 4**) and IL-6 (**Table 6**) were obviously elevated in the CPB group as compared with those in the sham group. At T3, the concentrations of TNF- α (**Table 3**), IL-1 β (**Table 4**) and IL-6 (**Table 6**) in the PRO group were significantly lower than those in the CPB group. Concentration of IL-22, a cytokine known to suppress inflammatory reactions, showed a tendency toward increase in the PRO group compared with that in the CPB group (**Figure 3**; **Table 5**).







Figure 2. Effect of Saccharomyces boulardii CNCM I-745 treatment on CPB-induced pathological lung injury. A. Hematoxylin and eosin (HE) staining of lung tissue sections from different groups (Scale bar = 100μ m). B. Lung injury score of rats in each group. *P<0.05 vs. CPB group. CPB: cardiopulmonary bypass.



Figure 3. Effect of Saccharomyces boulardii CNCM I-745 treatment on serum inflammatory cytokine levels after CPB. Comparisons of IL-1 β , TNF- α , IL-22 and IL-6 among groups at different time points. *P<0.05 vs. sham group; #P<0.05 vs. CPB group. CPB: cardiopulmonary bypass.

Saccharomyces boulardii CNCM I-745 inhibited ferroptosis in the lung after CPB

To investigate the involvement of ferroptosis in the protective effect of Saccharomyces boulardii CNCM I-745, the levels of ferroptosis markers, Acyl-CoA synthetase long chain family member 4 (ACSL4) and glutathione peroxidase 4 (GPX4), were detected by Western blot. The results showed that the level of ACSL4 was obviously increased, and the level of GPX4 was obviously decreased in the CPB group com-

Groups	T1	T2	T3	F	Р
Sham	266.43 ± 21.8	267.06 ± 21.32	262.6 ± 17.51	0.391	1.000
CPB	277.83 ± 19.37	525.17 ± 56.2 ^{#,a}	407.48 ± 34.66 ^{#,a,b}	127.292	<0.001
ANT	285.22 ± 12.34	629.92 ± 58.71 ^{#,*,&,a}	553.5 ± 38.58 ^{#,*,&,a,b}	207.812	<0.001
PRO	247.46 ± 5.99*	408.42 ± 9.69 ^{#,*,a}	309.43 ± 8.67 ^{#,*,a,b}	1096.810	<0.001
F	5.363	72.452	116.351		
Р	0.009	<0.001	<0.001		

Table 3. The levels of TNF- α in each group at different time points

°P<0.05 vs. T1; °P<0.05 vs. T2; "P<0.05 vs. Sham; *P<0.05 vs. CBP; °P<0.05 vs. PR0.

Table 4. The levels of IL-1 β in each group at different time points

Groups	T1	T2	ТЗ	F	Р
Sham	29.98 ± 0.89	30.03 ± 0.5	29.96 ± 0.91	0.017	1.000
CPB	30.99 ± 1.03	49.64 ± 9.54 ^{#,a}	55.42 ± 7.5 ^{#,a,b}	39.295	<0.001
ANT	31.57 ± 1.24	52.27 ± 5.39 ^{#,&,a}	$64.53 \pm 4.62^{\text{#,*,&,a,b}}$	124.472	<0.001
PRO	29.83 ± 1.21	37.27 ± 2.48 ^{*,a}	40.33 ± 1.75 ^{#,*,a,b}	144.400	<0.001
F	2.946	17.627	54.831		
Р	0.120	<0.001	<0.001		

°P<0.05 vs. T1; °P<0.05 vs. T2; *P<0.05 vs. sham; *P<0.05 vs. CBP; *P<0.05 vs. PRO.

Table 5. The levels of IL-22 in each group at different time points

Groups	T1	T2	Т3	F	Р	
Sham	29.82 ± 2.57	30.39 ± 2.53	31.71 ± 2.39	2.005	0.925	
CPB	30.07 ± 2.24	17.41 ± 1.52 ^{#,a}	11.49 ± 2.07 ^{#,a,b}	183.825	<0.001	
ANT	19.77 ± 3.13 ^{#,*,&}	14.46 ± 2.52 ^{#,&,a}	$8.39 \pm 1.4^{\text{#,\&,a,b}}$	54.185	<0.001	
PRO	46.94 ± 4.8 ^{#,*}	41.53 ± 3.93 ^{#,*,a}	32.55 ± 3.28 ^{*,a,b}	190.828	<0.001	
F	56.739	103.090	131.544			
Р	<0.001	<0.001	<0.001			

 $^{\rm a}P{<}0.05$ vs. T1; $^{\rm b}P{<}0.05$ vs. T2; $^{\rm e}P{<}0.05$ vs. sham; $^{\rm s}P{<}0.05$ vs. CBP; $^{\rm a}P{<}0.05$ vs. PR0.

Table 6. The levels of IL-6 in each group at different time points

Groups	T1	T2	Т3	F	Р
Sham	127.83 ± 10.24	129.15 ± 11.27	129.09 ± 9.84	0.056	1.000
CPB	125.98 ± 6.14	232.92 ± 19.31 ^{#,a}	205.41 ± 16.54 ^{#,a,b}	363.444	<0.001
ANT	131.71 ± 6.85	281.84 ± 10.09 ^{#,*,&,a}	303.2 ± 11.77 ^{#,*,&,a,b}	1544.962	<0.001
PRO	119.28 ± 6.06	167.65 ± 10.09 ^{#,*,a}	146.56 ± 12.46 ^{*,a,b}	167.749	<0.001
F	2.381	107.934	168.051		
Р	0.237	<0.001	<0.001		

 $^{\rm a}P{<}0.05$ vs. T1; $^{\rm b}P{<}0.05$ vs. T2; $^{\#}P{<}0.05$ vs. Sham; $^{*}P{<}0.05$ vs. CBP; $^{\rm a}P{<}0.05$ vs. PR0.

pared with those in the sham group (**Figure 4**). Saccharomyces boulardii CNCM I-745 and DFO, a specific inhibitor of ferroptosis, significantly reduced the level of ACSL4 and increased the level of GPX4 (**Figure 4**). These data suggested that Saccharomyces boulardii CNCM I-745 inhibited ferroptosis in the lung after CBP. Saccharomyces boulardii CNCM I-745 increased the ratio and maturity of cDCs in the gut after CPB

It is demonstrated that intestinal microorganisms-stimulated cDCs could produce hepcidin, a known endogenous suppressor of ferroptosis



Figure 4. Effect of probiotics treatment on the protein levels of ferroptosis markers. The levels of GPX4 and ACSL4 in lung tissue were measured by Western blot. **P<0.05 vs. sham group; ^aP<0.05 vs. CPB group; ^bP<0.05 vs. PRO group. GPX4: Glutathione peroxidase 4; ACSL4: Acyl-CoA synthetase long chain family member 4.

[23, 24]. Therefore, the effects of Saccharomyces boulardii CNCM I-745 on the ratio and maturity of cDCs were investigated. We firstly determined the flora in feces and proximal colon of rats in each group by using 16S rDNA amplicon sequencing method. As shown in **Figure 5**, Saccharomyces boulardii CNCM I-745 pretreatment obviously downregulated the relative abundance of Escherichia Shigella, which was increased by antibiotics treatment. Saccharomyces boulardii CNCM I-745 pretreatment also significantly increased the ratio and maturity of cDCs, while antibiotics treatment significantly reduced the ratio and maturity of cDCs (**Figure 6**).

Discussion

Our results showed that Saccharomyces boulardii CNCM I-745 treatment could alleviate lung injury in rats subjected to CPB. To best of our knowledge, this is the first report the described the beneficial role of probiotics in preventing lung injury after CPB. Previously, probiotics administration has been demonstrated to improve pulmonary function in children with ALI [25]. A previous study has also revealed that probiotics treatment attenuated lung injury by inhibiting lung inflammatory response in experimental sepsis [16]. Notably, probiotics was shown to reduce lung injury by reducing bacterial translocation in an experimental model of acute necrotizing pancreatitis [14]. Recently, Durmaz et al. reported that probiotics treatment alleviated lung injury by reducing oxidative stress, intestinal cellular damage and modulation of inflammatory processes in an experimental model of aortic clamping [15]. These findings indicate that the protective mechanism of probiotics on lung jury is complex and depends on the type of disease.

Ferroptosis has recently been recognized as an iron-dependent form of cell death [26-28], whereas GPX4 and ACSL4 have been demonstrated as the central regulators of ferroptosis [29-32]. GPX4 prevents ferroptosis by converting lipid hydroperoxides into non-toxic lipid alcohols [29, 30], while ACSL4 promoted the execution of ferroptosis by regulating lipid metabolism [31, 32]. Although ferroptosis has been shown to play a crucial role in lung injury and other lung diseases [33-35], the role of ferroptosis in CPB-induced lung injury remains unknown. In the present work, we firstly found that the GPX4 level was significantly increased, and the ACSL4 level was significantly decreased in the lung tissue after CPB, indicating that ferroptosis was implicated in the process of CPB-induced lung injury. The administration of Saccharomyces boulardii CNCM I-745 significantly reversed the protein levels of GPX4 and ACSL4, which were also clearly reversed by DFO (a ferroptosis inhibitor). These results indicated that probiotic Saccharomyces boulardii CNCM I-745 inhibited ferroptosis in the lung after CPB.

To date, little is known about how Saccharomyces boulardii CNCM I-745 inhibits ferroptosis in lungs. Affecting the gut-lung microbiota axis was demonstrated to be a key protective mechanism of probiotics in lung diseases [36-38]. Moreover, previous studies showed that intestinal microorganisms-stimulated cDCs could produce hepcidin, which was regarded as an endogenous protective molecule against ferroptosis [23, 24]. Thus, we hypothesized that probiotics may inhibit ferroptosis by regulating the ratio and maturity of cDCs. Our results showed that CPB decreased the ratio and maturity of cDCs in guts of rats, and the ratio and maturity of cDCs were then significantly increased by probiotics treatment. These data suggested that increasing the ratio



Figure 5. Effect of probiotics treatment on the flora in feces and proximal colon of rats. The species relative abundance of each sample at the gate level is shown.

and maturity of cDCs in guts may be the inhibitory mechanism of probiotics on ferroptosis in the lungs after CPB.

Antibiotics are used routinely before CPB in clinical cardiac surgery. However, the present study showed that CPB-induced lung injury was aggravated by antibiotics treatment. This treatment also up-regulated the ferroptosis level in the lung after CPB. Moreover, antibiotics treatment up-regulated the relative abundance of Escherichia Shigella and inhibited the ratio and maturity of cDCs in guts. These results indicate that antibiotics treatment may aggravate CPBinduced lung injury through the gut-lung microbiota axis. We found that the ratio of cDCs (%) and maturity of cDCs (%) in CBP group was almost as high as that in the PRO group. This may be due to the fact that in the process of CPB, the body can reduce the related injury by increasing the number and maturity of CDCs through self-compensation. At the same time, probiotics treatment promotes this compensation, while antibiotics treatment aggravates the injury. As probiotics treatment could attenuate lung injury after CPB in rat model, it should be interesting to further investigate the clinical use of probiotics instead of antibiotics before CPB in cardiac surgery.

There are several limitations in our study. Firstly, we demonstrated the protective effect

of Saccharomyces boulardii CNCM I-745 treatment against CPB in a rat model of CPB, it is still necessary to analyze the role of ferroptosis in the protective effect of Saccharomyces boulardii CNCM I-745 in vitro by using cell model. Secondly, there are many kinds of probiotics, and other probiotics has been shown to attenuate lung injury. Thus, it is highly possible that other kind of probiotics could attenuate CPBinduced lung injury in addition to Saccharomyces boulardii CNCM I-745, and further investigation is required to uncover other probiotics involved. Finally, further work on the mechanistic details of Saccharomyces boulardii CNCM I-745 on CPB-induced ferroptosis is required.

In conclusion, our results indicated that probiotic Saccharomyces boulardii CNCM I-745 treatment could attenuate ALI after CPB in a rat model. The protective effect of Saccharomyces boulardii CNCM I-745 was mainly associated with suppression of ferroptosis in lung through up-regulating the ratio and maturity of cDCs in gut. These findings suggest that Saccharomyces boulardii CNCM I-745 treatment is a potential therapeutic approach for the prevention of ALI after CPB.

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Disclosure of conflict of interest

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Address correspondence to: Dr. Qing-Xiu Wang, Department of Anesthesiology, East Hospital Affiliated to Tongji University, No. 150, Jimo Road, Shanghai 200120, China. E-mail: qxw1123@yeah.net

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