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Treatment of 5-fluorouracil-induced chemotherapeutic intestinal mucositis in mice by oral inulin via glycerophospholipid and retinol metabolic pathways

Hao Din[a,†], **Yue Xi**[b,†], **Man Yan**[c], **Chang Sun**[c], **Jiajun Tan**[c], **Zhengnan Yang**[d,*], **Liang Wu**[c,*]

[a] Department of Respiratory Medicine, Zhenjiang First People's Hospital, Zhenjiang 212002 China
[b] Medical Laboratory Department, Huai'an Second People's Hospital, Huai'an 223022 China
[c] Department of Laboratory Medicine, School of Medicine, Jiangsu University, Zhenjiang 212013 China
[d] Yizheng Hospital, Nanjing Drum Tower Hospital Group, Yizheng 210008 China

*Corresponding authors, e-mail: yang3638@sina.com, wlujs@ujs.edu.cn
† These authors contributed equally to this work.

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ABSTRACT: 5-Fluorouracil (5-FU) is a commonly used chemotherapy drug, but there is no effective prevention and treatment for the side effects of intestinal mucositis induced by 5-FU. Inulin is a type of fermentable dietary fiber, which is non-digestible, and can improve metabolic function by modulating intestinal microbiota. The current study is aimed at appraising the effect of inulin on 5-FU-induced intestinal mucositis in mice by non-targeted metabolomics. The results indicated that oral inulin significantly inhibited 5-FU-induced body weight loss and intestinal shortening in mice, inhibited NLRP3 inflammasome activation and IL-1β expression, and increased IL-10 and IgA expressions. Inulin could significantly reduce intestinal mucosal injury induced by 5-FU in mice and significantly affected the production of 27 different metabolites in mouse serum. These metabolites were closely related to glycerophospholipid metabolism and retinol metabolic pathways. The study provided a potential new method for the prevention and treatment of chemotherapeutic intestinal mucositis.

KEYWORDS: 5-fluorouracil (5-FU), inulin, chemotherapy intestinal mucositis, untargeted metabolomics

INTRODUCTION

5-Fluorouracil (5-FU) is a widely used and highly effective chemotherapy drug for treating gastrointestinal malignancies. It works by competitively inhibiting thymine nucleotide synthetase, thereby preventing DNA synthesis in tumor cells [1]. However, the use of 5-FU often leads to intestinal mucosal damage, and up to 60% of patients experience chemotherapeutic intestinal mucositis. This condition can cause severe vomiting and diarrhea, making it difficult to continue with chemotherapy and reducing the overall effectiveness and survival rate for tumor patients [2]. Unfortunately, there are currently no effective methods available to prevent or treat chemotherapeutic intestinal mucositis [3]. Therefore, it is crucial to discover an effective approach for relieving 5-FU induced intestinal mucositis in order to improve tumor treatment outcomes.

Inulin is a type of functional plant polysaccharide that falls under the category of water-soluble dietary fiber [4]. Unlike other carbohydrates, it is not easily broken down by gastric acid and can only be utilized by specific anaerobic bacteria in the colon. This promotes the growth of beneficial probiotics in the colon and helps maintain a healthy intestinal flora structure [5]. Research has demonstrated that inulin can enhance the levels of short-chain fatty acids (SCFAs) in the blood and cecum contents of mice [6–8]. Similarly, in the human body, inulin can be fermented by intestinal flora into SCFAs such as acetic acid, propionic acid, and butyric acid [9–11].

SCFAs have several beneficial effects such as reducing colon pH, inhibiting pathogen growth, decreasing the production of toxic metabolites by bacteria, and protecting the structure of the intestinal mucosa [12]. In previous studies, oral inulin has been found to significantly improve symptoms of inflammatory bowel disease and reduce inflammation in the intestinal mucosa [13, 14]. However, the potential of inulin in preventing and treating 5-FU-induced intestinal mucositis is still unclear. In this study, we administered inulin to mice through drinking water and then induced intestinal mucositis by injecting 5-FU into the abdominal cavity. We aimed to investigate the preventive and therapeutic effects of inulin on 5-FU-induced intestinal mucositis and analyze the underlying mechanisms using non-targeted metabolomics technology. The findings from this study will contribute to the development of inulin-based strategies for the prevention and treatment of chemotherapeutic-induced intestinal mucositis.

MATERIALS AND METHODS

Model of chemotherapy intestinal mucositis

Thirty SPF grade ICR mice, weighing 18–22 g, male, 6 weeks of age, were provided by the Center of Compara-

tive Medicine of Yangzhou University and kept in clean environment of Animal Laboratory Center, Jiangsu University. The mice were randomly divided into 3 groups with 10 mice in each group, including normal control group (NC group), 5-FU group (5-FU group), and inulin group (INU group). Mice in the INU group were given 1% (v/v) inulin solution (Guangdong Fengsheng Biotechnology Co., Ltd., Guangzhou, China) instead of drinking water 30 days in advance, and mice in other groups were given pure water. The inulin purity was greater than 90%, and the polymerization degree was 2–60. From day 31, mice in the 5-FU and INU groups were connected for 5 days by intrabitoneal injection of 5-FU (30 mg/kg) (Tianjin Jinyao Amino Acid Co., Ltd., Tianjin, China) to induce chemotherapeutic intestinal mucositis. Mice in the NC group did not receive any treatment. All mice were fed under standard laboratory condition, where the temperature was (25 ± 1) °C, the humidity was (50 ± 10)%, and the light condition was dark/light according to 12 h/12 h cycle. The weight of mice in each group was measured once every 7 days, and the weight change curve was drawn. The mice were sacrificed after fasting for 12 h, the sections from stomach to cecum were dissected, and the intestinal length of mice in each group was measured.

H&E staining and immunohistochemical stain

Mice were cut for about 1 cm proximal portion of the cecum and fixed in 4% paraformaldehyde for 48 h. After paraffin embedding, hematoxylin-eosin (H&E) staining was performed. The integrity of intestinal mucosal villous epithelium, separation of mucosa from lamina propria, edema of muscle layer and intestinal mucosal villi shedding were observed under microscope.

The expression of occludin in small intestinal mucosa was detected by immunohistochemical stain. The main steps are as follows: the slices were dewaxed into water, soaked in 3% hydrogen peroxide solution at room temperature for 10 min, and sealed in normal goat serum (Boster Biological Technology Co., Ltd., Wuhan, China) at room temperature for 20 min. Occludin antibody (Boster Biological Technology Co., Ltd.) was diluted at 1:100 ratio and incubated with small intestinal mucosal tissue overnight at 4 °C, and goat anti-rabbit IgG antibody was incubated at 37 °C for 20 min. The color was developed by DAB-H_2O_2 at room temperature and was finally observed under microscope after being sealed by neutral gum.

RNA extraction and qRT-PCR

Appropriate amount of mouse spleen tissues was collected, and 1 ml of lysate solution was added. The spleen tissues were ground on ice with nuclease-free plastic sticks until there was no visible tissue residue, and then the total RNA of spleen tissues was extracted by total RNA extraction kit (Nanjing Vazyme, Nanjing, China), according to the kit instructions. The cDNA was synthesized by HiScript III RT SuperMix for qPCR (+gDNA wiper) (Nanjing Vazyme) using oligo(dT) as primer, and mRNA expressions of the inflammatory rated factors were determined by qRT-PCR. The total system of qRT-PCR reaction was 20 µl, including 10 µl of SYBR Green Master premix, 0.4 µl of 10 µmol/l upper and downstream primers, and 2 µl of cDNA template. Reaction procedure: predenaturation at 95 °C for 5 min, denaturation at 95 °C for 3 s, annealing at 58 °C for 20 s, and extension at 72 °C for 30 s with a total of 40 reaction cycles. Using GAPDH as internal reference, mRNA relative expression was calculated by the formula $2^{-\Delta\Delta Ct}$. The sequences of primers for the qRT-PCR assay were synthesized by Suzhou GENEWIZ Company, and the sequences were shown in Table S1.

ELISA assay

Serum IL-1β and IgA concentrations were determined by ELISA kit (Meimian, Yangcheng, China). After the ELISA reaction, the absorbance values at 450 nm were determined with enzyme-labeled instrument, and the IL-1β and IgA concentrations in each group were calculated by standard curve method.

Untargeted metabololomics analysis

The blood samples of mice were collected at the end of the experiment. In order, about 1.5 ml of blood was collected in a tube without any coagulation element and then was left at room temperature for 30 min before centrifugation at the speed of 3000g for 5 min at 4 °C. After that, the serum was carefully transferred to another clean tube to be stored under −80 °C immediately.

About 100 µl of the re-melted stored plasma was absorbed and mixed with 300 µl of methanol. After centrifugation at 16,000g for 10 min, about 120 µl of supernatant was absorbed for detection, in which 10 µl from each sample was mixed and used as a quality control sample (QC). All samples were tested in Yangzhou University using UPLC–IMS-Q–Tof system (Waters Corporation, Milford, Massachusetts, USA) and Unifi software (Waters Corporation) to collect data and match metabolites to molecular formulas. The Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (OPLS-DA) were used to analyze the trend of metabolites by Progenesis QI software (Waters Corporation). The potential differential metabolites were screened according to VIP > 1 and $p < 0.05$ rules. The obtained differential metabolites were retrieved and confirmed in Human Metabolome Database (https://hmdb.ca/). Based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.ad.jp/kegg/kegg2.html), the related metabolic pathways of the potential differential metabolites were determined.

Statistical analysis

The data were collected in Excel sheet and analyzed using SPSS 22.0 software for windows, the numerical data were expressed as mean and standard deviation (SD), and mean differences were compared using one way ANOVA test with post hoc test (Tukey methods). All tests were two-sided with $p < 0.05$ considered significant. For mapping, Graphpad Prism 9.2 software was used.

RESULTS

Inulin reverses 5-FU induced weight loss and shortens intestinal length in mice

The weight of the NC group mice continued to increase throughout the experiment. Compared with the NC group, the body weight of mice in the 5-FU and INU groups decreased significantly on the 2nd day after injection of 5-FU ($p < 0.05$); the body weight of the 5-FU group mice continued to decrease throughout the experiment ($p < 0.05$); the body weight of mice in the INU group tended to be stable on the 3rd day after 5-FU injection, and there was no significant change ($p > 0.05$) (Fig. 1A). Compared with the NC group, intestinal length of mice in the 5-FU and INU groups was significantly shortened ($p < 0.05$). The intestinal length of mice in the INU group was significantly longer than that of 5-FU group ($p < 0.05$) (Fig. 1B).

Inulin inhibits the expressions of inflammation-related factors in spleen tissue and serum

The mRNA expressions of NLRP3 and IL-10 in spleen were significantly changed after 5-FU induced intestinal mucositis (Fig. 2A,B). Compared with the NC group, NLRP3 mRNA expression in spleen tissues of the 5-FU and INU groups was significantly increased ($p < 0.05$). The mRNA expression of IL-10 in spleen tissue in the 5-FU group was significantly decreased, while that in the INU group was significantly increased ($p < 0.05$). Compared with the 5-FU group, the mRNA expressions of NLRP3 and IL-10 in spleen tissues of the INU group were significantly decreased, and the mRNA expression of IL-10 was significantly increased ($p < 0.05$).

Serum IL-1β concentration reflected the systemic inflammatory response level of mice, and IgA concentration reflected the mucosal immunity level of mice. Compared with the NC group, serum IL-1β concentration in the INU and 5-FU groups was significantly increased ($p < 0.05$). Compared with the 5-FU group, serum IL-1β concentration in the INU group was significantly decreased ($p < 0.05$) (Fig. 2C). Compared with the NC group, IgA concentration in serum of mice in the 5-FU group was significantly decreased, while IgA concentration in the INU group was significantly increased ($p < 0.05$). Compared with the 5-FU group, IgA concentration in the INU group was significantly increased ($p < 0.05$) (Fig. 2D).

Inulin protects intestinal mucosal barrier integrity

In the NC group, the intestinal structure was intact, the intestinal mucosa was not damaged, the intestinal gland body was arranged neatly, and the mucosa lamina propria was not changed. The small intestine structure of the 5-FU group was severely necrotic with mucosal atrophy, villi shedding, glandular mutilation, and crypt disappearance. The intestinal mucosal injury of the INU group was better than that of the 5-FU group, and the mucosal villi shedding and mucosal layer necrosis of small intestine were improved (Fig. 3A).

In the NC group, the positive expression of occludin in small intestine was higher, and the mucosal epithelial cells were darker and brownish. The positive expression of occludin in small intestine of 5-FU group decreased obviously, and the coloration was lighter. The positive expression of occludin in small intestine of INU group was significantly higher than that of 5-FU group ($p < 0.05$) (Fig. 3B).

Inulin regulates metabolomics of 5-FU-induced intestinal mucositis in mice

Various metabolites in mice serum of the NC, 5-FU, and INU groups were analyzed by UPLC–IMS Q–TOF technique in ESI^+ mode and ESI^- mode. A total of 301 metabolites were detected in ESI^+ mode and 182 metabolites were detected in ESI^- mode. Unsupervised PCA model showed that serum metabolites in each group could be well clustered in the ESI^+ mode and the ESI^- mode, and samples in the 5-FU and NC groups were obviously separated, indicating that 5-FU could induce significant changes in serum endogenous metabolites in mice (Fig. 4).

To further verify the differences between serum samples from each group, the samples were analyzed by a supervised OPLS-DA model. The results showed that the samples of the NC, 5-FU, and INU groups could be separated significantly in the ESI^+ mode and the ESI^- mode, and the clustering of samples within the group was obvious, indicating that the differences between groups were more significant than the differences between individuals. The samples of the INU and 5-FU groups were well separated, and the samples of the INU group were closer to the samples of the NC group, suggesting that inulin had a reverse effect on the abnormal endogenous differential metabolites in the serum of 5-FU-induced intestinal mucosal inflammation mice. The verification parameters of OPLS-DA model were $R^2X = 0.666$, $R^2Y = 0.987$, $Q^2 = 0.849$ (ESI^+ mode) and $R^2X = 0.664$, $R^2Y = 0.996$, $Q^2 = 0.931$ (ESI^- mode); R^2 represents the interpretability of the variable, and Q^2 represents the predictability of the model, the closer to 1 indicating that the results of

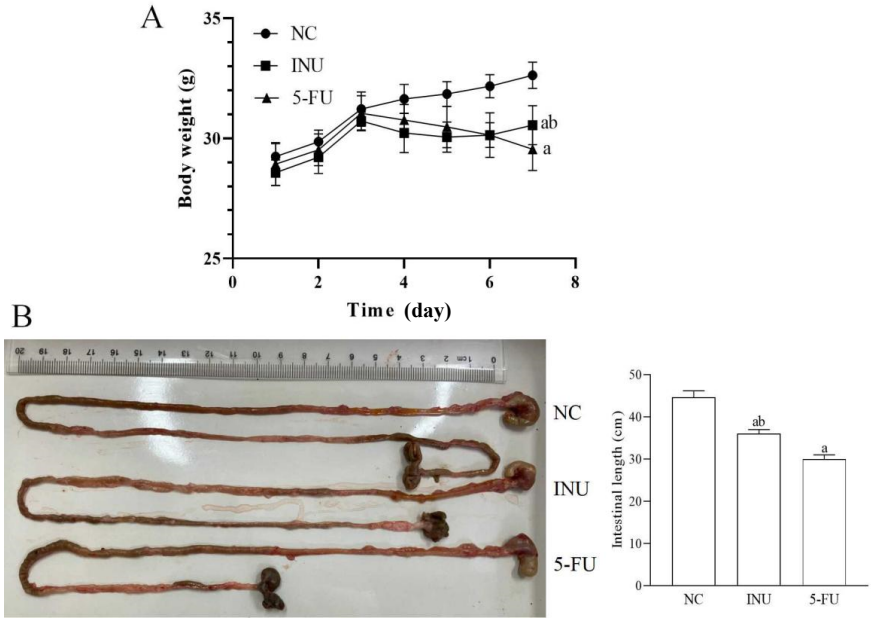

Fig. 1 The change of body weight and intestinal length. (A) The weight change and (B) intestinal length change. a: compared with the NC group, $p < 0.05$ and b: compared with the 5-FU group, $p < 0.05$. NC: normal control group, INU: 5-fluorouracil + inulin treated group, and 5-FU: 5-fluorouracil treated group.

Table 1 The differential metabolites in the 5-FU group vs. NC group and INU group vs. 5-FU group.

MODE	No	Metabolite	Formula	Library ID	RT (min)	M/Z	5-FU vs NC	INU vs 5-FU
ESI^+	1	LysoPC(20:4(5Z,8Z,11Z,14Z))	$C_{28}H_{50}NO_7P$	LMGP01050048	5.95	544.34	↑	↓
	2	PS(20:3(8Z,11Z,14Z)/19:1(9Z))	$C_{45}H_{80}NO_{10}P$	LMGP03010607	23.33	848.53	↑	↓
	3	PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z))	$C_{52}H_{80}NO_8P$	LMGP01011119	23.33	916.51	↑	↓
	4	Tryptophanol	$C_{10}H_{11}NO$	HMDB03447	8.31	184.07	↑	↓
	5	N1-Caffeoyl-N10-feruloylspermidine	$C_{26}H_{33}N_3O_6$	HMDB38843	23.33	984.50	↑	↓
	6	Vitamin A	$C_{20}H_{30}O$	HMDB00305	13.17	287.23	↓	↑
	7	Sciadonic acid	$C_{20}H_{34}O_2$	HMDB31058	16.32	307.26	↑	↓
	8	Glycerophosphocholine	$C_8H_{20}NO_6P$	HMDB00086	0.82	280.09	↑	↓
	9	L-Tryptophan	$C_{11}H_{12}N_2O_2$	HMDB00929	3.19	227.07	↑	↓
	10	Dihydrouracil	$C_4H_6N_2O_2$	HMDB00076	0.91	132.07	↑	↓
	11	Ginsenoside Rh6	$C_{36}H_{62}O_{11}$	HMDB39436	5.88	671.43	↑	↓
	12	PC(6:0/6:0)	$C_{20}H_{40}NO_8P$	LMGP01011229	5.95	476.23	↑	↓
	13	3-Dehydroxycarnitine	$C_7H_{15}NO_2$	HMDB06831	5.83	184.07	↑	↓
	14	PC(O-16:1(11Z)/0:0)	$C_{24}H_{50}NO_6P$	LMGP01060028	5.99	502.33	↑	↓
	15	1-Octene	C_8H_{16}	HMDB32449	4.90	242.28	↑	↓
	16	2,3-Dihydro-5-(5-methyl-2-furanyl)-1H-pyrrolizine	$C_{12}H_{13}NO$	HMDB40012	4.96	392.23	↑	↓
	17	Santene hydrate	$C_9H_{16}O$	HMDB37001	5.15	158.15	↑	↓
	18	Alfalone	$C_{17}H_{14}O_5$	HMDB38811	4.87	619.16	↓	↑
	19	(E)-2,4,4'-Trihydroxychalcone	$C_{15}H_{12}O_4$	HMDB29462	4.71	279.06	↓	↑
	20	Pelargonidin 3-sophoroside	$C_{28}H_{33}O_{14}^+$	HMDB33679	4.86	616.17	↓	↑
ESI^-	21	20-hydroxy-eicosanoic acid	$C_{20}H_{40}O_3$	LMFA01050075	23.21	309.27	↓	↑
	22	Pelargonaldehyde	$C_9H_{18}O$	LMFA06000040	23.22	283.26	↓	↑
	23	Ergosterol peroxide	$C_{28}H_{44}O_3$	HMDB37941	23.34	409.31	↓	↑
	24	Caffeic acid 3-sulfate	$C_9H_8O_7S$	HMDB41706	3.86	258.99	↑	↓
	25	Ethyl 1-(methylthio)ethyl disulfide	$C_5H_{12}S_3$	HMDB33046	3.38	188.98	↓	↑
	26	D-Ribose	$C_5H_{10}O_5$	HMDB00283	0.84	195.05	↓	↑
	27	1-(2-methoxy-13-methyl-tetradecanyl)-sn-glycero-3-phosphoethanolamine	$C_{21}H_{46}NO_7P$	LMGP02060011	5.95	476.27	↓	↑

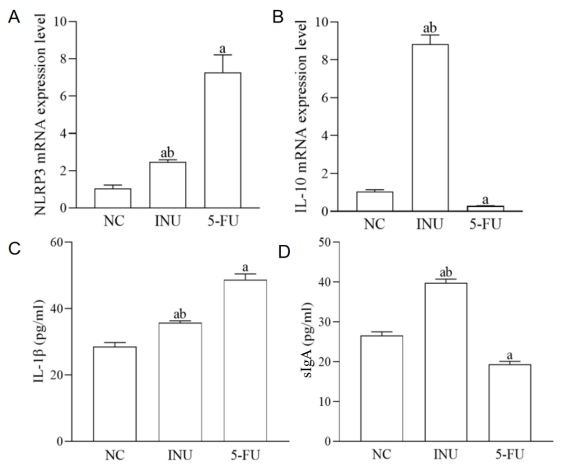

Fig. 2 The expression levels of NLRP3, IL-10, IL-1β, and IgA in spleen and serum of mice. (A) NLRP3 mRNA expression, (B) IL-10 mRNA expression, (C) IL-1β level, and (D) sIgA level. a: compared with the NC group, $p < 0.05$ and b: compared with the 5-FU group, $p < 0.05$. NC: normal control group, INU: 5-fluorouracil + inulin treated group, and 5-FU: 5-fluorouracil treated group.

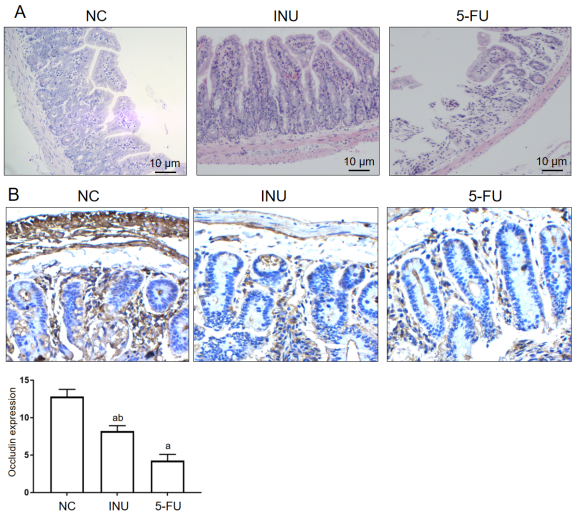

Fig. 3 Observation of intestinal histopathological morphology by HE and immunohistochemical staining. NC: normal control group, INU: 5-fluorouracil + inulin treated group, and 5-FU: 5-fluorouracil treated group.

OPLS-DA model are more reliable (Fig. 5).

According to VIP > 1.0 and $p < 0.05$, the standard screening for differential metabolites, 20 and 7 different metabolites were obtained in the ESI^+ mode and ESI^- mode, respectively. The cluster heat map was constructed to visually display the relative content changes of different metabolites in each group. Oral inulin can significantly reverse 5-FU-induced serum metabolite disturbance in mice. These metabolite products include LysoPC(20:4(5Z, 8Z, 11Z, 14Z), PS(20:3(8Z, 11Z, 14Z)/19:1(9Z)), PC(22:6(4Z, 7Z, 10Z, 13Z, 16Z, 19Z)), tryptophanol, vitamin A, sciadonic acid, glycerophosphocholine, L-Tryptophan, PC(6:0/6:0), 3-Dehydroxycarnitine, PC(O-16:1(11Z)/0:0), ergosterol oxidant, and 1-(2-methoxy-13-methyl-tetradecanyl)-sn-glycero-3-phosphoethanolamine. These metabolites are involved in multiple metabolic pathways, including glycerol phospholipid metabolism, retinol metabolism, pantothenic acid and CoA biosynthesis, sphingolipid metabolic pathways, etc., (Fig. 6 and Table 1).

DISCUSSION

Chemotherapy drugs are crucial in the treatment of malignant tumors. However, one significant drawback is their low selectivity [15]. These drugs cannot exclusively target tumor cells without harming normal tissue cells, particularly those with high metabolic rates such as bone marrow and mucosal cells [16]. Consequently, severe adverse reactions like myelosuppression and digestive tract mucositis may occur.

The European Association of Medical Oncology has defined chemotherapy-induced intestinal mucositis (CIM) as the development of intestinal inflammatory or ulcerative lesions in tumor patients undergoing chemotherapy. Common clinical manifestations of CIM include nausea, vomiting, abdominal pain, and mucous stools [17]. In severe cases, patients may experience water, electrolyte, and acid-base imbalances, malnutrition, and even multiple organ dysfunction syndrome, sepsis, systemic organ failure, or septic shock. CIM not only delays the chemotherapy cycle and reduces the quality of life for patients but also increases the risk of death and negatively impacts the efficacy of chemotherapy, ultimately worsening the prognosis of the disease [18].

5-FU, or 5-fluorouracil, is a widely used antitumor medication known to cause CIM. In fact, more than 50% of patients who undergo 5-FU chemotherapy experience severe CIM, which manifests primarily as symptoms like nausea, vomiting, diarrhea, and in some cases, even hematochezia [19]. Research conducted in the past has indicated that 5-FU has the ability to trigger apoptosis in small intestinal epithelial cells, disrupt the tight junctions between cells, and impair the mechanical barrier of the intestinal tract [20].

Dying cells release a significant amount of double-stranded DNA (dsDNA), which acts as an inflammatory factor known as damage-associated molecular patterns (DAMPs) [21, 22]. These DAMPs are recognized by pattern recognition receptors (PRRs), which are crucial for the body's initial immune response and play a key role in both natural and adaptive immune responses [23]. Activation of PRRs enhances natural immunity and modulates adaptive immunity, affecting infected

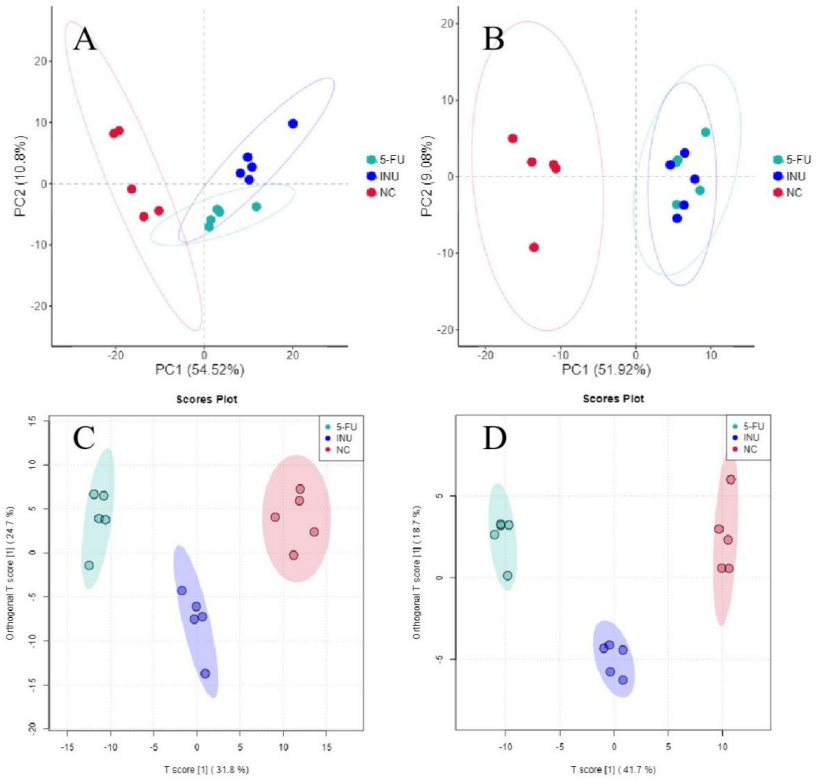

Fig. 4 Results of PCA and OPLS-DA analysis for all 3 groups. (A) The PCA score plot in ESI^+ model, (B) the PCA score plot in ESI^- model, (C) the OPLS-DA score plot in ESI^+ model, and (D) the OPLS-DA score plot in ESI^- model.

and non-infected immune tissue damage and leading to systemic inflammatory response (SIRS) [24]. The interaction between PRRs and DAMPs triggers the activation and up-regulation of transcription factors [25]. This, in turn, leads to the transcription of genes associated with inflammatory responses, including those that encode pro-inflammatory cytokines, chemokines, and proteins involved in PRR signaling [26]. Additionally, the use of 5-FU can disrupt the balance of intestinal microbes, reducing the diversity and abundance of beneficial bacteria and damaging the intestinal biological barrier. These findings suggest that 5-FU negatively impacts both the intestinal biological and immune barriers, emphasizing the importance of protecting the integrity of the intestinal mucosal barrier as a means to prevent and control CIM.

The NOD-like receptor protein 3 inflammasome (NLRP3) is a crucial member of the inflammasome family, particularly in gastrointestinal diseases. A study by Bauer et al [27] revealed that the NLRP3 inflammasome can contribute to inflammation in the gastrointestinal tract through IL-1β. Normally, the expression of NLRP3 inflammasome in intestinal mucosal epithelial cells and immune cells is low in the absence of intestinal infection. However, when an infection occurs, the expression of NLRP3 rapidly increases. This elevated expression leads to the activation of Caspase-1 protease and the maturation of IL-1β precursor and IL-18 precursor, ultimately triggering an inflammatory response [28, 29].

Our study discovered that 5-FU has the ability to activate the NLRP3 inflammasome in mice, leading to a notable increase in the expression of the pro-inflammatory cytokine IL-1β in the serum. However, we found that inulin can effectively inhibit the activation of the NLRP3 inflammasome, resulting in a decrease in IL-1β expression and an increase in the expression of the anti-inflammatory cytokine IL-10. These findings indicate that oral inulin can effectively prevent 5-FU-induced intestinal mucositis by inhibiting the activation of the NLRP3 inflammasome. This, in turn, reduces the secretion of pro-inflammatory cytokine IL-1β and promotes the expression of anti-inflammatory cytokine IL-10, thus maintaining immune balance *in vivo*.

Our study also utilized HE and immunohistochemical staining to examine the small intestinal mucosal tissue of mice. These analyses revealed that intraperitoneal injection of 5-FU caused severe damage to the intestinal villi and crypt structures, resulting in a significant negative impact on the intestinal mucosal barrier function and a decrease in nutrient absorption capacity.

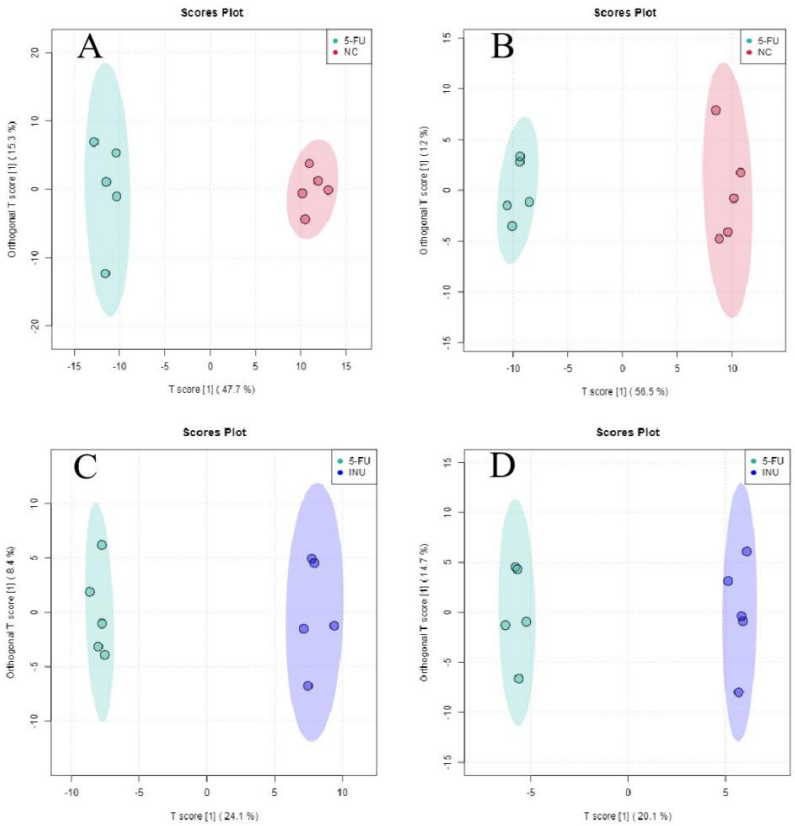

Fig. 5 Results of OPLS-DA analysis. (A) 5-FU group vs. NC group in ESI^+ model, (B) 5-FU group vs. NC group in ESI^- model, (C) 5-FU group vs. INU group in ESI^+ model, and (D) 5-FU group vs. INU group in ESI^- model.

These effects were further manifested by a decrease in body weight and a significant reduction in intestinal length. However, we observed that pre-administration of inulin through oral administration significantly alleviated the damage to the intestinal mucosal tissue induced by 5-FU in mice. This treatment helps to maintain the integrity of the intestinal mucosal barrier and the stability of the intestinal microecology.

Furthermore, our research revealed a significant decrease in the serum expression of secretory immunoglobulin IgA (sIgA) following the intraperitoneal injection of 5-FU. It is worth noting that both intestinal lamina propria and intestinal epithelial cells possess the ability to secrete sIgA, which plays a crucial role in the defense against pathogen adhesion and colonization in the intestinal mucosa [30]. The observed reduction in IgA concentration in the serum of 5-FU-treated mice indicates damage to the intestinal mucosal immune barrier, rendering them more susceptible to blood infections caused by various pathogens. Notably, we found that pre-administration of inulin via oral route significantly increased the serum IgA concentration in 5-FU-treated mice. This finding suggests that inulin can enhance intestinal mucosal immunity and provide protection against a wide range of infections.

Metabolomics results showed that 5-FU could cause the disorder of serum metabolites in mice, and inulin could effectively regulate these different metabolites in a variety of metabolic pathways. Phosphatidyl choline (PC) and lysophosphatidyl choline (LysoPC) are not only important components of cell membrane structure, but also closely related to intestinal inflammatory injuries [31]. LysoPC, as a chemotactic mediator, can participate in the inflammatory by activating and modifying immune cells through specific G-protein-coupled receptors [32]. Wu et al [33] found that some PC is an important biomarker of inflammation, and its content is sustainably changed. LysoPC is an endogenous lysophospholipid produced by PC through phospholipase A2. When the content is too high, it will cause certain damage to the cell membrane and further lead to the disorder of phospholipid metabolism [34]. We identified LysoPC(20:4(5Z, 8Z, 11Z, 14Z)), phosphoethanolamine phosphate, phosphocholine, glycerophosphocholine, PS(20:3(8Z, 11Z, 14Z)/19:1(9Z)), PC(22:6(4Z, 7Z, 10Z, 13Z, 16Z,

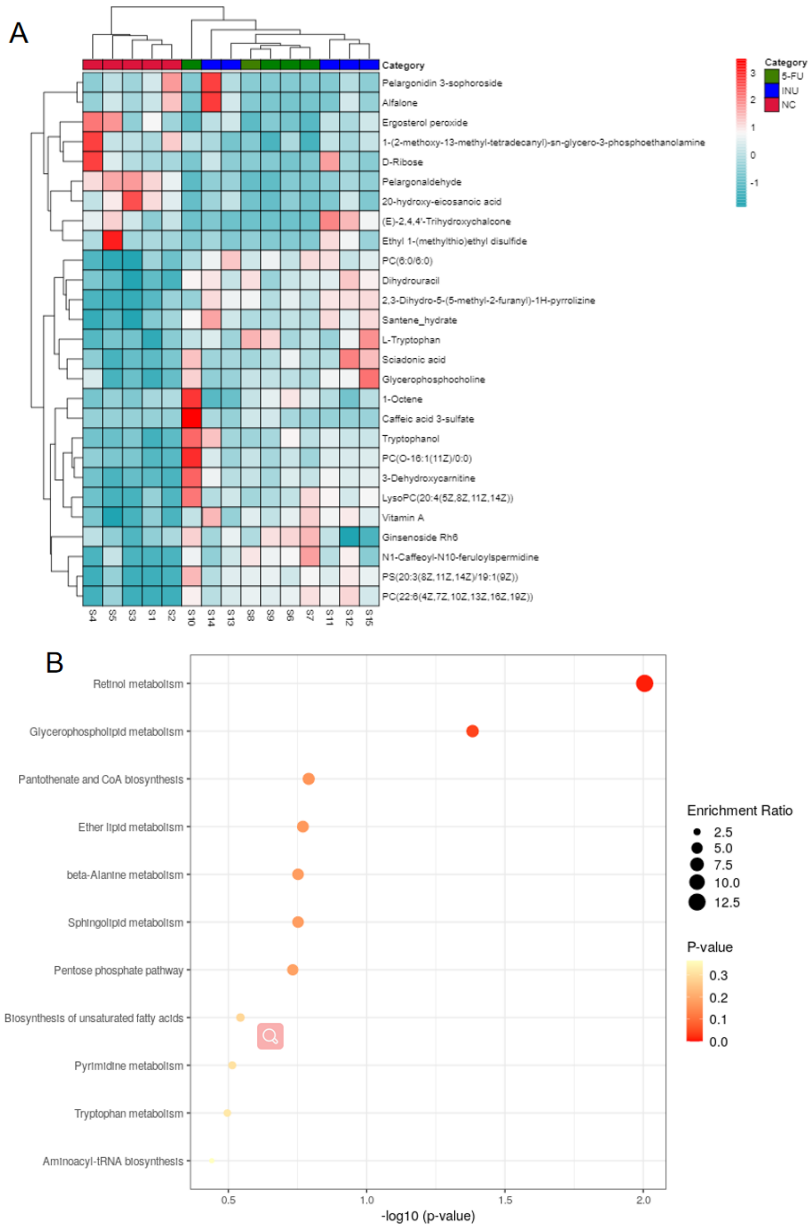

Fig. 6 The cluster heat map and metabolic pathway analysis.

19Z)), PC(6:0/6:0), PC(O-16:1(11Z)/0:0), and other differential metabolites affecting the metabolism of glycerol phospholipid and linoleic acid. Compared to the NC group, the levels of LysoPC(20:4(5Z, 8Z, 11Z, 14Z), glycerophosphocholine, PS(20:3(8Z, 11Z, 14Z)/19:1(9Z)), PC(22:6(4Z, 7Z, 10Z, 13Z, 16Z, 19Z)), PC(6:0/6:0), and PC(O-16:1(11Z)/0:0) in the 5-FU group were significantly increased, and the level of phosphoethanolamine was significantly decreased, which indicated that the metabolism of glycerol phospholipid compounds was disturbed. And after inulin intervention, LysoPC(20:4(5Z, 8Z, 11Z, 14Z)), glycerophosphocholine, PS(20:3(8Z, 11Z, 14Z)/19:1(9Z)), PC(22:6(4Z, 7Z, 10Z, 13Z, 16Z, 19Z)), PC(6:0/6:0), and PC(O-16:1(1)1Z)/0:0) decreased significantly, while phosphoethanolamine increased significantly, suggesting that inulin could improve the dysfunction of glycerophospholipid metabolism and linoleic acid metabolism in mice intestinal mucosal inflammation by regulating the levels of some glycerophospholipids.

Vitamin A is a crucial fat-soluble vitamin that consists of retinal, retinoic acid, and retinol. It plays a vital role in regulating cell differentiation and pro-

liferation as well as maintaining the integrity of epithelial cells and immune function [35]. Research has demonstrated that vitamin A is involved in preserving intestinal mucosal integrity and regulating normal immune function. Additionally, a study conducted by Pattanakitsakul et al [36] discovered that vitamin A can enhance the expression of tight junction proteins in the mucosa to repair damage caused by inflammation induced by LPS. In our study, we observed a significant decrease in serum vitamin A levels in mice from the 5-FU group. However, intervention with inulin significantly improved the vitamin A levels, indicating that inulin may have a protective effect on the intestinal mucosa by regulating retinol metabolism disorders.

In conclusion, our study found that 5-FU has the ability to activate the NLRP3 inflammasome in mice. This activation leads to an increase in the secretion of pro-inflammatory cytokines such as IL-1β, causing damage to the intestinal mucosal barrier. Additionally, 5-FU also reduces the secretion of IgA, resulting in a decrease in mucosal immunity.

It is well known that the microbiome exists as a symbiotic entity within humans, livestock, wild animals, birds, fish, reptiles, insects, and other organisms, playing an important role in host physiology, metabolism, and the regulation of the host immune system [37]. When microbial populations are influenced by external factors such as 5-FU, any changes in their composition or disruption of the symbiotic alliance with the host may ultimately lead to the occurrence of various types of pathological changes. Existing metabolomics can identify changes in the microbiome and their impact on the metabolic pathways related to host physiology, metabolism, and immune regulation.

Our research revealed that 5-FU affects mouse metabolic pathways, specifically glycerol phospholipid metabolism and linoleic acid metabolism. On the other hand, inulin, when administered orally, effectively inhibits the systemic inflammatory response caused by 5-FU in mice. It also plays a protective role in maintaining the structural integrity of the small intestinal mucosa.

The mechanism behind this protective effect of inulin may be linked to its ability to correct various endogenous metabolites in mouse serum. These metabolites are involved in glycerophospholipid metabolism and retinol metabolism.

CONCLUSION

Overall, our findings suggest that taking inulin orally prior to chemotherapy treatment with 5-FU can significantly reduce the occurrence of intestinal mucositis. This discovery provides a promising new approach for the treatment of this condition in clinical chemotherapy.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2025.015.

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REFERENCES

1. Torres DM, Tooley KL, Butler RN, Smith CL, Geier MS, Howarth GS (2008) Lyprinol only partially improves indicators of small intestinal integrity in a rat model of 5-fluorouracil-induced mucositis. *Cancer Biol Ther* **7**, 295–302.
2. Zhang T, Lu SH, Bi Q, Liang L, Wang YF, Yang XX, Gu W, Yu J (2017) Volatile oil from amomi fructus attenuates 5-fluorouracil-induced intestinal mucositis. *Front Pharmacol* **8**, 786.
3. Anderson PM, Lalla RV (2020) Glutamine for amelioration of radiation and chemotherapy associated mucositis during cancer therapy. *Nutrients* **12**, 1675.
4. Bhanja A, Sutar PP, Mishra M (2022) Inulin-A polysaccharide: review on its functional and prebiotic efficacy. *J Food Biochem* **46**, e14386.
5. Zeaiter Z, Regonesi ME, Cavini S, Labra M, Sello G, Di Gennaro P (2019) Extraction and characterization of inulin-type fructans from artichoke wastes and their effect on the growth of intestinal bacteria associated with health. *Biomed Res Int* **2019**, 1083952.
6. Matt SM, Allen JM, Lawson MA, Mailing LJ, Woods JA, Johnson RW (2018) Butyrate and dietary soluble fiber improve neuroinflammation associated with aging in mice. *Front Immunol* **9**, 1832.
7. Mistry RH, Gu F, Schols HA, Verkade HJ, Tietge U (2018) Effect of the prebiotic fiber inulin on cholesterol metabolism in wildtype mice. *Sci Rep* **8**, 13238.
8. Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C, Schmidt TM (2019) Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. *mBio* **10**, e02566-18.
9. Wang X, Gibson GR (1993) Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *J Appl Bacteriol* **75**, 373–380.
10. Timm DA, Stewart ML, Hospattankar A, Slavin JL (2010) Wheat dextrin, psyllium, and inulin produce distinct fermentation patterns, gas volumes, and short-chain fatty acid profiles *in vitro*. *J Med Food* **13**, 961–966.
11. Kaur A, Rose DJ, Rumpagaporn P, Patterson JA, Hamaker BR (2011) *In vitro* batch fecal fermentation comparison of gas and short-chain fatty acid production using "slowly fermentable" dietary fibers. *J Food Sci* **76**, H137–H142.
12. Roberfroid MB (2007) Inulin-type fructans: functional food ingredients. *J Nutr* **137**, 2493S–2502S.
13. Akram W, Garud N, Joshi R (2019) Role of inulin as prebiotics on inflammatory bowel disease. *Drug Discov Ther* **13**, 1–8.
14. Sun Q, Arif M, Chi Z, Li G, Liu CG (2021) Macrophages-targeting mannosylated nanoparticles based on inulin

for the treatment of inflammatory bowel disease (IBD). *Int J Biol Macromol* **169**, 206–215.
15. Chen F, Fang Y, Zhao R, Le J, Zhang B, Huang R, Chen Z, Shao J (2019) Evolution in medicinal chemistry of sorafenib derivatives for hepatocellular carcinoma. *Eur J Med Chem* **179**, 916–935.
16. Sougiannis AT, VanderVeen BN, Davis JM, Fan D, Murphy EA (2021) Understanding chemotherapy-induced intestinal mucositis and strategies to improve gut resilience. *Am J Physiol Gastrointest Liver Physiol* **320**, G712–G719.
17. Akbarali HI, Muchhala KH, Jessup DK, Cheatham S (2022) Chemotherapy induced gastrointestinal toxicities. *Adv Cancer Res* **155**, 131–166.
18. Prieto-Callejero B, Rivera F, Fagundo-Rivera J, Romero A, Romero-Martín M, Gómez-Salgado J, Ruiz-Frutos C (2022) Relationship between chemotherapy-induced adverse reactions and health-related quality of life in patients with breast cancer. *Medicine (Baltimore)* **99**, e21695.
19. Chen H, Zhang F, Li R, Liu Y, Wang X, Zhang X, Xu C, Li Y, et al (2020) Berberine regulates fecal metabolites to ameliorate 5-fluorouracil induced intestinal mucositis through modulating gut microbiota. *Biomed Pharmacother* **124**, 109829.
20. Ma J, Ma Y, Chen S, Guo S, Hu J, Yue T, Zhang J, Zhu J, et al (2021) SPARC enhances 5-FU chemosensitivity in gastric cancer by modulating epithelial-mesenchymal transition and apoptosis. *Biochem Biophys Res Commun* **558**, 134–140.
21. Wilkins AC, Patin EC, Harrington KJ, Melcher AA (2019) The immunological consequences of radiation-induced DNA damage. *J Pathol* **247**, 606–614.
22. Wu D, Wang S, Yu G, Chen X (2021) Cell death mediated by the pyroptosis pathway with the aid of nanotechnology: prospects for cancer therapy. *Angew Chem Int Ed Engl* **60**, 8018–8034.
23. Negi S, Das DK, Pahari S, Nadeem S, Agrewala JN (2019) Potential role of gut microbiota in induction and regulation of innate immune memory. *Front Immunol* **10**, 2441.
24. Tomalka JA, Suthar MS, Diamond MS, Sekaly RP (2022) Innate antiviral immunity: how prior exposures can guide future responses. *Trends Immunol* **43**, 696–705.
25. Zhang Y, Pfeiffer A, Tepperman JM, Dalton-Roesler J, Leivar P, Gonzalez Grandio E, Quail PH (2020) Central clock components modulate plant shade avoidance by directly repressing transcriptional activation activity of PIF proteins. *Proc Natl Acad Sci USA* **117**, 3261–3269.
26. Cronstein BN, Aune TM (2020) Methotrexate and its mechanisms of action in inflammatory arthritis. *Nat Rev Rheumatol* **16**, 145–154.
27. Reddy BS, Hamid R, Rao CV (1997) Effect of dietary oligofructose and inulin on colonic preneoplastic aberrant crypt foci inhibition. *Carcinogenesis* **18**, 1371–1374.
28. Bauer C, Duewell P, Mayer C, Lehr HA, Fitzgerald KA, Dauer M, Tschopp J, Endres S, et al (2010) Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. *Gut* **59**, 1192–1199.
29. Bruchard M, Rebé C, Derangère V, Togbé D, Ryffel B, Boidot R, Humblin E, Hamman A, et al (2015) The receptor NLRP3 is a transcriptional regulator of Th2 differentiation. *Nat Immunol* **16**, 859–870.
30. Macpherson AJ, Hunziker L, McCoy K, Lamarre A (2001) IgA responses in the intestinal mucosa against pathogenic and non-pathogenic microorganisms. *Microbes Infect* **3**, 1021–1035.
31. Clemente JC, Manasson J, Scher JU (2018) The role of the gut microbiome in systemic inflammatory disease. *BMJ* **360**, j5145.
32. Yuan Z, Yang L, Zhang X, Ji P, Hua Y, Wei Y (2020) Mechanism of Huang-lian-Jie-du decoction and its effective fraction in alleviating acute ulcerative colitis in mice: Regulating arachidonic acid metabolism and glycerophospholipid metabolism. *J Ethnopharmacol* **259**, 112872.
33. Nietgen GW, Durieux ME (1998) Intercellular signaling by lysophosphatidate. *Cell Adhes Commun* **5**, 221–235.
34. Wu X, Cao H, Zhao L, Song J, She Y, Feng Y (2016) Metabolomic analysis of glycerophospholipid signatures of inflammation treated with non-steroidal anti-inflammatory drugs-induced-RAW264.7 cells using (1)H NMR and U-HPLC/Q-TOF-MS. *J Chromatogr B Analyt Techno Biomed Life Sci* **1028**, 199–215.
35. Cho WH, Yeo HJ, Yoon SH, Lee SE, Jeon DS, Kim YS, Lee SJ, Jo EJ, et al (2015) Lysophosphatidylcholine as a prognostic marker in community-acquired pneumonia requiring hospitalization: a pilot study. *Eur J Clin Microbiol Infect Dis* **34**, 309–315.
36. Pattanakitsakul P, Chongviriyaphan N, Pakakasama S, Apiwattanakul N (2020) Effect of vitamin A on intestinal mucosal injury in pediatric patients receiving hematopoietic stem cell transplantation and chemotherapy: a quasai-randomized trial. *BMC Res Notes* **13**, 464.
37. Sharma S, Chaubey KK, Singh SV, Gupta S (2022) Symbiotic microbiota: A class of potent immunomodulators. *ScienceAsia* **48**, 855–865.

Appendix A. Supplementary data

Table S1 The primer sequences of qRT-PCR.

Gene	Primer sequence ($5' \rightarrow 3'$)
β-actin	F:ATGACCCAAGCCGAGAAGG R:CGGCCAAGTCTTAGAGTTGTTG
NLRP3	F:ATTACCCGCCCGAGAAAGG R:CATGAGTGTGGCTAGATCCAAG
IL-10	F:GAAGCTCCCTCAGCGAGGACA R:TTGGGCCAGTGAGTGAAAGGG