Three main short-chain fatty acids attenuate 5-FU-induced THP-1 cells inflammation via glycerolphospholipid and sphingolipid metabolism

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Short Report

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Abstract

5-Fluorouracil (5-FU) is a common antitumor drug, but there is no effective treatment for its side effect, intestinal mucositis. The inflammatory reaction of macrophages in intestinal mucosa induced by 5-FU is an important cause of intestinal mucositis. In this study, we investigated the anti-inflammatory effects of the three main short chain fatty acids (SCFAs), sodium acetate (NaAc), sodium propionate (NaPc), and sodium butyrate (NaB), on human mononuclear macrophage-derived THP-1 cells induced by 5-FU. The inhibition mechanism of macrophage inflammation was studied by untargeted metabolomics. The THP-1 cells were pre-incubated with 100 μmol/L of NaAc, NaPc, and NaB for 24 h, then treated with 2.5 mmol/L 5-FU for 24 h. The expressions of ROS, NF-κB p65, NLRP3 inflammasome, pro-inflammatory/anti-inflammatory cytokines were determined, and the cell metabolites was analyzed by untargeted metabolomics techniques. It is found that the three main SCFAs could inhibit the pro-inflammation factors expressions including NLRP3, Caspase-1, IL-1β, and IL-6 when treated with 5-FU. The ROS expression and NF-κB pathway activity of THP-1 cell were inhibited by the three main SCFAs pre-incubated. Our results indicated that the three main SCFAs can effectively suppress the THP-1 cell inflammation via ROS/NF-κB/NLRP3 pathway, and affect 20 kinds of THP-1 cell metabolites which belong to amino acids and phosphatidylcholine. These significantly altered metabolites were involved in amino acid metabolism, glycerolphospholipid metabolism and sphingolipid metabolism. This is the first time that the three main SCFAs had been found to inhibit 5-FU-induced macrophage inflammation through the above metabolic pathways through untargeted metabolomics.

Highlights

- We found for the first time that main SCFAs (NaAc, NaPc, NaB) could inhibit macrophage inflammation induced by 5-fluorouracil (5-FU) in vitro.
- The expression of NLRP3, Caspase-1, IL-1β, IL-6 of THP-1 cell induced by 5-FU were significantly inhibit when treated with the three main SCFAs.
- The three main SCFAs could suppress the THP-1 cell inflammation via ROS/NF-κB/NLRP3 pathway.
- It was found for the first time that the three main SCFAs could affect 20 kinds of metabolic products of THP-1 cells, which are related to amino acid metabolism, glycerolphospholipid metabolism, and sphingolipid metabolism.

Introduction

Short-chain fatty acids (SCFAs) are important metabolites of intestinal flora, which are produced by the fermentation of indigestible dietary fiber and resistant starch by certain specific anaerobic bacteria in the gut [1]. The number of carbon atoms of SCFAs is less than 6, and sodium acetate (NaAc), sodium propionate (NaPc), and sodium butyrate (NaB) together account for more than 90% of the total SCFAs, often in an ionic state in the intestinal tract [2]. SCFAs can promote the proliferation and differentiation of intestinal mucosa epithelial cells, prevent intestinal mucosa epithelial cells from atrophy and maintain a normal mucosa barrier. It also inhibits the production of pro-inflammatory cytokines and reduces inflammatory damage. There are studies showing that ingestion of probiotics or dietary fiber may be an adjunct to inflammatory bowel diseases, but the exact mechanism remains unclear [3].

5-Fluorouracil (5-FU) is a commonly used tumor chemotherapy drug that inhibits tumor cell proliferation by interfering with tumor cell DNA synthesis [4]. It is widely used in the treatment of a variety of cancers [5]. However, 40–80% of patients develop intestinal mucositis-like inflammatory bowel disease during treatment, with symptoms such as indigestion, diarrhea, and dehydration, which in severe cases can prevent chemotherapy from continuing [6]. Previous studies have shown that oral probiotics/prebiotics such as lactobacillus or dietary fiber can significantly reduce intestinal side effects and improve patients' tolerance to chemotherapy drugs, but the exact mechanism is still unclear [7].
chemotherapeutic intestinal mucositis, many pro-inflammatory cytokines secreted by activated macrophages in the mucosa are an important factor causing intestinal mucosal injury. Studying the exact mechanism intestinal microflora metabolites inhibiting macrophage inflammatory response provides a new approach for the prevention and treatment of chemotherapeutic intestinal mucositis. Our previous studies have shown that the three main SCFAs could reduce 5-FU-induced inflammatory response in THP-1 cells, and the expressions of NLRP3, caspase-1, and IL-1β of THP-1 cells were significantly reduced after treatment with three main SCFAs, as was production of important pro-inflammatory cytokine ROS [8]. However, the mechanism of the three main SCFAs inhibited the inflammatory response of THP-1 cells induced by 5-FU was unclear. In this study, we investigated the mechanisms by which the three main SCFAs inhibit 5-FU-induced THP-1 cells inflammation using untargeted metabolomics and explored the feasibility of probiotics/prebiotics to alleviate 5-FU-induced chemotherapy-induced intestinal mucosal inflammation.

Materials And Methods

Cell culture and processing

Human mononuclear macrophage (THP-1) cells were used in this experiment and the cultured and subcultured according to conventional methods (PMID: 23621670). The cells, in the logarithmic growth phase, were inoculated in a 6-well plate with 1×10⁶ cells in each well. A normal control group (NC), a 5-FU group (5-FU), a 5-FU + sodium acetate group (NaAc), a 5-FU + sodium propionate group (NaPc), and a 5-FU + sodium butyrate group (NaB) were set up in the experiment. The cells in the NC group did not undergo any treatment. The cells in the NaAc group, NaPc group, and NaB group were pre-treated with 100 µmol/L NaAc, NaPc, and NaB for 24 h, respectively. The cells in the 5-FU group, NaAc group, NaPc group, and NaB group were treated with 2.5 mmol/L 5-FU for 24 h.

Reactive oxygen species (ROS) detection

The 2-dichlorofluorescein yellow diacetate (DCFH-DA) probe kit (Beyotime Biotechnology, China) were used for the detection of intracellular ROS concentration (PMID: 31521245). The fluorescence intensity of DCFH-DAA in cells was detected by flow cytometry (Accuri C6 Plus, BD, USA).

Western blotting assessment

The process of Western blotting assessment was following Xi’s report (PMID: 35448938). The primary antibodies were diluted 1:1000 in TBST buffer and incubated overnight at 4 °C. The ECL chemiluminescence kit was applied (Millipore, USA), and the results were analyzed by Image J software. Total cellular protein expression and plasma protein analysis were performed using β-actin as a reference. The expression of nuclear protein analysis was performed using Histone H1 as a reference. All the experiments were replicated 3 times.

qRT-PCR analysis

Total RNA was purified from THP-1 cells using Trizol method, and cDNA was synthesized by reverse transcription with oligo(dT)ₙ as a primer. mRNA expression was determined by qRT-PCR. The qRT-PCR volume was 20 µL, including 10 µL of SYBR Green Master premixture, 0.4 µL of upstream primer, and 0.4 µL of downstream primer (10 µmol/L), 2 µL of cDNA template. The reaction procedure included 95 °C pre-denaturation for 5 min, 95 °C denaturation for 3 s, 58 °C annealing for 20 s, and 72 °C extension for 30 s. The whole qPCR reactions were 40 cycles. The GAPDH gene was the reference, the relative expression of mRNA was calculated by the 2⁻ΔΔCT method. The primer sequences are listed in Table 1.
Table 1
RT-PCR Primers sequence. FP, forward primer; RP, reverse primer.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH</td>
<td>FP:CATCACTGCCACCCAGAAGACTG</td>
</tr>
<tr>
<td></td>
<td>RP:ATGCCAGTGAGCTTCCCGTTCAG</td>
</tr>
<tr>
<td>NLRP3</td>
<td>FP:AACAGCCACCTCACTTCCAG</td>
</tr>
<tr>
<td></td>
<td>RP:CCAACCACAATCTCCGAATG</td>
</tr>
<tr>
<td>Caspase-1</td>
<td>FP: GCACAAGACCTCTGACAGCA</td>
</tr>
<tr>
<td></td>
<td>RP: TTGGGCAGTTTTGGTATTC</td>
</tr>
<tr>
<td>IL-1β</td>
<td>FP:CCTGTCTCGTGGTTGAAAGA</td>
</tr>
<tr>
<td></td>
<td>RP:GGGAACCTGGGCAGACTCAA</td>
</tr>
<tr>
<td>IL-6</td>
<td>FP:CCTCGGTCGGTGTTGAAAGA</td>
</tr>
<tr>
<td></td>
<td>RP:GAGGTGAGTGCTGTCTGT</td>
</tr>
<tr>
<td>IL-10</td>
<td>FP:TCTCGAGATGCGTTTACAGA</td>
</tr>
<tr>
<td></td>
<td>RP:TCAGACAAGCTTGGCAACCACCA</td>
</tr>
</tbody>
</table>

Metabolic characterization of cell sample preparation

Cell metabolomics analysis samples were prepared according to Liu’s method (PMID: 32692565). Metabolomics detection were performed by the Testing Center of Yangzhou University using chromatography-mass spectrometry platform.

Chromatographic separation of the metabolites was performed on a Waters UPLC system equipped with an ACQUITY UPLC® BEH C18 (100 mm × 2.1 mm i.d., 1.7 µm; Waters, Milford, USA). The mobile phases were composed of 0.1% formic acid in water (A) and acetonitrile (B) with a flow rate of 0.3 mL/min. The solvent gradient varies according to: 0–3 min, 99 – 80% A; 3–4 min, 80 – 30% A; 4–20 min, 30 – 25% A; 20–21 min, 25 – 1% A; and followed by 4 min for equilibrating the systems. A sample injection volume of 2 µL was used and column temperature was maintained at 40 ºC. All these samples were kept at 4 ºC during the analysis period.

The mass spectrometric data were collected using a Waters ACQUITY UPLC I-Class Plus/VION IMS QToF Mass Spectrometer. The parameter settings for the ESI ionization mode were set as follows: analyzer mode, sensitivity; capillary, 0.8 kV; source temperature, 120 ºC; desolvation temperature, 550 ºC (positive) and 450 ºC (negative); cone gas flow rate, 50 L/h; desolvation gas flow rate, 800 L/h; collision energy contained low energy 6 V and energy ramp 10 to 40 eV. Data were acquired with mass spectrometry Elevated Energy (MS²) technique. The full MS resolution was 50000. The mass range for detection is 50–1000 m/z.

Data processing and multivariate analysis

Raw data were imported into Progenesis QI 2.3 (Nonlinear Dynamics, Waters, USA) for peak detection and alignment following UPLC-MS/MS analyses. Preprocessing generated a data matrix consisting of the retention time (RT), mass-to-charge ratio (m/z) values, and peak intensity. Metabolic traits detected at least 80% were retained in any sample set. After filtration, the lowest metabolite values for a specific sample were estimated. Metabolite levels fell below the quantitative threshold, and metabolic characteristics were normalized according to the total amount. We used the
internal standard for QC data (reproducibility), metabolic characteristics, and discarded the relative standard deviation (RSD) of the >30% QC. After normalization procedures and imputation, statistical analysis was performed on log-transformed data to identify significant differences in metabolite levels between comparable groups. The accurate mass identified the mass spectra of these metabolic features, MS/MS fragments spectra, and isotope ratio difference by searching reliable biochemical databases such as the Human Metabolome Database (HMDB) (http://www.hmdb.ca/) and Lipid Maps (https://www.lipidmaps.org). The mass tolerance between the measured m/z values and the exact mass of the components of interest was ±10 ppm. For metabolites with MS/MS confirmation, only those with MS/MS fragment scores greater than 30 were considered confidently identified as metabolites.

In order to get an overview of the metabolic data, principal component analysis (PCA) using unsupervised method was applied, through the visualization of general clustering, trends or outliers. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to build a model for identifying variables. Significantly different metabolites were selected based on the variable weight value (VIP) obtained from OPLS-DA model and Student’s t test P value. Metabolites with VIP > 1.0 and P < 0.05 were considered potential biomarkers for the analysis.

Image J Software and GraphPad Prism 8.0 analysis software were used for image analysis and data processing after protein development. SPSS 22.0 software was used for performing the t-test and one-way ANOVA. A value for P < 0.05 was considered to be statistically significant.

Results

The expression of ROS in THP-1 cells

The expression of ROS in THP-1 cells was determined by flow cytometry (Fig. 1). Compared with the NC group, ROS expression in THP-1 cells was significantly increased in the 5-FU group (P < 0.05). Compared with the 5-FU group, the expression of ROS in the NaAc, NaPc, NaB groups were decreased significantly (P < 0.05).

The nuclear and cytoplasmic distribution of NF-κB p65 in THP-1 cells

The expressions of NF-κB p65 in the nucleus and cytoplasm of THP-1 cells were determined by Western blotting (Fig. 2). After treatment with 5-FU, NF-κB p65 expression in the nucleus was significantly up-regulated, and NF-κB p65 expression in plasma was significantly down-regulated (P < 0.05).

Compared with the NC group, the NF-κB p65 expressions in the nucleus of the 5-FU group, the NaAc, and NaB groups were significantly increased (P < 0.05), and the NF-κB p65 expressions in the cytoplasm of the 5-FU, the NaAc, NaPc, and NaB groups were significantly decreased (P < 0.05). In comparison with the 5-FU group, the expressions of NF-κB p65 in the nucleus NaAc, NaPc, and NaB groups decreased significantly. However, the NF-κB p65 expressions in cytoplasm were significantly upregulated (P < 0.05).

The expressions of pro-inflammatory factors in THP-1 cells

Compared with NC group, the mRNA expressions of NLRP3, Caspase-1, IL-1β, and IL-6 in the 5-FU group were significantly higher (Fig. 3). Compared with the 5-FU group, the mRNA expressions of NLRP3, Caspase-1, IL-1β, and IL-6 in the NaAc, NaPc, and NaB groups decreased significantly (P < 0.05). Compared with the 5-FU group, the mRNA expression of IL-10 in the NaAc, NaPc, and NaB groups increased significantly (P < 0.05).

Regulation of metabolomics in THP-1 cells
In this study, UPLC-MS/MS was used to comprehensively analyze metabolic profiling and metabolites among the NC, 5-FU, NaA, NaP, NaB groups. Two hundred forty-seven ions in the positive mode ions were detected in the cell samples. PCA was used for the first time for an unsupervised comprehensive observation of cell samples. Samples from the 5-FU group showed a deviation from the NC group in terms of metabolite profiles, suggesting that the endogenous metabolites in the cells have changed significantly after the model making (Fig. 4). Compared with the group of 5-FU, NaAc, NaPc, and NaB groups were closer to the NC group in the principal component direction. Simultaneously, the separating trends among the NaA, NaP, NaB, and 5-FU groups were clear. We then performed the OPLS-DA to further analyze and characterize group differences.

In the graphs of OPLS-DA scores, there was a clear separation between the NC, 5-FU, NaAc, NaPc, and NaB groups (Fig. 5). In the OPLS-DA spots, five clusters separated well and clustered distinctly in positive ion modes, reflecting that the group differences were more prominent than individual ones. The evaluation parameters of the OPLS-DA model were $R^2_X = 0.729$, $R^2_Y = 0.988$, $Q^2 = 0.976$ (positive ion mode), where $R^2$ represents the interpretability of the variables, $Q^2$ represents the predictability of the model, and the closer these two are to 1 represents a better OPLS-DA model explanation of the group differences. Cells after the administration of NaAc, NaPc, and NaB groups were significantly distinct from the 5-FU group, indicating that these SCFAs affected THP-1 cell metabolic profile, and the metabolite disorders were improved, skewing them toward normal cells (Fig. 6, 7, 8, 9).

As a supervised multivariate analysis method, OPLS-DA showed the distinct separation of the NC and 5-FU groups in the positive ionic modes (Fig. 10). The ions with VIP $> 1.0$ and $P < 0.05$ were significant differential metabolites in the present study. Twenty-four cell metabolites with significantly altered expression were screened between the 5-FU and NC cohorts. On applying the OPLS-DA model to evaluate the changes among the NaAc, NaPc, NaB, and 5-FU groups (Fig. 11, 12, 13), the metabolic profile between the 5-FU group and the NaAc, NaPc, NaB group were shown to be completely separated, and 20 significantly altered cellular metabolites were screened in the NaAc, NaPc, NaB and 5-FU groups (Table 2).
### Table 2
Cellular metabolites of THP-1 cells treated with SCFAs

<table>
<thead>
<tr>
<th>No.</th>
<th>Metabolite</th>
<th>Formula</th>
<th>Library ID</th>
<th>RT/min</th>
<th>M/Z</th>
<th>NaAc</th>
<th>NaPc</th>
<th>NaB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sphinganine</td>
<td>C18H39NO2</td>
<td>HMDB00269</td>
<td>5.02</td>
<td>302.31</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>2</td>
<td>Polyoxyethylene (600) mono- ricinoleate</td>
<td>C21H40O3</td>
<td>HMDB32476</td>
<td>21.65</td>
<td>703.57</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>3</td>
<td>(±)-(Z)-2-(5-Tetradecenyl)cyclobutanone</td>
<td>C18H32O</td>
<td>HMDB37543</td>
<td>12.70</td>
<td>282.28</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>4</td>
<td>Cytochalasin Opho</td>
<td>C28H37NO4</td>
<td>HMDB35366</td>
<td>5.66</td>
<td>452.27</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>5</td>
<td>N-docosahexaenoyl glutamic acid</td>
<td>C27H39NO5</td>
<td>LMFA08020089</td>
<td>5.96</td>
<td>480.27</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>6</td>
<td>Phytal acetate</td>
<td>C22H42O2</td>
<td>HMDB32470</td>
<td>5.52</td>
<td>356.35</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>7</td>
<td>Amantadine</td>
<td>C10H17N</td>
<td>HMDB15051</td>
<td>4.83</td>
<td>320.31</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>8</td>
<td>Octadecanamide</td>
<td>C18H37NO</td>
<td>HMDB34146</td>
<td>5.03</td>
<td>284.29</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>9</td>
<td>8Z-Heptadecene</td>
<td>C17H34</td>
<td>LMFA11000102</td>
<td>5.23</td>
<td>256.30</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>10</td>
<td>Glycerol 1-octadecanoate</td>
<td>C21H42O4</td>
<td>HMDB31075</td>
<td>21.64</td>
<td>359.31</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>11</td>
<td>PC (13:0/21:0)</td>
<td>C42H84N08P</td>
<td>LMGP01010466</td>
<td>21.75</td>
<td>784.58</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>12</td>
<td>PC (16:1(9Z)/14:0)</td>
<td>C38H74NO8P</td>
<td>LMGP01011475</td>
<td>21.77</td>
<td>726.50</td>
<td>↑</td>
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<td>↑</td>
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<tr>
<td>13</td>
<td>Annoglabasin E</td>
<td>C20H32O3</td>
<td>HMDB36262</td>
<td>9.71</td>
<td>658.51</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>14</td>
<td>Aminophenazone</td>
<td>C13H17N3O</td>
<td>HMDB15493</td>
<td>6.55</td>
<td>480.31</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>15</td>
<td>Zerumbone oxide</td>
<td>C15H22O2</td>
<td>HMDB36466</td>
<td>6.36</td>
<td>235.16</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>16</td>
<td>Bisnorbiotin</td>
<td>C8H12N2O3S</td>
<td>HMDB04821</td>
<td>6.53</td>
<td>234.09</td>
<td>↓</td>
<td>↓</td>
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</tr>
<tr>
<td>17</td>
<td>Monoethylhexyl phthalic acid</td>
<td>C16H22O4</td>
<td>HMDB13248</td>
<td>7.21</td>
<td>579.29</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>18</td>
<td>Vignatic acid A</td>
<td>C30H39N3O7</td>
<td>HMDB33599</td>
<td>6.11</td>
<td>554.28</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>19</td>
<td>TG (12:0/12:0/16:0)</td>
<td>C43H8206</td>
<td>LMGL03012632</td>
<td>3.56</td>
<td>712.64</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>20</td>
<td>3-Methylcyclohexanol</td>
<td>C7H140</td>
<td>HMDB31538</td>
<td>4.64</td>
<td>246.24</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

↑ indicates increase, ↓ indicates decrease, vs. 5-FU group.

Heat maps show trends in overall changes in common characteristics and metabolite levels among different populations (Fig. 14). Metabolites with similar abundance trends were placed closer together. According to the heat map results, the three major metabolites treated with SCFAs were closely clustered and separated from the 5-FU group.

We applied the online KEGG database was to pathway enrichment analysis, exploring the most relevant pathways as well as potential mechanisms. Variations in the 20 disturbed metabolites that the differences between 5-FU group and NaAc, NaPc, NaB groups were related to multiple metabolic pathways including D-glutamine and D-glutamate metabolism, glycerophospholipid metabolism, nitrogen metabolism, arginine metabolism, butanoate metabolism, histidine metabolism, sphingolipid metabolism, galactose metabolism, glutathione metabolism (Fig. 15). Callback trends
of disrupted metabolites suggested that the anti-inflammation mechanism of the three major SCFAs was related to the metabolic pathways.

**Discussion**

It is known that 5-FU not only interferes with DNA synthesis in tumor cells, but also in normal cells. This resulted in the production of a large number of ROS and the release of a large number of double-stranded DNA (dsDNA) [9], which acts as an important damage-associated molecular pattern (DAMPs). The dsDNA is a key factor in inducing inflammation and autoimmune injury [10]. The NLRP3 family is the most important member of the inflammasome family, and it can be activated by bacterial toxins, ATP, ROS, other pathogens, and dangerous signaling molecules. NLRP3 inflammasome is an important factor in anti-infective immunity and induction of inflammatory diseases [11]. In the absence of infection and intestinal inflammation, NLRP3 expression in intestinal mucosal epithelial cells and immune cells is very low. Upon activation, expression is up-regulated in intestinal immune cells. Then the assembly of activated protease caspase-1 promotes the cleavage and maturation of IL-1β and IL-18 precursors, triggering a severe inflammatory response [12]. In the study, 5-FU can up-regulate the expression of ROS from THP-1 cells, and then promote NF-κB p65 protein entry into the nucleus of the cell, and activate the NF-κB signal pathway and NLRP3 inflammasome. This ultimately leads to increased expression of various pro-inflammatory factors.

SCFAs are the major product of anaerobic fermentation, which is closely related to the regulation of the intestinal immune system. The SCFAs concentrations in the human intestinal tract range from 70 ~ 140 mmol/L and are rapidly absorbed by the blood, which mainly includes NaAc, NaPc, and NaB [13]. Besides providing energy for the proliferation of intestinal mucosal epithelial cells, SCFAs produced by fermentation in the intestinal tract can regulate the composition of human intestinal flora, reduce the growth of harmful bacteria, and prevent intestinal dysfunction [14]. Even more importantly, SCFAs may inhibit the release of various proinflammatory factors from immune cells to inhibit excessive intestinal inflammation, thereby reducing intestinal mucosal injury in patients with colitis [15, 16]. The three main SCFAs have been shown in previous studies to be able to reduce the release of pro-inflammatory factors from immune cells and inhibit inflammation, and to promote the expression of the anti-inflammation factor IL-10. In addition, NaAc and NaB can also play anti-inflammatory roles by inhibiting the activation of the NF-κB pathway [17], and our results support this conclusion. According to our results, the three main SCFAs could significantly suppress the expression of pro-inflammatory factor IL-6 and up-regulate the expression level of anti-inflammatory cytokine IL-10. These cytokines can significantly suppress the expression of pro-inflammatory factor IL-1β, ROS and NF-κB pathway activation and NLRP3 inflammasome.

In summary, our metabolomics results showed that 5-FU-induced THP-1 cell metabolites were disordered, and NaAc, NaPc, NaB can effectively regulate these differential metabolites across multiple metabolic routes. The biological functions of several different metabolites are described below. Sphingolipids, including ceramide, sphingosine-1-phosphate (S1P), sphingosine, sphingolipids, and glycosphingolipids, serve as basic components of organelles and membranes. Sphingolipids molecules have multiple biological functions and serve as essential components of organelles and membranes. For example, ceramide and sphingosine can effectively inhibit the growth of myeloma cells, rhabdomyosarcoma cells, renal tubular cells, and hippocampal nerve cells and induce their apoptosis [18, 19, 20], whereas S1P can inhibit the apoptotic macrophages, granule cells, and other cells [21, 22]. This study found that the three main SCFAs intervention in THP-1 cells reduces sphingosine generation. It is likely that SCFAs are obtained by influencing the sphingolipid metabolism, thereby promoting cell growth and reducing apoptosis.

Amino acids are important nutrients for immune cells and are the basic building blocks of human immune system. Multiple amino acids such as glutamate, arginine, tryptophan, leucine, methionine, and cysteine exert activation, differentiation, and function in T cells [23]. The enhancement of macrophage function by glutamine mainly promotes
antigen presentation, phagocytosis, and cytokine secretion [24], and glutamine supplementation reduces pro-inflammatory IL-6 and IL-8 production in lymphocytes and epithelial cells, and enhances the expression of anti-inflammatory IL-10 [25]. In the present study, NaAc can significantly up-regulate glutamate production and promote amino acid metabolism. It suggested that the mechanism by which NaAc ameliorates improves 5-FU-induced inflammation might be related to the regulation of amino acid metabolism.

In signal transduction, glycerophospholipids function as precursors for lipid mediators. In the cell membrane, phosphatidylcholine (PC) is the main glycerophospholipid. Phosphatidylcholine, a major constituent of the lipid bilayer structure of the cell membrane, plays a significant role in cell membrane fusion, pinocytosis, and membrane function. The gut microbiota was found to be able to regulate the metabolism of phosphatidylcholine by SCFAs. Acetate and propionate are mainly metabolized in the liver, and the substrates for gluconeogenesis serve as an energy source, and are involved in the fatty acid synthesis [26]. Kindta’s results confirmed that intestinal colonization of SCFA-producing bacteria such as Bacteroidetes could promote the synthesis of long-chain fatty acids (C16 and C18) and their precursors of phosphatidylcholine in the liver. In this study, the three main SCFAs on THP-1 cells can increase phosphatidylcholine (PC) production, indicating that the mechanisms of anti-inflammation induced by 5-FU may be related to the regulation of glycerol-phospholipid metabolism.

Conclusions

In conclusion, 5-FU can up-regulate the expression of ROS in THP-1 cells, activate NLRP3 inflammasome and the NF-κB pathway, and finally induce and aggravate inflammation; the three main SCFAs can inhibit the inflammation in THP-1 cells, and play a therapeutic role through amino acid metabolism, glycerol-phospholipid metabolism, and sphingolipid metabolism (Fig. 16). There are still shortcomings in this study. The three main SCFAs can inhibit the inflammation of macrophages, but the inhibitory effect of the mixtures of the three main SCFAs in different proportions remains to be further studied.

Declarations

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Author contributions Yanyan Zhang: Experimental design; Methodology, Validation, Data analysis, Investigation, Writing-Original Draft, Writing-Review & Editing; Liang Wu: Conceptualization, Methodology, Validation, Data analysis, Investigation, Writing-Review & Editing; Changshui Yang: Investigation, Data Curation, Funding acquisition; Haoyu Mao: Investigation, Data analysis; Chengyin Wang: Resources, Writing-Review & Editing, Supervision, Funding acquisition. All authors read and approved the final manuscript.

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Competing interests The authors declare that there are no conflicts of interest.

References


**Figures**

**Figure 1**

Detection of ROS expression in THP-1 cells by flow cytometry. The three main SCFAs (NaAc, NaPc, NaB) can significantly inhibit the ROS expression induced by 5-FU. Each experiment was repeated at least three times. Data were expressed as...
Effects of three main SCFAs (NaAc, NaPc, NaB) on NF-κB p65 expressions. Western blotting assay: the cells were pretreated with 100 μmol/L of SCFAs (NaAc, NaPc, NaB) and then stimulated with 5-FU (2.5 mmol/L). The expressions of NF-κB p65 in cell nuclear and cytoplasmic lysates were determined by Western blotting. Each experiment was repeated at least three times. Data were expressed as the mean ± SEM. \(^{a}P < 0.05\), vs. the NC group; \(^{b}P < 0.05\), vs. the 5-FU group.
The mRNA expressions of NLRP3, Caspase-1, IL-1β, IL-6 and IL-10 were determined by qRT-PCR. The mRNA expressions of NLRP3, Caspase-1, IL-1β and IL-6 were significantly increased when treated by 5-FU, and the three main SCFAs (NaAc, NaPc, NaB) can inhibit these factors expressions induced by 5-FU. The mRNA expression of IL-10 was increased when treated with NaAc, NaPc and NaB. Each experiment was repeated at least three times. Data were expressed as the mean ± SEM. \(^{a} P < 0.05\), vs. the NC group; \(^{b} P < 0.05\), vs. the 5-FU group.
Figure 4

PCA score plot in the positive ion mode analysis the effects of SCFAs on cellular metabolites.
Figure 5

OPLS-DA score plot in the positive ion mode analysis the effects of SCFAs on cellular metabolites.
Figure 6

PCA score plot analysis the NC and 5-FU group.
Figure 7

PCA score plot analysis the NaAc and 5-FU group.
Figure 8

PCA score plot analysis the NaPc and 5-FU group.
Figure 9

PCA score plot analysis the NaB and 5-FU group.
Figure 10

OPLS-DA score plot of the NC and 5-FU group.
Figure 11

OPLS-DA score plot of the NaAc and 5-FU group.
Figure 12

OPLS-DA score plot of the NaPc and 5-FU group.
Figure 13
OPLS-DA score plot of the NaB and 5-FU group.
Figure 14

Heat maps of differential metabolites from cells. Rows: metabolites; Columns: samples. On the top is the cluster of samples, and on the left is the cluster of metabolites. Red means the metabolites was expressed at a higher level, and blue means the metabolites was expressed at a lower level.
Figure 15

Main metabolic pathway impact analysis. The smaller the P value, the more significant the enrichment. The magnitude of the enrichment factor indicates the reliability of significance.
Figure 16

Mechanism of intestinal SCFAs inhibiting 5-FU-induced mucositis.