**An inulin-type fructan CP-A from *Codonopsis pilosula* alleviated** **5-fluorouracil-induced intestinal mucositis via** **E****RK/MLCK/MLC2 pathway and regulation of gut microbiota**

Jiangtao Zhou 1, 2, 3, #, Deyun Li 1, #,Jiajing Wang 1, Zhuoyang Cheng 1, 2, 3, Changjian Wang 1, Xuepeng Zhang 1, Xiexin Xu 1, Jianping Gao 1, 2, 3, \*

1. School of Pharmacy, Shanxi Medical University, Taiyuan 030001, China

2. Medicinal Basic Research Innovation Center of Chronic Kidney Disease, Ministry of Education, Shanxi Medical University, Taiyuan 030001, China

3. Shanxi Provincial Key Laboratory of Drug Synthesis and Novel Pharmaceutical Preparation Technology, Shanxi Medical University, Taiyuan 030001, China

\*Corresponding author: Jianping Gao (School of Pharmaceutical Science, Shanxi Medical University, 55 Wenhua Street, Yuci District, Jinzhong 030600, Shanxi, P. R. China)

E-mail: [**jpgao123@163.com**](mailto:jpgao123@163.com)

# These authors contributed equally to this work

**Table S1** Sequences of the primers used in the RT-PCR

|  |  |  |
| --- | --- | --- |
| Gene | Primer | Gene sequence (5’-3’) |
| IL-4 | Forward | ACA AGG AAC ACC ACG GAG AAC G |
| Reverse | TCT TCA AGC ACG GAG GTA CAT CAC |
| IL-6 | Forward | GCC TTC TTG GGA CTG ATG TTG TTG |
| Reverse | GTC TGT TGT GGG TGG TAT CCT CTG |
| IL-10 | Forward | AAG GCA GTG GAG CAG GTG AAG |
| Reverse | TGA GTA TCA CGT AGG CTT CTA TGC |
| TNF-α | Forward | CCG AGA TGT GGA ACT GGC AGA G |
| Reverse | CCA CGA GCA GGA ATG AGA AGA GG |
| ERK1/2 | Forward | GTT CCC AAA CGC TGA CTC CAA AG |
| Reverse | GCC AGA GCC TGT TCA ACT TCA ATC |
| MLCK | Forward | TTC AAG ATG GTG GTG GCT GTG G |
| Reverse | TGC TTG CTC CTT GTT CTC CTC AG |

**Table S2** Relative abundance of the most representative phyla in experimental rats

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Phylum | Control | 5-FU | BTC | CP-A L | CP-A M | CP-A H | Variation (5-FU) |
|  | Relative abundance (%) | | | | | |
| Firmicutes | 89.49±2.13\*\* | 62.86±12.86 | 80.66±3.20\* | 85.25±4.69\* | 86.32±2.08\*\* | 89.90±2.01\*\* | ¯ |
| Proteobacteria | 2.00±0.14\*\* | 27.03±11.78 | 7.58±3.80\* | 6.71±3.02\* | 4.50±1.14\*\* | 1.67±0.21\*\* | ­↑ |
| Bacteroidetes | 6.97±1.74 | 6.54±2.54 | 9.95±2.23 | 3.24±1.29 | 5.26±2.09 | 6.63±1.83 | ­↑ |
| Actinobacteria | 0.67±0.17 | 2.57±0.94 | 1.01±0.65 | 3.93±1.34 | 2.64±0.19 | 1.15±0.60 | ­↑ |
| Tenericutes | 0.16±0.10 | 0.22±0.19 | 0.06±0.01 | 0.29±0.11 | 0.41±0.23 | 0.12±0.07 | ­↑ |
| Cyanobacteria | 0.17±0.02 | 0.16±0.02 | 0.15±0.01 | 0.16±0.02 | 0.18±0.03 | 0.11±0.01 | ¯ |
| Verrucomicrobia | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 | 0.03±0.02 | 0.06±0.02 | 0.01±0.01 | ¯ |
| TM7 | 0.01±0.01 | 0.00±0.00 | 0.00±0.00 | 0.07±0.06 | 0.02±0.01 | 0.01±0.00 | ¯ |
| Elusimicrobia | 0.01±0.01 | 0.00±0.00 | 0.00±0.00 | 0.07±0.06\* | 0.02±0.01 | 0.01±0.00 | ¯ |

Values were represented the mean±SEM.

Kruskal-Wallis test was applied for statistics and \**p* < 0.05 and \*\**p* *<* 0.01 versus 5-FU group (n = 6).

**Table S3** Relative abundance of the most representative classes in experimental rats

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phylum | Class | Control | 5-FU | BTC | CP-A L | CP-A M | CP-A H | Variation (5-FU) |
| Relative abundance (%) | | | | | |
| Firmicutes | Clostridia | 35.97±3.48 | 43.28±6.54 | 27.71±8.12 | 44.58±9.58 | 46.50±9.99 | 24.43±2.61 | ­↑ |
|  | Bacilli | 50.23±5.06\*\* | 13.38±5.85 | 50.28±10.24\*\* | 37.41±12.35\* | 30.32±7.72 | 61.39±4.20\*\* | ¯ |
|  | Erysipelotrichi | 2.86±1.12 | 5.97±1.91 | 2.39±0.85 | 3.01±1.28 | 9.23±2.67 | 3.81±2.52 | ­↑ |
| Actinobacteria | Coriobacteriia | 0.31±0.04\* | 1.99±0.89 | 0.89±0.66 | 1.18±0.53 | 0.85±0.19 | 0.57±0.29 | ­↑ |
|  | Actinobacteria | 0.35±0.15 | 0.57±0.49 | 0.12±0.03 | 2.75±1.45\* | 1.79±0.22 | 0.57±0.33 | ­↑ |
| Proteobacteria | Alphaproteobacteria | 0.43±0.08\*\* | 0.22±0.02 | 0.24±0.02 | 0.28±0.03 | 0.32±0.04 | 0.21±0.01 | ¯ |
|  | Betaproteobacteria | 0.42±0.04 | 0.25±0.03 | 0.28±0.03 | 0.52±0.21 | 0.37±0.06 | 0.18±0.02 | ¯ |
|  | Gammaproteobacteria | 0.59±0.08\*\* | 25.44±11.94 | 6.03±3.42\* | 1.95±0.63\*\* | 2.47±1.37\*\* | 0.74±0.14\*\* | ­↑ |
|  | Deltaproteobacteria | 0.53±0.14 | 0.99±0.28 | 0.95±0.33 | 3.92±2.44 | 1.06±0.33 | 0.47±0.14 | ­↑ |
| Bacteroidetes | Bacteroidia | 6.96±1.74 | 6.53±2.54 | 9.95±2.23 | 3.23±1.29 | 5.25±2.09 | 6.60±1.83 | ¯ |

Values were represented the mean±SEM.

Kruskal-Wallis test was applied for statistics and \**p* < 0.05 and \*\**p* *<* 0.01 versus 5-FU group (n = 6).

**Table S4** Relative abundance of the most representative orders in experimental rats

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phylum | Order | Control | 5-FU | BTC | CP-A L | CP-A M | CP-A H | Variation (5-FU) |
| Relative abundance (%) | | | | | |
| Firmicutes | Clostridiales | 35.97±3.48 | 43.27±6.54 | 27.70±8.12 | 44.58±9.58 | 46.49±9.99 | 24.43±2.61 | ­↑ |
|  | Lactobacillales | 50.07±5.08\*\* | 12.96±5.68 | 50.22±10.23\*\* | 37.23±12.36\* | 29.97±7.64 | 60.60±4.82\*\* | ¯ |
|  | Erysipelotrichales | 2.86±1.12 | 5.97±1.91 | 2.39±0.85 | 3.01±1.28 | 9.23±2.67 | 3.81±2.52 | ­↑ |
| Proteobacteria | Desulfovibrionales | 0.52±0.14 | 0.99±0.28 | 0.95±0.33 | 3.92±2.44 | 1.05±0.33 | 0.47±0.14 | ­↑ |
|  | Burkholderiales | 0.42±0.04 | 0.25±0.03 | 0.28±0.03 | 0.51±0.21 | 0.37±0.06 | 0.18±0.02 | ¯ |
|  | Enterobacteriales | 0.27±0.08\*\* | 25.11±11.93 | 5.79±3.43\* | 1.33±0.69\*\* | 2.20±1.38\*\* | 0.45±0.16\*\* | ­↑ |
|  | Pseudomonadales | 0.28±0.02 | 0.23±0.03 | 0.20±0.03 | 0.57±0.33 | 0.21±0.04 | 0.22±0.04 | ¯ |
| Actinobacteria | Coriobacteriales | 0.31±0.04 | 1.99±0.89 | 0.89±0.66 | 1.18±0.53 | 0.85±0.19 | 0.57±0.29 | ­↑ |
|  | Actinomycetales | 0.32±0.16 | 0.50±0.45 | 0.07±0.01 | 2.71±1.45\* | 1.09±0.36 | 0.38±0.30 | ­↑ |
| Bacteroidetes | Bacteroidales | 6.96±1.74 | 6.53±2.54 | 9.95±2.23 | 3.23±1.29 | 5.25±2.09 | 6.60±1.83 | ¯ |

Values were represented the mean±SEM.

Kruskal-Wallis test was applied for statistics and \*P<0.05 and \*\**p* *<* 0.01 versus 5-FU group (n = 6).

**Table S5** Relative abundance of the most representative families in experimental rats

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phylum | Family | Control | 5-FU | BTC | CP-A L | CP-A M | CP-A H | Variation (5-FU) |
| Relative abundance (%) | | | | | |
| Firmicutes | Lactobacillaceae | 49.29±5.19\*\* | 11.60±5.33 | 49.90±10.17\*\* | 33.05±13.36\* | 27.86±7.54 | 59.44±5.59\*\* | ¯ |
|  | S24-7 | 4.82±0.88 | 3.43±1.25 | 7.23±1.78\* | 1.97±0.53 | 3.77±1.26 | 5.45±1.54 | ¯ |
|  | Peptostreptococcaceae | 2.84±0.97 | 4.52±2.36 | 2.32±0.97 | 5.73±1.55 | 3.70±1.09 | 2.39±0.63 | ­↑ |
|  | Clostridiaceae | 1.04±0.28 | 1.29±0.56 | 0.36±0.06 | 0.74±0.20 | 0.64±0.14 | 0.53±0.13 | ­↑ |
|  | Erysipelotrichaceae | 2.86±1.12 | 5.97±1.91 | 2.39±0.85 | 3.01±1.28 | 9.23±2.67 | 3.81±2.52 | ­↑ |
|  | Ruminococcaceae | 16.33±2.24 | 8.27±3.28 | 7.56±2.20 | 16.81±4.27 | 16.60±7.39 | 7.40±1.77 | ¯ |
|  | Lachnospiraceae | 8.78±2.44\* | 23.20±6.25 | 11.74±5.17 | 11.55±4.13 | 17.21±5.44 | 9.08±1.16\* | ­↑ |
| Proteobacteria | Enterobacteriaceae | 0.27±0.08\*\* | 25.11±11.93 | 5.79±3.43\* | 1.33±0.69\*\* | 2.20±1.38\*\* | 0.45±0.16\*\* | ­↑ |
|  | Desulfovibrionaceae | 0.52±0.14 | 0.99±0.28 | 0.94±0.33 | 3.92±2.44 | 1.05±0.33 | 0.47±0.14 | ­↑ |
| Actinobacteria | Coriobacteriaceae | 0.31±0.04\*\* | 1.99±0.89 | 0.89±0.66 | 1.18±0.53 | 0.85±0.19 | 0.57±0.29 | ­↑ |

Values were represented the mean±SEM.

Kruskal-Wallis test was applied for statistics and \**p* < 0.05 and \*\**p* *<* 0.01 versus 5-FU group (n = 6).

**Table S6** Relative abundance of the most representative families in experimental rats

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phylum | Genus | Control | 5-FU | BTC | CP-A L | CP-A M | CP-A H | Variation (5-FU) |
| Relative abundance (%) | | | | | |
| Firmicutes | Lactobacillus | 48.89±5.22\*\* | 11.34±5.21 | 49.42±10.08\*\* | 32.92±13.33 | 27.66±7.49 | 59.21±5.60\*\* | ¯ |
|  | Oscillospira | 0.60±0.22 | 2.45±1.71 | 0.77±0.40 | 3.21±1.37 | 1.17±0.25 | 0.84±0.47 | ­↑ |
|  | Blautia | 4.19±1.69 | 10.69±5.44 | 3.06±0.89\* | 1.84±0.58\* | 4.68±1.70 | 3.72±0.97 | ­↑ |
|  | Dorea | 0.42±0.20 | 2.35±0.79 | 2.25±1.77 | 1.53±1.05 | 0.54±0.26 | 0.29±0.08 | ­↑ |
|  | Ruminococcaceae\_Ruminococcus | 1.79±0.73\* | 0.39±0.16 | 1.22±0.48 | 0.69±0.25 | 0.77±0.18 | 0.87±0.26 | ¯ |
|  | Subdoligranulum | 2.30±1.93 | 0.44±0.23 | 1.08±1.00 | 1.71±1.01 | 1.54±1.18 | 0.62±0.32 | ¯ |
| Bacteroidetes | Bacteroides | 0.10±0.03\* | 1.88±1.23 | 0.78±0.27 | 0.47±0.36 | 0.86±0.62 | 0.25±0.03 | ­↑ |
| Proteobacteria | Shigella | 0.16±0.04\*\* | 24.25±11.64 | 5.45±3.33\* | 1.13±0.67\*\* | 2.00±1.34\*\* | 0.36±0.16\*\* | ­↑ |
|  | Desulfovibrio | 0.45±0.13 | 0.79±0.22 | 0.67±0.19 | 2.33±1.17\* | 0.91±0.29 | 0.38±0.12 | ­↑ |
| Tenericutes | Allobaculum | 2.21±1.14 | 5.60±1.82 | 1.59±0.86 | 2.36±1.36 | 8.54±2.80 | 2.74±2.64 | ­↑ |

Values were represented the mean±SEM.

Kruskal-Wallis test was applied for statistics and \**p* < 0.05 and \*\**p* *<* 0.01 versus 5-FU group (n = 6).