Clinical effects of ursodeoxycholic acid on patients with ulcerative colitis may improve via the regulation of IL-23-IL-17 axis and the changes of the proportion of intestinal microflora

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Abstract

Background: We aimed to evaluate the therapeutic effect of additional ursodeoxycholic acid (UDCA) with mesalazine, compared to mesalazine alone in patients with ulcerative colitis (UC). The mechanism was evaluated by monitoring the changes of IL-23-IL-17 axis and the intestinal microflora.

Methods: In this prospective, single center study, patients with UC were randomly assigned to the Mesalazine group ($n=20$) or the UDCA + Mesalazine group ($n=20$). Mayo score and Inflammatory Bowel Disease Questionnaire (IBDQ), and fecal samples for 16S rRNA sequencing and blood samples for IL-23 and IL-17 ELISA were collected for analysis.

Results: Mayo scores and IBDQ score of the UDCA + Mesalazine group were significantly better than those of the Mesalazine group ($P = 0.015$ and $P < 0.001$, respectively). At post-treatment week 4, IL-23 and IL-17 levels were significantly lower in the UDCA + Mesalazine group compared to those in the Mesalazine group (both $P < 0.038$). In patients with UC after treatment, *Firmicutes* in the UDCA + Mesalazine group was higher than those in the Mesalazine group ($P < 0.001$). The UDCA + Mesalazine group showed lower percentage of *Proteobacteria* compared to those in the Mesalazine group ($P < 0.001$).

Conclusion: Additional UDCA could provide better therapeutic effects than mesalazine alone, possibly due to the change of IL-23 and IL-17 and the proportional distribution of intestinal microflora.

Keywords: IL-23, IL-17, mesalazine, microflora, ulcerative colitis, ursodeoxycholic acid

INTRODUCTION

Ulcerative colitis (UC) is a chronic non-specific intestinal inflammatory disease, with the main clinical manifestations of UC including diarrhea, mucopurulent hematochezia, and abdominal pain. Although the etiology remains unclear, the pathological changes of intestine are wide and frequently recur, indicating the long disease duration and the probable life-long effect on the quality of life.[1] Some of the conventional drugs used to treat UC are

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ineffective, so many new biologics targeting the immune mechanism of UC were developed, based on the findings of the intestinal mucosal immune system dysfunction and pathological T cell responses that were considered as the direct cause of UC.[2-4] In particular, IL-23/IL-17 axis can regulate pro-inflammatory factors and is positively correlated with the disease activity of UC.[5-7] Although these immune-centered treatments are effective, the high prices may limit the use and side effects may not be tolerated by some patients.[8,9] Thus, the need for new therapeutic medications is of utmost importance.

Ursodeoxycholic acid (UDCA) is a secondary bile acid formed by colonic bacteria. UDCA has shown good cytoprotective, anti-inflammatory properties and therapeutic effects in a rat colitis model.[10] In liver diseases, UDCA improved the stability of microbiota in patients with hepatic insufficiency and primary biliary cholangitis.[11,12] In intestinal diseases, UDCA can prevent recurrent infection caused by *Clostridium difficile* by inhibiting the activity of other secondary bile acids.[13] In addition, UDCA has proved that it can reverse the physiological imbalance within hepatocytes caused by apoptosis.[14]

Previous studies indicated that compared to patients with UC in remission, the ecological imbalance of the intestinal microflora is more pronounced in active phase patients,[15] and further aggravates the intestinal mucosa and the systemic inflammatory response.[16-19] UDCA as a phospholipase A2 inhibitor, probably can improve the symptoms of UC and have therapeutic effect on UC through restoration of the intestinal mucus phosphatidylcholine content by reducing the impacts from ectophospholipase-containing intestinal microflora, which is considered as one of the important mechanism of pathogenesis of UC.[20] UDCA derivative, UDCA-LPE has shown to improve the mucosal inflammation in a genetic mouse model of UC via changing the pattern of colonic microbiota distribution.[21]

In the present study, we examined the therapeutic effects of additional UDCA, compared to mesalazine only. For investigating the mechanism of UDCA, we also compared the change of IL-23/IL-17 axis and the distribution of intestinal microflora in patients with UC between the UDCA + Mesalazine and the Mesalazine groups.[10-14,22]

METHODS

Participants
The study protocol was approved by the institutional review board of Fuzhou General Hospital (IRB No.

2016-020), and was registered in Chinese Clinical Trial Registry (ChiCTR200038316). All participants signed a written consent. Newly diagnosed patients with UC were enrolled at the Department of Gastroenterology and Outpatient Clinic of Fuzhou General Hospital. Diagnoses were established based on the consensus guideline published by the British Society of Gastroenterology.[23] Disease severity was defined according to the improved Truelove and Witts disease Degree classification combined with endoscopy.[24,25] Inclusion criteria were: (1) age from 18 to 75 years; (2) disease severity-mild to moderate. No UC treatment was prescribed before baseline for all patients. Patients were randomly assigned to the Mesalazine group and the UDCA + Mesalazine group, *n* = 20 in each group. Mesalazine (Heilongjiang Timehome Pharmaceutical Co., Ltd , China) was prescribed as 1 g, QID based on the British Gastroenterology Society guidelines, and UDCA (Daewoong Pharmaceutical Co., Ltd, Seoul, South Korea) was 200 mg, BID used the suggestion for reflux esophagitis treatment listed in the package insert as reference. Twenty healthy volunteers were also included as an individual group. Patients with underlying disease had to be controlled well by maintaining appropriated medications. Immunosuppressive agents, steroid hormones, non-steroidal anti-inflammatory drugs and other types of aminosalicylic acid were prohibited during the study period. Antibiotics and probiotics were prohibited for 3 months before UC treatment. The demographical and clinical information, fecal and blood samples were collected at baseline, post-treatment 1 week and 4 weeks, for further analysis.

Evaluations of clinical status and quality of life of patients with UC
Colonoscopy examination was performed at baseline, post-treatment 1 and 4 weeks, using a standard colonoscope (Olympus, Tokyo, Japan). Mayo score[25] was evaluated at baseline and post-treatment 4 weeks,[25] Inflammatory Bowel Disease Questionnaire (IBDQ)[26] were evaluated at baseline and post-treatment 4 weeks.[26]

Measurement of IL-17 and IL-23
Serum samples were collected after overnight starvation for the measurement of IL-17 and IL-23 using ELISA kit (Shanghai Xitang Biotechnology Co., Ltd., Shanghai, China) according to the manufacturer's instruction. The absorbance was measured as the optical density at 450 nm using a microplate reader to calculate the concentrations of IL-17 or IL-23 in the samples.

Evaluation of intestinal microflora
Fecal samples were collected at baseline and post-treatment 1 and 4 weeks, stored at -80°C until analysis was performed.

Bacterial 16S ribosomal RNA (rRNA) sequencing was performed to identify the intestinal microflora after constructing a sequencing library using MetaVx Library Construction Kit (GENEWIZ, Inc., South Plainfield, NJ, USA). The quality of the library was evaluated by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), and next generation sequencing was performed using Illumina MiSeq sequencer (Illumina, San Diego, CA, USA) and aligned with SILVA 128 rRNA databases.

Statistical analysis

Because of the small sample size, all the continuous data were presented by median and interquartile range (IQR), and the corresponding analyses were performed by non-parametric tests. For comparison between the two treatment groups, Mann-Whitney test was performed. For the comparisons of two repeated measurements within group, Wilcoxon signed ranks test was performed. Bonferroni correction was applied in the above analyses to avoid type I error of multiple comparisons. Categorical data (gender) were presented by count and percentage, and the association of gender and treatments were tested with Fisher's exact test. All analyses were performed with IBM SPSS Statistic 25.0. A two-sided *P* value less than 0.05 indicated statistical significance.

RESULTS

Patient characteristics

The Mesalazine group included 12 males and 8 females, with ages of 22 to 74 years (median of 50.0 years). The UDCA + Mesalazine group comprised 10 male and 10 female patients, with ages of 22 to 64 years (median of 50.5 years). The baseline characteristics including age, gender, Montreal classification, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) levels were all comparable between the two treatment groups (*P* > 0.05), [Table 1]. No drug-related adverse event was found during the study period in both treatment groups.

Mayo score

To evaluate the efficacy of treatments for patients with UC, Mayo scores were calculated before and after treatment,

based on gastrointestinal symptoms, endoscopic findings, and physician evaluation. Mayo scores at baseline of the two treatment groups were comparable. After treatment, Mayo scores were significantly decreased in both groups. In patients of the Mesalazine group, Mayo scores were significantly decreased from medians of 7.0 at baseline to 5.5 and 3.5 at post-treatment weeks 1 and 4, respectively. Mayo score in patients of the UDCA + Mesalazine group were significantly decreased from medians of 8.5 at baseline to 2.0 at post-treatment week 4, and also significantly decreased from medians of 4.0 at post-treatment week 1 to 2.0 at post-treatment week 4. For the comparisons between the two treatment groups, at post-treatment weeks 1 and 4, Mayo scores in patients of the UDCA + Mesalazine group were significantly lower than those in the Mesalazine group (both *P* = 0.015, medians of 4.0 vs. 5.5 at post-treatment week 1 and medians of 2.0 vs. 3.5 at post-treatment week 4) [Table 2]. Endoscopic Mayo sub-scores were comparable between the two groups at baseline. In the Mesalazine group, there was no significant change of endoscopic Mayo sub-scores from baseline to post-treatment week 4. In the UDCA + Mesalazine group, endoscopic Mayo sub-score at post-treatment week 4 was significantly lower than that at baseline and post-treatment 1 week (medians of 1.0 at post-treatment week 4 vs. 2.0 and 2.0 at baseline and post-treatment week 1, both *P* < 0.001). Endoscopic Mayo sub-scores of the UDCA + Mesalazine group were significantly lower than those in the Mesalazine group at post-treatment weeks 4 (medians of 1.0 vs. 2.0, *P* = 0.017) [Table 2].

IBDQ score

To better clarify the impact of treatment on quality of life in patients with UC, IBDQ scores before and after treatment were measured. At baseline, the scores of social ability, emotional ability, systemic symptoms, intestinal symptoms, and total score were comparable between the two treatment groups. At post-treatment week 4, the results illustrated that the scores of social ability (medians of 30.0 vs. 25.0, *P* = 0.014), emotional ability (medians of 64.5 vs. 59.0, *P* = 0.009), and systemic

Table 1: Comparisons for the characteristics of the two treatment groups in patients with UC

		Patients with UC		Healthy control group (*n*=20)
		Mesalazine (*n*=20)	UDCA+Mesalazine (*n*=20)	
Age (years)		50.0 (42.0, 57.5)	50.5 (40.5, 59.0)	42.5 (30.0, 50.5)
Gender	Male	12 (60.0%)	10 (50.0%)	9 (45.0%)
	Female	8 (40.0%)	10 (50.0%)	11 (55.0%)
Montreal classification	E1 (proctitis)	13 (65.0%)	13 (65.0%)	NA
	E2 (left-sided colitis)	7 (35.0%)	7 (35.0%)	NA
	E3 (pancolitis)	0	0	NA
Baseline CRP (mg/mL)		8.5 (6.9, 12.2)	9.0 (8.0, 12.2)	NA
Baseline ESR (mm/hour)		24.6 (19.3, 27.8)	23.8 (17.2, 27.1)	NA

Continuous data are presented by medians and IQR. Gender is presented by count and percentage. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; UC, ulcerative colitis. NA: not available

ability (medians of 29.0 vs. 25.5, $P = 0.012$) were all significantly higher in patients of the UDCA + Mesalazine group, compared to those in the Mesalazine group. Meanwhile, the total IBDQ scores at post-treatment week 4 were significantly higher in the UDCA + Mesalazine group compared to those in the Mesalazine group (182.5 vs. 163,5, $P < 0.001$) [Table 2].

Serum IL-23 and IL-17 levels

At baseline, serum IL-23 and IL-17 levels had no significant difference between the two treatment groups. Serum IL-23 and IL-17 levels were both decreased after treatment. In the Mesalazine group, IL-23 levels at post-treatment week 4 were significantly lower than those at post-treatment 1 week (medians of 669.2 pg/mL vs. 891.7 pg/mL). In the UDCA + Mesalazine group, IL-23 levels at post-treatment week 4 were significantly lower than those at baseline (medians of 437.7 pg/mL vs. 990.4 pg/mL). IL-17 levels of both treatment groups were significantly decreased. From baseline to post-treatment week 1, in the Mesalazine group, medians decreased from 6.26 pg/mL to 4.53 pg/mL; and in the UDCA + Mesalazine group, from 6.60 pg/mL to 3.66 pg/mL. IL-17 levels were further decreased from post-treatment week 1 to post-treatment week 4, In the Mesalazine group medians decreased from 4.53 pg/mL to 2.94 pg/mL, and in the UDCA + Mesalazine group, from 3.66 pg/mL to 2.67 pg/mL (all $P < 0.05$). At post-treatment week 4, IL-23 and IL-17 levels were significantly lower in patients of the UDCA + Mesalazine group, compared to those in patients of the Mesalazine group, with $P < 0.001$ for IL-23 and $P = 0.038$ for IL-17 [Table 3].

Intestinal microflora before treatment

The Healthy individual group included 11 males and 9 females, with median age of 42.5 years [Table 1]. In the Healthy individual group, the most frequent intestinal microflora was *Firmicutes*, followed by *Bacteroidetes* and *Bacteroides,* with medians of 53.99%, 24.17%, and 5.03%, respectively. In patients with UC before treatment, the most frequent intestinal microflora was *Bacteroidetes*, followed by *Bacteroides* and *Proteobacteria,* with medians of 57.31%, 33.82%, and 25.78%, respectively. Percentages of *Bacteroidetes*, *Bacteroides*, *Proteobacteria,* and *Fusobacteria* were significantly higher in patients with UC, compared to those in the healthy individuals, and the percentages of *Firmicutes* and *F. prausnitzii* were significantly lower in patients with UC, compared to those in the healthy individuals. The differences in the percentages of *Escherichia-Shigella*, *Actinobacteria*, *prevotella_9*, *Fusobacterium,* and *Roseburia* between the healthy individuals and patients with UC did not obtain any statistical significance [Figure 1].

Treatment effects on intestinal microflora in patients with UC
Bacteroidetes

Both treatment groups showed significant decreases at post-treatment week 4 compared to those at baseline and post-treatment week 1. In the Mesalazine group, medians of percentages were decreased from 57.9% at baseline to 44.9% and 22.0% at post-treatment 1 and 4 weeks, respectively (both $P < 0.05$). In the UDCA + Mesalazine group, medians of percentages were decreased from 56.7%

Table 2: Change trends of Mayo and IBDQ scores from baseline to post-treatment week 4 in patients with UC of the two treatment groups

		Patients with UC		P
		Mesalazine (*n*=20)	UDCA+Mesalazine (*n*=20)	
Mayo score	Baseline	7.00 (5.00, 9.00)	8.50 (7.00, 10.50)	0.183
	Post-treatment week 1	5.50 (4.00, 9.00)[a]	4.00 (2.50, 6.00)	0.015*
	Post-treatment week 4	3.50 (2.00, 6.50)[a]	2.00 (1.00, 3.50)[ab]	0.045*
Endoscopic Mayo	Baseline	2.0 (2.0, 2.0)	2.0 (2.0, 2.0)	>0.999
sub-score	Post-treatment week 1	2.0 (1.0, 3.0)	2.0 (2.0, 2.0)	0.650
	Post-treatment week 4	2.0 (1.0, 2.0)	1.0 (1.0, 2.0)[ab]	0.017*
IBDQ score				
Total	Baseline	134.0 (122.5, 140.0)	142.0 (132.5, 151.0)	0.064
	Post-treatment week 4	163.5 (149.5, 167.5)[a]	182.5 (173.5, 191.5)[a]	<0.001*
Social ability	Baseline	19.5 (16.0, 22.0)	22.5 (19.5, 26.0)	0.072
	Post-treatment week 4	25.0 (18.0, 30.0)[a]	30.0 (26.0, 32.0)[a]	0.014*
Emotional ability	Baseline	50.5 (40.5, 60.0)	52.0 (44.0, 62.0)	0.774
	Post-treatment week 4	59.0 (52.0, 62.0)[a]	64.5 (59.0, 76.5)[a]	0.009*
Systemic symptoms	Baseline	21.5 (19.5, 22.5)	23.5 (20.0, 27.0)	0.083
	Post-treatment week 4	25.5 (23.5, 26.5)[a]	29.0 (25.5, 32.0)[a]	0.012*
Intestinal symptoms	Baseline	43.5 (38.0, 47.0)	48.0 (36.5, 52.0)	0.310
	Post-treatment week 4	54.5 (46.5, 59.0)[a]	57.5 (51.5, 61.5)[a]	0.180

Data are presented by medians and IQR.

*P<0.05 indicates a statistically significant difference between the two treatment groups. [a]indicates a statistically significant change compared to baseline within group. [b]indicates a statistically significant change compared to post-treatment 1 week within group. IBDQ, Inflammatory Bowel Disease Questionnaire; UC, ulcerative colitis
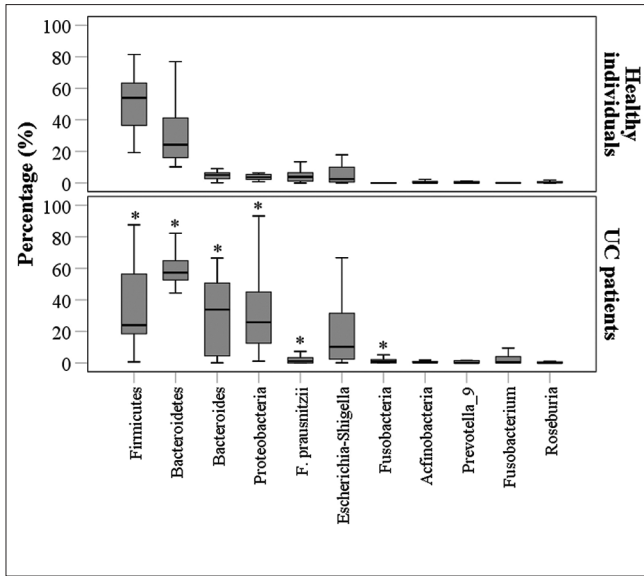
Figure 1: Comparisons of intestinal flora between patients with UC (n = 40, at baseline before treatment) and healthy individuals (n = 20). * *P* < 0.0045 (0.05/11) indicates a statistically significant difference between patients with UC and the healthy individuals by Bonferroni correction

at baseline to 55.2% and 40.8% at post-treatment 1 and 4 weeks, respectively (both *P* < 0.05). The differences of *Bacteroidetes* percentages between the two treatment groups did not obtain any statistical significance at each time point [Table 4].

Firmicutes

Percentage of *Firmicutes* in patients of the UDCA + Mesalazine group remained stable from baseline to post-treatment week 1 with medians of 40.3% and 39.7%, respectively, but were significantly increased to 61.0% at post-treatment 4 weeks, compared to baseline. In the UDCA + Mesalazine group, medians of percentages were significantly higher than those of the Mesalazine group at post-treatment week 4 (61.0% vs. 28.7%, p < 0.001).

Proteobacteria

Both treatment groups showed significant decreases at

post-treatment 1 and 4 weeks, compared to baseline. In the Mesalazine group, medians of percentages were decreased from 45.0% at baseline to 31.6% at post-treatment 1 week and 17.3% at post-treatment 4 weeks (both *P* < 0.05). In the UDCA + Mesalazine group, medians of percentages were decreased from 18.4% at baseline to 3.9% at post-treatment 1 week and 3.7% at post-treatment 4 weeks (both *P* < 0.05). The UDCA + Mesalazine group showed significantly lower percentage of *Proteobacteria* compared to the Mesalazine group at post-treatment 1 and 4 weeks (both *P* < 0.001) [Table 4].

Fusobacteria

Percentages of *Fusobacteria* in both treatment groups showed significant decreases at post-treatment 1 and 4 weeks, compared to baseline. Medians of percentages were decreased from 0.57% at baseline to 0.03% at post-treatment 1 week and 0.01% at post-treatment 4 weeks in the Mesalazine group, and from 1.01% at baseline to 0.04% at post-treatment 1 week and 0.01% at post-treatment 4 weeks in the UDCA + Mesalazine group, no other significant difference was found between the two treatment groups at each time point [Table 4].

Actinobacteria

In the UDCA + Mesalazine group, percentages of *Actinobacteria* were significantly increased at post-treatment 1 week, compared to baseline, with medians of 0.38% vs. 1.63%. Percentages of *Actinobacteria* in the UDCA + Mesalazine group were also significantly higher than those in the Mesalazine group, with medians of 1.63% vs. 0.53% at post-treatment 1 week (*P* = 0.004). At post-treatment 4 weeks, no significant difference between the two treatment groups was found [Table 4].

Bacteroides

Percentages of *Bacteroides* in the Mesalazine group were significantly decreased from medians of 27.42% at baseline to 4.16% at post-treatment 1 week, then significantly increased to a median of 28.73% at post-treatment 4 weeks. Percentages of *Bacteroides* in the UDCA + Mesalazine

Table 3: Change trends of IL-23 and IL-17 levels from baseline to post-treatment 4 weeks in patients with UC of the two treatment groups

		Patients with UC		*P*
		Mesalazine (*n*=20)	UDCA+Mesalazine (*n*=20)	
IL-23 (pg/mL)	Baseline	962.2 (631.2, 1,477.6)	990.4 (427.3, 1,450.1)	0.914
	Post-treatment 1 week	891.7 (860.6, 935.4)	651.4 (241.6, 1,107.8)	0.130
	Post-treatment 4 weeks	669.2 (635.7, 708.9)[b]	437.7 (393.8, 471.0)[a]	<0.001*
IL-17 (pg/mL)	Baseline	6.26 (4.65, 8.98)	6.60 (4.78, 7.88)	0.808
	Post-treatment 1 week	4.53 (3.78, 5.63)[a]	3.66 (2.80, 4.84)[a]	0.079
	Post-treatment 4 weeks	2.94 (2.50, 3.61)[ab]	2.67 (1.86, 2.98)[ab]	0.038*

Data are presented by medians and IQR.

*Indicates a significant difference between the two treatment groups. [s]Indicates a significant change compared to baseline level within group. [b]Indicates a significant change compared to post-treatment 1 week within group

Table 4: The treatment effects of Mesalazine and UDCA+Mesalazine on intestinal microflora in patients with UC

		Patients with UC		P
		Mesalazine (*n*=20)	UDCA+Mesalazine (*n*=20)	
Bacteroidetes	Baseline	57.9 (52.0, 63.3)	56.7 (52.7, 68.4)	0.779
	Post treatment 1 week	44.9 (25.4, 54.9)	55.2 (47.7, 60.3)	0.040
	Post treatment 4 weeks	22.0 (12.6, 39.0) [ab]	40.8 (31.2, 49.0) [ab]	0.013
Firmicutes	Baseline	21.3 (17.2, 33.7)	40.3 (22.2, 62.0)	0.026
	Post treatment 1 week	26.2 (18.7, 35.7)	39.7 (25.8, 46.7)	0.055
	Post treatment 4 weeks	28.7 (22.7, 47.8)	61.0 (43.6, 72.3) [ab]	<0.001*
Proteobacteria	Baseline	45.0 (21.9, 60.9)	18.4 (11.6, 30.5)	0.005
	Post treatment 1 week	31.6 (21.8, 44.7) [a]	3.9 (2.6, 9.3) [a]	<0.001*
	Post treatment 4 weeks	17.3 (11.6, 22.7) [ab]	3.7 (2.5, 5.1) [a]	<0.001*
Fusobacteria	Baseline	0.57 (0.00, 2.77)	1.01 (0.08, 1.98)	0.472
	Post treatment 1 week	0.03 (0.01, 0.12) [a]	0.04 (0.01, 0.11) [a]	0.752
	Post treatment 4 weeks	0.01 (0.00, 0.05) [a]	0.01 (0.00, 0.02) [a]	0.638
Actinobacteria	Baseline	0.45 (0.21, 1.43)	0.38 (0.22, 0.83)	0.351
	Post treatment 1 week	0.53 (0.17, 1.05)	1.63 (0.68, 2.87) [a]	0.004*
	Post treatment 4 weeks	0.53 (0.24, 1.13)	0.82 (0.17, 1.43)	0.516
Bacteroides	Baseline	27.42 (4.04, 50.05)	34.69 (5.25, 52.99)	0.465
	Post treatment 1 week	4.16 (1.35, 15.22) [a]	5.27 (2.01, 7.01) [a]	0.931
	Post treatment 4 weeks	28.73 (4.31, 41.94) [b]	6.22 (4.05, 22.88) [a]	0.250
Escherichia-Shigella	Baseline	10.17 (4.43, 31.36)	10.87 (0.72, 32.28)	0.952
	Post treatment 1 week	6.94 (1.79, 30.49)	2.62 (0.56, 9.66) [a]	0.104
	Post treatment 4 weeks	4.09 (2.09, 8.96) [ab]	1.06 (0.15, 4.85)	0.032
F. prausnitzii	Baseline	0.82 (0.01, 3.15)	1.10 (0.01, 3.35)	0.856
	Post treatment 1 week	2.12 (0.88, 3.75)	3.49 (2.15, 5.63) [a]	0.052
	Post treatment 4 weeks	3.96 (1.41, 4.84) [ab]	3.25 (2.13, 4.02) [a]	0.654
Prevotella_9	Baseline	0.29 (0.00, 4.37)	0.03 (0.00, 0.85)	0.406
	Post treatment 1 week	0.09 (0.00, 0.75)	0.02 (0.00, 0.14)	0.231
	Post treatment 4 weeks	2.19 (0.76, 4.69) [b]	0.01 (0.00, 0.36)	<0.001*
Roseburia	Baseline	0.01 (0.00, 0.24)	0.17 (0.00, 1.53)	0.046
	Post treatment 1 week	0.19 (0.05, 0.73)	0.00 (0.00, 0.11) [a]	<0.001*
	Post treatment 4 weeks	0.11 (0.02, 0.27)	0.00 (0.00, 0.02) [a]	<0.001*

Data are presented by medians and IQR.

* Because of Bonferroni correction, $P<0.0045$ (0.05/11) indicates a significant difference between the two treatment groups. [a]Indicates a significant change compared to baseline level within group. [b] Indicates a significant change compared to post-treatment 1 week within group

group also showed significant decreases from baseline to post-treatment 1 week, with the medians from 34.69% to 5.27%, then remained stable to post-treatment 4 weeks. No significant difference was found between the two treatment groups at each time point [Table 4].

Escherichia-Shigella

The differences between the two treatment groups did not obtain any statistical significance at each time point. Percentages of *Escherichia-Shigella* in the UDCA + Mesalazine group showed significant decreases from baseline to post-treatment 1 week, with medians from 10.87% to 2.62%. Percentage of *Escherichia-Shigella* in patients of the Mesalazine group at post-treatment 4 weeks showed significant decreases, compared to those at baseline and post-treatment 1 week, with medians of 4.09% vs. 10.17% at baseline and 6.94% at post-treatment 1 week, (both $P < 0.05$) [Table 4].

F. prausnitzii

The differences between the two treatment groups did not obtain any statistical significance at each time point. Compared to baseline, percentages of *F. prausnitzii* in the

UDCA + Mesalazine group showed significant increases at post-treatment 1 and 4 weeks, with medians of 1.10% vs. 3.49% and 3.25%, respectively. In the Mesalazine group, percentages of *F. prausnitzii* at post-treatment 4 weeks showed significant increases compared to those at baseline and post-treatment 1 week, with medians of 3.96% vs. 0.82% at baseline and 2.12% at post-treatment 1 week [Table 4].

Prevotella_9

The differences between the two treatment groups did not obtain any statistical significance at baseline and post-treatment 1 week. At post-treatment 4 weeks, percentages of *Prevotella_9* in the UDCA + Mesalazine group were significantly lower than those in the Mesalazine group, with medians of 0.01% vs. 2.19% ($P < 0.001$). Only those in the Mesalazine group showed significant increases at post-treatment 4 weeks compared to those at post-treatment 1 week [Table 4].

Roseburia

The differences between the two treatment groups did not obtain any statistical significance at baseline. At

post-treatment 1 and 4 weeks, percentages of *Roseburia* in the UDCA + Mesalazine group were significantly decreased compared to those at baseline. Significant difference between the two treatments were found at post-treatment 1 and 4 week, with medians of 0.0% vs. 0.19% and 0.0% vs. 0.11%, respectively [Table 4].

DISCUSSION

Based on the changes of Mayo and IBDQ scores, additional UDCA could provide significantly better therapeutic effects than mesalazine alone, possibly due to the changes of IL-23 and IL-17 related immune responses, because at post-treatment 4 weeks, IL-23 and IL-17 levels were significantly lower in the UDCA + Mesalazine group compared to those in the Mesalazine group. The proportional distribution of the intestinal microflora was different between patients with UC and the healthy individuals. Percentages of *Bacteroidetes*, *Bacteroides*, *Proteobacteria,* and *Fusobacteria* were significantly higher, and percentage of *Firmicutes* and *F. prausnitzii* were significantly lower in patients with UC, compared to those in the healthy individuals. In patients with UC, after treatment, the percentages of *Firmicutes* in the UDCA + Mesalazine group was significantly higher than that in the Mesalazine group, and the UDCA + Mesalazine group showed a significantly lower percentage of *Proteobacteria* compared to that in the Mesalazine group.

IL-23/IL-17 axis is one of the key pro-inflammatory signaling pathways and has been increasingly considered as the leading cause of chronic intestinal inflammation and other chronic autoimmune inflammatory diseases.[27-32] Furthermore, the level of IL-23 and IL-17 has been reported to correlate with the disease activity of UC.[6,7,33] In UC, IL-23 may induce more Th17 responses after receiving specific stimuli and increases the level of IL-17 that is secreted mainly by CD 4 effectors, further activating neutrophils and causing damag to endothelial cells and tissue, thus inducing inflammation.[7,33] One of the stimuli is the antigen from the intestinal microflora. Bacterial translocation can cause epithelial barrier dysfunction and increases intestinal permeability, thereby aggravat UC symptoms.[34-38]

In the present study, before treatment, the differences in the percentages of *Escherichia-Shigella*, *Actinobacteria*, *prevotella_9*, *Fusobacterium,* and *Roseburia* between the healthy individuals and UC patients did not obtain statistical significance. In those with significant difference at baseline, after treatment, *Bacteroidetes, Proteobacteria,* and *Bacteroides* were significantly decreased, and *Firmicutes* was increased,

which is consistent with previous studies.[39-41] Furthermore, comparing the results of the two treatment groups, the percentages of *Firmicutes* was higher and *Proteobacteria* was lower significantly in the UDCA + Mesalazine group.

Proteobacteria is one of the most abundant bacteria in the intestine, and is considered as a potential marker of intestinal microbial instability.[42] Studies focusing on the changes in the proportions of the intestinal microflora during IBD have shown that *Proteobacteria* usually proliferate more under these conditions of IBD, compared to others.[43,44] During intestinal inflammation, epithelial cells reduce β-oxidation due to an increase in oxygen supply, which is thought to promote stunting and is associated with the proliferation of *Proteobacteria*.[36,41,43-47] It has been known that in patients with UC and UC mouse models, the abundance of *Firmicutes* was decreased, compared to the healthy individuals,[40] and significantly increased after *Akkermansia muciniphia* treatment, accompanied with the amelioration of mucosal inflammation and reduction of weight loss, colon length shortening and histopathology scores in a UC mouse model.[48]

The possible mechanism of UDCA on the changes of the proportions of *Proteobacteria* and *Firmicutes* needs more molecular examinations to clarify in the future. Speculating from previous studies of UDCA, phosphatidylcholine contents in the patients with UC can be restored by UDCA through inhibiting activity of phospholipase.[21] The distribution of intestinal commensal microbiota is changed probably due to the different phospholipase activity borne by each bacterial strain.[36,49-51] The study has some limitations. The fecal microbiota cannot fully reflect the intestinal microbiota. While there were no significant differences in age and gender among the study groups, our results may have been affected by external environmental and other factors. Since the albumin and fecal calprotectin levels are important indicators in patients with UC, this information may provide valuable reference in future studies. Patients enrolled in this study were newly diagnosed with UC, therefore, it was not possible to observe differences among disease stages and the possible effects of other medications for UC such as anti-TNF agents, before UDCA treatment. Investigation of the main microflora in the feces of patients with UC identified a positive effect of short-term use of UDCA; however, these findings need to be further verified in a large independent cohort study.

In conclusion, UDCA combined with mesalazine had better therapeutic effects and quality of life on patients with UC compared to those with mesalazine alone. Regulation of the IL-23/IL-17 axis may be one of the mechanisms of

these effects. UDCA may play a positive role in the balance of intestinal microflora in patients with UC. A larger, well-designed randomized controlled trial with extensive follow-up is likely to verify and update the results of this analysis.

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