Original Research Chaihu-Guizhi-Ganjiang Decoction is more efficacious in treating irritable bowel syndrome than Dicetel according to metabolomics analysis

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Research Article

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Abstract

Background

Chaihu-Guizhi-Ganjiang Decoction (CGGD) is a traditional Chinese medicine (TCM) prescription used to treat viral influenza in China. There is evidence that CGGD can be used to treat irritable bowel syndrome (IBS) but the potential mechanism of action and metabolites produced upon CGGD treatment are not known.

Methods

Patients with IBS were treated with pinaverium bromide (Dicetel™) and then CGGD after a washout period of 1 week. Both treatments lasted for 30 days. Efficacy and changes in metabolites in plasma after the two treatments were compared. Plasma samples were acquired before and after each treatment, and untargeted metabolites analysis undertaken.

Results

Efficacy was measured by two systems: the Rome IV criteria and TCM theory. Irrespective of whether the Rome IV criteria or TCM theory were used, CGGD showed significantly better efficacy than Dicetel against IBS. CGGD also had a greater influence on plasma metabolism than Dicetel. Dicetel treatment led to increased tryptophan metabolism (increased levels of 5-Hydroxyindoleacetaldehyde) and increased protein metabolism (increased levels of L-arginine). CGGD use led to increased carnitine metabolism, with increased levels of L-carnitine and acylcarnitine. Such changes in these metabolites could suppress IBS by improving gastrointestinal motility and suppressing pain, depression, and inflammation.

Conclusions

CGGD appeared to be more efficacious than Dicetel in treating IBS. Metabolomics analysis helped to reveal the biomolecular basis of this beneficial effect.

Background

Irritable bowel syndrome (IBS) is a debilitating, chronic, and highly prevalent disorder of gut–brain interactions [1, 2]. In routine clinical practice, IBS symptoms are recurrent disordered defecation and abdominal pain [3].

The Rome IV criteria have been selected to diagnose IBS in clinical and research settings. The Rome IV criteria were derived by consensus from a multinational group of experts in disorders of gut–brain interactions [4] and stated that patients with IBS report abdominal-pain symptoms at least once a week on average [4].
IBS is a common disease with a prevalence of approximately 4.4–4.8% in the USA, UK, and Canada. It affects most commonly individuals younger than 50 years and women [5]. IBS causes a significant burden to healthcare systems worldwide. Direct medical costs attributed to IBS in the USA (excluding prescription and over-the-counter medications) have been estimated to be $1.5–$10 billion per year [6]. The direct and indirect costs related to IBS are estimated to be up to ¥123 billion in China [7]. According to a meta-analysis of 80 clinical studies involving 260,960 individuals in 2014 [8], the IBS prevalence in China was 6.5%. The overall prevalence of IBS in the Chinese population was ≤ 10% in 2016, and the peak age of onset was 30–59 years. Being a woman, consuming alcohol, having a history of intestinal infection, anxiety, depression, or food allergy, and living in certain locations in China are risk factors for IBS in the Chinese population [8–11]. Therefore, treating IBS and finding important metabolites to predict disease development are very important.

The factors leading to susceptibility to IBS are diverse and the pathology of IBS is complicated. In general, it is believed that IBS is a result of a combination of multiple factors, including mental/psychological disorders, visceral hypersensitivity, intestinal infection, and disorders in gastrointestinal (GI) motility [12–14]. In routine practice, drugs such as pinaverium bromide (Dicetel™) and Alosetron™ regulate intestinal movement. They reduce visceral hypersensitivity, improve emotions, and regulate intestinal flora. Dicetel and Alosetron are used commonly to relieve IBS symptoms [15–18]. Dicetel reduces the plateau phase of slow waves in the electrical activity of the smooth muscle cells, thereby inhibiting influx of calcium ions (Ca2+) and preventing consequent contractions of intestinal smooth muscle [19, 20]. Therefore, Dicetel is recommended as first-line therapy for short-term relief from IBS symptoms [21]. However, due to the limited efficacy and serious side-effects of Dicetel and Alosetron, efficacious treatment of IBS is lacking.

Traditional Chinese medicine (TCM) formulations have value in IBS treatment [22–24]. According to TCM theory, the etiology of IBS is attributed mainly to the weakness of Pi (spleen) and Wei (stomach) or damage to the Gan (liver) [7, 25], which is summarized as “Dan Re Pi Han” in TCM theory.

Recently, Chaihu Guizhi Ganjiang Decoction (CGGD) has been developed and used to treat liver/spleen deficiency, abdominal pain, and diarrhea [26]. This prescription comprises Chinese thorowax, Ramulus cinnamomi, Rhizoma Zingiberis, root of Trichosanthes species, Scutellaria baicalensis, oyster shell, and Radix Glycyrrhizae Preparata. CGGD has been shown to have a promising effect against IBS, but its pharmacological mechanism of action is not known.

Metabolomics analysis is a comprehensive study of low-molecular-weight metabolites. It offers phenotypic information not captured by genetic profiling, and has become the focus of systems biology [27]. TCM theory on the diagnosis and treatment of diseases is based on understanding of the overall dynamics of patients. Hence, metabolomics analysis could provide more direct and important information for deciphering the pharmacological mechanism of action of TCM formulations [28]. In particular, the effect of CGGD on the plasma metabolome of IBS patients has not been reported.
First, we determined the effect of CGGD against IBS compared with that of Dicetel. Subsequently, we analyzed the effect of CGGD on the plasma metabolome of IBS patients and explored the potential pharmacological mechanism of action of CGGD against IBS.

Materials And Methods

Ethical approval of the study protocol

Ethical approval (2019SL033) of the study protocol was granted by the Biomedical Research Ethics Committee of Shanghai Changzheng Hospital (Shanghai, China). Written consent was obtained from all participants [10].

Study design

This was a prospective study (Fig. 1A). Patients were treated with two drugs successively and placed into groups: positive drug and CGGD control. The duration of medication taking was 1 month. A washout period of 1 week was setup to determine the Symptom Index score. The positive drug was Dicetel. The plasma of patients was collected across four time periods.

Diagnosis of IBS

The diagnosis of IBS was based on the Rome IV criteria. The typical clinical manifestations of IBS are recurrent abdominal pain (≥ 1 day a week in the last 3 months) accompanied by changes in defecation frequency and fecal traits (appearance). Symptoms appear for ≥ 6 months and persist for the previous 3 months [29].

The diagnosis of IBS based on TCM theory was also used for efficacy evaluation. It was formulated according to the Clinical research of new Chinese medicine and the consensus of TCM Diagnosis [30] and Treatment of irritable bowel syndrome (2017) [31]. The main criteria were: (1) pain or swelling of the chest; (2) distension/pain in the abdomen, emotional relapse/aggravation; (3) fecal abnormalities; (4) dry throat with a bitter taste, or dizziness. Secondary criteria were: (i) indigestion and anorexia; (ii) fatigue; (iii) tongue quality. Patients with (3) and at least one of (1), (2), or (4) of the main criteria were diagnosed as having IBS. Patients with two features of secondary criteria and more than one of (1), (2) and (4) of the main criteria were said to have IBS.

Inclusion criteria

The inclusion criteria were: (1) meeting the diagnostic criteria of IBS; (2) age > 18 years and < 75 years; (3) providing written informed consent.

Exclusion criteria

The exclusion criteria were: (1) constipation in IBS; (2) patients with other chronic or serious diseases (e.g., heart disease, renal failure); (3) patients with other diseases that affect normal communication
between the physician and patient; (4) combination of two or more diseases meeting the inclusion criteria; (5) long-term use of similar drugs used in our study.

**Elimination criteria**

The criteria for elimination from this study were: (1) patients not treated as planned; (2) patients taking drugs other than the study drugs (including TCM formulations or drugs with anti-tumor effects); (3) treatment ceased due to suspected safety issues.

**Collection of plasma samples and treatment**

Thirty-three patients aged 23–72 years diagnosed with IBS were enrolled from 2020 to 2021. The study lasted 17 weeks: Dicetel treatment (4 weeks), washout period (1 week), CGGD treatment (4 weeks), and follow-up (8 weeks). During the entire observation period, living conditions and dietary habits remain unchanged according to self-reporting by patients (Table.1).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Basic information of patients</th>
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<tr>
<td>Age</td>
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<tr>
<td></td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>Female</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
</tr>
</tbody>
</table>

After enrollment, patients were treated with Dicetel (50 mg, t.d.s., p.o. (with food); Abbott Healthcare SAS, Paris, France). The treatment spanned 4 weeks. Then, they rested for 1 week without treatment. After this, they were treated with CGGD (1 bag, t.d.s., p.o. (post-prandial; Guangdong Yifang Pharmaceuticals, Foshan, Guangdong) for 4 weeks. Before and after each treatment, the Discomfort Index of patients according to TCM theory and the Rome IV criteria was obtained. The Discomfort Index was completed by patients and follow-up was conducted in the outpatient setting, inpatient setting, and by telephone conversations. Data input and verification were completed and checked by two individual researchers. Finally, clinical data were obtained for statistical analyses and clinical observations documented.

Simultaneously, plasma samples (5 mL) from each patient at day (D)0 (before Dicetel treatment), D30 (after Dicetel treatment), D37 (before CGGD treatment), and D67 (after CGGD treatment) were collected.
Samples were stored at −80°C for subsequent analyses.

Metabolomics analysis

Untargeted metabolomic analysis was modified according to our previous work [32]. Sample pretreatment was carried out by protein precipitation. Ultra-high-performance liquid chromatography-quadruple-time of flight-mass spectrometry (UHPLC-Q-TOF-MS) was undertaken on an UHPLC system (1290 series; Agilent Technologies, Santa Clara, CA, USA) coupled with a Q-TOF LC/MS system (6530 Accurate-Mass; Agilent Technologies) in positive electrospray-ionization mode (Dual Jet Stream; Agilent Technologies). The column was HSS T3 (3.5 µm, 2.1 × 100 mm; Waters, Milford, MA, USA). Mobile phase A was water with formic acid (0.1% v/v). Mobile phase B was acetonitrile with formic acid (0.1% v/v). The temperature was maintained at 30°C. The gradient started with 5% B, increased to 10% at 3.5 min, 40% at 6 min, 60% at 16 min, 80% at 20 min, 100% at 20.3 min, and a post-run of 5 min. Quality control (QC) samples were injected at the beginning of the run and after every eight samples during sequence analysis to assess the analytical performance.

Statistical analyses

Semi-quantitative levels of the plasma metabolome were extracted by MSDial 4.48 (http://prime.psc.riken.jp). All ions were normalized to the internal standard. Ion peaks with a coefficient of variation < 30% in QC samples and co-expressed with additional ions were filtered. Filtered ions were identified by MSFINDER 3.50 (http://prime.psc.riken.jp). Metabolites with the highest identification score encoded by the Human Metabolome Database (https://hmdb.ca) and METLIN (http://metlin.scripps.edu/) were selected for subsequent analyses. Then, data were normalized with quantile normalization, log₂ transformation, and Pareto Norm sequentially, followed by filtering of metabolites with large within-group variance using interquartile ranges. K-nearest neighbor [33] was applied for imputation and limma [34] for differential analyses. All clinical covariates were adjusted. P < 0.05 was considered the threshold for significance. Gephi v0.9.2 (https://gephi.org) was used to generate a network based on the results of correlation analyses [35]. The Student's t-test was used to screen CGGD- and Dicetel-related endogenous metabolites. Statistical analyses were carried out using R v3.6.3 (R Institute for Statistical Computing, Vienna, Austria). P < 0.05 was considered significant.

Results

Beneficial effect of CGGD against IBS compared with Dicetel

Irrespective of whether the Rome IV criteria or TCM theory were used, CGGD showed significantly better efficacy than Dicetel against IBS. Efficacy assessment according to the Rome IV criteria is based on five criteria (Fig. 1B): Dicetel improved four of them and CGGD improved all five of them. In addition, for each criterion, CGGD caused larger changes with greater statistical significance than Dicetel. The maximal, median, and minimal degree in improvement of all Rome IV criteria for Dicetel was 0.63, 0.38, and 0.29, and for CGGD it was 1.90, 1.81, and 1.64, respectively.
For Dicetel, the least-improved criterion was “Inconvenience of intestinal symptoms on life” (average Symptom Index score decreased from 3.08 to 2.75). The most-improved criterion was “Abdominal pain” (average Symptom Index score decreased from 3.29 to 2.65).

For CGGD, the least-improved criterion was “Satisfaction of stool condition” (average Symptom Index score decreased from 3.42 to 1.52). The most-improved criterion was “Abdominal pain” (average symptom index decreased from 3.29 to 2.65).

Similar results were obtained using TCM theory. Efficacy assessment using TCM theory contains seven criteria (Fig. 1B): Dicetel and CGGD improved all of them. For each criterion, CGGD caused larger changes with greater statistical significance than Dicetel. The maximal, median, and minimal degree of improvement of all TCM-theory criteria for Dicetel was 1.21, 0.75, and 0.42, and for CGGD it was 2.58, 2.45, and 1.45, respectively.

For Dicetel, the least-improved criterion was “Tongue quality” (average Symptom Index score decreased from 2.83 to 2.42). The most-improved criterion was “Pain or swelling of the chest” (average Symptom Index decreased from 3.83 to 2.63).

For CGGD, the least-improved criterion was “Indigestion and anorexia” (average Symptom Index score decreased from 2.10 to 0.65). The most-improved criterion was “Dry throat with a bitter taste” (average symptom index decreased from 4.06 to 1.48).

Influence of Dicetel and CGGD on the plasma metabolome

Untargeted metabolome analysis led to detection of 643 plasma metabolites. Among them, four types of endogenous metabolites were related to the effect of Dicetel (significant difference between D30 vs. D0). The largest group was organic acids and derivatives, which contained metabolites such as N-acetyltryptophan, and L-arginine. The second largest group was lipids and lipid-like molecules, and contained metabolites such as malonylcarnitine (AC\textsubscript{4}-OH) and pimelylcarnitine (AC\textsubscript{6}-COOH). The third largest group was organoheterocyclic compounds, which contained metabolites such as 5-Hydroxyindoleacetaldehyde (5-HIAA). The plasma levels of most of these metabolites were also increased upon Dicetel treatment.

Seven types of endogenous metabolites were related to the effect of CGGD (significant difference between D67 vs. D37). The largest group was lipids and lipid-like molecules, which contained metabolites such as hydroxyisovaleroyl carnitine (AC\textsubscript{4}-CH\textsubscript{3}), 3-hydroxyoctanoyl carnitine (AC\textsubscript{8}-OH), phosphatidylethanolamine (35:0), 3-hydroxydecanoyl carnitine (AC\textsubscript{10}-OH), and phosphatidylcholine (40:7). The second largest group was organic acids and derivatives, which contained metabolites such as N-Acetylisoleucine and L-arginine. The third largest group was organoheterocyclic metabolites, which contained metabolites such as 5-HIAA. The plasma levels of most of these metabolites were increased upon CGGD treatment.
According to plots of principal components analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) drawn based on these treatment effect-related metabolites, CGGD-group samples showed better separation compared with Dicetel-group samples (Fig. 1B). PCA plots revealed the value of PC1 to be 33.5% for the CGGD group, which was smaller than that in the Dicetel group (37.8%). PLS-DA plots revealed the value of component 1 to be 32.6% in the CGGD group, which is smaller than that in the Dicetel group (36.5%).

Analysis of enrichment of biological pathways on CGGD and Dicetel-related differential metabolites

Differential metabolites were subjected to bioinformatics analysis to reveal their biological function in a phenotypic context. In this way, we clarified the reason why the efficacy of CGGD was different to that of Dicetel for treating IBS.

Analysis of enrichment of biological pathways was carried out by Home - Reactome Pathway Database (https://reactome.org/) for treatment effect-related metabolites for the Dicetel group and CGGD group (Fig. 3). All enriched biological pathways could be divided into one category: “Metabolism of amino acids and derivatives”. All enriched biological pathways were upregulated in the CGGD group and Dicetel group (Fig. 3A). “Phase I – Functionalization of metabolites” and “Biological oxidations” were the shared biological pathways between the CGGD group and Dicetel group.

The metabolites from shared biological pathways between the Dicetel group and CGGD group were subjected to subsequent analysis. 5-HIAA and arginine were enriched in the CGGD group and Dicetel group. Phenylethylamine (PEA) was enriched only in the CGGD group.

Correlation analyses between CGGD- and Dicetel-related differential metabolites

We wished to understand the correlation between CGGD- and Dicetel-related differential metabolites. The correlation among these metabolites was analyzed, and a network diagram of metabolites with a significant correlation (p < 0.05) created. Finally, the metabolites from the Dicetel group and CGGD group were drawn as two network diagrams (Fig. 4A and B).

The most correlated metabolites in the Dicetel group were N-acetyltryptophan, AC₆-COOH, and arginine. The most correlated metabolites in the CGGD group were biotin sulfone, leucylproline, and AC₄-CH₃. These highly correlated metabolites may have important roles in the pharmacological effect of Dicetel and CGGD against IBS.

Discussion

Conventional mechanism of action of IBS

Traditionally, the diagnosis of IBS has been based on identification of symptoms that correlate with several different syndromes associated with disorders such as IBS diarrhea, IBS constipation, functional diarrhea, functional constipation, chronic functional abdominal pain, or bloating [36]. Several peripheral
and central mechanisms initiate disorders of GI motor and sensory functions leading to IBS symptoms [37]. The predominant pathophysiological mechanisms in IBS are abnormalities of gut smooth muscle, visceral hypersensitivity, and central nervous system (CNS) hypervigilance. IBS symptoms are not specific to a single etiologic mechanism but are manifestations of several peripheral mechanisms that perturb motor and sensory functions [38]. Our study lacked a healthy control group to elucidate directly the metabolomic basis of IBS. Nevertheless, anti-IBS drugs induced metabolomics changes in regulation of biological pathways based on abnormalities of gut smooth muscle and CNS hypervigilance. These biological pathways included the metabolism of tryptophan, arginine, and L-carnitine-regulated lipid metabolism.

Pharmacological mechanism of action of Dicetel in the body

Dicetel is a GI-selective antagonist of Ca\(^{2+}\) channels. It has highly selective spasmolytic activity in the GI tract. Dicetel helps to lessen the discomfort and abdominal pain associated with functional intestinal disturbances (e.g., IBS) by inhibiting Ca\(^{2+}\) influx into intestinal smooth muscle cells [39]. Dicetel also inhibits the contractile effect of digestive hormones and proinflammatory mediators such as cholecystokinin, gastrin, and substance P. These metabolites play a key part in the contraction of intestinal smooth muscles, and are linked to defecation-associated abdominal pain and discomfort in patients with IBS.

The beneficial effect of Dicetel arose from two biological pathways. First, Dicetel improved tryptophan metabolism, especially the production of 5-HIAA and N-acetyltryptophan (Fig. 5). Tryptophan can be converted to N-acetyltryptophan by N-Acetyltransferase or into 5-hydroxytryptophan (5-HTP) by tryptophan hydroxylase (TPH)1 and TPH2. Dicetel is a blocker of L-type Ca\(^{2+}\) channels, and Ca\(^{2+}\) influx through L-type high-voltage-activated calcium channels is essential for full activation of TPH [40, 41]. Hence, inhibiting Ca\(^{2+}\) influx using Dicetel would lead to more N-acetyltryptophan. Conversely, 5-HTP can be converted to 5-hydroxytryptamine (5-HT). Once bound to target receptors or taken-up by 5-HT transporters, internalized 5-HT can be metabolized by monoamine oxidase, thereby leading to 5-HIAA generation. Often, the 5-HIAA concentration is used to detect changes in the whole-body 5-HT level [42]. An increased 5-HIAA level cannot be explained directly by the negative influence on TPH by Dicetel. Another compensatory mechanism (e.g., suppressed 5-HT internalization by cells) may also lead to an increased plasma level of 5-HIAA. The Ca\(^{2+}\)-channel blockers isradipine and darodipine can increase the 5-HIAA:5-HT ratio in mouse brains [43].

A low 5-HT level can cause depression [44], which is one of the causes leading to IBS. IBS has been postulated to be the most clinically important peripheral disease associated with 5-HT levels [45]. In addition, 5-HIAA and N-acetyltryptophan cause cell toxicity at high concentrations. 5-HIAA can inhibit the growth of the ovary cells of Chinese hamsters [46] and suppresses normal neuronal function by inducing production of oligomerized alpha-synuclein [47]. N-acetyltryptophan is classified as a “uremic toxin” if present in high abundance in sera or plasma [48, 49]. N-acetyltryptophan has been identified as a catabolite of tryptophan generated by the gut microbiota. After absorption through the intestinal
epithelium, tryptophan catabolites enter the bloodstream and are excreted subsequently in urine [50]. Uremic toxins are a diverse group of endogenously produced molecules that, if not cleared appropriately or eliminated by the kidneys, can cause kidney damage, cardiovascular disease, and neurological deficits [51]. The overall negative influence of Dicetel on neurons may suppress IBS-related CNS hypervigilance, and improve GI motility and secretion of the enterochromaffin cells [52, 53].

Apart from tryptophan metabolism, Dicetel can alter the arginine level in blood, which can increase protein synthesis and suppress inflammation. These actions also help to relieve IBS symptoms [54].

Potential pharmacological mechanism of action of CGGD

CGGD has been used to treat liver/spleen deficiency, abdominal pain, and diarrhea [26]. The potential pharmacological mechanism of action of CGGD is more extensive and complex than that of Dicetel. The beneficial effect of CGGD arose from three biological pathways. In addition to increased metabolism of tryptophan and arginine (which were also documented for Dicetel), CGGD improved carnitine-mediated lipid metabolism.

With respect to tryptophan metabolism, CGGD could increase the blood level of PEA, which functions as a neuromodulator or neurotransmitter [55]. PEA is a direct stimulator of 5-HT biosynthesis, thereby regulating GI transit and colonic secretion in vivo and ex vivo. PEA can improve depression [56, 57]. It can activate the G-protein coupled receptor trace amine-associated receptor 1 (TAAR1) which, in turn, mediates 5-HT biosynthesis by stimulating TPH1/ amino acid decarboxylase activities. Zhai and colleagues [58] showed that PEA production stimulates 5-HT biosynthesis to accelerate GI transit via a TAAR1-dependent mechanism. They demonstrated that PEA could relieve constipation [58]. CGGD also increases the blood level of 5-HIAA, which can suppress CNS hypervigilance. Research has shown that baicalin and Chinese thorowax have a positive influence on the concentration of monoamines, including PEA and 5-HT [59], but the exact biological mechanism is under investigation. It has been speculated that the Ca\textsuperscript{2+} present in oyster shells can also promote tryptophan to be converted to 5-HTP by TPH1/2 [60, 61], thereby resulting in increased metabolic output [62].

With regard to carnitine-mediated lipid metabolism, the overall effect of CGGD seemed to increase the availability of acetyl groups, which can suppress pain and depression. L-carnitine is transported across cell membranes primarily by two organic cation transporters (OCTNs): OCTN1 and OCTN2. Once inside a cell, the main physiological function of L-carnitine is to shuttle long-chain fatty acids across mitochondrial membranes with the aid of carnitine palmitoyl transferase 1 (CPT1). The latter is located on the internal side of the external mitochondrial membrane and converts activated fatty acyl-coenzyme A (acyl-CoA) from acyl-CoA to acyl-carnitine. Carnitine translocase exchanges acyl-carnitine for carnitine from the matrix via the internal mitochondrial membrane. On the internal side of the inner mitochondrial membrane, CPT2 catalyzes acyl-CoA synthesis from acylcarnitine and a matrix pool of CoA [73]. Acyl-CoA is processed by beta-oxidation to produce energy in the form of adenosine triphosphate [63]. As demonstrated in vitro and in vivo, S. baicalensis and baicalin can activate CPT directly and accelerate the
Conclusions

CGGD was more efficacious than Dicetel for treating IBS according to metabolomics analysis. Dicetel could induce tryptophan metabolism leading to increased blood levels of N-acetyltryptophan and 5-HIAA. Dicetel could also improve arginine metabolism. These Dicetel-related biological pathways could be 

\[
\beta\text{-oxidation of lipids [70–72]. CGGD increases the blood level of long-chain lipids such as PE(35:0) and PC(40:7), as well as acylcarnitine. Acetyl-CoA and acetyl-L-carnitine are important providers of acetyl groups. The latter can activate the glutamate receptor metabotropic 2 gene through epigenetic regulation to suppress pain and depression [64, 65]. Studies [65, 66] have shown that carnitine supplementation can improve the depressive state of male patients suffering from uremia and cancer patients. In addition, the root of }\text{Trichosanthes}\text{ species and oyster shells can supplement levels of lysine and aspartate as synthetic substrates of carnitine, which can increase the carnitine content directly [67, 68].}
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CGGD can also increase the blood level of arginine. The latter can activate adenosine monophosphate kinase, which then stimulates fatty-acid oxidation in skeletal muscle and glucose uptake in muscles [57]. This phenomenon can be explained (at least in part) by the existence of baicalin. Baicalin can increase expression of arginase-1 [69], which converts arginine into ornithine. Ornithine has been shown to have a role in regulation of cellular immunity in the microenvironment [70], and its metabolites can increase protein synthesis and suppress inflammation. Arginine and its metabolites also have important roles in regulation of esophageal, gastric, and intestinal motility [58].

In addition to the three major biological pathways stated above, two metabolomics changes associated with CGGD may explain its beneficial effect against IBS. First, Rhizoma Zingiberis can supplement ergothioneine directly [71]. According to Cheah and colleagues, ergothioneine can relieve the symptoms of several cognitive diseases [72]. Fond and coworkers have suggested that ergothioneine expression through OCTN1 transporters can protect the nervous system from oxidative stress, maintain energy reserves, provide nutritional and neuroprotective factors, and inhibit abnormal brain excitation, combined with the risk factors of IBS [73], which is also an important reason to relieve patients' symptoms. Second, Radix Glycyrrhizae Preparata also has roles in treating depression [59] and IBS [74].

Collectively, combined with the risk factors of IBS, CGGD can improve the mental state of patients by regulating lipid metabolism. We elucidated the pharmacological mechanism of action of CGGD in regulating lipid metabolism through network pharmacology and lipidomics. This strategy may contribute to the discovery of new drugs and clinical application of CGGD in IBS as well as diseases associated with disorders of lipid metabolism.

Limitations

Our study had four main limitations. First, the study population was small. Second, the patients were enrolled in one center. Third, intrinsic and environmental factors that could influence IBS were not assessed. Fourth, only positive results were compared with other positive results from the literature.

Conclusions

CGGD was more efficacious than Dicetel for treating IBS according to metabolomics analysis. Dicetel could induce tryptophan metabolism leading to increased blood levels of N-acetyltryptophan and 5-HIAA. Dicetel could also improve arginine metabolism. These Dicetel-related biological pathways could
suppress CNS hypervigilance and inflammation, and increase protein synthesis. CGGD elicited similar changes to pathways involving acetyltryptophan and 5-HIAA, but also induced carnitine-mediated lipid metabolism to suppress pain and depression. The most efficacious active ingredient of CGGD may be baicalin.

**Declarations**

**Ethics approval and consent to participate**

Ethical approval (2019SL033) of the study protocol was granted by the Biomedical Research Ethics Committee of Shanghai Changzheng Hospital (Shanghai, China) consent for publication. Written informed consent was obtained from all patients.

**Consent for publication**

Written informed consent was obtained from all patients before writing this manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

The data generated in this study are available from the corresponding author upon request.

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**Authors' contributions**

WSC and XL conceived and designed this study. XL recruited participants. MML and XL designed and carried out the research. TY and YD collected and prepared samples. MML, JNC, and JWZ contributed to statistical and bioinformatics analyses. MML and JWZ drafted the manuscript. FZ, SQ, and XL provided constructive suggestions for the study. All authors contributed to the writing of this manuscript. All authors approved the final version of the manuscript.
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**Figures**
A. Study design and procedure

Figure 1

Experimental design and collection of clinical data. (A) Study design. Patients were enrolled for eligibility assessment based on inclusion criteria. Thirty-five patients were enrolled. "D0–D30" denotes from Day 0 (D0) to Day 30 (D30), and represents differences in patients before and after Dicetel treatment in terms of the Symptom Index score as well as collected blood samples before and after treatment. Eventually, 24 patients provided Discomfort Index scores before and after treatment. D37–D67 represents differences in
patients before and after CGGD treatment in terms of the Symptom Index score and collected blood samples before and after treatment. In the middle of these two periods is a 1-week washout period. Thirty-one patients provided scores for the Discomfort Index before and after treatment. “D30 vs. D0” denotes the procedure of exploring the therapeutic effect of Dicetel and the effect of the human metabolome. “D67 vs. D37” refers to the procedure of exploring the therapeutic effect of CGGD and the effect of the human metabolome.

(B) Clinical data. Images include the numerical mean of the Symptom Index score and error bar, and P-value. *Represents differences between different groups of the same target. *p < 0.05, **p < 0.01, ***p < 0.001. P-value was calculated by the Student’s t-test. Y-axis represents the symptom under different criteria. The X-axis represents the average Symptom Index score when taking different drugs at different stages. Criteria are divided into Rome IV and TCM. The Symptom Index score of patients under different criteria (Rome IV or TCM) is selected, followed by selected by different reference indicators under these two criteria (Table 2). First, the enrollment time was recorded as “D0” and the Symptom Index was recorded. Second, after 4 weeks of treatment with Dicetel, the Symptom Index score of patients condition at this time was recorded as “D30”. Third, after the washout period (1 week), the Symptom Index score was recorded as “D37”. Finally, after 4 weeks of treatment with CGGD, the Symptom Index score was recorded as “D67”.
A. Number of before and after treatment related compounds in different stage

B. Sample distributions based on tumor staging-related metabolites

Figure 2

Differences in metabolites after treatment with Dicetel and CGGD

(A) Number of metabolites associated with a significantly altered treatment effect. The sample was divided into a Dicetel group (D30 vs. D0) and CGGD group (D67 vs. D37). The part of the axis that is >0 indicates the number of metabolites whose concentration exhibits an increasing trend. The part of the
axis that is <0 indicates the number of metabolites whose concentration exhibits a decreasing trend. The ordinate expresses a simplified “super-class” of metabolites. The abscissa expresses the number of these simplified super-classes exhibiting high/low expression at different stages. These metabolites were divided into seven species according to their superclass from an Internet website (https://hmdb.ca/): benzenoids; lipids and lipid-like molecules; organic acids and their derivatives; organoheterocyclic metabolites; organic nitrogen metabolites; organic oxygen metabolites; alkaloids and their derivatives.

(B) Sample distributions based on differences in efficacy-related metabolites. The positions of the different-colored spheres represent the distribution of different metabolites at different stages. PCA and PLS-DA plots express different sample distributions of differential metabolites in the Dicetel group and CGGD group.

Figure 3
Analysis of enrichment of biological pathways

(A) Analysis of enrichment of biological pathways in the CGGD group and Dicetel group. The p-value was set at 0.05. The dot border color represents the significance value of pathway enrichment. “Pathway weight” is defined as the number of entities found/total number of entities. The size of the dot represents the pathway weight. “Pathway direction” is the median log_{10} fold change (FC) of metabolites in the pathway (red = upregulated). *p < 0.05 for D67 vs. D37 and D30 vs. D0.

(C) Analysis of enrichment of biological pathways in the CGGD group and Dicetel group according to differential metabolites. The evaluation criteria were identical to those shown in Figure 1B. The number on the Y-axis and length of the column represent the concentration of the metabolite at this stage. The X-axis represents the different stages (D0, D30, D37, and D67).

A. Correlation analyses

Figure 4
Correlation analyses

The correlation network reflects the correlation of compounds in a pathway. The network comprised only paired metabolites with a significant correlations ($p \leq 0.05$). The degree of thickness, degree of edge, and diameter of the node show the importance of metabolites.

Figure 5
Metabolism and related metabolic processes

Solid arrows denote the positive connections of substrate and products of a single biochemical reaction. Dotted arrows denote positive connections among metabolites, enzymes, and physiological functions. Blue arrows denote the concentration change of metabolites in the CGGD group. Blue arrows denote the concentration change of metabolites in the Dicetel group. The direction of arrows denotes the change in trend.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable.xlsx