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A Phase I Trial of Berberine in Chinese with Ulcerative Colitis

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ABSTRACT

The Chinese natural product, berberine, has biological properties that support its potential efficacy as a colon cancer prevention agent. Its longstanding use in China to treat gastrointestinal tract and rheumatologic disorders is generally regarded as safe, supporting initial investigations in an at-risk population, such as individuals with ulcerative colitis. However, the safety of berberine in this population is not established. Individuals living in China with biopsy-proven ulcerative colitis, ≤grade 2 dysplasia, and with a ulcerative colitis disease activity index (UCDAI) score ≤1 on mesalamine, were randomized 3:1 in a double-blind phase I trial to berberine 900 mg/day or placebo for 3 months, with the primary objective of assessing safety. Blood samples and biopsies of the colorectum, from prespecified locations, were collected prior to and following therapy. Secondary endpoints included changes in UCDAI score, and in tissue and plasma markers of inflammation. Of toxicities at least possibly related, one episode of grade 3 elevation in transaminases and one episode of grade 1 nausea were observed among 12 individuals on berberine, and none were observed among 4 on placebo. The mean plasma berberine concentration was 3.5 nmol/L after berberine treatment, significantly higher than 0.5 nmol/L with placebo. Berberine significantly decreased the Geboes grade in colonic tissue, but had a nonsignificant effect on other tissue or blood biomarkers related to cell growth and inflammation. The combination of berberine and mesalamine is well tolerated in Chinese with ulcerative colitis and may enhance mesalamine's anti-inflammatory effects in colonic tissue.

Introduction

Colorectal cancer ranks high in cancer incidence and mortality worldwide, including in the United States and China (1). It is the third most common type of cancer in both United States and China and accounts annually for approximately 11% and 14% of all cancer deaths, respectively (2–5). Thus, identifying strategies to reduce its incidence is important. Ulcerative colitis is a chronic intestinal inflammatory process, places individuals at high risk for colorectal cancer, and individuals with ulcerative colitis therefore represent a good cohort to evaluate the efficacy of potential cancer prevention agents (6–10). Globally, the incidence of colorectal cancer in patients with ulcerative colitis is estimated to be 2 to 5 times higher than that in the general population of the same age group (11). The incidence of colorectal cancer in patients with ulcerative colitis in the United States from 1998 to 2010 was 76.0 per 100,000 person-years (12), whereas in China from 1990 to 2003, it was 30.8 (13). The vast majority of patients with ulcerative colitis in both China and the United States are maintained on therapy based upon 5-aminosalicylic acid (5-ASA), commonly in the form of mesalamine, a second-generation 5-ASA (14, 15). Although mesalamine effectively reduces periods of active disease and extends remission time (16, 17), ulcerative colitis often becomes refractory, and with prolonged ulcerative colitis, there is an increased risk of colorectal cancer (2% at 10 years, 8% by 20 years, and 18% by 30 years; ref. 18). Moreover, once colorectal cancer develops, treatment remains suboptimal and its prognosis is poorer for patients with ulcerative colitis compared with those without (19). Therefore, the development of new therapies that

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combine high treatment efficacy, convenient dosing, low side effects, and, more importantly, colorectal cancer prevention ability, is an important goal for ulcerative colitis management and its associated colorectal cancer incidence reduction.

A cancer prevention agent must meet a highly restrictive set of criteria. It must modulate the progression of early carcinogenesis for an extended period of time. For prevention, an acceptable toxicity profile implies infrequent, mild side effects. Thus, natural products with low toxicity make for attractive, novel cancer prevention agents (20). They represent compounds with a diverse array of bioactivity, and humans have consumed a large number of these for extended periods with little apparent toxicity. Another feature of natural products is a tendency to exert pharmacologically weak effects on several targets. Agents that are prospectively synthesized and designed to act upon a single target, but turn out to act upon several different targets are viewed as pharmacologically impure and are considered undesirable. When this perspective is applied to natural products, they are not well received. However, an agent that has the ability to weakly modulate more than one pharmacologic target, but whose combined effects offer selective therapeutic pressure upon a given biological process, constitutes a strategy that can achieve selective biological efficacy and low long-term toxicity. Through their interface with complex biological systems over very long periods of time, natural products offer access to such a potential.

Conversely, natural products have several limitations. Primarily, many mechanistic studies, especially those using cell lines, use concentrations that are far above those attained in humans, a likely explanation for the high degree of nonspecificity observed in these studies. The prediction of target modulation in humans from *in vitro* data is therefore difficult. Well-designed animal studies can provide an important measure of efficacy in the context of a whole system and provide preliminary estimates of safety or toxicity. Prospective studies of natural product use in humans usually are lacking, and reported experience is usually historical and anecdotal. These caveats notwithstanding, natural products are generally well tolerated, and given evidence of medicinal effects, even if anecdotal, are worthy of consideration.

Compounds used in Chinese traditional medicine represent a rich source of bioactive compounds that have been used in humans for extended periods. Berberine, a main constituent of *Coptidis* rhizome, is one such compound and has many desirable properties related to potential colorectal cancer prevention. A large body of evidence indicates that it has the potential to inhibit colorectal carcinogenesis in humans, mainly through its anti-inflammation effects. Berberine is widely used to treat gastroenteritis and diarrhea in China (21–24). In four different clinical trials involving adult patients with acute diarrhea, berberine decreased stool volume and was nontoxic (25–28). In a separate trial, berberine was shown to reduce acute radiation intestinal syndrome in people receiving abdominal radiotherapy (29). Berberine is efficacious across different animal models of intestinal inflammation (30–32). Of particular relevance, it inhibits colon carcinogenesis in preclinical models. It has high activity in preventing colitis induced by dextran sulphate sodium and trinitrobenzene sulfonic acid in mice and rats (33–36). Furthermore, it prevents azoxymethane-induced colon carcinogenesis in rats (37), with such effects being largely attributed to its anti-inflammatory properties.

Several lines of evidence support the notion that a primary effect of berberine on the intestine relates to anti-inflammatory activity. In China, berberine is widely used clinically to treat a range of inflammatory ailments, while specific anti-inflammatory activity has been demonstrated across of variety of models. Specifically, berberine is efficacious in adjuvant-induced murine models of arthritis and in experimental models of osteoarthritis in the rat (38–42). It suppresses autoimmune nephritis in BALB/c mice (43) and attenuates lipopolysaccharide-induced extracellular matrix accumulation and inflammation in rat mesangial cells (44). In addition to these rigorously conducted and physiologically relevant studies, berberine's basic anti-inflammatory action has been loosely linked to a number of downstream processes, including antioxidatic, antiapoptotic, and antitumor activities in a wide array of reports (reviewed in refs. 45, 46). However, the design of such studies (typically involving very high concentrations of agent *in vitro*) places limitations on whether such mechanisms are operative in systemic models.

Another attractive and very important feature of berberine relates to its ability to provide low systemic delivery of agent, but high delivery to the target organ, in this case the intestine. The systemic delivery of any agent provides an opportunity for systemic toxicity, and this increases with length of agent exposure. However, a central goal of cancer prevention agents is having little to no toxicity in the face of extended administration times. A consideration of these factors, and the fact that a given at-risk organ can be targeted through local drug delivery, is serving to drive the investigation of local delivery strategies in the field of cancer prevention (47). In the case of berberine, studies in rodents indicate that oral berberine is absorbed through the intestinal lining, undergoes rapid first-pass metabolism in the liver and has a systemic delivery of below 0.001% (48–50). That is, oral administration provides for delivery to colonic epithelium and the supporting organ, with very little systemic delivery. Studies in humans support this by demonstrating maximum plasma concentrations of 1.1 nmol/L after 400 mg doses, with 300 mg doses being used more typically (51).

Taken together, studies support the notion that berberine has a favorable therapeutic effect upon inflammatory states in the colon, has activity in colorectal cancer prevention models, has a pharmacologic profile that provides for selective targeting of the intestines, and widespread use in the Chinese population shows it is well tolerated. We hypothesize that berberine has high potential to prevent colorectal cancer in those with ulcerative colitis. Individuals with ulcerative colitis are at high risk for developing colorectal cancer and constitute an

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important target cohort for investigating cancer prevention strategies, including therapeutic. It is important to test new therapeutics in a medically stable cohort, especially so for those focused on prevention. However, ulcerative colitis causes symptoms and induces instability in the function of the gastrointestinal system. As treatment with mesalamine readily reverses symptoms and normalizes function, a cohort with ulcerative colitis medically stabilized with mesalamine represents a cohort well suited for testing new prevention agents. Berberine was tested at a dose of 300 mg three times per day, corresponding to its widespread over the counter use at this dose and schedule in China. At higher doses, reports describe gastrointestinal side effects in about a third, with most requiring dose reduction (52). Finally, it is recognized that in final application as a cancer prevention therapeutic, it is very likely that berberine will need to be administered over a long duration, either continuously or intermittently. However, the combination of berberine plus mesalamine has never before been formally evaluated. Therefore, the primary endpoint of the current study is toxicity, and in recognition of the need to optimize patient safety, the duration for testing this first time combination was set at three months. Also, with a central focus on safety, and considering that it is widely used in China and that drug metabolism is not uniform across races, the study was conducted in China, in a cohort of Chinese with ulcerative colitis.

We implemented a phase I randomized double-blind trial of berberine versus placebo in China in people with clinically stable ulcerative colitis taking maintenance mesalamine. This study was designed to address the hypothesis that berberine would be well tolerated in patients with ulcerative colitis, when administered with mesalamine. We demonstrate that berberine was well tolerated in Chinese patients with ulcerative colitis in clinical remission on mesalamine. Oral dosing was associated with very low nanomolar mean plasma concentrations of berberine. In colon tissue, berberine significantly decreased the Geboes grade, which is a composite measure of tissue inflammation used in ulcerative colitis. Measurements of other biomarkers of proliferation and inflammation conducted on blood and colon tissue provide prospective data for sample size estimates that can be used to design larger studies in the future. Findings from this study support future investigation of berberine as a potential colorectal cancer prevention agent.

Materials and Methods

Study design

The study is a double-blind placebo-controlled phase I trial of berberine in participants with ulcerative colitis maintained on mesalamine. All participants were recruited and treated at Xijing Hospital (Xi'an, China), and all were native Chinese. The study was reviewed and approved by the Institutional Review Board of Northwestern University (Chicago, IL) and the Human Ethics Committee of Xijing Hospital. All participants gave verbal and written informed consent. The study was conducted in a manner that adhered to all Good Clinical Practice as per the International Conference on Harmonisation and Declaration of Helsinki ethical principles (53). The United States NCI (Bethesda, MD), Division of Cancer Prevention, sponsored and monitored the trial, performed statistical and scientific reviews of the protocol, and housed the data. In a blinded fashion, all study data were quality checked, through on-site monitoring by dedicated personnel from Northwestern University and Oregon Health and Science University (Portland, OR), and by the NCI, and then locked. It was then unblinded and released to the study biostatistician for analysis. The study was registered with ClinicalTrials.gov (NCT02365480).

Participants were eligible if they were 18–70 years old, had a pathologic diagnosis of ulcerative colitis, were in clinical remission as defined by a ulcerative colitis disease activity index (UCDAI) score of $\leq$1 for at least 3 months, on maintenance mesalamine for at least 3 months, had an Eastern Cooperative Oncology Group (ECOG) performance status of $\leq$1, had intact end organ function, and were able to read, understand, and willing to give written informed consent. Participants were excluded who had immunomodulatory treatment within 3 months, or who had a dysplasia-associated mass or lesion.

Participants underwent pretreatment baseline colonoscopy with biopsy and blood draw for biomarker analysis and were randomized 3:1 to 300 mg oral berberine three times per day or matching placebo. After 3 months, posttreatment colonoscopy with biopsy and blood draw were repeated. They also underwent history and physical examination at baseline, monthly during treatment, and at 1 month after, and were contacted by telephone at day 7. All baseline symptoms and adverse events were recorded and graded. Berberine, isolated from *Coptidis* rhizome, was supplied as >98% pure berberine hydrochloride in 100 mg tablets by Shanghai SINE TianPing Pharmaceutical Co., who also provided matching placebo.

The primary objective was to determine the safety of berberine in Chinese participants with ulcerative colitis in clinical remission on maintenance mesalamine. Secondary objectives included plasma-based measures of inflammation, including C-reaction protein level, erythrocyte sedimentation rate, and cytokines such as TNFα, IL4, IL6, IL8, and IL10, tissue-based measures of inflammation, including TNFα, cyclooxygenase (COX)-2, and NF-κB, and of anticancer action, including Ki-67, activated caspase-3, and DNA methylation on SFRP1, TCERGIL FBN2, and TFP12. Other secondary measures included ulcerative colitis–related symptoms, measured by the UCDAI score (54), and histologic inflammation, measured by the Geboes grading system (55).

Colonoscopy

At baseline, participants fasted after midnight, and used PEG-4000 for GI prep. Seven biopsies were taken, close to an inflammatory area, and the area marked by ink. At 3 months, seven biopsies were taken $\leq$5 cm from the initial site.

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IHC and histologic analysis of tissue

The IHC assays were performed in batches after completion of accrual; pre- and postintervention samples were processed together in the same batch. Appropriate positive and negative controls were included for all antigens. Formalin-fixed, paraffin-embedded tissue was sectioned and subjected to the IHC evaluation as reported previously (56). Five-micron sections were mounted on Vectabond-coated Superfrost slides, deparaffinized by heating at 60°C for 30 minutes, rehydrated by a graded series of ethanol rinses, and washed in PBS. Unmasking of the antigen epitope for Ki-67 was carried out by heating sections in EDTA buffer (pH 9) in a pressure microwave (NordicWare) for a total of 9 minutes at high heat setting. For COX-2 and NF-κB, antigens were unmasked using antigen unmasking solution (Vector Laboratories) and pressure microwave the slides for a total of 9 minutes at high heat setting, per the manufacturer's instructions. Endogenous peroxidase activity was blocked in 1% H_2O_2 (in methanol) for 10 minutes and nonspecific binding sites were blocked with 5% horse serum in PBS. Primary antibodies COX-2 and NF-κB (Santa Cruz Biotechnology) were used at 1:100 and Ki-67 (Dako) was used at 1:200. After washing in PBS, the samples were incubated with biotinylated secondary antibodies (1:2,000), followed by development of avidin–biotin peroxidase complex according to the manufacturer's recommendations (using the Vectastain Elite ABC Kit from Vector Laboratories).

A semiquantitative scale was used to evaluate immunoreactivity of epithelial cells. For COX-2 and NF-κB, the extent of staining was graded and scored as 0 (negative staining), 1+ (10% stained cells), 2+ (10%–50% stained cells), and 3+ (50% stained cells). For Ki-67, staining was graded and scored as 0 (negative staining), 1+ (1/3 of cells stained), 2+ (1/2 of cells stained), and 3+ (≥2/3 of cells stained). Histologic analysis of tissue inflammation was performed on hematoxylin and eosin (H&E)–stained tissue, and quantified using the Geboes grading system, as described previously (55). All IHC and H&E sections were scored by the study pathologist (Z.-S. Li), who was blinded to the study group. A second pathologist (G.-Y. Yang) participated in a consensus read of all sections.

Measurement of plasma cytokines

ELISA Kits (R&D Systems) were used to measure plasma concentrations of TNFα, IL4, IL6, IL8, and IL10, according to the manufacturer's instructions.

Plasma berberine concentrations

Patients were instructed to take study medications one hour after eating, along with 8 ounces of water. Blood draws were in the morning, timed to be 1–2 hours after the first dose, with time of dosing and blood draw recorded. The pharmacology of berberine in humans is poorly understood. A comprehensive pharmacokinetic analysis of berberine in rodents has been reported by several different investigators, with associated peak blood concentrations ranging from 1.3 to 2 hours post oral dosing and a half-life ranging between 5 and 14 hours (49, 50). On the basis of this information, the current study was designed to measure berberine concentration in the blood at a time corresponding to estimated peak concentrations.

Plasma berberine concentrations were run in duplicate, as reported previously (57). In brief, HPLC [using an Agilent eclipse plus C18 (2.1 × 150 mm, 3.5 μm) column] coupled to an Agilent 6410 Triple Quadrupole MS equipped with an electrospray ionization source (Agilent Technologies) was used. We used 500 μL plasma for extraction, and an aliquot of 10 μL was injected into the LC/MS-MS 6410 system. A calibration curve was constructed from the peak heights against the concentrations using unweighted linear regression. The concentrations of berberine in the samples were determined using the regression parameters obtained from the calibration curve. Calibration standards were included in every analytical batch of samples. All data were acquired employing Agilent 6410 Quantitative Analysis version analyst data processing software. The limit of detection (LOD) was determined as the lowest concentration that could be detected with acceptable accuracy and precision, which is achieved from the plot three times above the noise level. The limit of quantification (LOQ) in samples was defined as the lowest concentration on the calibration curve for which assay imprecision (coefficient of variation) is lower than 10% and is at least 10 times as much as the LOD. The LOQ values for berberine in plasma were as follows: upper LOQ, 40 ng/mL; lower LOQ, 0.1 ng/mL.

Statistical analysis

The study statistician assigned the initial randomization and performed all of the analyses. Means, medians, SDs, minimum, and maximum values were computed for all variables, as well as differences between means of groups. For all experiments unless otherwise stated, comparisons within groups (within berberine or within placebo) between pre- and posttreatment were evaluated with the two-sided Wilcoxon matched-pairs signed rank test. Differences between groups in change from pre- to posttreatment were evaluated with the Wilcoxon rank-sum test. Data from all 16 observations were included in the analysis.

Results

Between August 2016 and October 2017, 20 participants were consented, 2 were found to be ineligible, 2 withdrew after starting intervention, and 16 went on to complete the study, as shown in **Fig. 1**. Of the two who withdrew after starting intervention, both were receiving berberine, and neither reported adverse events: one was on agent for 2.5 months and was not willing to come to the next study visit, and the other was on agent for 1 month, and withdrew because of changes happening at home.

The characteristics of participants are shown in **Table 1**. No significant differences were observed between placebo and berberine arms for any characteristics. The mean age was 49 and 45 for placebo and berberine arms, respectively, reflecting

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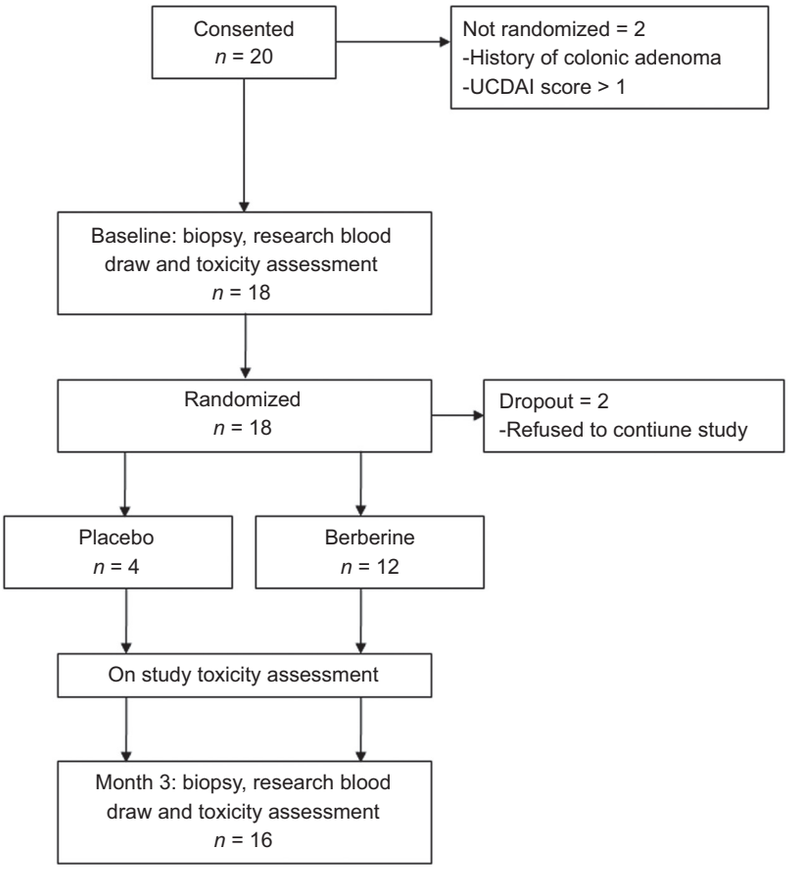

Figure 1.
Consort diagram.

the demographics of ulcerative colitis. The stable clinical status of participants is reflected by the presence of a normal BMI of 22 and an ECOG performance status of 0, with 4 of 4 (100%) and 10 of 12 (83%) participants having a UCDAI score of 1 in placebo and berberine arms, respectively, and the remainder having a score of 0. Although the average duration of disease was 3.0 ± 0.5 (mean ± SEM) and 9.5 ± 4.8 years for berberine and placebo groups, respectively, this was not statistically significant (Student *t* test). In this regard, it is important to consider that there are only four placebo patients, and there was a wide range in the duration their disease: 1.6, 5.9, 9.6, and 20.8 years.

Table 1. Clinical characteristics of participants.

	Placebo	Berberine
Number	4	12
Age, mean (range)	49 (33–55)	45 (24–63)
Gender		
Female, number (%)	2 (50)	6 (50)
Male, number (%)	2 (50)	6 (50)
BMI, mean (range)	22.7 (18.1–27.1)	22.3 (16.3–25)
UCDAI score		
0	0	2
1	4	10
ECOG performance status, mean (range)	0 (0,0)	0 (0,0)
Duration of ulcerative colitis (years), mean (range)	9.5 (1.6–20.8)	3.0 (0.5–5.8)
Baseline symptoms and history, patient reported		
None[a]	3	10
Suspected cholangitis in the past	1	0
Hypertension	0	1
Abdominal distension, epigastric discomfort	0	1

Abbreviation: BMI, body mass index.
[a]Does not include changes in stool.

The primary endpoint of this study was toxicity. Given that this was a chemoprevention study, adverse events of all grades were tracked, and are shown in **Table 2**. A total of 4 and 14 participants on placebo and berberine arms, respectively, were evaluable for adverse events during the three months of time on agent, and the 1-month posttreatment follow-up period. Two participants withdrew from study, at 1 and 2.5 months, both were on the berberine arm, neither reported adverse events, and these participants were not included in the analysis presented in **Table 2**. A total of seven different adverse events were observed in 5 different participants, and all were on the berberine arm. One patient had increased aspartate aminotransferase (AST, grade 2) and increased alanine aminotransferase (ALT, grade 3) observed on a single test performed at month three on agent, both of which were resolved at the follow-up visit a month later. These were thought to be probably related to

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Table 2. Adverse effects.

	Number evaluable[a]	Adverse event	Attrition	Number of patients	Comment
Berberine	12				
		Infection (grade 1)	Not related	1	Upper respiratory tract; resolved
		Pancreatitis (grade 2)	Not related	1	Began 7 days after stopping agent; not resolved at the 1-month post Follow-up
		Increased transaminases: AST (grade 2), ALT (grade 3)	Probably	1	Observed at month three on agent, resolve at 1-month post follow-up
		Diarrhea (grade 1)	Not related	1	Two separate events, each lasting 1 day; participant had abdominal discomfort for 3 years prior to study entry and at baseline
		Nausea (grade 1)	Possible	1	Occurred the day berberine was started; never occurred again
Placebo	4				No adverse events reported

[a]Number evaluable after 3 months. Two participants on berberine withdrew from study at 1 and 2.5 months, did not report any adverse events, and are not included in this analysis.

study treatment. Another patient experienced diarrhea (grade 1) twice: episodes were 1 month apart, each lasted 1 day, and this participant had baseline epigastric discomfort at the time of study entry, which had been present for several years. Thus, the two episodes of diarrhea were deemed to be unrelated to treatment. Another patient experienced a single episode of grade 1 nausea, thought to be possibly related, which was experienced shortly after starting berberine on day 1; it was transient and never occurred again. One patient experienced infection (grade 1, unrelated) and another patient experienced pancreatitis (grade 2, unrelated). All adverse events resolved within the time frame of the study, except pancreatitis, which developed one week after treatment was stopped, and was still present at the one-month follow-up time point.

Measurement of plasma concentrations of berberine demonstrated a significant increase in concentration to 1.16 ± 0.40 ng/mL (mean ± SEM; 3.46 ± 1.19 nmol/L) in the berberine posttreatment group at month 3, compared with either the placebo posttreatment ($P = 0.020$) or berberine pretreatment ($P < 0.001$) groups (**Fig. 2**). The increase in the berberine posttreatment group represented a 6.5-fold increase compared with the placebo posttreatment group and 5.8-fold increase compared with the berberine pretreatment group. Blood draws for measurement of berberine concentration occurred 1.9 ± 0.1 hours (mean ± SEM) after morning dosing.

Secondary endpoints can be categorized as measures of effects on clinical status, tissue histology, tissue IHC, and plasma cytokines, shown in **Table 3**. With the exception of Ki-67, all of these markers provide measures of the state of inflammation. Ki-67 provides a measure of cell proliferation. The UCDAI score provides a measure of the clinical status of a patient with ulcerative colitis (54). There was a 40% and 50% decrease in the UCDAI score in berberine and placebo groups, respectively. Using the nonparametric Wilcoxon matched-pairs signed rank test, this was not statistically significant in either group. Using the parametric Student two-sided paired *t* test, the decrease in the berberine group was significant ($P = 0.04$), whereas that in the placebo group was not. That there are only 4 participants in the placebo group is a factor in lack of significance. The observed decrease in both groups is consistent with the fact that participants on both groups are also being treated with mesalamine.

The Geboes scoring system provides a measure of tissue inflammation (55). There was a 30% decrease in the Geboes score after berberine treatment, and this was statistically significant, **Fig. 3**. In the placebo group, there was a 5% nonsignificant decrease. There was not a significant difference between berberine and placebo groups.

Tissue-based IHC markers consisted of COX-2, NF-κB, and Ki-67 expression in colonic epithelial cells. No significant changes were observed. With Ki-67, the 30% decrease in both placebo and berberine groups at month 3 supported the notion that mesalamine may be mediating a uniform effect. A comparison of all 16 participants (placebo + berberine)

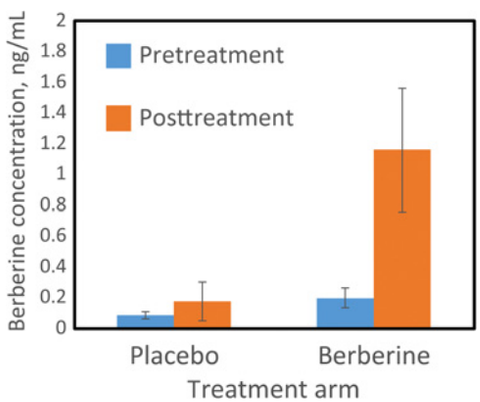

Figure 2.
Plasma concentrations of berberine. Data represent the mean ± SEM plasma concentration of berberine measured at baseline, pretreatment, and after 3 months of treatment with either berberine or placebo, post-treatment. *, P value ≤ 0.05 for groups indicated by line.

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Table 3. Secondary endpoints.

Category of test	Parameter measured	Treatment	Pretreatment Mean ± SEM	Posttreatment Mean ± SEM	Ratio post/pre	*P* value for change Within group[a]	*P* value for change Between groups[b]
Clinical assessment	UCDAI score	Placebo	1.0 ± 0.0	0.5 ± 0.3	0.5	NS	NS
		Berberine	0.8 ± 0.1	0.5 ± 0.2	0.6	NS	
Histologic assessment	Geboes score	Placebo	4.68 ± 0.39	4.45 ± 0.62	0.95	NS	NS
		Berberine	3.19 ± 0.44	2.22 ± 0.47	0.70	0.04	
Tissue IHC	COX-2	Placebo	1.0 ± 0.5	1.5 ± 0.3	1.5	NS	NS
		Berberine	1.6 ± 0.2	2.0 ± 0.2	1.3	NS	
	NF-κB	Placebo	1.8 ± 0.6	2.8 ± 0.3	1.6	NS	NS
		Berberine	2.3 ± 0.5	2.5 ± 0.6	1.1	NS	
	Ki-67	Placebo	2.3 ± 0.3	1.5 ± 0.6	0.7	NS	NS
		Berberine	1.8 ± 0.3	1.3 ± 0.1	0.7	NS	
Plasma cytokines	IL2, pg/mL	Placebo	1.12 ± 0.72	4.32 ± 3.51	3.84	NS	NS
		Berberine	3.79 ± 2.27	3.99 ± 2.00	1.05	NS	
	IL4, pg/mL	Placebo	2.25 ± 0.00	2.25 ± 0.00	1.00	NS	NS
		Berberine	2.56 ± 0.30	2.41 ± 0.16	0.94	NS	
	IL6, pg/mL	Placebo	3.42 ± 0.18	5.22 ± 3.41	1.53	NS	NS
		Berberine	147.48 ± 150.55	4.26 ± 0.94	0.03	NS	
	IL8, pg/mL	Placebo	12.42 ± 5.83	69.33 ± 104.68	5.58	NS	NS
		Berberine	39.68 ± 26.03	34.74 ± 24.87	0.88	NS	
	IL10, pg/mL	Placebo	0.55 ± 0.00	5.03 ± 5.18	9.15	NS	NS
		Berberine	13.42 ± 5.10	15.95 ± 5.40	1.19	NS	
	TNFα, pg/mL	Placebo	58.68 ± 14.90	54.60 ± 2.14	0.93	NS	NS
		Berberine	97.78 ± 38.58	62.81 ± 8.11	0.64	NS	

[a]Wilcoxon matched-pairs signed rank test; NS, not significant (i.e., $P > 0.05$).
[b]Wilcoxon rank-sum test; NS, not significant (i.e., $P > 0.05$).

demonstrated a statistical trend, $P = 0.06$, using the nonparametric Wilcoxon matched-pairs signed rank test, and statistical significance, $P = 0.04$, using the parametric Student two-sided paired *t* test, in Ki-67 expression at month 3, compared with pretreatment.

With respect to plasma cytokines, TNFα, IL6, and IL8 have been linked to proinflammatory activity in the pathogenesis of ulcerative colitis in humans, whereas IL10 and IL4 are considered anti-inflammatory (58). Overall, there were no significant changes, nor were there any apparent trends. The large decrease in IL6 observed with berberine treatment is driven by a single value at the pretreatment time point that was over 500-fold above the mean of the other values. Removal of this outlier yields a post/pretreatment ratio of 1.1 in the berberine group, without statistical significance.

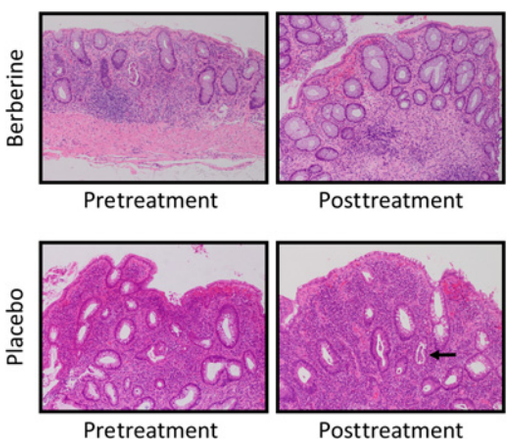

Figure 3.
Representative photomicrographs of colonic mucosa. Representative photomicrographs of H&E-stained tissues were taken under light microscopy. For berberine, the pretreatment slide depicts tissue with a Geboes score of 5.1, showing crypt destruction and heavy influx of inflammatory cells, and the posttreatment slide depicts tissue with a Geboes score of 2.2, showing diffuse architectural abnormalities and moderate influx of inflammatory cells. For placebo, the pretreatment slide depicts tissue with a Geboes score of 4.1, showing crypt destruction and distortion, and heavy influx of inflammatory cells, and the posttreatment slide depicts tissue with a Geboes score of 5.4, showing glandular architectural distortion, extensive influx of inflammatory cells, and crypt abscess formation (arrow).

Discussion

This is the first study to prospectively examine berberine in combination with mesalamine. This study demonstrates that berberine is well tolerated when taken along with mesalamine by Chinese patients with ulcerative colitis. The grade 3 increase in transaminase levels was detected one time in 1 participant. This one-time finding coupled with the fact that berberine is in widespread use in China and has not been associated with altered liver function support the notion that berberine did not have a causal role. In fact, a meta-analysis of the effect of berberine for the treatment of nonalcoholic fatty liver disease, which included over 500 individuals, indicated that berberine had a positive therapeutic effect upon liver function, including decreases in transaminases (59). The single episode of grade 1 transient nausea observed in one participant on the day they started berberine was scored as possibly due to agent, based on

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timing. Considering that this person also had ulcerative colitis, which causes such symptoms and that berberine has been historically used to treat symptoms of the gastrointestinal tract, the causal link between berberine and transient nausea is not at all clear. If further studies did support causality, its mild, transient nature would not be considered prohibitory to use.

One goal of this study was to advance a strategy that minimizes systemic toxicity by developing cancer prevention agents that optimize local delivery to the at-risk target organ but have minimal systemic absorption (47). Berberine possesses these properties as they relate to high exposure to the intestine and poor systemic absorption (48). In the current study, we demonstrate that, in people with ulcerative colitis (i.e., who have an inflamed intestinal lining), mean blood concentrations of berberine are very low after a total daily dose of 900 mg (3.5 nmol/L, or 1.16 ng/mL). These findings approximate the 1.1 nmol/L concentrations previously reported after a total daily dose of 400 mg (51). It is important to consider that the pharmacokinetics of berberine in humans is poorly defined, and that studies involving a comprehensive pharmacokinetic analysis of berberine in rodents yield a range of findings (48–50). These factors notwithstanding, plasma concentrations in the current study were measured on average 1.9 hours postdosing. With rodent studies indicating a time to peak blood concentration of 1.3–2.0 hours postdose, the values measured in the current study most likely approximate peak concentrations. Although reports of berberine's half-life in preclinical studies similarly span a range, that is, 5–14 hours, one report in rats demonstrated that there was no change in cytochrome P450 profiles with chronic administration (60). These findings are consistent with the notion that little changes in berberine pharmacology would be expected with long-term administration in humans. It will be important for future studies to comprehensively evaluate the pharmacokinetics of berberine in humans.

The incorporation of monitoring of drug concentrations into the study design is an important feature, especially when the agent is widely available over the counter. Our analysis of plasma berberine concentrations did not find clear evidence of drop-in or drop-out and demonstrated nearly a 6-fold increase in concentration with treatment, compared with pretreatment values. It is unclear whether the low baseline values of berberine detected in the plasma represents consumption in the diet, and/or use of berberine taken outside of the study, albeit at much lower doses. Future studies, inclusive of those in different populations, should take this into consideration.

In considering findings from this study, particularly those related to secondary endpoints, it is important to consider that only 12 and 4 participants were on the berberine and placebo arms, respectively. These small numbers place inherent limits on data interpretation. The finding of greatest interest relates to the Geboes score. It decreased by 30% with berberine treatment, and this was statistically significant. When compared with placebo, however, effects were not statistically significant. It is important to consider that the low number of placebo participants places significant limits on the rigor of statistical comparison between berberine and placebo groups. Of high interest, there was only a 5% decrease in the Geboes score in placebo participants. Together, these findings should be considered hypothesis generating and support the hypothesis that berberine is exerting an anti-inflammatory effect on the colonic mucosa in people with ulcerative colitis.

Improvements in UCDAI score were seen in both arms, but were not statistically significant using the nonparametric Wilcoxon matched-pairs signed rank test. The magnitude of the decreases and their presence in both groups, that is, a 40% and 50% decrease in berberine and placebo groups, respectively, prompted us to examine significance using the parametric Student two-sided paired *t* test, which demonstrated a significant decrease in the berberine group but not the placebo. However, given that there was a 50% decrease in the placebo group, it is very likely that with increased numbers, this would be statistically significant. As all participants are on mesalamine, it is likely positively affecting participants in both arms. The data are insufficient to conclude that berberine is providing additional benefit.

Tissue-based biomarkers COX-2 and NF-κB relate to inflammation, whereas Ki-67 relates to cell proliferation. No significant changes were observed. With Ki-67, there was a trend for decrease in both groups at month 3, compared with pretreatment, which was statistically significant when evaluated using the parametric Student two-sided paired *t* test. This likely reflected the effect of mesalamine, and the similar changes between placebo and berberine groups do not support the notion that berberine was exerting any additional effect.

The cytokines TNFα, IL6, and IL8 have been linked to proinflammatory activity in the pathogenesis of ulcerative colitis in humans, whereas IL10 and IL4 are considered anti-inflammatory (58). No significant changes in the plasma concentrations of these cytokines were identified in the current study. Given that significant changes in measures of inflammation were observed with other biomarkers, one potential explanation may be that they were altered at the tissue level, but such changes were not observed systemically, potentially supporting the notion of local therapy to the gut with this oral agent that has poor absorption into the circulation. Finally, although not statistically significant, the difference in mean duration of disease between berberine and placebo groups was 6.5 years. Although notable for its magnitude, given only four placebo patients, a relatively wide range of duration, that is, 1.6–20.8 years, and lack of statistical significance, any attribution of significance should be approached with extreme caution. This should be evaluated in future larger studies.

This study demonstrates for the first time, to our knowledge, that it is possible to conduct an early-phase cancer prevention trial in China, with NCI sponsorship. This is important for several reasons. The study of specific populations is of high importance related to understanding cancer biology, prevention, and therapeutic modulation. China, for example, has high esophageal and low prostate cancer rates compared with

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Western countries (1), thereby providing an important opportunity to identify underlying causes. Furthermore, differences in metabolic processes and mechanisms of carcinogenesis, which can impact efficacy and toxicity of therapeutics, justify a cohort-specific examination, as was undertaken in the current study. Conversely, there are practical difficulties related to the study of different populations. With China as an example, they include distance, differences in societal norms and language and meeting legal and institutional requirements across two countries. We overcame these barriers by arranging for a U.S.-based researcher (R. Bergan) to frequently visit the research institution in China, becoming familiar with research practices and researchers. In addition, a member of the China-based team (L. Xu) spent several years in the United States, learning U.S.-based research methods. This study demonstrates that such barriers can be overcome, resulting in potentially important advances.

In summary, we demonstrate the feasibility of conducting an early-phase cancer prevention trial in China. We demonstrate that oral berberine only delivers low nanomolar concentrations to the blood. Berberine is shown to be well tolerated by Chinese with ulcerative colitis receiving mesalamine. Finally, berberine was shown to significantly decrease colonic tissue inflammation, as measured by the Geboes score. These findings warrant continued investigation of berberine as a potential cancer prevention agent for colorectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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