Chronoefficacy of the herbal medicines *Puerariae radix* and *Coptidis rhizoma* in mice: A potential role of REV-ERBα

Jinming Liu
Zhongshan Torch Devlopment Zone Hospital
Haiman Xu
Jinan University
Li Zhang
Jinan University
Shuai Wang
Jinan University
Danyi Lu
Jinan University
Min Chen
Jinan University
Baojian Wu (bj.wu@hotmail.com)
Jinan University

Research

Keywords: Chronoefficacy, dosing time, Puerariae radix, Coptidis rhizoma, hyperhomocysteinemia, chronic colitis

Posted Date: January 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-151541/v1

Abstract

Background

Identifying drugs with circadian time-dependent efficacy (chronoefficacy) and understanding the underlying mechanisms would help to improve drug treatment outcome. Here, we aimed to determine chronoefficacy of the herbal medicines *Puerariae radix* (PR) and *Coptidis rhizoma* (CR), and investigate a potential role of REV-ERBα as a drug target in generating chronoefficacy.

Materials and methods

PR and CR efficacy were assessed based on the diseases hyperhomocysteinemia and chronic colitis, respectively. The efficacy of PR against hyperhomocysteinemia in mice was evaluated by measuring total homocysteine, triglyceride levels and lipid accumulation. The efficacy of CR against chronic colitis in mice was evaluated by measuring disease activity index, colon length, malondialdehyde/myeloperoxidase activities and IL-1β/IL-6 levels. The underlying mechanisms related to REV-ERBα target genes were analyzed by qPCR. Puerarin in PR and berberine in CR were analyzed by UPLC-QTOF/MS.

Results

PR dosed at ZT10 generated a better efficacy against hyperhomocysteinemia than drug dosed at ZT2. Furthermore, PR increased the expressions of REV-ERBα target genes *Bhmt, Cbs* and *Cth* (encoding three key enzymes responsible for homocysteine catabolism), thereby alleviating hyperhomocysteinemia in mice. Moreover, CR attenuated chronic colitis in mice in a circadian time-dependent manner. ZT10 dosing generated a better anti-colitis efficacy as compared to ZT2 dosing. This was accompanied by lower production of colonic inflammatory cytokines (i.e, *Nlrp3*, *IL-1β*, *IL-6*, *Tnf-α* and *Ccl2*, REV-ERBα target genes) in colitis mice at ZT10 dosing. The circadian patterns of PR and CR effects were respectively consistent with those of puerarin (a main active constituent of PR, a REV-ERBα antagonist) and berberine (a main active constituent of CR, a REV-ERBα agonist).

Conclusion

The therapeutic effects of PR and CR depend on dosing time in mice, which are probably attributed to circadian expression of REV-ERBα as the drug target. Our findings have implications for improving therapeutic outcomes of herbal medicines with a chronotherapeutics approach.

Background

Homocysteine (Hcy) is a sulfur-containing amino acid derived from the methionine metabolism. It exists in both free and protein-bound forms, and can be oxidized in plasma to the disulfides Hcy-Hcy and Hcy-cysteine [1]. Free and protein-bound Hcy and its disulfides are globally referred to as total homocysteine (tHcy) [1]. The intracellular metabolism of Hcy occurs through two major pathways, namely, remethylation and transsulfuration. Deficiency of the metabolic enzymes [e.g, betaine homocysteine methyltransferase (BHMT), cystathionine β-synthase (CBS), and cystathionine g-lyase (CTH)] involved in these two pathways can lead to hyperhomocysteinemia [2]*. Elevated Hcy is considered to be an independent risk factor for cardiovascular and cerebrovascular diseases such as stroke and dementia, and therefore represents a major health problem* [3,4]*. Puerariae radix (PR, named "Gegen" in Chinese), the root of Pueraria lobata (Wild.) Ohwi, is a commonly used crude herb in oriental medicine. It is used clinically to treat various cardiovascular diseases such as hypertension, angina pectoris and type 2 diabetes mellitus* [5]*.In our recent study, puerarin, a major active ingredient of PR, alleviates hyperhomocysteinemia in a dosing time-dependent manner* [6]*.* However, it remains to be determined whether PR can be used to combat *hyperhomocysteinemia.*

Inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease, are chronic relapsing idiopathic diseases characterized by epithelial barrier damage and disruption of inflammatory homeostasis in the intestinal tract [7,8]*. Abdominal pain, diarrhea, rectal bleeding and weight loss are the most common symptoms in IBD patients* [9,10]*. Although the pathogenic mechanism of IBD remains largely unknown, it is generally believed that the disease is associated with dysregulated immune responses* [11]*. During the development of IBD, a number of proinflammatory molecules [e.g, tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), and interleukin-6 (IL-6)] are expressed at higher levels* [12,13]*. These cytokines have been shown to play important roles in mediating inflammatory responses. Currently, the main drugs for IBD management are anti-inflammatory agents, immune system suppressors and antibiotics. However, these drugs have some limitations such as poor treatment outcome and major adverse effects* [14]*. Therefore, novel strategies are clearly needed to improve treatment outcome for IBD.*

Coptidis rhizoma (CR), also known as Huanglian in China, is the dried rhizome of medicinal plants from the family Ranunculaceae, including Coptis chinensis Franch (Weilian in Chinese), C. deltoidea C.Y. Cheng et Hsiao (Yalian in Chinese), and C. teeta Wall (Yunlian in Chinese) [15,16-17]*.It is a well-known herbal medicine and commonly used to treat digestive diseases (e.g, diarrhoea and chronic colitis), respiratory diseases (e.g, tuberculous empyema and whooping cough), paediatric diseases (e.g, dyspepsia and hyperthermia of infantile external sensation), and dermatological diseases (e.g, acne, psoriasis, and dermatitis) [17,18]*. To date, over 100 chemical constituents have been isolated and identified from CR. Alkaloids such as berberine are the most abundant among these chemical components and are regarded as the main active ingredients of CR [17]. In fact, berberine has been developed as a drug to treat gastrointestinal diseases including acute enteritis, diarrhea and colitis [19][20]-[21]. In a recent study, we

revealed circadian pharmacological effects of berberine on chronic colitis in mice [22]. However, it remains unknown whether CR effects on colitis are circadian time-dependent or not.

Physiology and behaviors of organisms are subjected to circadian (~24 h) rhythms caused by the rotation of Earth around its own axis [23]. Studies over the last two decades have identified components of the endogenous circadian clock systems that govern ~24 h rhythms that are persistent even in the absence of external cues (e.g, light) [24]. The circadian clock systems, consisting of several negative feedback loops, are present in hypothalamic suprachiasmatic nuclei (SCN) as well as in most peripheral tissues (e.g, liver and intestine). The core feedback loop comprises the core clock genes *BMAL1* (brain and muscle Arnt-like protein-1) and *CLOCK* (circadian locomotor output cycles kaput), which form a heterodimer and transcriptionally regulate rhythmic expressions of clock-controlled genes (CCGs) including *PER* (period), *CRY* (cryptochrome), and *REV-ERBα* [6]. The SCN central clock, entrained by time cues (e.g, light), synchronizes the clocks in peripheral tissues via neural and endrocrine signals [25]. REV-ERBα is a component of circadian clockwork and a ligand-responsive nuclear receptor. The synthetic compounds SR9009, SR8278 and GSK4112 as well as the natural compounds puerarin and berberine have been identified as REV-ERBα ligands [6,22].

Chronotherapy, an emerging concept in the field of therapeutics, refers to a treatment strategy that aims to improve the overall control of a disease and to minimize side effects by altering the timing of medication [26]. It thus provides an approach to improve pharmacotherapy by administering drugs at the time-of-day when they are best effective and/or lowest toxic. Circadian variations in drug targets, severity of disease and drug pharmacokinetics are thought to be important factors affecting the efficacy of drugs [6,22]. For instance, circadian expression of the drug target REV-ERBα accounts for chronoefficacy of the drugs such as puerarin and berberine [6,22]. In the present study, we aims to determine circadian pharmacological effects of PR and CR, two common herbal medicines. PR and CR efficacy were assessed based on the diseases *hyperhomocysteinemia and chronic colitis*, respectively. We for the first time unraveled circadian time-dependent efficacy (chronoefficacy) of PR and CR, which is potentially attributed to circadian expression of the drug target REV-ERBα. It is proposed that therapeutic outcomes of PR and CR efficacy may be improved via timed delivery.

Materials And Methods

Materials

Puerariae radix (PR) and *Coptidis rhizoma* (CR) were obtained from the First Affiliated Hospital of Jinan University (Guangzhou, China). Puerarin and L-methionine (also referred as methionine in this paper) were purchased from Aladdin Chemicals (Shanghai, China). Berberine was purchased from TopScience Co. (Shanghai, China). Dextran sulfate sodium (DSS, molecular weight of 36-50 kDa) was purchased from MP Biomedicals (Irvine, CA). Assay kits for total homocysteine (tHcy), triglyceride (TG), malondialdehyde (MDA), and myeloperoxidase (MPO) were purchased from Jiancheng Bioengineering

Institute (Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) kits for IL-1β and IL-6 were purchased from Neobioscience Technology Company (Shenzhen, China).

Animals

Wild-type C57BL/6 mice (male, 5-6 weeks of age) were purchased from HFK Biotechnology (Beijing, China). Mice were maintained under a 12 h light/12 h dark cycle {lights on at 7:00 AM [=Zeitgeber time 0 (ZT0)] and lights off at 7:00 PM (=ZT12)}, with free access to food and water.

Extraction of herbal medicines

Extracts of PR and CR were prepared according to previously reported methods [27,28]. Concentrated extractions were stored at 4°C before use. Puerarin accounts for 2.69% (w/w) of total PR exact, and berberine accounts for 4.66% (w/w) of total CR exact (Fig. 1).

Sample preparation for qualitative analysis

Approximately 0.4 g of PR and CR extracts were dissolved in 10 ml 50% methanol (*v/v*), followed by vortex and centrifugation (13,000 g, 15 min). The supernatant was filtered through 0.22 μm membranes, and then diluted to appropriate concentration (i.e, 2 mg/ml) for analysis.

UPLC-QTOF/MS analysis

Puerarin in PR and berberine in CR were quantified as previously described [6,22]. Peak areas of puerarin and berberine were recorded with extract masses of *m/z* 417.05 ± 0.05 Da and 336.06 ± 0.05 Da, respectively. Representative extracted ion chromatograms are provided in Figure 1.

Methionine-induced hyperhomocysteinemia and drug treatment

Hyperhomocysteinemia was induced by feeding mice with 0.5% methionine in drinking water for eight weeks as previously described [6]. Normal control mice were provided with pure drinking water. Hyperhomocysteinemia mice were divided into eight groups randomly (n = 5 per group) to assess the effects of PR on hyperhomocysteinemia. For dose-response study, four groups of mice were respectively gavaged with 1 g/kg PR, 2 g/kg PR, 3 g/kg PR and vehicle at ZT10 once a day for 7 days. On day 8, mice were sacrificed at ZT2 to collect plasma and liver samples. For chronoefficacy study, the remaining groups of mice were gavaged with 2 g/kg PR or vehicle at ZT2 or ZT10 per day for 7 days. On day 8, mice were sacrificed at ZT2 to collect plasma and liver samples.

DSS-induced colitis and treatment

Chronic colitis was induced in mice via three cycles of 2% DSS (in drinking water) feeding as previously described [22]. Control mice were fed with pure drinking water. The colitis mice were divided into eight groups (namely, groups 1-8, *n* = 6 per group). Group 1, group 2 and group 3 of mice were respectively gavaged with 100, 200 and 400 mg/kg CR at ZT10 once a day for 7 days. Group 4 of mice were treated with vehicle. On day 8, CR-, vehicle-treated and normal control mice were sacrificed at ZT2 to collect plasma and colon samples. The remaining groups of mice were gavaged with 400 mg/kg CR or vehicle at ZT2 or ZT10 once a day for 7 days. On day 8, mice were sacrificed at ZT2 to collect plasma and colon samples.

Biochemical analysis

Plasma and hepatic tHcy levels were quantified by performing enzymatic cycling assays with a homocysteine kit according to the manufacturer's protocol (Jiancheng Bioengineering Institute, Nanjing, China). Plasma and hepatic TG levels were measured using a TG assay kit according to the manufacturer's protocol (Jiancheng Bioengineering Institute, Nanjing, China). Colonic MDA and MPO activities were measured using malondialdehyde and myeloperoxidase assay kits according to the manufacturer's protocol (Jiancheng Bioengineering Institute, Nanjing, China). Colonic IL-1β and IL-6 levels were quantified with ELISA kits according to the manufacturer's protocol (Neobioscience, Shenzhen, China).

Oil red O staining

Oil red O staining was performed to analyze hepatic lipid accumulation as previously described [6]. In brief, liver samples were fixed in 10% paraformaldehyde and embedded in paraffin. Paraffin sections (10 μm) were sequentially stained with oil red O and hematoxylin. Images were obtained using a Zeiss Axio Imager M1 microscope (Carl Zeiss AG, Oberkochen, Germany).

Macroscopic scoring of colitis

Changes in body weight, diarrhea and bleeding in colitis mice were recorded on a daily basis after drug treatment. Scoring was performed according to the criteria described previously [29]. Body weight changes were calculated relative to day 1. Disease activity index (DAI) is the sum of the weight loss, diarrhea, and bloody stool scores. After the mice were sacrificed, colons lengths (as an indirect marker of colonic inflammation) were immediately measured with a centimeter ruler.

H&E staining

Colon tissues were fixed in 4% paraformaldehyde and embedded in paraffin, followed by hematoxylin-eosin (H&E) staining. Histological damage was scored based on enterocyte loss, crypt inflammation, *lamina propria* mononuclear cells, neutrophils infiltration, and epithelial hyperplasia as previously described [30]. The total histological score ranged from 0 (no changes) to 6 (extensive cell infiltration and tissue damage).

qPCR

qPCR assays were performed as described in our previous publication [31]. Amplification reaction consisted of an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. Primers are listed in Table 1.

Statistical analysis

Data are presented as mean ± SD (standard deviation). Student's t test was used to test for statistical differences between two groups. One-way or two-way ANOVA followed by Bonferroni post hoc test was used for multiple group comparisons. The level of significance was set at $p < 0.05$ (*).

Results

PR dose-dependently alleviates hyperhomocysteinemia in mice

Hyperhomocysteinemia in mice was induced by feeding 0.5% methionine in drinking water for 8 weeks as described in our prior study (Fig. 2A) [6]. Mice with hyperhomocysteinemia showed increased circulating and hepatic levels of both tHcy and TG, suggesting successful construction of hyperhomocysteinemia model (Fig. 2B/C). Elevated lipid accumulation in the liver was confirmed by oil red O staining (Fig. 2D). Puerarin, a major *constituent of PR, was shown to possess anti-*hyperhomocysteinemia effect in our prior study [6]. We thus investigated whether PR alleviates hyperhomocysteinemia. The daily therapeutic dose of PR is 10-15 g for adults (Chinese Pharmacopoeia, 2015). According to dose translation between humans and mice, the equivalent dose for mice was estimated to be 2.05-3.075 g/kg per day [32]. Therefore, three different doses (i.e, 1, 2 and 3 g/kg) were selected to test the effects of PR on hyperhomocysteinemia. Hyperhomocysteinemia mice were treated with RP or vehicle by oral gavage at ZT10 once a day for 7 days (Fig. 1A). PR significantly decreased the levels of plasma and hepatic tHcy in a dose-dependent manner (Fig. 2B). In the meantime, plasma and hepatic levels of TG were reduced by PR (Fig. 2C). Reduction of hepatic lipid accumulation by PR (2 g/kg) in hyperhomocysteinemia mice was illustrated by oil red O staining (Fig. 2D). Altogether, these data indicate alleviation of hyperhomocysteinemia by PR in mice.

Chronoefficacy of PR against hyperhomocysteinemia in mice

We next examined potential effects of dosing time on PR efficacy because the major constituent puerarin displays circadian time-dependent efficacy (chronoefficacy) [6]. To this end, hyperhomocysteinemia mice were treated with PR (2 g/kg, once daily for 7 days) at each of two circadian time points ZT2 (corresponding to morning) and ZT10 (corresponding to evening). PR dosed at either ZT2 or ZT10 decreased the plasma and hepatic levels of both tHcy and TG (Fig. 3). However, ZT10 dosing generated a superior efficacy (reflected by higher percentages of change in tHcy and TG) as compared to ZT2 dosing, indicating chronoefficacy of PR against hyperhomocysteinemia (Fig. 3). The time-varying effect of PR was in full agreement with that of puerarin, the main active compound of PR [6]. Puerarin acts on the circadian protein REV-ERBα (whose expression oscillates with times of the day with a higher level at ZT10 and a lower level at ZT2) as an antagonist to increase hepatic expressions of three key enzymes involved in homocysteine catabolism (i.e, BHMT, CBS and CTH), thereby alleviating hyperhomocysteinemia in mice [2,6]. Because the drug target is an oscillating protein, puerarin displays a chronoefficacy (with a superior efficacy at ZT10 than at ZT2). We thus determined the expressions of hepatic *Bhmt, Cbs* and *Cth* in PR- or vehicle-treated hyperhomocysteinemia mice. PR treatment dose-dependently increased the levels of *Bhmt, Cbs* and *Cth* in hyperhomocysteinemia mice (Fig. 4A). Moreover, ZT10 dosing generated higher levels of *Bhmt, Cbs* and *Cth* than at ZT2 dosing in hyperhomocysteinemia mice (Fig. 4B). The higher *Bhmt, Cbs* and *Cth* expressions for ZT10 dosing may contribute to the stronger drug effects. Taken together, the efficacy of PR against hyperhomocysteinemia is dosing time-dependent in mice. This is potentially attributed to circadian expression of the drug target REV-ERBα.

CR dose-dependently attenuates chronic colitis in mice

To assess the therapeutic efficacy of CR against chronic colitis, we established mouse model with DSS-induced chronic colitis following a published protocol (Fig. 5A) [22]. The mice showed significant loss of body weight after the second cycle of DSS treatment (Fig. 5A). After three cycles of DSS administration, mice with colitis showed reduced colon length (a marker of intestinal inflammation) as well as increased levels of DAI, MDA (a measure of the colonic oxidative insult), MPO (an index of neutrophil accumulation), and inflammatory factors (IL-1β and IL-6) (Fig. 5B/C/D). The colonic injury in colitis mice was also confirmed by H&E staining (Fig. 5E). As expected, CR treatment dose-dependently increased the colon length in colitis mice (Fig. 5B). Moreover, CR-treated colitis mice exhibited a decreased DAI score compared with vehicle-treated mice (Fig. 5C). In addition, biochemical assessments showed that colonic MDA and MPO activities as well as colonic IL-1β and IL-6 levels were dose-dependently reduced by CR (Fig. 5D). Alleviation of colitis by CR was further verified by histopathological analysis of the colon sections (Fig. 5E). To be specific, enterocyte loss, crypt inflammation, *lamina propria* mononuclear cells, neutrophils infiltration, and epithelial hyperplasia were significantly lower in CR-treated than in vehicle-

treated colitis mice (Fig. 5E) [30]. Taken together, these findings indicate that CR can reduce the severity of chronic colitis in mice.

Chronoefficacy of CR against chronic colitis in mice

We next examined potential effects of dosing time on CR efficacy because the major constituent berberine displays circadian time-dependent efficacy [22]. To this end, colitis mice were gavaged with CR (400 mg/kg, once daily for 7 days) at ZT2 or ZT10. CR dosing at ZT2 and at ZT10 significantly decreased colonic MDA and MPO activities (Fig. 6A/B). Additionally, CR dosed at either ZT2 or ZT10 reduced the levels of IL-1β and/or IL-6, (Fig. 6C/D). In order to assess circadian pharmacological effects of CR, the percentages of change in colonic MDA and MPO activities as well as IL-1β and IL-6 levels were calculated for each timed treatment. We found that the anti-colitis effects (positively related to the percentages of change) of CR were stronger when the herbal medicine was dosed at ZT10, and were weaker when drug was dosed at ZT2 (Fig. 6). Therefore, CR attenuates chronic colitis in a circadian time-dependent manner in mice with a better efficacy at ZT10.

Berberine acts on the circadian protein REV-ERBα as an agonist to restrain colonic inflammation via intercepting NF-κB signaling and NLRP3 inflammasome activation [22,[33]]. Because the drug target is an oscillating protein, berberine displays a chronoefficacy against chronic colitis (with a superior efficacy at ZT10 than at ZT2). To determine whether the circadian anti-colitis effects of CR in mice are associated with berberine and EV-ERBα, we examined the mRNA levels of REV-ERBα target genes (i.e, *Nlrp3, IL-1β, IL-6, Tnf-α,* and *Ccl2)* which are key inflammatory cytokines in colon tissue [8,22]. CR dose-dependently down-regulated *Nlrp3, IL-1β, IL-6, Tnf-α* and *Ccl2* mRNA levels in colitis mice (Fig. 7A). Moreover, CR (400 mg/kg) dosing at ZT2 and ZT10 caused decreases in *Nlrp3, IL-1β, IL-6, Tnf-α* and *Ccl2* mRNAs (Fig. 7B). However, the changes were more evident for ZT10 dosing (Fig. 7B). This may contribute to the superior anti-colitis effects of CR at ZT10 consistent with the expression of REV-ERBα (a higher expression at ZT10 than at ZT2) and the circadian berberine effects. Overall, the efficacy of CR against chronic colitis is dosing time-dependent in mice, which is potentially attributed to circadian expression of the drug target REV-ERBα.

Discussion

In this study, we have observed chronoefficacy of PR against hyperhomocysteinemia in mice (Fig. 3). PR dosed at ZT10 generated a better efficacy against hyperhomocysteinemia than drug dosed at ZT2 (Fig. 3). Moreover, PR increased the expression levels of REV-ERBα target genes *Bhmt*, *Cbs* and *Cth* (three key genes involved in homocysteine catabolism), thereby down-regulating homocysteine level and alleviating hyperhomocysteinemia in mice (Fig. 4). We also found that CR attenuates chronic colitis in mice in a circadian time-dependent manner (Fig. 6). ZT10 dosing generated a stronger anti-colitis effect as compared to ZT2 dosing (Fig. 6). This is accompanied by lower production of colonic inflammatory cytokines (i.e, *Nlrp3*, *IL-1β*, *IL-6*, *Tnf-α* and *Ccl2*, all these are REV-ERBα target genes) in ZT10-treated than

in ZT2-treated colitis mice (Fig. 7). In our previous studies, puerarin (a main active constituent of PR) acts on the circadian protein REV-ERBα as an antagonist to alleviate hyperhomocysteinemia, whereas berberine (a main active constituent of CR) acts on REV-ERBα as an agonist to attenuate chronic colitis [6,22]. The circadian patterns of PR and CR effects are respectively consistent with those of puerarin and berberine [6,22]. Therefore, we propose that the time-varying PR and CR effects may be attributed to the rhythmic expression of REV-ERBα as the drug target of the two herbal medicines.

The findings from current and our previous studies support the notion that targeting REV-ERBα represents a promising approach for prevention and management of colitis and hyperhomocysteinemia [6,22,31]. Besides these two types of diseases, REV-ERBα has been implicated in the development of many other disorders such as hypercholesterolemia, obesity, dyslipidemia, and diabetes [2,[34],[35]]. It was thus reasonable to postulate that PR and CR may also show pharmacological effects on these REV-ERBα-regulated diseases because they can alter REV-ERBα activities via their chemical constituents including puerarin and berberine. However, whether this is the case or note awaits further investigations.

Searching for the most appropriate timing for drug administration is the main goal of chronotherapeutics. Chronotherapy schedules with optimal dosing times have been developed and shown to be more effective than conventional treatment for various diseases such as cancers, cardiac and vascular disease [[36],[37]]. IBD is a chronic relapsing idiopathic disease characterized by epithelial barrier damage and inflammatory homeostasis disruption in the intestinal tract [7,8]. The pathogenesis of IBD remains poorly understood, and pharmacotherapy for the disease is far from optimal. In this regard, chronotherapy may provide a new means to improve treatment outcome against colitis. This is based on the facts that colitis displays a circadian rhythm in disease severity and that the efficacy of drugs (both berberine and CR) depends on the dosing time (Fig. 6) [22]. However, it is acknowledged that chronoefficacy of CR and its active constituent berberine requires validations in humans as current findings are based on experimental animals.

Anti-hyperhomocysteinemia effects of PR were assessed by measuring body tHcy and TG levels as previously reported [6]. We used the doses of PR extract at 1, 2 and 3 g/kg based on dose translation between humans and mice [32]. PR dose-dependently decreased the plasma and hepatic levels of both tHcy and TG in hyperhomocysteinemia mice (Fig. 2). Although changes in tHcy and TG between 3 g/kg PR and 2 g/kg PR treatment are noted, the changes are not statistically significant (Fig. 2). This suggests potential saturation in the pharmacological effects of PR. The exact reasons why this occur are unknown. However, it is speculated that the saturation in solubility (i.e, the active ingredients may be not fully dissolved in the gastrointestinal tract at the dose of 3 g/kg) may play a contributing role.

In the present study, CR doses (100, 200, and 400 mg/kg) and treatment duration (7 days) for efficacy assessment against chronic colitis in mice were formulated according to a previous report [[38]]. The efficacy of CR against chronic colitis was accessed based on DAI, colon length, MDA/MPO activities and IL-1β/IL-6 levels as well as histopathological analysis. The parameters for DAI scoring here are comprehensive functional measures that are somewhat analogous to clinical symptoms observed in

human IBD, and the scoring method has been validated by prior studies [39]. Colon length is significantly shortened in colitis mice, and represents a marker of intestinal inflammation. MPO is an enzyme existing in neutrophil leucocytes and has been found to be a reliable marker for the neutrophil accumulation in inflamed tissues [40]. MDA is a product derived from the lipoperoxidative processes that take place as a consequence of the colonic oxidative insult [22,40]. The MDA level is much higher in colitis and often times used as an index of oxidative status in colitis [22].

Conclusion

In conclusion, the efficacy of the herbal medicines PR and CR depends on dosing time in mice. The chronoefficacy is probably attributed to circadian expression of REV-ERBα as the drug target. Our findings have implications for improving therapeutic outcomes of herbal medicines with a chronotherapeutics approach.

Abbreviations

BMAL1, brain and muscle Arnt-like protein-1; BHMT, betaine homocysteine methyltransferase; CBS, cystathionine β-synthase; CTH, cystathionine γ-lyase; *CLOCK*, circadian locomotor output cycles kaput; CR, *Coptidis rhizoma*; *CRY*, cryptochrome; DAI, disease activity index; DSS, dextran sulfate sodium; IBD, inflammatory bowel diseases; IL-1β, interleukin-1beta; IL-6, interleukin-6; MDA, malondialdehyde; MPO, myeloperoxidase; *PER*, period; PR, *Puerariae radix*; tHcy, total homocysteine; TG, triglyceride; TNF-α, tumor necrosis factor-alpha; ZT, zeitgeber time.

Declarations

Acknowledgements

Not applicable.

Fundings

This work was supported by the National Natural Science Foundation of China (No. 81722049) and the Guangzhou Science and Technology Project (No. 201904010472).

Author contributions

Participated in research design: Liu, Xu, Chen and Wu.

Conducted experiments: Liu, Xu, Chen and Zhang

Performed data analysis: Liu, Xu, Wang, Lu, Chen and Wu.

Wrote the manuscript: Chen and Wu

Ethics approval and consent to participate

All animal experiments were performed using protocols approved by the Institutional Animal Care and Use Committees of Guangzhou University of Chinese Medicine (Appr. date: 2020-09-09; IACUC Issue No:ZYD-2020-64).

Availability of data and materials

The datasets used in this study are available from the corresponding author upon reasonable request.

Consent for publication

Not applicable.

Conflict of interest

The authors report no conflict of interest.

References

1. Cattaneo M. Hyperhomocysteinemia, atherosclerosis and thrombosis. Thromb Haemost. 1999;81(2):165–76.
2. Zhang T, Chen M, Guo L, Yu F, Zhou C, Xu H, et al. Reverse erythroblastosis virus α antagonism promotes homocysteine catabolism and ammonia clearance. Hepatology, 2019;70(5),1770–1784.
3. Mayer E, Jacobsen D, Robinson, K. Homocysteine and coronary atherosclerosis. J Am Coll Cardiol. 1996;27(3):517–27.
4. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. The New England journal of medicine, 2002;346(7),476–483.
5. Zhang Z, Lam TN, Zuo Z. Radix Puerariae: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. Journal of clinical pharmacology, 2013;53(8),87–811.
6. Chen M, Zhou C, Xu H, Zhang T, Wu, B. Chronopharmacological targeting of Rev-erbα by puerarin alleviates hyperhomocysteinemia in mice. Biomedicine & pharmacotherapy, 2020;125,109936.
7. Gupta RB, Harpaz N, ItzkowitzmS, Hossain S, Matula S, Kornbluth A, et al.Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. Gastroenterology, 2007;133(4),1099–1341.

8. Wang L, Xie H, Xu L, Liao Q, Wan S, Yu Z,et al. rSj16 Protects against DSS-Induced Colitis by Inhibiting the PPAR-α Signaling Pathway. Theranostics, 2017;7(14),3446–3460.
9. Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. Gastroenterology, 2011;140(6),1785–1794.
10. Sandborn WJ, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, et al. Subcutaneous golimumab maintains clinical response in patients with moderate-to-severe ulcerative colitis. Gastroenterology, 2014;46(1),96-109.
11. De Souza HS, Fiocchi C. Immunopathogenesis of IBD: current state of the art. Nature reviews. Gastroenterology & hepatology,2016;13(1),13–27.
12. Hart AL, Al-Hassi HO, Rigby RJ, Bell SJ, Emmanuel AV, Knight SCet al. Characteristics of intestinal dendritic cells in inflammatory bowel diseases. Gastroenterology, 2005;129(1),50–65.
13. Brand S, Beigel F, Olszak T, Zitzmann K, Eichhorst ST, Otte JM, et al. IL-22 is increased in active Crohn's disease and promotes proinflammatory gene expression and intestinal epithelial cell migration. American journal of physiology. Gastrointestinal and liver physiology, 2006;290(4),G827–G838.
14. Baumgart DC, Sandborn, WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet (London, England), 2007;369(9573),1641–1657.
15. Ma BL, Yao MK, Zhong J, Ma YM, Gao CL, Wu JS, et al. Increased systemic exposure to rhizoma coptidis alkaloids in lipopolysaccharide-pretreated rats attributable to enhanced intestinal absorption. Drug metabolism and disposition: the biological fate of chemicals,2012;40(2), 381–388.
16. Chen J, Zhao H, Wang X, Lee FS, Yang H, Zheng L. Analysis of major alkaloids in Rhizoma coptidis by capillary electrophoresis-electrospray-time of flight mass spectrometry with different background electrolytes. Electrophoresis, 29(10), 2008;2135–2147.
17. Wang J, Wang L, Lou GH, Zeng HR, Hu J, Huang QW, et al. Coptidis Rhizoma: a comprehensive review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. Pharmaceutical biology, 2019;57(1),193–225.
18. Wu S, Xu L, Liu H, Tong X. Clinical application and dosage of Coptidis Rhizoma. Chin Clin Doctor, 2015;43,92–94.
19. Zhou H, Mineshita, S. The effect of berberine chloride on experimental colitis in rats in vivo and in vitro. The Journal of pharmacology and experimental therapeutics, 2000; 294(3), 822–829.
20. Tang J, Feng Y, Tsao S, Wang N, Curtain R, Wang Y. Berberine and Coptidis rhizoma as novel antineoplastic agents: a review of traditional use and biomedical investigations. Journal of ethnopharmacology, 2009;126(1),5–17.
21. Lee IA, Hyun YJ, Kim DH. Berberine ameliorates TNBS-induced colitis by inhibiting lipid peroxidation, enterobacterial growth and NF-κB activation. European journal of pharmacology, 2010;648(1-3),162–170.
22. Zhou Z, Lin Y, Gao L, Yang Z, Wang S, Wu B. Circadian pharmacological effects of berberine on chronic colitis in mice: Role of the clock component Rev-erbα. Biochemical pharmacology,

2020;172,113773.

23. Foster RG, Roenneberg T. Human responses to the geophysical daily, annual and lunar cycles. Current biology : CB, 2008;18(17),R784–R794.
24. Reppert, S. M, & Weaver, D. R. (2002). Coordination of circadian timing in mammals. Nature, 418(6901), 935–941.
25. Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annual review of physiology, 2010;72,517–549.
26. Kaur G, Phillips C, Wong K, Saini, B. Timing is important in medication administration: a timely review of chronotherapy research. International journal of clinical pharmacy, 2013;35(3),344–358.
27. Paradkar PN, Blum PS, Berhow MA, Baumann H, Kuo SM. (). Dietary isoflavones suppress endotoxin-induced inflammatory reaction in liver and intestine. Cancer letters, 2004;215(1), 21–28.
28. Chang BY, Lee DS, Lee JK, Kim YC, Cho HK, Kim SY. Protective activity of kudzu (Pueraria thunbergiana) vine on chemically-induced hepatotoxicity: in vitro and in vivo studies. BMC complementary and alternative medicine, 2015;16(1), 1-8.
29. Sann H, von Erichsen J, Hessmann M, Pahl A, Hoffmeyer A. Efficacy of drugs used in the treatment of IBD and combinations thereof in acute DSS-induced colitis in mice. Life sciences, 2013;92(12),708–718.
30. Song Y, Kim YR, Kim SM, Ain QU, Jang K, Yang CS,et al. RNAi-mediated silencing of TNF-α converting enzyme to down-regulate soluble TNF-α production for treatment of acute and chronic colitis. Journal of Controlled Release, (2016;239, 231–241.
31. Guo L, Yu F, Zhang T, Wu B. The clock protein Bmal1 regulates circadian expression and activity of sulfotransferase 1a1 in mice. Drug Metabolism and Disposition, 2018;46(10),1403–1410.
32. Nair A, Morsy MA, Jacob S. Dose translation between laboratory animals and human in preclinical and clinical phases of drug development. Drug development research, 201879(8), 373-382.
33. Wang S, Lin Y, Yuan X, Li F, Guo L, Wu, B. REV-ERBα integrates colon clock with experimental colitis through regulation of NF-κB/NLRP3 axis. Nature communications, 2018;9(1), 1-12.
34. Yuan X, Dong D, Li Z, Wu B. Rev-erbα activation down-regulates hepatic Pck1 enzyme to lower plasma glucose in mice. Pharmacological Research, 2019;141,310-318.
35. Zhang T, Zhao M, Lu D, Wang S, Yu F, Guo L, et al. REV-ERBα regulates CYP7A1 through repression of liver receptor homolog-1. Drug Metabolism and Disposition, 2018;46(3),248-258.
36. Altinok A, Lévi F, Goldbeter A. Identifying mechanisms of chronotolerance and chronoefficacy for the anticancer drugs 5-fluorouracil and oxaliplatin by computational modeling. european journal of pharmaceutical sciences, 2009;36(1), 20–38.
37. Smolensky MH, Hermida RC, Geng YJ. Chronotherapy of cardiac and vascular disease: timing medications to circadian rhythms to optimize treatment effects and outcomes. Current Opinion in Pharmacology, 2020;57,41-48.

38. Jeong JJ, Jang SE, Hyam SR, Han MJ, Kim DH. The rhizome mixture of Anemarrhena asphodeloides and Coptidis chinensis ameliorates acute and chronic colitis in mice by inhibiting the binding of lipopolysaccharide to TLR4 and IRAK1 phosphorylation. Evidence-Based Complementary and Alternative Medicine, 2014.
39. Murthy S, Murthy NS, Coppola D, Wood DL. The efficacy of BAY y 1015 in dextran sulfate model of mouse colitis. Inflammation research,1997;46(6),224-233.
40. Wang X, Zhao L, Han T, Chen S, Wang J. Protective effects of 2, 3, 5, 4'-tetrahydroxystilbene-2-O-beta-d-glucoside, an active component of Polygonum multiflorum Thunb, on experimental colitis in mice. European Journal of Pharmacology, 2008;578(2-3), 339-348.

Figures

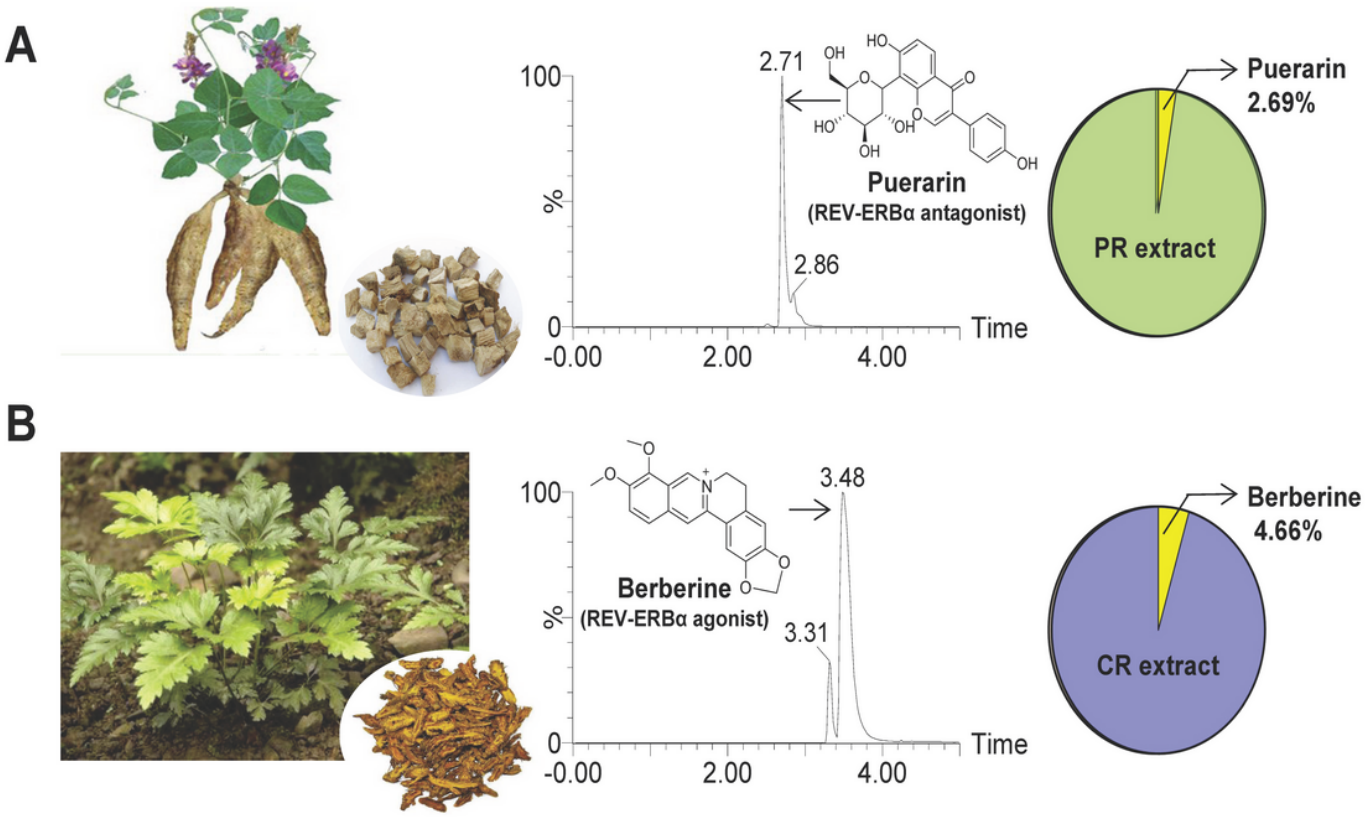

Figure 1

Characterization of PR and CR. (A) Whole plant and rhizome of PR (left), representative extracted ion chromatogram of puerarin (middle) and the percentage of puerarin in PR extract derived from UPLC-QTOF/MS analysis (right). (B) Whole plant and rhizome of CR (left), representative extracted ion chromatogram of berberine (middle) and the percentage of berberine in CR extract derived from UPLC-QTOF/MS analysis (right).

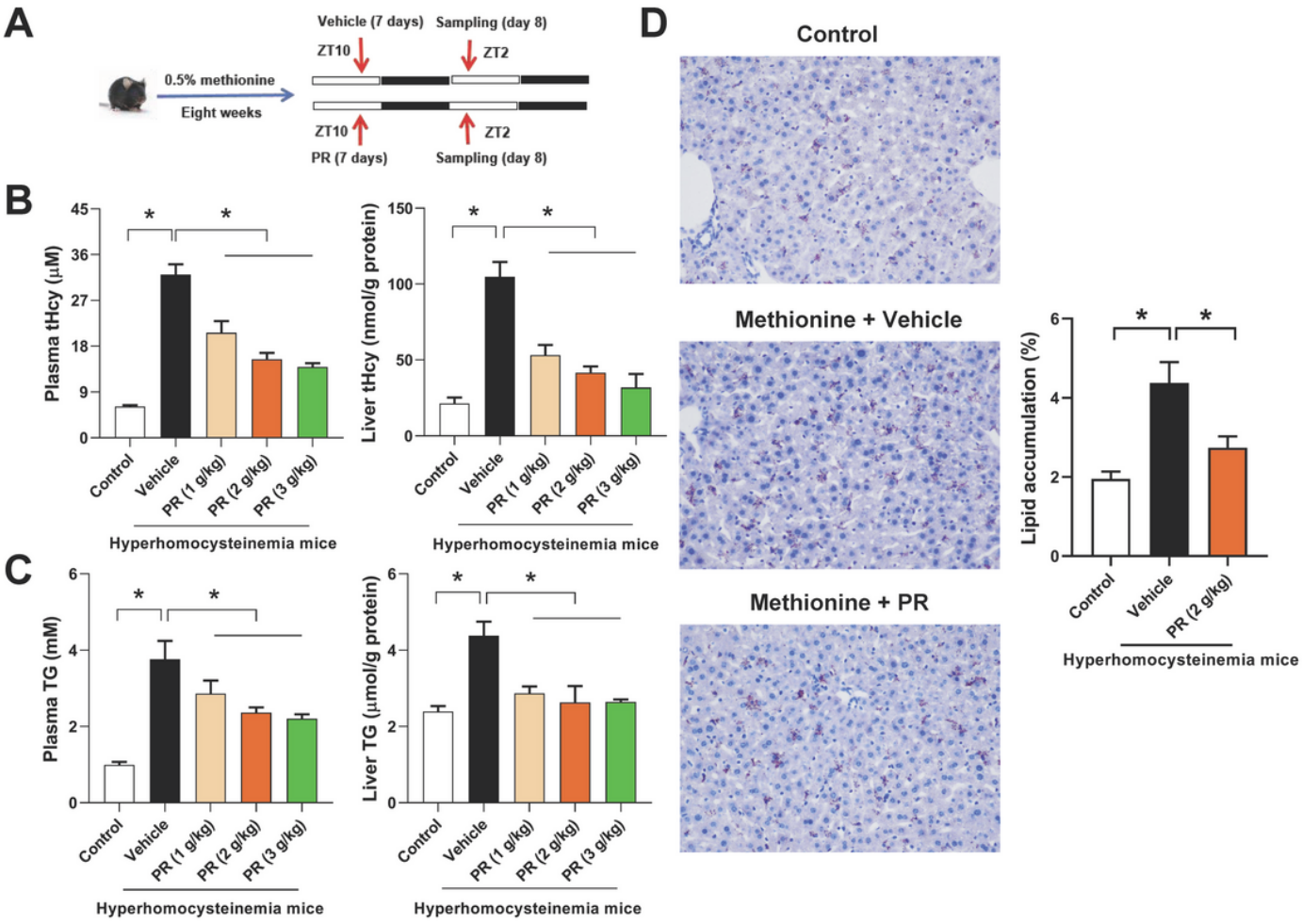

Figure 2

PR dose-dependently alleviates hyperhomocysteinemia in mice. (A) Schematic diagram for the experimental protocol of hyperhomocysteinemia model establishment and PR treatment. (B) Plasma and hepatic tHcy levels in each group of mice. Data are mean ± SD (n = 5). *p < 0.05 (one-way ANOVA with Bonferroni post hoc test). (C) Plasma and hepatic TG levels in each group of mice. Data are mean ± SD (n = 5). *p < 0.05 (one-way ANOVA with Bonferroni post hoc test). (D) Oil red O staining of livers from control and hyperhomocysteinemia mice. Data are mean ± SD (n = 5). *p < 0.05 (t-test).

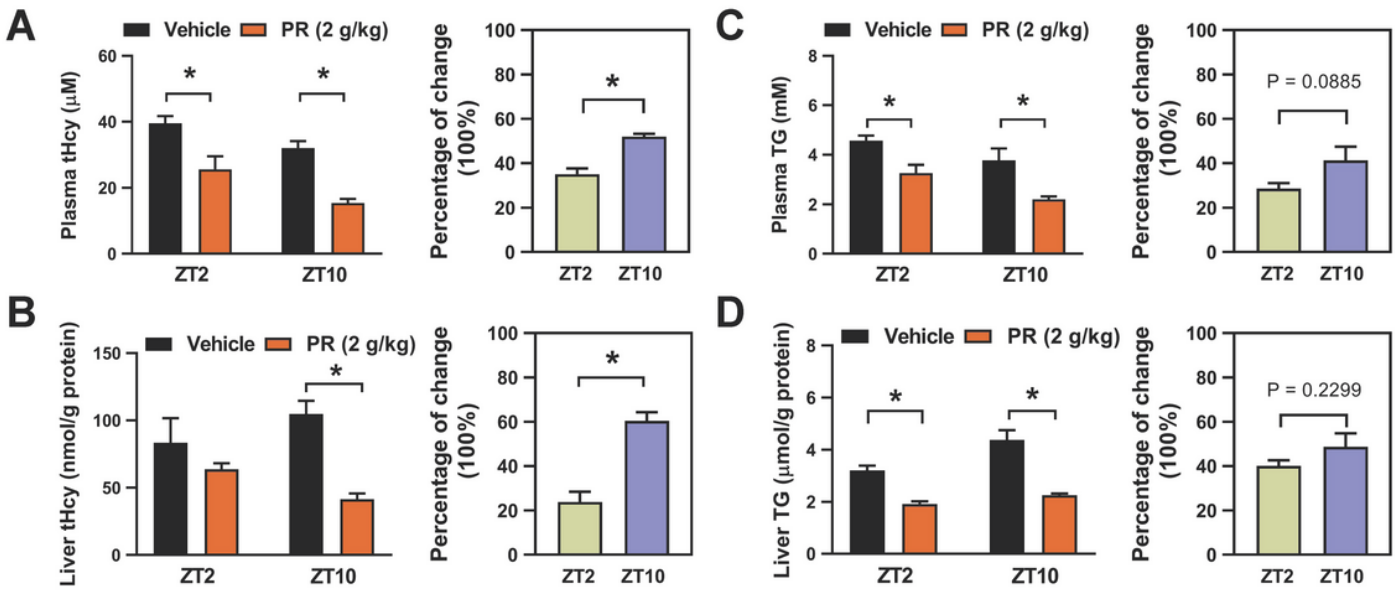

Figure 3

Chronoefficacy of PR against hyperhomocysteinemia in mice. (A) Effects of PR on plasma tHcy levels at two different dosing times ZT2 and ZT10. The right panel shows the percentages of change in plasma tHcy levels. (B) Effects of PR on hepatic tHcy levels at two different dosing times ZT2 and ZT10. The right panel shows the percentages of change in hepatic tHcy levels. (C) Comparisons of plasma TG levels for drug treatments at ZT2 and ZT10. The right panel shows the percentages of change in plasma TG levels. (D) Comparisons of hepatic TG levels for drug treatments at ZT2 and ZT10. The right panel shows the percentages of change in hepatic TG levels. Data are mean ± SD (n = 5). *p < 0.05 (t-test).

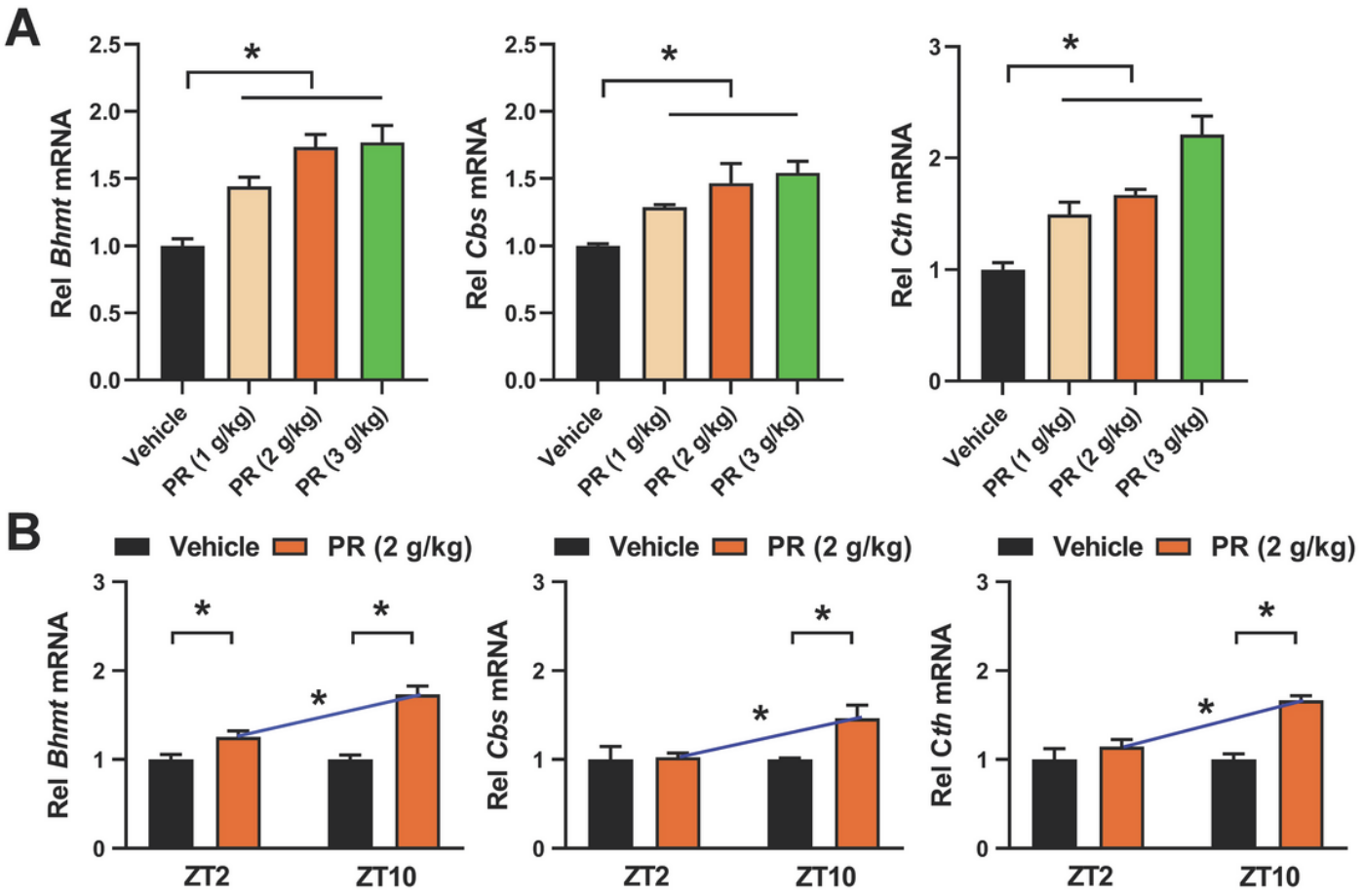

Figure 4

PR induces hepatic Bhmt, Cbs and Cth expressions in hyperhomocysteinemia mice. (A) Effects of PR (1, 2, and 3 g/kg) on Bhmt, Cbs, and Cth mRNA expressions in hyperhomocysteinemia mice. *p < 0.05 (one-way ANOVA with Bonferroni post hoc test). Rel, Relative. (B) Effects of PR (2 g/kg) on Bhmt, Cbs, and Cth mRNA expressions at ZT2 and ZT10. Data are mean ± SD (n = 5). *p < 0.05 (two-way ANOVA with Bonferroni post hoc test). Rel, Relative.

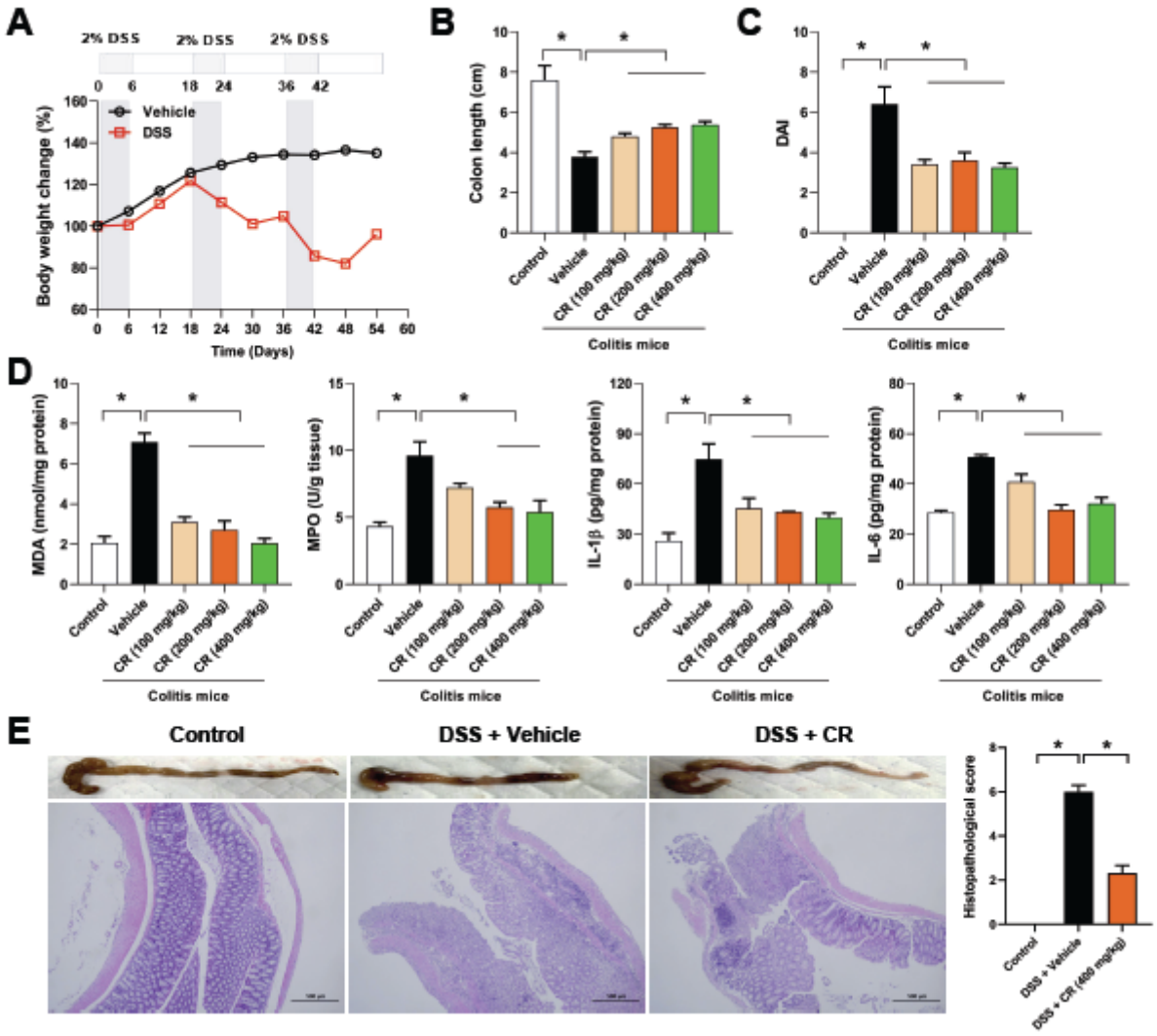

Figure 5

CR dose-dependently attenuates chronic colitis in mice. (A) Schematic diagram for the experimental protocol of chronic colitis establishment with mice. The bottom panel shows the mean weight change in control and DSS groups of mice. (B) Colons lengths of control mice and colitis mice treated CR. Data are mean ± SD (n = 6). *p < 0.05 (one-way ANOVA with Bonferroni post hoc test). (C) DAI scores of control mice and colitis mice treated CR. DAI was scored based on diarrhea, bleeding and body weight loss. Data are mean ± SD (n = 6). *p < 0.05 (one-way ANOVA with Bonferroni post hoc test). (D) Measurements of colonic MDA/MPO activities and IL-1β/IL-6 levels. Data are mean ± SD (n = 6). *p < 0.05 (one-way ANOVA with Bonferroni post hoc test). (E) Macroscopic appearance and H&E staining of colons as well as histopathological scores, showing an anti-colitis effect of CR (400 mg/kg). Data are mean ± SD (n = 6). *p < 0.05 (t-test).

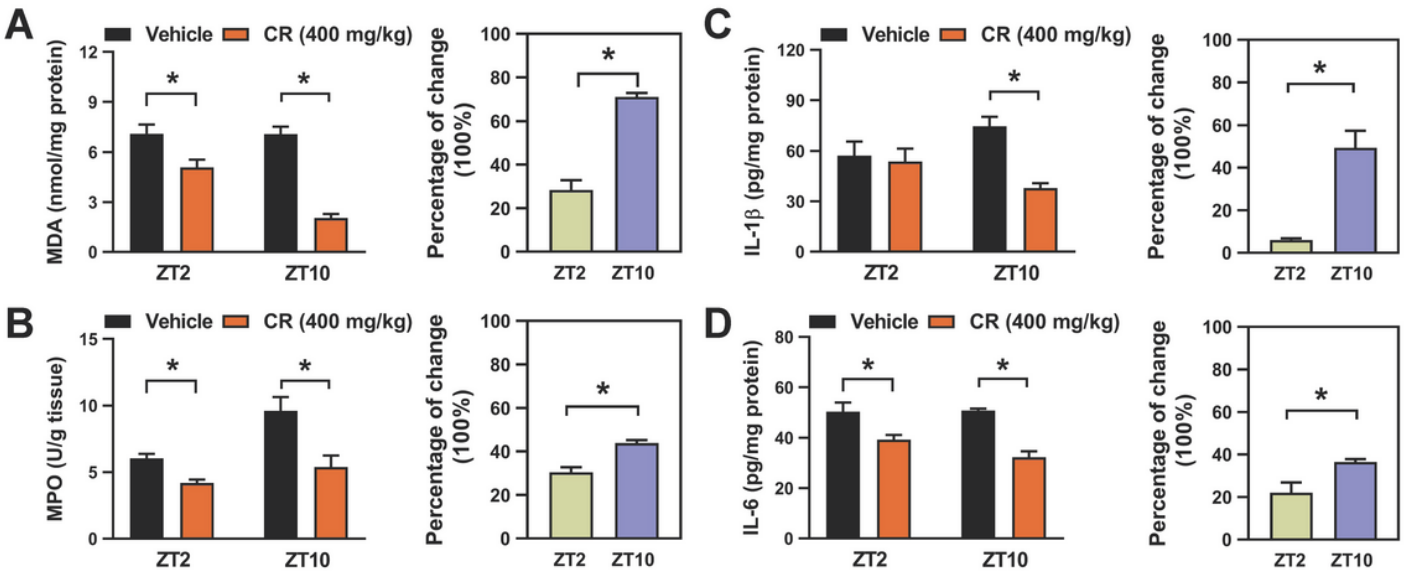

Figure 6

Circadian time-dependent effects of CR on chronic colitis in mice. (A) Effects of CR on colonic MDA activities at ZT2 and ZT10. The right panel shows the percentages of change in colonic MDA activities. (B) Effects of CR on colonic MPO activities at ZT2 and ZT10. The right panel shows the percentages of change in colonic MPO activities. (C) Comparisons of colonic IL-1β levels for drug treatments at ZT2 and ZT10. The right panel shows the percentages of change in colonic IL-1β levels. (D) Comparisons of colonic IL-6 levels for drug treatments at ZT2 and ZT10. The right panel shows the percentages of change in colonic IL-6 levels. Data are mean ± SD (n = 6). *p < 0.05 (t-test).

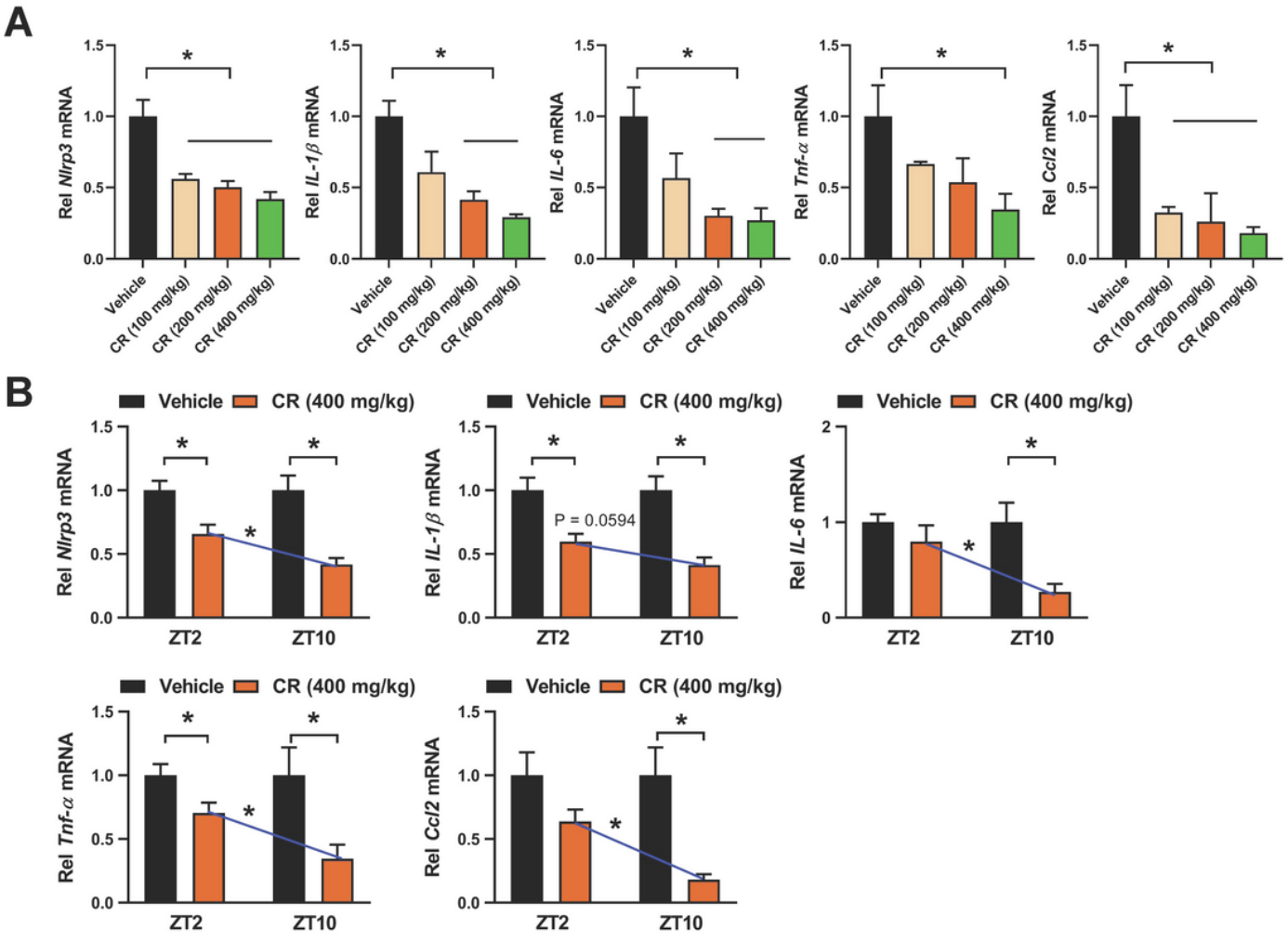

Figure 7

CR down-regulates colonic inflammatory cytokines in colitis mice. (A) Effects of CR (100, 200, and 400 mg/kg) on Nlrp3, IL-1β, IL-6, Tnf-α and Ccl2 mRNA expressions in colitis mice. *p < 0.05 (one-way ANOVA with Bonferroni post hoc test). (B) Effects of CR (400 mg/kg) on Nlrp3, IL-1β, IL-6, Tnf-α and Ccl2 mRNA expressions at ZT2 and ZT10. Data are mean ± SD (n = 6). *p < 0.05 (two-way ANOVA with Bonferroni post hoc test). Rel, Relative.