Int J Clin Exp Med 2016;9(6):10747-10756
www.ijcem.com /ISSN:1940-5901/IJCEM0022444

Original Article

Shenhuang Powder reduces the intestinal inflammatory response and ameliorates impaired gastrointestinal motility in a rat model of postoperative ileus

Xiao-Hong Xu[1], You-Lei Chen[1], Xing Wei[2], Qiu-Hua Sun[1], Hua-Sen Qiu[1,2]

[1]First Clinical Medical College, Zhejiang Chinese Medical University, Hangzhou 310053, China; [2]Department of Gastrointestinal Surgery, The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou 310053, China

Received December 22, 2015; Accepted March 21, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: Postoperative ileus (POI) is a temporary disturbance in gastrointestinal motility following surgery, and there is no ideal pharmacological agent available to shorten POI. We aimed to investigate whether Shenhuang Powder (SHP) can ameliorate the intestinal inflammation and improve gastrointestinal transit in the rats. POI was induced in adult male rat by standardized small intestinal manipulation (IM). SHP was administered transdermally 24 h prior to the surgery. POI in rat was characterized by decreased gastrointestinal transit, reduced body weight and marked upregulation of mRNA expression of inflammatory mediators including TNF-α, IL-1β, IL-6, and IL-12, IL-10, iNOS and ICAM-1. SHP treatment could ameliorate the intestinal inflammatory response and delayed intestinal transit induced by IM, as well as promote the recovery of normal body weight in POI animals. Our study provides evidence that SHP has a potential clinical utility to shorten POI.

Keywords: Postoperative ileus, Shenhuang Powder, gastrointestinal transit, inflammatory mediators, body weight

Introduction

Postoperative ileus (POI) is the generalized gastrointestinal (GI) dysfunction with impaired bowel mobility that develops as an aftermath of the abdominal surgery or other surgical procedures. POI significantly prolongs the length of hospital stay and increases the risk of developing the serious complications, thereby conferring a substantial financial and resource-intensive burden on both healthcare institutions and patients [1]. Unfortunately, there is no specific pharmacological agent available to shorten POI, and the treatment of POI is mainly limited to supportive measures to correct nutrition and fluid deficits and promoting GI function by early feeding, ambulation, or selective use of nasogastric decompression to alleviate the symptoms. The non-specific pharmacological agents, such as commonly used prokinetic drugs and/or selective periphery-restricted opioid receptor antagonists [2], although proved to be effective in shortening POI, have undesirable side effects, and higher cost [3, 4]. Therefore, novel specific therapeutic strategies for POI with fewer side effects warrant further investigation.

The pathogenesis of POI is multifactorial, and inflammatory mechanisms in response to the surgical trauma play a central role in the late phase of POI [5, 6]. The complex inflammatory cascade in POI is believed to begin with intestinal dendritic cells (DC), which release interleukin-12 (IL-12) once insulted by the surgical trauma. DC-derived IL-12 then activates T helper type1 memory cells which finally activate resident muscularis macrophages via interferon γ. Activated macrophages not only release nitric oxide to directly paralyze smooth muscle cell [6], but also secrete a wide variety of proinflammatory cytokines (IL-1β, IL-6) and chemokines leading to the upregulation of adhesion molecules (ICAM-1). All these activated signaling cascades can finally result in a full-blown POI-associated inflammation in the manipulated site [6]. Moreover, those activated T helper type 1 memory cells also migrate to un-manipulated

parts of the gastrointestinal tract through the portal vein, and cause the dissemination of POI over the entire intestinal tract [7]. The anti-inflammatory mediators , such as IL-10 has also been critically involved in the progress of POI [8]. Since the inflammatory mediators play critical roles in the initiation and development of POI, manipulation of these mediators confers the most promising strategy in prevention or treatment of the clinically relevant prolonged form of POI.

Shenhuang Powder (SHP) is a traditional Chinese medicine formula that is widely used to treat the abdominal obstruction and inflammatory bowel disease with prominent clinical efficacy [9]. It is composed of seven herbs that are well documented since the earliest Chinese pharmaceutical monograph "ShenNong Ben Cao Jing" [10], including Sun-dried Panax ginseng, Salviae Miltiorrhzae Radix et Rhizoma, Rhubarb, Fructus Aurantii Immaturus, Cortex Magnoliae Officinalis, Flos Caryophyllata, and Frutcus evodiae. These herbs or their constituent chemicals have been reported to exhibit anticytotoxic [11], antioxidant [12, 13], gastroprotective [14-16], prokinetic [17], and anti-inflammatory [13, 18] properties. Since POI is characterized by impaired gut motility and intestinal inflammation, we investigated the effect of SHP on mRNA expression of POI-associated inflammatory mediators and gut function in an animal model.

Materials and methods

Animals and experimental design

Adult male Sprague-Dawley rats were obtained from the Animal Center of Zhejiang Chinese Medicine University at an initial body weight of 280 to 320 g. All rats were single-housed in our laboratory and provided with standard rat chow and tap water *ad libitum* at 21-23°C and a 12-h light/dark cycle. Upon arrival, all rats were allowed to acclimate for at least 48 hours before the surgery. The experiment protocol was approved by the Institutional Animal Care and Use Committee at Zhejiang Chinese Medicine University. Rats were randomly divided into 4 groups (n = 6): control group, in which the rats' abdominal wall was opened and closed without further manipulation; SHP group, in which the rats were transdermally treated with SHP 24 h before their abdominal walls were opened and closed without further manipulation; IM group, in which the rats were treated with transdermal agents only 24 hours before intestinal manipulation; IM+SHP group, in which the rats were transdermally treated with SHP 24 h before intestinal manipulation. This experiment design applies for all three independent experiments: gastrointestinal transit assessment, body weight measurement and RT-PCR. Prior to surgery all rats were fasted for 20 to 22 h with free access to water.

Preparation of Shenhuang Powder and transdermal agent

SHP is the mixed powder from several Traditional Chinese herbs medicines (TMC), including Panax ginseng (Shengshaisen; sun-dried root of *Panax japonicus* (T. Nees) C.A. Mey), Salviae Miltiorrhzae Radix et Rhizoma (Danshen; dried roots of *Salvia miltiorrhiza* f. alba C.Y. Wu & H.W. Li), Rhubarb (Shendahuang; dried roots of *Rheum undulatum* var. *longifolium* C.Y. Cheng & T.C. Kao), Fructus Aurantii Immaturus (Zhishi; dried fruits of *Citrus aurantium* var. daidai Makino), Cortex Magnoliae Officinalis (Houpu; dried bark of *Magnolia officinalis* Rehder & E.H. Wilson), Fructus Evodiae (Wuzhuyu, dried fruits of *Evodiae Rutaecarpae* (Juss) Benth) and Flos Caryophyllata (Dinxiang; dried flower bud of *Syzygium abortivum* (Gagnep.) Merr. & L.M. Perry).

All herbs used in this study were purchased from Eastern China Medical Corp. (Zhejiang Province, China), which were of high quality and authenticated by Dr. Zhiying Xu, Department of Pharmacognosy of Zhejiang Chinese Medical University. SHP was prepared as in our previous study [9]. In brief, the crude herbs (Sun-dried Panax ginseng 300 g, Rhubarb 300 g, Salviae Miltiorrhzae Radix et Rhizoma 300 g, Fructus Aurantii Immaturus 200 g, Cortex Magnoliae Officinalis 150 g, Fructus Evodiae 75 g and Flos Caryophyllata 75 g) were washed fast with water and dried at 50°C in an oven, and then were mixed and porphyrized into cell level ultra-micropowder that could pass through a sieve with 100000 meshes per cm^2. The superfine powder was packaged (3.0 g/pouch) with a packing machine in a clean environment. The transdermal agent was made as following: the coarse particles of Cortex Magnoliae Officnalis (50 g), Fructus Evodiae (25 g) and Flos Caryophyllata (25 g)

were extracted with 90% ethanol through heating reflux in a round-bottom flask for 12 times. The extracting solution was filtered and concentrated in vacuum until no alcohol taste. The concentrated solution was diluted into a proper concentration with 100% ethanol (3.35 g crude drug per 100 ml solvents), and then subpackaged into 5 ml diluent per bottle.

At 24 hours prior to the surgery, the skin around back areas was first shaved, and then one pouch of SHP (3.0 g) mixed with 3 ml transdermal agent was applied to shaved areas and covered with a transdermal adhesive patch (5 cm × 5 cm).

Three-dimensional HPLC

One pouch of SHP (3.0 g/pouch) was immersed in 10 ml transdermal agent for 30 min, and the mixed solution was first filtered and then analyzed by HPLC (Agilent Technologies 1200 Series, Germany) under the conditions described as follows. Sample (10 μl) was applied to an Agilent zorbax SB-C18 column (4.6 mm × 250 mm, 5 μm, USA). The mobile phase was methanol(B)/acetic acid solution (0.2%/D) in a linear gradient as below: 0-10 min (B: 10%-15%), 10-20 min (B: 15%-20%), 20-30 min (B: 20%-30%), 30-40 min (B: 30%-40%), 40-50 min (B: 40%-50%), 50-60 min (B: 50%-60%), 60-100 min (B: 60%-100%), 100-110 min (B: 100%). The flow rate was 1.0 ml/min and the oven temperature was 30°C. HPLC patterns were analyzed by absorbance at 203-360 nm.

Intestinal Manipulation (IM) for induction of POI

The rat model of POI was induced by intestinal manipulation, following the previous study [19]. Briefly, after full anesthesia with isoflurane (2%-3%) inhalation, the abdomen was shaved first and then treated with povidone-iodine topical antiseptic for disinfection. A midline incision was made to expose the viscera. The small intestine and cecum were exteriorized, manipulated with moderate compression from the ligament of Treitz to the terminal ileum using two moist cotton-tipped applicators. The running procedure was repeated three times and then covered with gauze soaked in saline for an additional 10 min. After the manipulation the small intestine and the cecum were placed back into the abdominal cavity, and the incision was closed with running silk sutures. The surgical procedure lasted 25 to 30 min and was always performed from 8:30 to 10:30 AM.

Gastrointestinal transit time

Gastrointestinal transit was measured 48 h following the surgery by evaluating the digestive tract location of FITC-dextran (70000 MW, Life Technology, USA) [20]. Animals were lightly anaesthetized and were orally administered with 200 μl of FITC-dextran (6.25 mg/ml) via a gastric tube. Animals were then euthanized 30 min after administration, and the entire gastrointestinal tract was divided into 15 segments: a single stomach segment (Sto), 10 equal-length segments of small intestine (SI1-SI10), a single cecum segment (Cec) and three colon segments (Co1-Co3). FITC-dextran was washed out by saline. The supernatant from each bowel segment were centrifuged at 12000 rpm and the fluorescence of the cleared supernatants was read at 494 nm/521 nm wavelength in a fluorescence reader (Thermo Electron Corporation, USA). The data were expressed as the percentage of fluorescence signals per segment and plotted along the gastrointestinal tract. Gastrointestinal transit was calculated as the geometric centre (GC) of distribution of FITC-dextran using the following formula: GC = $\sum$ (% of total fluorescent signal per segment x segment number)/100. The gastric empty rate was calculated as total fluorescence signals subtracted by the signals remained in the stomach segment (Sto) [21].

We performed colonic transit measurement after intestinal manipulation as previously described [7, 22, 23]. Briefly, after animals were slightly anesthetized with isoflurane, we first checked the colon patency of each animal with a polished metal rod, and then retrogradely inserted a 2-mm glass ball 3cm deep into the colon of each animal. The time between the end of surgery and the appearance of glass ball in the fecal pellet was measured. The control rats, not subjected to IM, were studied on each experimental day together with the rats with IM.

Body weight

Body weight were first recorded at 6:00 to 8:00 AM before the animals were fasted, on the day of experiment right prior to the surgery, and then at 3-h intervals during the first 12 h follow-

Table 1. Nucleotide sequences of rat oligonucleotide primers

Gene	Sense primer	Antisense primer
IL-6	5'-CCCACAACAGACCAGTA-3'	5'-CAGGTAGAAACGGAACT-3'
IL-1β	5'-TGAAATGCCACCTTTTGACAG-3	5'-CCACAGCCACAATGAGTGATAC-3'
ICAM-1	5'-CAAACGGGAGATGAATGG-3'	5'-TGGCGGTAATAGGTGTAAAT-3'
TNF-α	5'-GTAGCAAACCACCAAGCG-3'	5'-GGTATGAAATGGCAAATCG-3'
IL-12	5'-GGGACATCATCAAACCG-3'	5'-TACGAGGAACGCACCTT-3'
iNOS	5'-TCCCGAAACGCTACACTT-3'	5'-GCGGCTGGACTTC TCACT-3'
GAPDH	5'-GGCATTGCTCTCAATGACAA-3'	5'-TGTGAGGGAGATGCTCAGTG-3'
IL-10	5'- ATAACTGCACCCACTTCCCA-3'	5'- TTTCTGGGCCATGGTTCTCT-3'

ing the surgery, and at Day 1, Day 2, Day 3 and Day 7 postsurgery. Changes in body weight are expressed as body weight gain compared with the weight of the fasted animals measured before the surgery.

Real-time reverse transcriptase-polymerase chain reaction

All chemicals and reagents were of analytical grade, if not otherwise specified. Isolated intestinal muscularis of rats was harvested 48 h following the surgery and stored at -80°C until analysis (n = 6 for each group). Total RNA was extracted from the isolated muscularis. Contaminating DNA was eliminated by DNA-free solution (Ambion). Target mRNAs of TNF-α, IL-1β, IL-6, IL-10, ICAM-1, IL-12 and iNOS were separately reverse-transcribed into complementary DNA (cDNA) with the High Capacity Reverse Transcription kit following manufactures instruction and measured using real-time PCR. mRNA was quantified in triplicate by a SYBR Green-PCR, using Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression as a standard. PCR was performed on an Abi Prism 7900HT in Universal PCR Mastermix by amplification of cDNA for 40 cycles (95°C × 15 sec, 60°C × 1 min). Dissociation of the PCR products by a melting curve analysis protocol consistently showed specific single melting peaks for all used primer pairs. Relative quantifications and calculations were performed by the 2-ΔΔCT method to analyze gene expression [24]. Primers were designed according to published sequences and GenBank accession numbers using Primer Express software (PE Applied Biosystems, Foster City, CA). The primer sequences were shown in **Table 1**.

Data analysis and statistics

The data were expressed as the mean ± S.E.M. Differences between groups were assessed for statistical significance by Student's *t* test and by one- or two-way ANOVA. A level of $P<0.05$ was considered statistically significant.

Results

Effect of SHP on gastrointestinal transit time in POI

Intestinal manipulation (IM) caused a significant delay *in vivo* gastrointestinal transit as evidenced by a much more proportion of dextran signals detected in the upper part of GI tract 48 h following the surgery compared with control animals (**Figure 1A**). In control animals, most of dextran had progressed to the colon 48 h after intragastric infusion, whereas in rats with IM, the majority of dextran signals was still localized at the GI tract above the upper jejunal segments (**Figure 1A**). The calculated geometric center (GC) in IM animals was 4.6 out of 15 gastrointestinal sections (10.4 out of 15 gastrointestinal segments in control, $F(1,12) = 423$, $P< 0.0001$). IM was also found to delay gastric emptying ([$F(1,12) = 34.2$, $P<0.0001$; **Figure 1B**) and colonic transit, causing a significant increase of the time to the first bowel movement registered by the excretion of the glass ball (501.5±74.3 s in IM versus 70.7±19.0 s in control; $P<0.001$; **Figure 1C**). These results indicated that a POI model was successfully established on rats with our protocol.

Although SHP had no effect on GC in control, it could significantly ameliorate delayed gastrointestinal transit in IM animals ($F(1, 20) = 34.13$, $P\leq0.001$; **Figure 1A**, **1D**). In IM animals treated with SHP, a large proportion of dextran signals can reach mid-jejunal segments, and thus a significantly less proportion of dextran signals were detected in upper parts of intestinal 48 h following the surgery compared with IM group rats (**Figure 1A**). There was also a significant effect on gastric emptying rate [$F(1, 20) = 22.3$, $P<0.0001$], indicating that SHP treatment also ameliorated delayed gastric emptying in IM animals (**Figure 1B**).

Effect of SHP on body weight in POI

IM significantly decreased body weight gain in manipulated animals [$F(1, 15) = 34.9$, $P<$

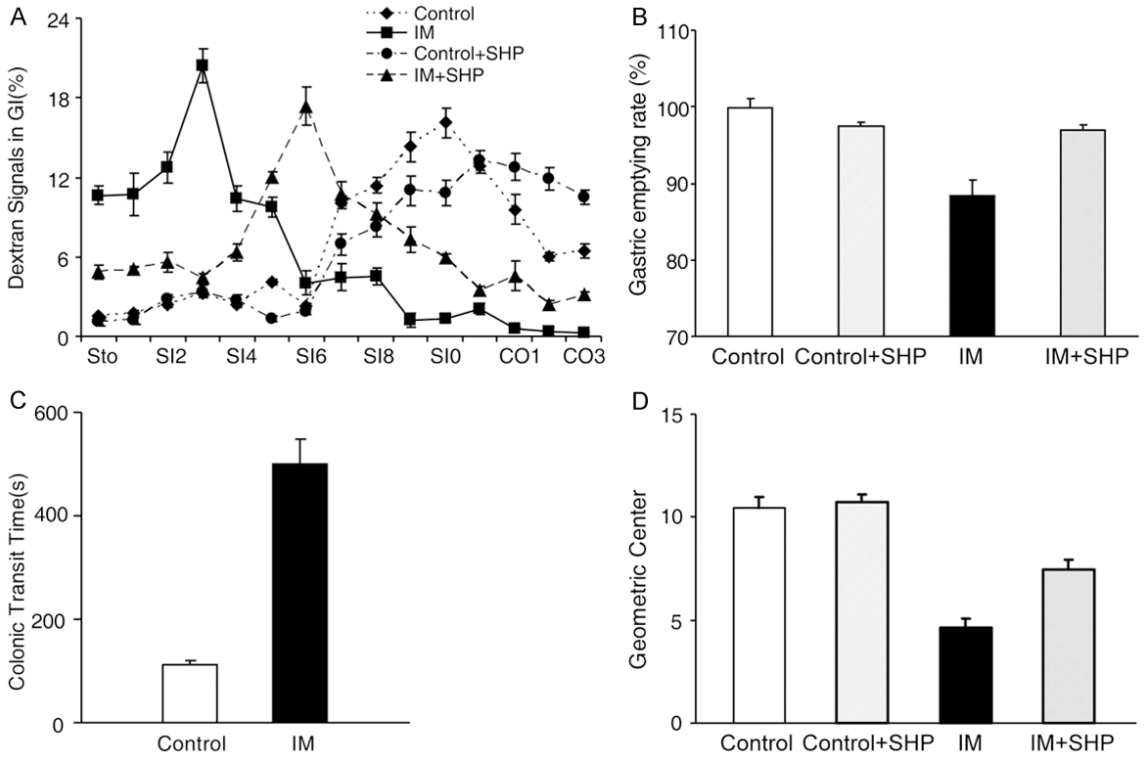

Figure 1. A. Distribution of non-absorbable FITC-dextran signals along the gastrointestinal tract at 48 h following the surgery. B. Gastric emptying rate of four groups of rats. C. The colonic transit time in control and IM rats. D. Geometric centre of FITC-dextran signals in four groups of rats. The data is represented as mean ± S.E.M.

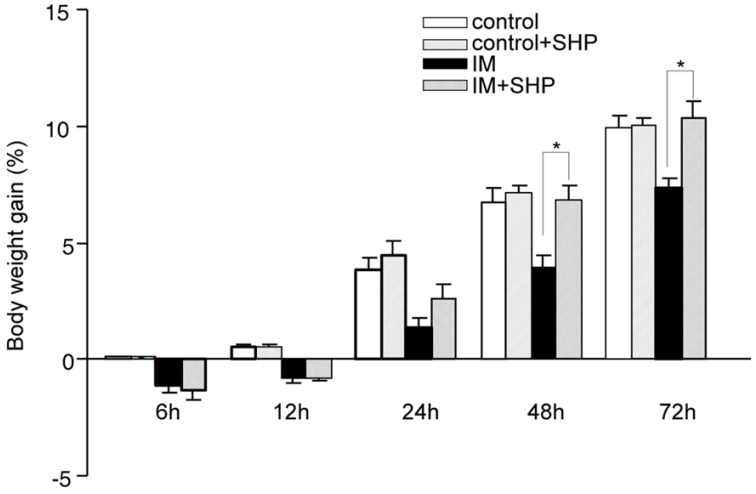

Figure 2. Body weight gain of four groups of rats. Body weight gain is represented as weight gain (g)/100 g body weight. Data are presented as mean ± S.E.M. "*" denote the significant differences between IM group and IM+SHP group.

0.001] [25]. This effect still maintained at 72 hours [F (1, 15) = 4.7, P = 0.04] following the surgery (**Figure 2**), but became insignificant 1 week following the surgery (P = 0.69). SHP alone did not produce a significant effect on body weight gain at any time point of the measurement (**Figure 2**). However, the IM animals receiving SHP treatment appeared to gain more weight than IM group (**Figure 2**). On 48 h following the surgery, there was a significant IM x SHP interaction on body weight gain [F (1, 25) = 4.8, P = 0.04]. The IM animals that received SHP treatment gained significantly more weight than the manipulated animals that received only transdermal agents. This was still the case on 72 hours following the surgery [F (1, 25) = 7.3, P = 0.01]; **Figure 2**), and body weight in IM animals that received SHP treatment already caught up to that in control non-IM animals (**Figure 2**).

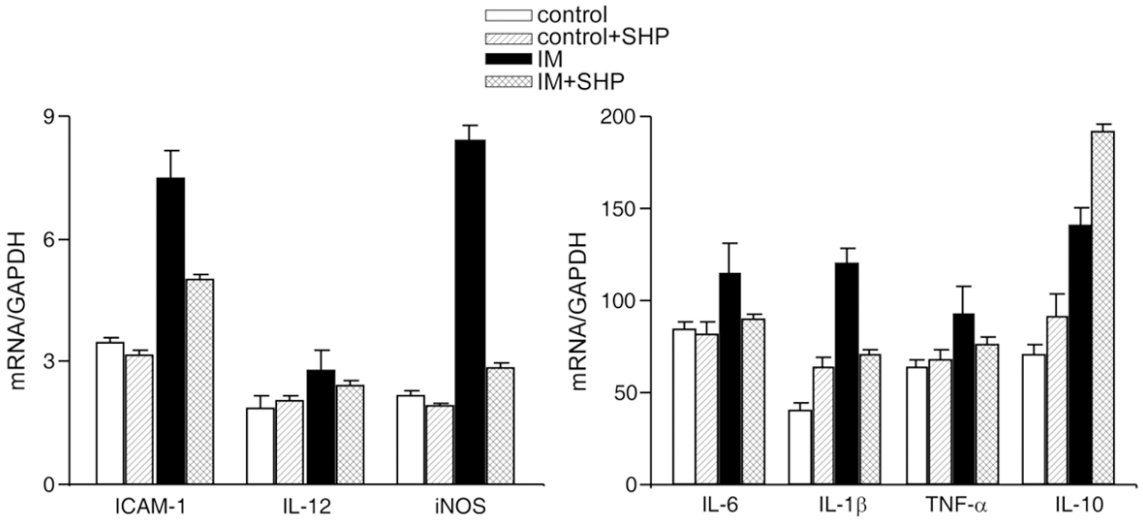

Figure 3. Effects of SHP on mRNA expression of inflammatory mediators in muscle layer of small intestine in rat POI model. Left: quantitative results of mRNA expression of ICAMP-1, IL-12 and iNOS. Right: quantitative results of mRNA expression of TNF-a, IL-6, IL-1b, IL-10. Bars indicate mean ± SEM from n = 6/group.

Effect of SHP on mRNA expression of inflammatory mediators in POI

SHP significantly ameliorated delayed gastrointestinal transit and gastric emptying induced by IM, and accelerated body weight normalization of IM animals, indicating that SHP might have a therapeutic effect on POI. Since the complex inflammatory response in intestinal muscularis servers as caustic mechanism for the late phase of POI [6], we thus investigated whether SHP could ameliorate the inflammatory response by examining the effect of SHP on mRNA expression of several critical POI-associated inflammatory mediators in the intestinal muscularis. The results showed that intestinal manipulation significantly upregulated the mRNA expressions of ICAM-1 [F (1, 12) = 65.1, $P<0.0001$], iNOS [F (1, 12) = 131, $P<0.0001$], IL-10 [F (1, 12) = 73, $P<0.0001$], IL-1β [F (1, 12) = 30, $P<0.0001$]. The mRNA expression of IL-6, IL-12 and TNF-α also showed an upward trend in IM animals 48 h following the surgery, but the significant level was not reached (IL-12 [F (1, 12) = 3.4, $P = 0.08$; P = 0.20, IL-6 [F (1, 12) = 3.6, $P = 0.08$; TNF-α [F (1, 12) = 1.8, $P = 0.18$), probably due to that the upregulated mRNA expression of these mediators began to decline 48 h following the surgery. SHP had no effect of the mRNA expression of any of these mediators in control rats. However, there was a significant SHP x IM interaction on the mRNA expression of IL-1β [F (1, 20) = 20.8, $P<0.001$], ICAM-1 [F (1, 20) = 8.7, $P<0.01$], iNOS [F (1, 20) = 67.2, $P<0.001$]. SHP significantly suppressed the increased mRNA expression of IL-1B, ICAM-1 and iNOS, and further upregulated the expression of IL-10 in manipulated animals [F (1, 12) = 14.6, $P = 0.001$]. The mRNA expression of IL-6 and TNF-α in manipulated animals also exhibited a downward trend after SHP treatment (IL-6; $P = 0.26$, TNF-α; $P = 0.16$) (**Figure 3**).

Three-dimensional HPLC

SHP is enriched with the high concentration of bioactive constituents that have therapeutic potentials for many inflammatory diseases. The HPLC profile of SHP revealed that the prepared SHP contained hesperidin, quercetin, ginsenoside-Rb1, aloeemodin, ginsenoside-Rg1, magnolol and chrysophanol (**Figure 4**).

Discussion

In this POI animal model, we observed a significant delay in gastrointestinal transit and colon transit, a decreased gastric emptying rate 48 h following intestinal manipulation (IM). We also observed that IM induced marked inflammatory responses characterized by an elevated mRNA level of a wide variety of inflammatory mediators in intestinal tissue, and reduced body weight gain, which are consistent with earlier reports [19, 26]. SHP could significantly ameliorate delayed gastrointestinal transit and

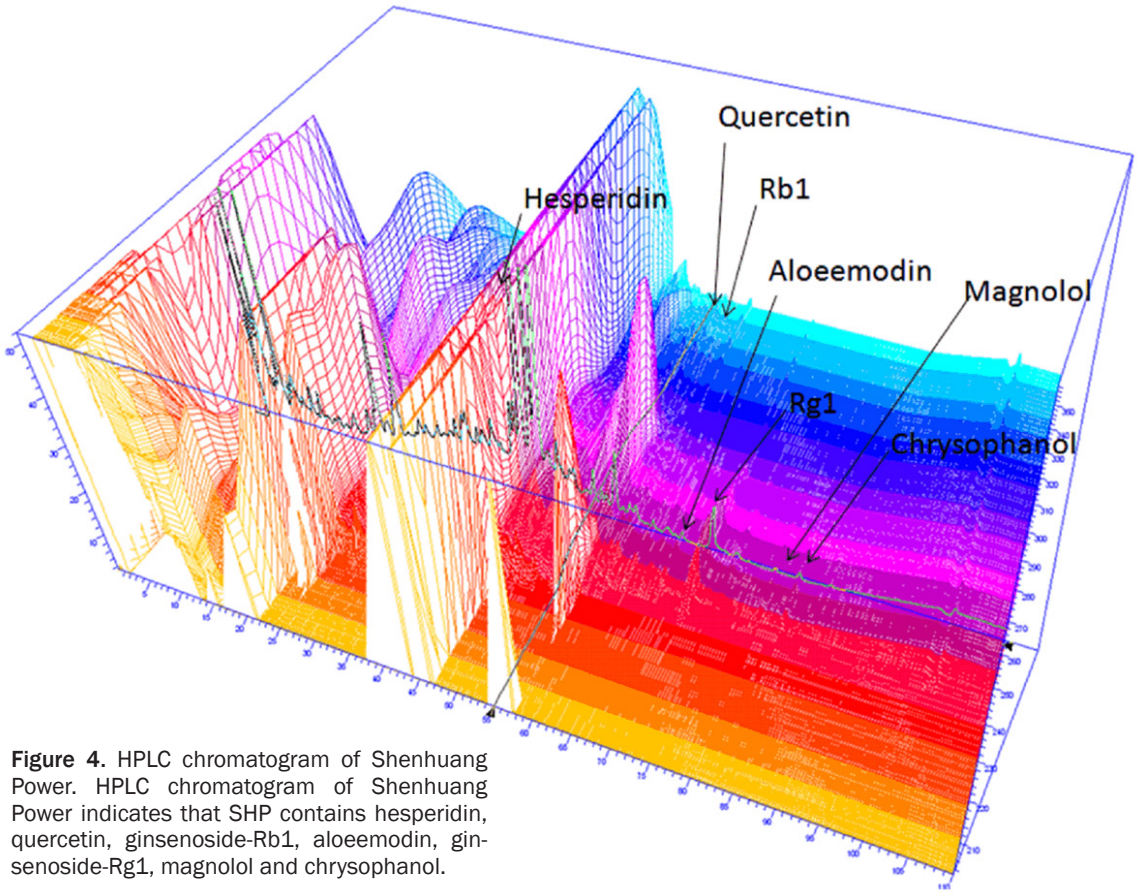

Figure 4. HPLC chromatogram of Shenhuang Power. HPLC chromatogram of Shenhuang Power indicates that SHP contains hesperidin, quercetin, ginsenoside-Rb1, aloeemodin, ginsenoside-Rg1, magnolol and chrysophanol.

gastric emptying rate, reduced the inflammatory response by regulating the inflammatory mediators critical for the induction and progress of POI, as well as promote the normalization of reduced body weight of POI animals. These results suggest that SHP might have a clinical utility to shorten POI.

A post-operative increase in intestinal inflammation has a positive correlation with the severity of POI, and decreasing intestinal inflammation can significantly improve POI [6]. During the induction of the POI, the pro-inflammatory cytokine IL-12, TNF-α, IL-1β and IL-6 are rapidly produced , and adhesion molecular ICAM-1 that mediates influx of leucocytes to the manipulated site are also released [5, 6]. These mediators initiate a full-blown POI and contribute to the gastrointestinal dysmotility through their direct cytotoxic action or their effect on the production of nitric oxide by iNOS and prostanoids [6]. In contrast to these pro-inflammatory mediators, anti-inflammatory cytokine IL-10 actively down-regulates intestinal inflammatory process to prevent an excessive and prolonged inflammation in POI [8]. Consistent with these previous studies, the present study found that POI rats showed significantly higher mRNA levels of pro-inflammatory mediators IL-12, TNF-α, IL-1β, IL-6, iNOS and ICAM-1 in the ileum tissue, while these elevated levels were reduced by SHP. In addition, while the mRNA level of anti-inflammatory cytokine IL-10 was significantly increased in POI rats, SHP further increased its elevated level in the ileum tissue. These results suggested that SHP could reduce intestinal inflammation not only by decreasing the level of pro-inflammatory mediators, but also increasing the level of anti-inflammatory cytokine in animal model of POI. The reduced intestinal inflammation by SHP may subsequently result in improved GI tract transit and increased food intake in POI rats, and ultimately leading to accelerated body weight recovery.

The bioactive constituents of SHP have been exhibited anti-inflammatory and anti-oxidative

actions. For instance, fructus aurantii immaturus is enriched with hesperidin that has anti-inflammatory activity and prokinetic actions on GI tract [17, 27]. Cortex magnoliae officinalis is enriched with honokiol and magnolol [28] that have antioxidative and anti-inflammatory effects [29]. Magnolol could modulate GI motility and attenuate sepsis-induced intestinal dysmotility by inhibiting the expression of TNF-α, iNOS, and increasing expression of IL-10, as well as blocking of oxidative stress in the intestine [18]. The major bioactive constituent of panax ginseng, ginsenosides, have exhibited anti-inflammatory properties in different studies [30], and among these subtypes, ginsenoside-Rb1 have been shown to promote the recovery of POI by inhibiting pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and upregulating anti-inflammatory cytokines IL-10 [13]. Rhubarb contains flavonoids such as quercetin, aloe-emodin and chrysophanol, all of which have anti-oxidative and anti-inflammatory effects, suppressing the production of NO, IL-6, and IL-1β and TNF-α [31, 32]. To examine potential chemical constituents responsible for the therapeutic effect of SHP, we performed HPLC and identified many bioactive constituents, including hesperidin, ginsenoside-Rb1, ginsenoside-Rg1, magnolol, quercetin, aloe-emodin and chrysophanol, suggesting that the effects on POI we observed may be attributed to the synergistic effects of these constituents. However, current knowledge is limited about which above-mentioned bioactive constituents of SHP could be efficiently absorbed transdermally with aid of transdermal agents, the bioactive constituents of SHP responsible for the effect of SHP remain to be determined.

Conclusions

In summary, our results indicate that transdermally administered SHP could reduce the intestine inflammatory response, ameliorate delayed gastrointestinal motility, and subsequently promote the recovery of reduced body weight in an animal model of POI, indicating its potential clinical utility to shorten POI. Given that orally administered drugs are not a feasible option for most of POI patients, transdermally administered SHP may confer great clinical potential to shorten POI in a safe, convenient and efficient way.

Acknowledgements

This work was supported by the grant from the National Natural Science Foundation of China (81173425 and 81470194).

Disclosure of conflict of interest

None.

Abbreviations

POI, postoperative ileus; SHP, Shenhuang Powder; GI, gastrointestinal; TCM, Traditional Chinese Medicine; IM, intestinal manipulation; iNOS, inducible nitro oxide synthesize; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-12, interleukin-12; IL-10, interleukin-10; ICAM-1, intercellular adhesion molecule-1; FITC, fluorescein isothiocyanate; DC, dendritic cells; IBS, inflammatory bowel disease; SI, small intestine; CO, colon; STO, stomach; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; GC, geometric center.

Address correspondence to: Hua-Sen Qiu, First Clinical Medical College, Zhejiang Chinese Medical University, Hangzhou 310053, China; Department of Gastrointestinal Surgery, The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou 310053, China. E-mail: qiuhuasen86@126.com; Qiu-Hua Sun, First Clinical Medical College, Zhejiang Chinese Medical University, Hangzhou 310053, China. E-mail: sqh807@163.com

References

[1] Iyer S, Saunders WB, Stemkowski S. Economic burden of postoperative ileus associated with colectomy in the United States. J Manag Care Pharm 2009; 15: 485-494.

[2] Fukuda H, Suenaga K, Tsuchida D, Mantyh CR, Pappas TN, Hicks GA, Dehaven-Hudkins DL, Takahashi T. The selective mu opioid receptor antagonist, alvimopan, improves delayed GI transit of postoperative ileus in rats. Brain Res 2006; 1102: 63-70.

[3] Becker G, Blum HE. Novel opioid antagonists for opioid-induced bowel dysfunction and postoperative ileus. Lancet 2009; 373: 1198-1206.

[4] Yeh YC, Klinger EV, Reddy P. Pharmacologic Options to Prevent Postoperative Ileus. Ann Pharmacother 2009; 43: 1474-1485.

[5] Vather R, O'Grady G, Bissett IP, Dinning PG. Postoperative ileus: mechanisms and future

directions for research. Clin Exp Pharmacol Physiol 2014; 41: 358-370.
[6] Wehner S, Vilz TO, Stoffels B, Kalff JC. Immune mediators of postoperative ileus. Langenbecks Arch Surg 2012; 397: 591-601.
[7] Engel DR, Koscielny A, Wehner S, Maurer J, Schiwon M, Franken L, Schumak B, Limmer A, Sparwasser T, Hirner A, Knolle PA, Kalff JC, Kurts C. T helper type 1 memory cells disseminate postoperative ileus over the entire intestinal tract. Nat Med 2010; 16: 1407-1413.
[8] Stoffels B, Schmidt J, Nakao A, Nazir A, Chanthaphavong RS, Bauer AJ. Role of interleukin 10 in murine postoperative ileus. Gut 2009; 58: 648-660.
[9] Wei X, Qiu HS, Zhang Q, Li DC, Sun YS, Li G, Chen DX, Zhang B. [Effect of shenhuang ointment in promoting the rehabilitation of postoperative gastrointestinal dysfunction patients of Qi stagnation blood stasis syndrome: a clinical observation]. Zhongguo Zhong Xi Yi Jie He Za Zhi 2014; 34: 661-5.
[10] Sun J, Hu S, Song X. Adjuvant effects of protopanaxadiol and protopanaxatriol saponins from ginseng roots on the immune responses to ovalbumin in mice. Vaccine 2007; 25: 1114-1120.
[11] Ding RB, Tian K, Cao YW, Bao JL, Wang M, He C, Hu Y, Su H, Wan JB. Protective effect of panax notoginseng saponins on acute ethanol-induced liver injury is associated with ameliorating hepatic lipid accumulation and reducing ethanol-mediated oxidative stress. J Agric Food Chem 2015; 63: 2413-22.
[12] Chan LW, Cheah EL, Saw CL, Weng W, Heng PW. Antimicrobial and antioxidant activities of Cortex Magnoliae Officinalis and some other medicinal plants commonly used in South-East Asia. Chin Med 2008; 3: 15.
[13] Tan S, Yu W, Lin Z, Chen Q, Shi J, Dong Y, Duan K, Bai X, Xu L, Li J, Li N. Anti-inflammatory Effect of Ginsenoside Rb1 Contributes to the Recovery of Gastrointestinal Motility in the Rat Model of Postoperative Ileus. Biol Pharm Bull 2014; 37: 1788-1794.
[14] Yu H, Jin H, Gong W, Wang Z, Liang H. Pharmacological actions of multi-target-directed evodiamine. Molecules 2013; 18: 1826-1843.
[15] Takase H, Yamamoto K, Hirano H, Saito Y, Yamashita A. Pharmacological Profile of Gastric Mucosal Protection by Marmin and Nobiletin from a Traditional Herbal Medicine, <I>Aurantii Fructus Immaturus</I>. Jpn J Pharmacol 1994; 66: 139-147.
[16] Yu X, Wu DZ, Yuan JY, Zhang RR, Hu ZB. Gastroprotective Effect of Fructus Evodiae Water Extract on Ethanol-Induced Gastric Lesions in Rats. Am J Chin Med 2006; 34: 1027-1035.
[17] Fang YS, Shan DM, Liu JW, Xu W, Li CL, Wu HZ, Ji G. Effect of Constituents from Fructus Aurantii Immaturus and Radix Paeoniae Alba on Gastrointestinal Movement. Planta Med 2009; 75: 24-31.
[18] Yang TC, Zhang SW, Sun LN, Wang H, Ren AM. Magnolol attenuates sepsis-induced gastrointestinal dysmotility in rats by modulating inflammatory mediators. World J Gastroenterol 2008; 14: 7353-60.
[19] Kalff JC, Schraut WH, Simmons RL, Bauer AJ. Surgical manipulation of the gut elicits an intestinal muscularis inflammatory response resulting in postsurgical ileus. Ann Surg 1998; 228: 652-663.
[20] Moore BA, Peffer N, Pirone A, Bassiri A, Sague S, Palmer JM, Johnson DL, Nesspor T, Kliwinski C, Hornby PJ. GLP-2 receptor agonism ameliorates inflammation and gastrointestinal stasis in murine postoperative ileus. J Pharmacol Exp Ther 2010; 333: 574-83.
[21] Venkova K, Fraser G, Hoveyda HR, Greenwood-Van Meerveld B. Prokinetic effects of a new ghrelin receptor agonist TZP-101 in a rat model of postoperative ileus. Dig Dis Sci 2007; 52: 2241-8.
[22] Kalff JC, Schwarz NT, Walgenbach KJ, Schraut WH, Bauer AJ. Leukocytes of the intestinal muscularis: their phenotype and isolation. J Leukoc Biol 1998; 63: 683-91.
[23] Schaefer N, Tahara K, Schmidt J, Wehner S, Kalff JC, Abu-Elmagd K, Hirner A, Türler A. Resident macrophages are involved in intestinal transplantation-associated inflammation and motoric dysfunction of the graft muscularis. Am J Transplant 2007; 7: 1062-70.
[24] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402-8.
[25] Venkova K, Mann W, Nelson R, Greenwood-Van Meerveld B. Efficacy of ipamorelin, a novel ghrelin mimetic, in a rodent model of postoperative ileus. J Pharmacol Exp Ther 2009; 329: 1110-1116.
[26] Fraser GL, Venkova K, Hoveyda HR, Thomas H, Greenwood-Van Meerveld B. Effect of the ghrelin receptor agonist TZP-101 on colonic transit in a rat model of postoperative ileus. Eur J Pharmacol 2009; 604: 132-137.
[27] Guardia T, Rotelli AE, Juarez AO, Pelzer LE. Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. Farmaco 2001; 56: 683-687.
[28] Wang X, Wang Y, Geng Y, Li F, Zheng C. Isolation and purification of honokiol and magnolol from cortex Magnoliae officinalis by high-speed counter-current chromatography. J Chromatogr A 2004; 1036: 171-175.

[29] Miao B, Zhang S, Wang H, Yang T, Zhou D, Wang BE. Magnolol pretreatment prevents sepsis-induced intestinal dysmotility by maintaining functional interstitial cells of Cajal. Inflammation 2013; 36: 897-906.
[30] Lee DC, Lau AS. Effects of Panax ginseng on tumor necrosis factor-alpha-mediated inflammation: a mini-review. Molecules 2011; 16: 2802-2816.
[31] Hu B, Zhang H, Meng X, Wang F, Wang P. Aloe-emodin from rhubarb (Rheum rhabarbarum) inhibits lipopolysaccharide-induced inflammatory responses in RAW264.7 macrophages. J Ethnopharmacol 2014; 153: 846-853.
[32] Kim SJ, Kim MC, Lee BJ, Park DH, Hong SH, Um JY. Anti-Inflammatory activity of chrysophanol through the suppression of NF-kappaB/caspase-1 activation in vitro and in vivo. Molecules 2010; 15: 6436-6451.