Berberine Hydrochloride Prevents Postsurgery Intestinal Adhesion and Inflammation in Rats

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ABSTRACT

Intestinal adhesion, characterized by connection of the loops of the intestine with other abdominal organs by fibrous tissue bands, remains an inevitable event of abdominal operations and can cause a number of complications. Berberine hydrochloride (berberine), a natural plant alkaloid derived from Chinese herbal medicine, is characterized by diverse pharmacological effects, such as anticancer and lower elevated blood glucose. This study is designed to investigate the effects of berberine on adhesion and inflammation after abdominal surgeries and the underlying molecular mechanisms. Adhesion severity grades and collagen deposition were assessed 14 days after surgery. We evaluated the levels of intercellular adhesion molecule-1 (ICAM-1) and transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), and examined transforming growth factor-activated kinase 1 (TAK1)/c-Jun N-terminal kinase (JNK) and TAK1/nuclear factor κB (NF-κB) signaling. The surgery group experienced the most severe adhesions, and berberine strikingly reduced the density and severity of adhesion. Results showed significant lower expression of IL-1β, IL-6, TGF-β, TNF-α, and ICAM-1, in berberine groups compared with the operation group. Activities of phosphorylated JNK and phosphorylated NF-κB were inhibited in the berberine groups compared with the surgery group. Our novel findings identified berberine hydrochloride as a promising strategy to prevent adhesion by downregulating ICAM-1 and reduce inflammation by inhibiting the TAK1/JNK and TAK1/NF-κB signaling after abdominal surgery, which brought out a good therapeutic approach for the development of clinical application for postoperative abdominal adhesion and inflammation.

Introduction

Intestinal adhesion, a common problem after abdominal surgery (Kosaka et al., 2008), is characterized by bands of fibrous tissue that connect the loops of the intestine to other abdominal organs or the abdominal wall (van Goor, 2007; Takagi et al., 2013), where slight bleeding cannot be completely prevented. Although surgical procedures and technologies have been improved to reduce adhesion formation, such as laparoscopic surgery (van’t et al., 2002), minimally invasive surgery is not always available (van’t et al., 2002; Ten Broek et al., 2013a). Thus, intraperitoneal adhesion formation after surgeries remains an inevitable event of most abdominal procedures (Wiseman et al., 1999). Studies have reported that the incidence of adhesions is over 90% (Chaturvedi et al., 2013; Takagi et al., 2013), and adhesions can also develop after abdominal bacterial infections, such as peritonitis (van Goor, 2007). Peritoneal adhesion is one of the leading causes of intestinal obstruction, up to 40% of all cases (Ellis, 1997), with 60–70% of the cases involving the small bowel (Townsend et al., 2011). Postsurgical adhesions are a principal factor that contribute to increased mortality and morbidities (Emans et al., 2009; Yigitler et al., 2012), such as life-threatening ileus, chronic abdominopelvic discomfort, chronic pelvic pain, infertility, dyspareunia, and intestinal obstruction, accounting for approximately 75% of all cases (Miller et al., 2000; Ghosheh and Salameh, 2007; Izumi et al., 2007).

An increasing number of strategies to reduce adhesions have been clinically available, including fibrinolytic agents, anti-inflammatory drugs, and barrier/separation materials. However, only a few antiadhesion agents can be applied...
effectively during laparoscopic surgery (Dupre et al., 2013), and few of the approaches work well to a satisfactory level (Sahin et al., 2007; Takagi et al., 2013). Drugs such as corticosteroids inhibit fibrinolytic activity and may actually support adhesion formation (Ward and Panitch, 2011), which might contribute to an unacceptably high incidence of anastomotic leaks. Undoubtedly, there is an urgent need for an effective formula of antiadhesion and anti-inflammation without similar clinical disadvantages.

Berberine hydrochloride (berberine; 5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo [5,6-a]quinolinizinium) (Fig. 1), an isoquinoline alkaloid initially derived from Rhizoma Coptidis, Hydrastis canadensis, and Berberis aquifolium, has been used in traditional Chinese medicine for many decades (Kong et al., 2004). Berberine extracts have had a long history of significant medicinal use for antimicrobial activity (Gray and Lachance, 1956; Feng et al., 2012) and anti-inflammatory effect (Cao et al., 2013; Mo et al., 2014). The predominant clinical uses of berberine include gastroenteritis, intestinal parasite infections, bacterial diarrhea, and ocular trachoma infections (Yao et al., 2013). These facts prompted us to propose that berberine may be used as a therapeutic strategy for preventing postsurgical intestinal adhesions because of its anti-inflammatory properties.

Adhesion molecules are key mediators of intestinal adhesion. Intercellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin superfamily necessary for the adhesion of leukocytes to the capillary endothelium (Jung et al., 2012). ICAM-1 has been implicated in the development of intestinal adhesion, and is proved to play an essential role in stabilizing cell-cell interactions (Sigal et al., 2000). ICAM-1 also participates in maintaining a proinflammatory environment conducive to leukocyte endothelial transmigration (Proud and Leigh, 2011). This study was set to investigate the antiadhesion efficacy of berberine and delineating the underlying mechanisms in a rat model of intestinal adhesions induced by abrasive abdominal surgery.

Materials and Methods

Animals. Fifty healthy male Sprague-Dawley rats (200–250 g body weight) were used for this study. Animals were housed under standard laboratory conditions at a constant room temperature (22 ± 0.5°C) and relative humidity (65–70%) with a 12-hour light and dark cycle. They were fed a commercial rat diet and water ad libitum. All rats were acclimated to the laboratory environment for 7 days before the operation. They were deprived of food for 12 hours before the experiment but had free access to water. All animal procedures were in accordance with the Declaration of Helsinki and were reviewed and approved by the Animal Care and Use Committee of the Harbin Medical University.

Experimental Design. The rats were allocated randomly into five groups (n = 10): sham control, surgery, berberine-1, berberine-2, and Interceed groups. The animals were anesthetized intraperitoneally with sodium pentobarbital (3%) at a concentration of 0.13 ml/100 g body weight. The abdomen was then shaved and prepared with alcohol and iodine solution. After drying, a 3-cm anterior midline ventral laparotomy through the abdominal wall and peritoneum was performed under sterile conditions to gain access to the abdominal cavity. We isolated the cecum and induced intestinal adhesion by abrading the cecum with a toothbrush, such that a homogeneous surface of petechial hemorrhage was created (Supplemental Fig. 1). After the cecum was returned to the abdomen, the midline incision was closed in two layers with 5-0 nylon (Townsend et al., 2011). For sham control animals, approximately 2.0 ml of normal saline was dropped into the abdominal cavity after the laparotomy surgery. For the surgery group, approximately 2.0 ml of normal saline was dropped into the abdominal cavity immediately after the abrasive surgery. For the berberine-1 group, berberine hydrochloride in normal saline (1.5 mg/ml) was dropped into the abdominal cavity immediately after the abrasive surgery. For the berberine-2 group, berberine hydrochloride in normal saline (0.75 mg/ml) was dropped into the abdominal cavity immediately after the abrasive surgery. Interceed is an adhesion barrier used as a positive control in the current study; for the Interceed group, a 3.0 × 2.0 cm piece of Interceed was attached to the abdominal wall defect, anterior to the abraded cecum, and approximately 2.0 ml of normal saline was added onto the Interceed.

Reagents. Berberine was obtained from Sigma-Aldrich (St. Louis, MO). The Interceed Absorbable Adhesion Barrier was purchased from Ethicon, Inc. (Somerville, NJ).

Evaluation of Adhesion Formation. On postoperative day 14, animals were anesthetized intraperitoneally with sodium pentobarbital (3%) at a concentration of 0.13 ml/100 g body weight. A laparotomy was undertaken to assess adhesion formation. The incision was performed at a position remote to the original laparotomy scar to prevent interfering with any adhesions between the abdominal wall and viscera. The abdominal wall was then opened fully to examine the extent of adhesions. The adhesions were graded by the scoring system developed from previously validated comparisons (Townsend et al., 2011). This method, taking into account number, strength, and distribution, provides a more objective measurement of adhesion formation. We adopted the macroscopic adhesion measurement score as our scoring system for assessing the severity of adhesion formation (Supplemental Table 1). Application of the scoring system was performed by an observer blinded to the study design. Photographs of adhesions were taken for each animal to allow grading of the adhesions by another independent observer (Supplemental Fig. 2).

Samples. After 14 days, the animals were euthanized, and blood serum was collected for enzyme-linked immunosorbent assay measurement. The cecum samples were dissected for a series of experiments.

RNA Extraction and Real-Time Reverse-Transcription Polymerase Chain Reaction Analysis for mRNAs. Interleukin-1β (IL-1β), IL-6, transforming growth factor β (TGF-β), tumor necrosis factor-α (TNF-α), and ICAM-1 mRNA expression was analyzed. The animals were humanely killed 14 days after the surgery, and the cecum was immediately dissected, frozen in liquid nitrogen, and stored at −80°C. The tissue was carefully transferred into a RNase-free tube for extraction of RNA. Thawed tissues were homogenized in 1 ml of TRIzol reagent (Invitrogen, Carlsbad, CA), and total RNA in the tissue was isolated. cDNA synthesis was performed by the reverse transcription kit (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. The SYBR Green polymerase chain reaction (PCR) Master Mix Kit (Applied Biosystems) was used for relative quantification of RNAs (Zhang et al., 2010). GAPDH was used as an internal control.
Real-time reverse-transcription PCR was performed on a 7500 FAST Real-Time PCR System (Applied Biosystems). The primers used in our study were provided in Supplemental Table 2 (You et al., 2011; Werry et al., 2012).

Enzyme-Linked Immunosorbent Assay Measurement. Levels of IL-1β, IL-6, TGF-β, ICAM-1, and TNF-α in serum and secreted from the cecum were detected using a rat IL-1β enzyme-linked immunosorbent assay (ELISA) kit, rat IL-6 ELISA kit, rat TGF-β ELISA kit, rat ICAM-1 ELISA kit (Wuhan Boster Biological Technology, Ltd., Wuhan, Hubei, China), and rat TNF-α ELISA kit (Beijing 4A Biotech Co., Ltd., Beijing, China), respectively.

Western Blot Analysis. The protein samples were extracted from rat cecum for immunoblotting analysis of p-TAK1, p-JNK, p-NF-kB p65, TAK1, JNK, and NF-kB p65. The protein content was determined using the BCA Protein Assay kit (Bio-Rad, Mississauga, ON, Canada). Equal amounts of protein (60 µg) were loaded on a 10% SDS-PAGE gel. The lysates were resolved by electrophoresis (70 V for 30 minutes and 100 V for 1.2 hours) and transferred onto nitrocellulose membranes. After blocking in 5% nonfat milk for 2 hours at room temperature, the membranes were treated with antibodies (Zhang et al., 2013). Membranes were blotted with antibodies against p-JNK (Cell Signaling Technology, Danvers, MA), JNK (Cell Signaling Technology), NF-kB (Santa Cruz Biotechnology, Santa Cruz, CA), p-NF-kB (Cell Signaling Technology), β-actin (Cell Signaling Technology), p-TAK1 (Cell Signaling Technology), and TAK1 (Cell Signaling Technology), according to the manufacturer's instructions. The next day, the membrane was washed in phosphate-buffered saline with Tween-20 three times (15 minutes each) and incubated for 1 hour with the fluorescein-conjugated antirabbit IgG secondary antibody (1:10,000) in the blocking buffer. Western blot bands were quantified using Odyssey v1.2 software (LI-COR Biosciences, Valencia, CA) by measuring the band intensity (area × outer diameter) for each group and normalized by β-actin. The final results are expressed as fold changes by normalizing the data to the control values.

Tissue Handling and Immunohistochemistry Assessment. The resected adhesion samples were fixed in a 10% formalin solution, and following dehydration, the samples were processed and embedded in paraffin using tissue-processing equipment. The sections were stained with H&E and Masson trichrome staining to assess the degree of fibrosis, inflammation, and vascular proliferation, using a previously reported semiquantitative scoring system. The fibrosis grading system was as follows: 0, nil; 1, minimal and loose; 2, moderate; and 3, florid and dense. Inflammation was scored as follows: 0, nil; 1, giant cells with occasional scattered lymphocytes and plasma cells; 2, giant cells with increased numbers of admixed lymphocytes, plasma cells, eosinophils, and neutrophils; and 3, numerous admixed inflammatory cells with microabscesses present. Vascular proliferation was scored as follows: 0, no vascular proliferation; 1, mild proliferation; 2, moderate proliferation; and 3, intense proliferation (Townsend et al., 2011).

For immunohistochemical analysis, cecum samples were fixed with 4% buffered paraformaldehyde embedded in paraffin, and tissue slices were dehydrated in ascending series of ethanol, cleared in xylene. All sections were incubated with ICAM-1 antibody at 4°C overnight. After incubation with the second antibody immunoglobulin, the sections were stained with diaminobenzidine.

Measurement of Collagen Content. Total collagen content was measured by a Biocolor Collagen Assay Kit (Biocolor LTD, Carrickfergus, UK) according to the manufacturer's instructions (Shan et al., 2009). Lysates (100 µl) were stained with 1 ml of Biocolor dye reagent. The unbound dye solution was removed, and 1 ml of the alkali reagent was added. The absorbance was read at 540 nm. Total collagen (milligrams) was calculated according to a linear calibration curve from standards (Vitrogen 100; Angiotech BioMaterials, Palo Alto, CA) and normalized to the total protein (milligrams) of each lysate.

Statistical Analysis. Data are given as means ± S.E.M. Comparisons of adhesion scores between different groups were made using the Kruskal–Wallis H test and the Mann–Whitney U test by SPSS 19.0 (SPSS Inc., San Diego, CA). One-way analysis of variance was applied for statistical comparisons between different experimental groups in Prism version 5 (GraphPad Software Inc., San Diego, CA). A value of P < 0.05 was considered statistically significant.

Results

The Antiadhesion Formation Properties of Berberine. To determine the severity and incidence of adhesion, the adhesion formation was classified into six grades, using a predetermined adhesion measurement score (Townsend et al., 2011): grade 0, no adhesions; grade 1, loose, filmy adhesions that could be separated by blunt dissection; grade 2, adhesions requiring <50% sharp dissection for separation; grade 3, adhesions requiring >50% sharp dissection for separation; grade 4, serosal injury; and grade 5, full-thickness injury (as described in Supplemental Table 1). Examples of photographic images at post-mortem evaluation of adhesions on day 14 after the surgery are displayed in Supplemental Fig. 2. The results on adhesion severity and adhesion area are presented in Table 1. Statistically significant differences were found among the groups in terms of the incidence and severity of adhesion formation. Rats of the abrasive surgery group showed the most severe adhesions between the abraded cecum and the abdominal wall lesion, of which four had an adhesion score over 4 with full-thickness injury. The adhesion was remarkably alleviated by berberine at concentrations of 1.5 and 0.75 mg/ml, compared with the nontreated surgery group. Moreover, berberine was as effective as the Interceed as a positive control agent.

Cellular Mechanisms for the Antiadhesion Properties of Berberine. Histopathology revealed significant morphologic differences in myodegeneration and a subacute to chronic inflammatory response within the abraded muscle. Similar changes were observed in the sham (Fig. 2, A–C) and berberine treatment groups (Fig. 2, G–I), indicating that the changes were a response to the surgical procedure. The microscopic alterations were evaluated for fibrin presence, inflammatory infiltrates (polymorphonuclear or mononuclear), giant cell presence, angiogenesis, fibroblast proliferation, hemorrhage, and necrosis. As shown in Fig. 2, in the surgery group (Fig. 2, D–F), the connective tissue and fibrin were florid and dense, and giant cells had increased numbers of admixed lymphocytes, plasma cells, eosinophils, and neutrophils, and even numerous admixed inflammatory cells with microabscesses present. There was also intense vascular proliferation and congestion, indicating the severe inflammatory process occurring in the surgery group compared with the sham group (Fig. 2, A–C). Berberine and Interceed treatment (Fig. 2, G–L) reduced the inflammatory responses as indicated by the scattered lymphocytes and plasma cells, and the mild proliferation of the blood vessel. Results of Masson trichrome staining in the surgery group showed massive amounts of collagen production and deposition (blue), mainly in the lamina propria, submucosal areas, and muscularis propria in the cecum. The berberine and Interceed groups remarkably attenuated fibrin and collagen deposition of the cecum samples (Fig. 3, A–D). Measurement of collagen verified decreased mRNA levels of collagen 1 (Fig. 3E) and collagen 3 (Fig. 3F) in berberine and Interceed groups compared with the surgery group by real-time reverse-transcription PCR.
Statistically significant differences in collagen content were discovered among the groups; the result showed collagen deposition increased in the surgery group and was attenuated with berberine and Interceed treatment (Fig. 3G).

Molecular Mechanisms for Antiadhesion Properties of Berberine. ICAM-1, also known as CD54, encodes a cell surface glycoprotein that is typically expressed in endothelial cells and immune cells and plays an important role in stabilizing cell-cell interactions and facilitating leukocyte endothelial transmigration. ICAM-1 also participates in a positive-feedback loop to maintain a proinflammatory environment conducive to leukocyte endothelial transmigration (Rothlein et al., 2011). We measured both circulating and tissue ICAM-1 levels and found that serum ICAM-1 was significantly elevated (~6.5-fold) in the surgery group and was reduced in the berberine-1 (~20.8% of the surgery group), berberine-2 (~31.2% of the surgery group), and Interceed (~37.1% of the surgery group) groups (Fig. 4A). Similarly, differential changes in cecum ICAM-1 levels were also consistently observed. ICAM-1 expression was significantly upregulated in the surgery group, from 347.7 ± 63.6 ng/ml in the sham group to 1028.7 ± 112.6 ng/ml, and the effect was nearly eliminated after treatment with berberine-1 (367.6 ± 77.1 ng/ml), berberine-2 (471.9 ± 76.7 ng/ml), or Interceed (511.7 ± 76.5 ng/ml) (Fig. 4B). In the immunohistochemical analysis, expression of ICAM-1 in the surgery group increased remarkably, and berberine reduced ICAM-1 and ameliorated adhesion (Fig. 4, C–F). To further investigate the role of ICAM-1 in mediating berberine’s antiadhesion effect, we applied an intestinal HT29 cell line, which was reported to have higher expression of ICAM-1 (Gallicchio et al., 2008). Treatment with berberine or ICAM-1 small interfering RNA (siRNA) led to a decrease of ICAM-1 expression. ICAM-1 relative protein expression and mRNA level in cells, as well as ICAM-1 concentration in cultural supernatants, were all significantly decreased in the berberine high-dose group and ICAM-1 siRNA group. The cell adhesion assay exhibited obvious reduction in relative adherent cells with berberine treatment and ICAM-1 siRNA transfection compared with the negative control (NC) group (Supplemental Fig. 3).

The pathogenesis of inflammation is a result of complex interactions between circulating inflammatory factors. Recent studies have revealed essential roles of cytokines, the

| Groups       | Adhesion Score |   |   |   |   | 0 | 1 | 2 | 3 | 4 | 5 |
|--------------|----------------|---|---|---|---|---|---|---|---|---|---|---|
| Sham         | 10             | 0 | 0 | 0 | 0 | 0 |   |   |   |   |   |   |
| Surgery**    | 0              | 1 | 2 | 0 | 3 | 4 |   |   |   |   |   |   |
| Berberine-1##| 5              | 2 | 2 | 1 | 0 | 0 |   |   |   |   |   |   |
| Berberine-2##| 4              | 2 | 1 | 2 | 1 | 0 |   |   |   |   |   |   |
| Interceed##  | 4              | 2 | 2 | 1 | 1 | 0 |   |   |   |   |   |   |

##P < 0.01, surgery vs. berberine and Interceed groups; **P < 0.01, sham vs. surgery.

Fig. 2. Histopathology assessment results of the five groups. (A–C) The sham group. (D–F) The surgery group. (G–I) The berberine groups. (J–L) The Interceed group (n = 3). Arrows represent vascular proliferation and congestion. Yellow asterisks represent giant cells with increased numbers of admixed lymphocytes, plasma cells, eosinophils, and neutrophils and even numerous admixed inflammatory cells with microabscesses. The box shows that the connective tissue and fibrosis is florid and dense. Scale bars: 10 μm.
chemical messengers between immune cells, such as TNF-α, IL-6, IL-1β, and TGF-β, in mediating inflammatory and immune responses (Holmdahl and Ivarsson, 1999; Kosaka et al., 2008; Kim et al., 2011; Morin et al., 2012). We observed that secretion of inflammatory cytokines IL-1β, TGF-β, TNF-α, and IL-6 was significantly decreased from 54.8 ± 8.4, 102.8 ± 14.6, 106.1 ± 23.6, and 496.3 ± 50.1 ng/ml to 28.1 ± 1.1, 26.9 ± 4.6, 48.0 ± 2.4, and 84.3 ± 3.5 ng/ml in the berberine-1 groups compared with the surgery group, respectively (Fig. 5).

Consistently, the quantitative PCR results of IL-1β, TGF-β, TNF-α, and IL-6 exhibited higher expression levels in cecum tissue in the surgery group compared with the sham animals. Treatment with berberine substantially reversed the overexpression of the cytokines (Fig. 6).

**Signaling Mechanisms for the Antiadhesion Properties of Berberine.** TAK1/JNK and TAK1/NF-κB signaling pathways have been known to play a critical role in the inflammatory response (Gonzalez-Guerrero et al., 2013). Our data suggest that these two signaling pathways were also involved in the postsurgical intestinal adhesion. As illustrated in Fig. 7, our Western blot results revealed that TAK1 was highly activated in the surgery group as reflected by the increased phosphorylated form of TAK1, and this activation was suppressed by berberine. As expected, the activities of
JNK and NF-κB signaling were decreased by berberine as indicated by the decreased phosphorylation of JNK and NF-κB.

Discussion

Here, we have presented results from our experimental investigation on the efficacy of berberine on adhesion formation in a rat model of intestinal adhesions developed following abrasive abdominal surgery. Our data unraveled berberine as an antiadhesion agent capable of inhibiting postabdominal surgery adhesion formation and inflammatory responses. Blockade of ICAM-1 secretion, downregulation of expression of various inflammatory molecules, and inhibition of TAK1/JNK and TAK1/NF-κB signaling may account for the antiadhesion properties of berberine, as summarized schematically in Fig. 8.

Intestinal adhesion after abdominal surgery is a continuous state of fibrosis and inflammation that concomitantly facilitates the progression of a variety of complications, such as chronic abdominopelvic discomfort, pain, and infertility. Berberine has recently become a useful anti-inflammatory drug for cardiovascular, endocrine, and intestinal disorders, because of its antitumor and antilipase effects (Jeong et al., 2009; Jiang et al., 2011; Meng et al., 2012; Yao et al., 2013). Here, we identified berberine as an antiadhesion agent that effectively protects against postabdominal surgery adhesion and inflammation, with several lines of evidence including reduced adhesion scores from direct assessment of adhesions, histopathology measurement of lymphocytes and vascular proliferation, decreased circulating concentration of ICAM-1, downregulated expression levels and secretion of various proinflammatory cytokines in both serum and cecum, and suppression of TAK1, JNK, and NF-κB signaling.

ICAM-1 is a key proadhesive protein that is markedly elevated in inflammatory diseases. Berberine effectively decreased ICAM-1 level in serum and cecum. In the immunohistochemical analysis, expression of ICAM-1 in this study indicates that suppression of ICAM-1 underlies the antiadhesive action of berberine on postsurgery tissues, as ICAM-1 is involved in stabilizing cell-cell adhesions and participates in maintaining a proinflammatory environment conducive to leukocyte endothelial transmigration. In vitro, treatment of the intestinal HT29 cell line, which is reported to have higher expression of ICAM-1 with berberine and ICAM-1 siRNA, led to a decrease of ICAM-1 expression. Expression of ICAM-1 was significantly decreased in the berberine high-dose group and ICAM-1 siRNA group. The cell adhesion assay exhibited obvious reduction of relative adherent cells with berberine treatment and ICAM-1 siRNA transfection. Nevertheless, because inflammatory cytokines were inhibited by berberine, suppression of ICAM-1 may be an indirect effect of berberine by inhibition of inflammation. Until this is accomplished, the role of ICAM-1 in mediating berberine’s antiadhesion...
effects could be fully established. The IL-1β, IL-6, TGF-β, and TNF-α cascade has been shown to play a critical role in the pathogenesis of tissue inflammation. A recent study demonstrated that Cicaderma ointment, a mix of natural extracts, modulates inflammation, promotes re-epithelialization, and accelerates skin wound healing through the modulation of a few cytokines, including TNF-α, macrophage inflammatory protein-1α, IL-12, IL-4, and macrophage–colony-stimulating factor (Morin et al., 2012). The increase of TNF-α could participate in the upregulation of several inflammatory

![Fig. 5. Effects of berberine on inflammatory cytokine secretion in serum. (A) Concentration of IL-1β in serum in the five groups (n = 7). (B) Concentration of TGF-β in serum. (C) Concentration of TNF-α in serum. (D) Concentration of IL-6 in serum. #P < 0.05; ##P < 0.01, surgery vs. berberine; *P < 0.05; **P < 0.01, sham vs. surgery; mean ± S.E.M.](image)

![Fig. 6. mRNA expression of inflammatory cytokine genes in cecum. (A) IL-1β mRNA relative level in the five groups (n = 7). (B) TGF-β mRNA relative level. (C) TNF-α mRNA relative level. (D) IL-6 mRNA relative level. #P < 0.05; ##P < 0.01, surgery vs. berberine; **P < 0.01, sham vs. surgery; mean ± S.E.M.](image)
cytokines, i.e., IL-1, IL-6, IL-12, and IL-17, but also the synthesis of cell surface adhesion molecules involved in adhesion to the endothelium (Mahdavian Delavary et al., 2011). Moreover, TAK1, a member of the mitogen-activated protein kinase kinase kinase family (Goldmann et al., 2013), is considered a key intermediate in a multitude of innate immune signaling pathways by directly regulating the JNK and extracellular signal-regulated protein kinase pathways.

Fig. 7. Results of Western blot on the TAK1/JNK and TAK1/NF-κB pathway. Activity of the TAK1/JNK and TAK1/NF-κB signaling was performed by the Western blot in the five groups (n = 3). (A) Representative Western blot bands. Relative protein expression of p-TAK1 (B), TAK1 (C), p-p65 NF-κB (D), p65 NF-κB (E), p-JNK (F), and JNK (G). ##P < 0.01, surgery vs. berberine; **P < 0.01, sham vs. surgery; mean ± S.E.M.
by activating regulators of JNK and ERK signaling SEK and MEK1/2, respectively (Gonzalez-Guerrero et al., 2013). TAK1 has also been demonstrated to be a major upstream activator of JNK, p38 mitogen-activated protein kinase, and NF-κB signaling in the obstructed kidney, and activation of JNK, p38, and the transcription factor NF-κB drives renal inflammation and fibrosis (Ma et al., 2011). Therefore, we hypothesize that the suppression of TAK1 cascade may be one underlying mechanism of berberine’s beneficial effects on postsurgery intestinal inflammation. Our data indicate that treatment of abrasive surgery rats with berberine significantly inhibited the upregulation of the active form of TAK1, phospho-TAK1, while having no effect on the total TAK1, the expression of which was not altered after abrasive surgery. JNK and its downstream transcription factor c-Jun have been associated with inflammation in several diseases, including asthma (Alrashdan et al., 2012), arthritis (Guma et al., 2011), and renal diseases, in association with renal inflammation, interstitial fibrosis, and decline of renal function (de Borst et al., 2009). In the present study, we found that the expression of phospho-JNK in the cecum of model rats was significantly upregulated, and this was attenuated by treatment with berberine. Another TAK1 cascade, NF-κB, a protein responsible for cellular responses to stimuli such as cytokines, UV irradiation, free radicals, and viral or bacterial antigens and that plays a crucial role in regulating the immune response to infection (Brasier, 2006; Perkins, 2007; Yadav et al., 2013; Dediego et al., 2014), was also implicated in the anti-inflammation effect of berberine. Our results showed that the activation of NF-κB induced by abrasive surgery was significantly inhibited by berberine, as indicated by a decreased phospho–NF-κB expression. These results strongly indicate that the inhibition of TAK1/JNK and TAK1/NF-κB accounts for the antiadhesion and anti-inflammation action of berberine.

Accumulated evidence implicated the utilization of mesh barrier materials or anti-inflammatory drugs to prevent the adhesion formation and directly interfere with the inflammatory process. Research to date has identified four main aspects of methods to prevent the formation of intestinal adhesions after abdominal surgery: 1) use of minimally invasive surgical procedures, such as laparoscopic surgery to reduce peritoneal trauma; 2) prevention of fibrin formation with pharmacological agents, such as heparin or tissue plasminogen activator; 3) reduction of the contact between organs and intra-abdominal contents by using biodegradable barriers such as Seprafilm; and 4) a wide range of biologically active substances in the form of simple fluids, gels, and solids, either combined or alone, have been investigated both clinically and experimentally to reduce or prevent abdominal adhesions (Tingstedt et al., 2007; Lauder et al., 2010; Takagi et al., 2013; Ten Broek et al., 2013a,b). An ideal barrier should be biodegradable, biocompatible, and surgically easy to handle and should act locally to avoid side effects. However, only certain materials meet these requirements, and no large prospective, randomized, double-blind human studies have demonstrated their efficacy. Some of them are effective in the short term, but the protective effects are lost in the long term; some have no effect on inflammation, and others have a severe detrimental effect. Thus, few of these materials have been widely adopted by surgeons, indicating that the materials only decrease adhesion severity, not incidence (Lauder et al., 2010). Interceed (TC7) Absorbable Adhesion Barrier is a preformed sheet of oxidized regenerative cellulose, and its safety and efficacy have been documented in several controlled clinical studies (Wiseman et al., 1999; Tsapanos et al., 2002). The data presented in our study demonstrated that administration of berberine decreased collagen synthesis, adhesion, and inflammation and had no detrimental effect on the wound-healing process. These results indicate that berberine may be an effective drug in the prophylaxis of adhesion formation with minimal side effects, which overcomes the shortage of current antiadhesion methods.

In this study, we have unraveled a novel pharmacological effect of berberine: effectively inhibiting adhesion and inflammation after abdominal surgery with few side effects. Meanwhile, we have also deciphered the potential molecular mechanisms underlying the antiadhesions properties of berberine. Our findings also strongly suggest that berberine is a promising candidate agent for clinical application to preventing adhesion after surgery. However, we are well aware that additional studies looking at the effects after a longer term are needed to obtain more definitive evidence for the sustained efficacy and the lack of toxic effects of berberine. Moreover, our results do not allow us to deduce a definite mechanism for the formation of adhesions and inflammations and for the antiadhesion properties of berberine, which merits future investigations to advance and deepen our understanding of the issue.

Authorship Contributions

Participant in research design: Y. Zhang, B. Yang.
Conducted experiments: Y. Zhang, X. Li, L. Yang, Ju, Du, Chen, Cheng, Liu, Q. Zhang, J. Li, Bulal.
Performed data analysis: X. Li, Xu, Wei.
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Supplemental Data

Berberine Hydrochloride Prevents Post-Surgery Intestinal Adhesion and Inflammation in Rats

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Supplementary methods

HT29 cell culture and transfection. Intestinal HT29 Cells were seeded in DMEM containing 10% fetal bovine serum (FBS) and used for all experiments. Cells were transfected with optimem medium (invitrogen) and lipo2000 (invitrogen). Cells was divided into four groups. Negative control group was transfected with ICAM-1 siRNA negative control synthesized by Ribo Life Science Co.,Ltd, berberine high dose group: berberine 1 (100uM), berberine low dose group: berberine 2 (20uM), and siRNA group which was transfected with ICAM-1 siRNA synthesized by Ribo Life Science Co.,Ltd, cultured in 37 °C for 24 hours.

HT29 cell adhesion assay. The cells were incubated with 50uM fibronectin (BD™ Fibronectin, human) diluted in PBS for 1h and then blocked with BSA at 37°C for 2 h. The cell adhesion and viability was detected by measuring the absorbance of each well at 490 nm.
Table S1. The scoring system used for the macroscopic evaluation of the adhesion formation

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no adhesions</td>
</tr>
<tr>
<td>1</td>
<td>loose filmy adhesions that could be separated by blunt dissection</td>
</tr>
<tr>
<td>2</td>
<td>adhesions requiring $&lt;$50% sharp dissection for separation</td>
</tr>
<tr>
<td>3</td>
<td>adhesions requiring $&gt;$50% sharp dissection for separation</td>
</tr>
<tr>
<td>4</td>
<td>serosal injury</td>
</tr>
<tr>
<td>5</td>
<td>full-thickness injury</td>
</tr>
</tbody>
</table>
Table S2. Nucleotide sequence of the forward and reverse primers for the Real-Time PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>GenBank No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat-IL-1β</td>
<td>Forward CCCTGCAGCTGGAGAGTGTGG</td>
<td>NM 031512.2</td>
</tr>
<tr>
<td></td>
<td>Reversed TGTGCTCTGCTTGAGGATGTTGCT</td>
<td></td>
</tr>
<tr>
<td>rat-IL-6</td>
<td>Forward TTCCTACCCCAACTTCCAATG</td>
<td>NM 012589.1</td>
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<tr>
<td></td>
<td>Reversed ATGAGTTGGATGGTCTTGTC</td>
<td></td>
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<tr>
<td>rat-TNF-α</td>
<td>Forward GACCCCTCACACTCAGATCATCTTCT</td>
<td>NM 012675.3</td>
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<tr>
<td></td>
<td>Reversed TGCTACGACGTGGGCTACG</td>
<td></td>
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<tr>
<td>rat-TGF-β</td>
<td>Forward GCGAGCGAAGCGACGAGGAG</td>
<td>NM 031131.1</td>
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<td></td>
<td>Reversed TGGGCGGGATGGCATCAAGGTA</td>
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<tr>
<td>rat-ICAM-1</td>
<td>Forward TCATGCCCGTGAAATTATGGT</td>
<td>NM_012967.1</td>
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<td></td>
<td>Reversed AGGACCCCTTCTAAGTGTTGGA</td>
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<td>rat-GAPDH</td>
<td>Forward AAGAAGGTGGTGGAAGCGAGGC</td>
<td>NM 017008.3</td>
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<td></td>
<td>Reversed TCCACCACCCAGTTGCTGA</td>
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<tr>
<td>rat-Col-1</td>
<td>Forward CAATGGCAGGCTGTTGCG</td>
<td>NM_053304.1</td>
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</table>
Reversed CACTCGCCCTCCCCGTCTTTGG

rat-Col-3  Forward TGAATGGTGGTTTTCAGTTCAG  NM_032085.1

Reversed GATCCCATCAGCTTCAGAGACT
Supplemental Figure 1

Fig. S1. Surgical Procedure:  (A) Sham: approximately 2.0 ml of normal saline was dropped into the abdominal cavity without the surgery. (B) Approximately 2.0 ml of normal saline was dropped into the abdominal cavity immediately after the surgery of abrasion. (C) Berberine hydrochloride in normal saline (1.5mg/ml) dropped into the abdominal cavity immediately after the surgery of abrasion. (D) Berberine hydrochloride in normal saline (0.75mg/ml) dropped into the abdominal cavity immediately after the surgery of abrasion. (E) a 3.0 × 2.0-cm piece of Interceed was attached to the abdominal wall defect of abrasion
Supplemental Figure 2

Fig. S2. Photographic images at post mortem evaluation of adhesions on 14 days postoperation. (A). Adhesion score 0 in a sham model. (B). Adhesion score 1 in a berberine-1 (1.5mg/ml) model. (C). Adhesion score 2 in a berberine-2 (0.75mg/ml) model. (D). Adhesion score 3 in an abrasion surgery model. (E). Adhesion score 4 in an abrasion surgery model. (F). Adhesion score 5 in an abrasion surgery model.
Fig.S3. Expression of ICAM-1 in HT29 cell line and results of cell adhesion assay.

(A). Relative protein expression of ICAM-1 in HT29 cell line in NC, berberine 1, berberine 2 and siRNA groups. (B). Concentration of ICAM-1 in cell supernatant in the 4 groups. (C). Relative mRNA expression in HT29 cell line. (D). Cell adhesion assay. n=3. *p, #p<0.05, **p, ###p<0.01; mean±SEM. *p: sham vs surgery. #p: surgery vs Berberine.