Aspirin prevents postoperative peritoneal adhesions by inhibiting the TGF-β1/Smad signaling pathway in rats

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Abstract. Postoperative peritoneal adhesion (PPA) is a common complication following abdominal surgery and effective preventive strategies remain limited. The present study aimed to investigate the protective effect of aspirin against PPA in rats and to elucidate the underlying mechanism involving the transforming growth factor-β1 (TGF-β1)/Smad signaling pathway. A total of 32 male Sprague-Dawley rats were randomly divided into the four following groups: Sham-operated group, model group, low-dose aspirin group (10 mg/kg) and high-dose aspirin group (30 mg/kg). A PPA model was established by cecal wall abrasion. The aspirin-treated groups received daily intragastric administration for 8 consecutive days post-surgery. All animals were euthanized on day 8. Adhesion severity was assessed using the Nair scoring system; histopathological changes were examined by Masson's trichrome staining; the expression levels of proteins related to the TGF-β1/Smad pathway and to markers of fibrosis were detected by western blot, immunohistochemical (IHC) and reverse transcription-quantitative PCR (RT-qPCR) analyses. In addition, rat primary peritoneal mesothelial cells (RPMCs) were treated with different concentrations of aspirin to evaluate the expression levels of the relevant proteins. The results indicated that compared with the model group, aspirin administration significantly reduced PPA scores. Histological examination revealed that aspirin treatment alleviated collagen deposition in adhesion tissues. The results derived from western blotting, IHC and RT-qPCR analyses demonstrated that aspirin downregulated the expression levels of TGF-β1, phosphorylated (p)-Smad2/3, alpha smooth muscle actin (α-SMA) and collagen type I, alpha 1 (COL1A1), and inhibited the phosphorylation of Smad2/3. Moreover, treatment of RPMCs with different concentrations of aspirin led to a dose-dependent decrease in the protein expression levels of TGF-β1, COL1A1, α-SMA, p-Smad2 and p-Smad3, while simultaneously upregulating the expression levels of the endogenous inhibitory factor Smad6 in this pathway. In conclusion, the data demonstrated that aspirin effectively prevented the formation of PPA in rats by suppressing peritoneal fibrosis, an effect likely mediated via inhibition of the TGF-β1/Smad signaling pathway. These findings suggest that aspirin is a promising candidate for the clinical prevention of PPA.

Introduction

Postoperative peritoneal adhesion (PPA) represents one of the most common complications following abdominal and pelvic surgery, profoundly affecting patients' postoperative recovery and long-term prognosis. Epidemiological data indicate that the overall incidence of PPA in patients undergoing intra-abdominal procedures ranges from 90 to 95% (1,2). PPA is defined as pathological fibrous connections formed between the peritoneal serosa, intestinal walls, omentum and other intra-abdominal tissues after surgical trauma. These adhesions not only cause immediate postoperative discomfort but also give rise to a spectrum of serious long-term sequelae, including recurrent chronic abdominal pain, repeated intestinal obstruction, female infertility due to pelvic adhesions and increased surgical risks during subsequent reoperations (3-5). In clinical practice, intra-abdominal adhesions substantially increase the difficulty of secondary laparoscopic and open surgeries, prolong operative time, and elevate the risks of intraoperative bleeding, visceral injury and postoperative infection. These complications impose immense physical suffering, financial burdens and psychological stress on patients, while also placing a heavy strain on healthcare systems worldwide (6,7). Despite the availability of various preventive strategies in clinical settings, effective interventions remain limited (8-10). Therefore, identifying reliable pharmacological targets and effective therapeutic agents to suppress the formation and progression of PPA is an urgent and valuable research direction.

The pathogenesis of PPA is a multifactorial, multi-stage process involving acute inflammation, fibrin exudation and abnormal deposition, excessive extracellular matrix (ECM)

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accumulation, collagen proliferation and remodeling, and aberrant angiogenesis. Among these, the imbalance of fibrin metabolism and subsequent peritoneal overfibrosis represent the core pathological events leading to irreversible adhesion formation (11,12). Under normal physiological conditions, the peritoneal mesothelium possesses robust barrier and fibrinolytic activities, maintaining a dynamic equilibrium between fibrin production and degradation. However, surgical mechanical injury, peritoneal ischemia and inflammatory stimuli disrupt the integrity of the mesothelial barrier, triggering extensive inflammatory cell infiltration and the release of proinflammatory cytokines. This promotes massive fibrin exudation while simultaneously suppressing endogenous fibrinolysis. The undegraded fibrin matrix subsequently serves as a scaffold for fibrous tissue proliferation, inducing the activation and differentiation of peritoneal fibroblasts, which ultimately culminate in abundant collagen deposition and peritoneal fibrosis, resulting in permanent postoperative adhesions (13).

Transforming growth factor-β1 (TGF-β1) is widely recognized as the most critical profibrotic cytokine in peritoneal fibrosis and PPA formation, playing a dominant regulatory role throughout the pathological cascade (14). Both clinical and basic research have consistently demonstrated that surgical peritoneal injury markedly upregulates the local expression and secretion of TGF-β1 in peritoneal tissues, and that elevated TGF-β1 levels are positively correlated with the severity of peritoneal adhesions and fibrosis (15,16). The canonical TGF-β1/Smad signaling pathway is the major downstream mediator of TGF-β1-induced fibrotic effects. Upon binding to its specific membrane receptors, TGF-β1 activates the phosphorylation of downstream Smad2 and Smad3 proteins. Phosphorylated Smad2/3 then form complexes with Smad4 and translocate into the nucleus, where they transcriptionally activate profibrotic genes. Numerous previous studies have confirmed that targeted inhibition of aberrant TGF-β1/Smad pathway activation effectively attenuates peritoneal fibrosis and blocks PPA formation (17-19).

Aspirin, a classic and widely used non-steroidal anti-inflammatory drug (NSAID), possesses well-established anti-inflammatory, analgesic, antipyretic and anticoagulant pharmacological properties (20). In recent years, growing evidence has revealed that aspirin exerts significant antifibrotic bioactivity in various organ fibrosis models. Clinical studies have demonstrated that aspirin can reduce hepatic fibrosis markers in patients with chronic liver disease (21) and ameliorate cardiac fibrosis (22,23), These findings collectively highlight the broad-spectrum antifibrotic potential of aspirin across diverse tissue microenvironments. Given that peritoneal fibrosis is the central pathological mechanism underlying PPA, we hypothesized that aspirin may alleviate adhesion formation by inhibiting postoperative peritoneal fibrosis. However, the effect of aspirin on PPA and its regulatory role in the TGF-β1/Smad pathway remain largely unexplored.

In the present study, a stable rat PPA model was established using the cecal abrasion method. Through both *in vivo* animal experiments and *in vitro* cellular assays, the preventive efficacy of different doses of aspirin on PPA in rats was systematically evaluated. Concurrently, the study focused on the core TGF-β1/Smad signaling cascade and examined the expression levels of key pathway proteins and fibrosis-related markers to elucidate the molecular mechanisms by which aspirin inhibits peritoneal fibrosis and ameliorates PPA. The findings aim to provide robust experimental evidence and theoretical support for the clinical translation and application of aspirin as an affordable, safe and effective agent for the prevention of postoperative peritoneal adhesions.

Materials and methods

Animals. A total of 32 healthy male Sprague-Dawley rats (6-week-old, weighing 200-220 g) were supplied by Speyford Biotechnology Co., Ltd. The animals were housed under standard conditions at a temperature of 20-25˚C and relative humidity of 40-50%, with a 12/12-h light/dark cycle. Food and water were provided *ad libitum*. The present study was approved by the Animal Ethics Committee of the First Affiliated Hospital of Yangtze University (approval no. KY20435; Jingzhou, China).

Drugs. Aspirin was purchased from Bayer AG. It was dissolved in dimethyl sulfoxide (DMSO) and subsequently diluted in culture medium to final concentrations of 1 mmol/l and 3 μmol/l, respectively.

Cell culture. Rat primary peritoneal mesothelial cells (RPMCs; cat no. CP-R157) were obtained from Wuhan Pricella Biotechnology Co., Ltd. (https://www.biomart.cn/infosupply/119487195.htm). The cells were received at passage 1. They were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 μg/ml streptomycin (Beyotime Institute of Biotechnology) at 37˚C in a humidified atmosphere containing 5% CO_2. Based on a previous study (24) and cell viability assays (Fig. S1), the cells at passages 2-4 were treated with 1 mmol/l or 3 mmol/l aspirin for 24 h. Control cells were treated with an equal volume of culture medium containing 0.1% DMSO.

Establishment of PPA model and drug administration. SD rats were randomly assigned to four groups (n=8 per group). Following fasting for 12 h, the rats were anesthetized by intraperitoneal injection of 3% sodium pentobarbital at a dose of 30 mg/kg (0.1 ml/100 g). Following hair removal and disinfection, a midline abdominal incision (~2 cm) was made to expose the cecum. In the model group and in the aspirin-treated groups, a sterile dry gauze (2x2 cm, 12 layers) was used to rub the cecal wall and the corresponding parietal peritoneum back and forth 15 times under moderate pressure (~0.5 N) until petechiae appeared on the surface. The abraded tissues were left exposed for 5 min prior returning to the abdominal cavity. The muscle layer and skin were sutured layer by layer. In the sham-operated group, only laparotomy and closure were performed without any cecal abrasion. Based on previous findings (25), the postoperative treatments were as follows: Both the sham and model groups were administered 2 ml normal saline daily by gavage for 8 consecutive days; the low-dose aspirin group received aspirin at 10 mg/kg (dissolved in 2 ml normal saline); and the high-dose aspirin group received aspirin at 30 mg/kg (dissolved in 2 ml normal saline). Both aspirin groups were treated for 8 consecutive days. The total experimental duration from surgery to euthanasia was 8 days.

Table I. Peritoneal adhesion scoring system according to the Nair scoring system.

Grade	Description of adhesive bands
0	No adhesions
1	A single, filmy adhesive band; easily separable by blunt dissection
2	More than one filmy adhesion; blunt dissection is slightly difficult but achievable
3	Dense adhesion; requires sharp dissection
4	Very dense adhesions; severe distortion of visceral anatomy due to adhesions, making separation extremely difficult

Table II. Primer sequences for reverse transcription-quantitative PCR.

Gene name	Primer sequence (5'-3')	Product size, bp
COL1A1	F: GAGAGAGCATGACCGATGGA	251
	R: CGTGCTGTAGGTGAATCGAC	
α-SMA	F: TGTGCTGGACTCTGGAGATG	148
	R: GAAGGAATAGCCACGCTCAG	
GAPDH	F: GACATCAAGAAGGTGGTG	124
	R: CAGCATCAAAGGTGGAAG	

F, forward; R, reverse; COL1A1, Collagen α-1(I) chain; α-SMA, α-smooth muscle actin.

Animal health and behavior were monitored twice daily throughout the experimental period. Humane endpoints were defined in accordance with the ARRIVE guidelines (26): (i) Body weight loss exceeding 20% within 48 h; (ii) inability to access food or water due to severe abdominal distension or immobility; (iii) persistent hunched posture, piloerection, or lethargy unresponsive to environmental enrichment; (iv) signs of severe pain (for example, vocalization, self-mutilation, or aggressive behavior); (v) respiratory distress or cyanosis. Any animal meeting one or more of these criteria was immediately deeply anesthetized with intraperitoneal sodium pentobarbital (30 mg/kg) and then euthanized by cervical dislocation. No animals reached these endpoints or succumbed unexpectedly during the study; all 32 rats completed the experiment and were included in the final analysis. To minimize suffering, all surgical procedures were performed under deep anesthesia induced by sodium pentobarbital (30 mg/kg, intraperitoneal injection). No postoperative analgesics were administered, as pilot studies indicated that the model induced only mild and transient discomfort. Nevertheless, the animals were closely monitored, and any signs of pain would have prompted immediate analgesic intervention (buprenorphine 0.05 mg/kg, subcutaneous injection) or euthanasia according to the humane endpoints. At 6 h after the last administration (day 8), all rats were anesthetized with 3% sodium pentobarbital (0.1 ml/100 g, intraperitoneal injection) and subsequently euthanized by cervical dislocation. Death was confirmed by observing (i) cessation of heartbeat and respiration for at least 5 min, (ii) fixed and dilated pupils, and (iii) absence of pedal and corneal reflexes. All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

Evaluation of adhesion/severity. On the 8th postoperative day, the rats were euthanized and a U-shaped incision was made to open the abdominal cavity. The severity of adhesions was assessed in a blinded manner by two independent observers according to the Nair scoring system (Table I) (27). Following adhesion scoring, the entire adhesion tissue was excised. Each sample was bisected longitudinally: One-half was fixed in 4% paraformaldehyde for 24 h for histological and immunohistochemical (IHC) analyses and the one-other half was snap-frozen in liquid nitrogen and stored at -80˚C for western blotting and reverse transcription-quantitative PCR (RT-qPCR) analyses. All sample divisions were performed by the same researcher using standardized anatomical landmarks.

Histopathology and IHC analysis. The adhesion tissues were fixed in 4% paraformaldehyde for 24 h, embedded in paraffin and sectioned at a thickness of 4 μm. Collagen deposition was detected using a Masson's trichrome staining kit (Wuhan Saiwei Biotechnology Co., Ltd.) following the manufacturer's instructions: Sections were dewaxed, rehydrated and stained for 15 min. For IHC, sections were sequentially dewaxed in xylene and dehydrated through a graded ethanol series. Antigen retrieval was performed by microwave heating in citrate buffer (pH 6.0) for 15 min. Following blocking with 10% bovine serum albumin (Beijing Dingguo Changsheng Biotechnology Co., Ltd.) at room temperature for 15 min, the sections were incubated overnight at 4˚C with the following primary antibodies: Rabbit anti-TGF-β1 (1:200; cat. no. BM3901; Boster Biological Technology), rabbit anti-COL1A1 (1:200; cat. no. F1228; Selleck Chemicals), rabbit anti-alpha smooth muscle actin (α-SMA; 1:2,000; cat. no. 14395-1-ap; Proteintech Group, Inc.), rabbit anti-p-Smad2 (1:200; cat. no. AY0742; Shanghai Abways Biotechnology Co., Ltd.) and rabbit anti-p-Smad3 (1:200; cat. no. CY5140; Shanghai Abways Biotechnology Co., Ltd.). Following washing, the sections were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (1:500; cat. no. PN0046; Wuhan Pinwofei Biotechnology Co., Ltd.) at 37˚C for 1 h. Color development was performed using 3,3'-diaminobenzidine for 90 sec, followed by counterstaining with hematoxylin for 5 min. The sections were subsequently dehydrated, cleared and mounted. The images of three random fields per section were captured using a Nikon Digital Eclipse C1 confocal microscope. The positively stained areas were quantified

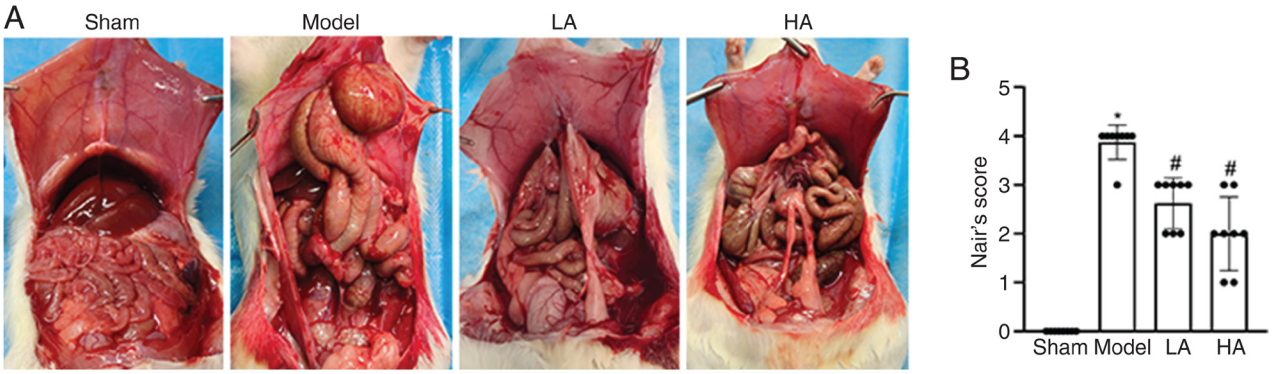

Figure 1. Aspirin attenuates the severity of postoperative peritoneal adhesion. (A) Representative images of peritoneal adhesions in different groups. (B) Statistical analysis of adhesion scores based on the Nair scoring system for each group (n=8). *P<0.05 vs. sham group; #P<0.05 vs. model group. HA, high-dose aspirin; LA, low-dose aspirin.

using ImageJ software (version 1.44p; National Institutes of Health), and the mean value of the three fields was used for statistical analysis.

Western blot analysis. Total protein was extracted from adhesion tissues using RIPA lysis buffer (Beyotime Institute of Biotechnology) containing protease and phosphatase inhibitors. Protein concentration was determined using the BCA assay (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Equal amounts of protein (20 μg per lane) were separated on 10% gels using SDS-PAGE and transferred onto polyvinylidene difluoride membranes. Following blocking with 5% skimmed milk for 1 h at 4˚C, the membranes were incubated overnight at 4˚C with the following primary antibodies: Rabbit anti-TGF-β1 (1:1,000; cat. no. ab215715; Abcam), rabbit anti-COL1A1 (1:1,500; cat. no. ab316222; Abcam), rabbit anti-α-SMA (1:2,000; cat. no. ab32575; Abcam), rabbit anti-p-Smad2 (1:1,000; cat. no. 3108; Cell Signaling Technology, Inc.), rabbit anti-Smad2 (1:500; cat. no. 1641-1; Epitomics; Abcam), rabbit anti-p-Smad3 (1:1,000; cat. no. 1880-1; Epitomics; Abcam), rabbit anti-Smad3 (1:500; cat. no. 1735-1; Epitomics; Abcam), rabbit anti-Smad6 (1:200; cat. no. sc-13048; Santa Cruz Biotechnology, Inc.) and rabbit anti-GAPDH (1:20,000; cat. no. KC-5G4; Kangcheng Biotechnology). Following washes with TBST containing 0.1% Tween-20, the blots were treated with HRP-conjugated goat anti-rabbit IgG secondary antibody (1:5,000; cat. no. ZB-5301; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.) for 1 h at room temperature. The protein signals were developed using an ECL chemiluminescence substrate (Thermo Fisher Scientific, Inc.) and captured with a ChemiDoc™ imaging system (Bio-Rad Laboratories, Inc.).

RT-qPCR. Total RNA was isolated from cells using TRIzol® reagent (Life Technologies; Thermo Fisher Scientific, Inc.), following the provided protocol. A PrimeScript® RT reagent kit (Takara Bio, Inc.) was employed for cDNA synthesis. Quantitative PCR was conducted with a SYBR Green PCR Master Mix-PLUS kit (Toyobo Co., Ltd.) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.) The experimental procedures were performed as previously described (18). All primer sequences (listed in Table II) were obtained from Sangon Biotech Co., Ltd. GAPDH was used as the internal reference gene. The relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (28).

Statistical analysis. The measurement data are expressed as the mean ± standard deviation. The Shapiro-Wilk test was used to assess normality and the Levene's test was used to evaluate homogeneity of variances. For data satisfying both assumptions, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed. For data that did not meet these assumptions, the Kruskal-Wallis test followed by Dunn's post hoc test was applied. The graphs were generated using GraphPad Prism 9.0 software (Dotmatics) and statistical analysis was conducted using SPSS 27.0 software (IBM Corp.). P<0.05 was considered to indicate a statistically significant difference.

Results

Aspirin attenuates the severity of PPA in rats. Representative images illustrating the formation of intraperitoneal adhesions in each group are shown in Fig. 1A and B. No apparent adhesions were observed in the sham-operated group. In contrast to these observations, the model group exhibited extensive, dense and severe adhesions, which led to distortion of the visceral anatomy and made the adhesions difficult to separate. Aspirin treatment significantly reduced the adhesion score compared with the model group.

Aspirin attenuates PPA fibrosis in rats. Masson's trichrome staining revealed extensive collagen deposition (blue staining) in the peritoneal adhesion tissues of the model group compared with that of the sham-operated group (Fig. 2A). Aspirin treatment significantly reduced the levels of collagen deposition, with the high-dose aspirin group indicating a more pronounced inhibitory effect than the low-dose group (Fig. 2B). IHC staining was further performed to assess the expression of fibrosis markers, α-SMA and collagen type I α1 chain (COL1A1). The results demonstrated markedly enhanced positive staining for both markers in the model group. In contrast to these observations, the aspirin-treated groups exhibited significantly lower positivity in both area and intensity for COL1A1 and α-SMA compared with that of the model group (Fig. 3A-C). To validate these findings, RT-qPCR and western blot analyses were conducted. The

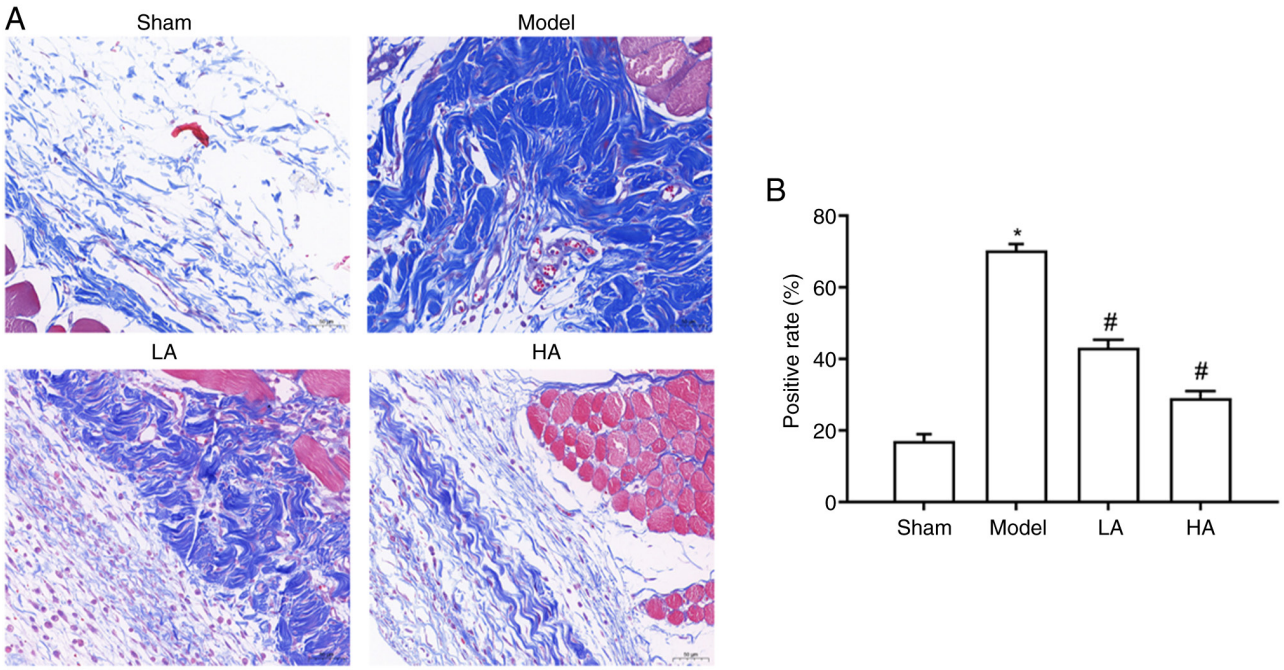

Figure 2. Aspirin reduces collagen deposition in postoperative peritoneal adhesion. (A) Representative images of Masson's trichrome staining of adhesion tissues. Collagen fibers are stained blue (magnification, x200; scale bar, 50 μm). (B) Quantification of collagen deposition indicates a significant reduction in the aspirin-treated group (n=8) compared with the model group (n=8). *P<0.05 vs. the sham group; $^{\#}$P<0.05 vs. the model group. HA, high-dose aspirin; LA, low-dose aspirin; MT, Masson's trichrome.

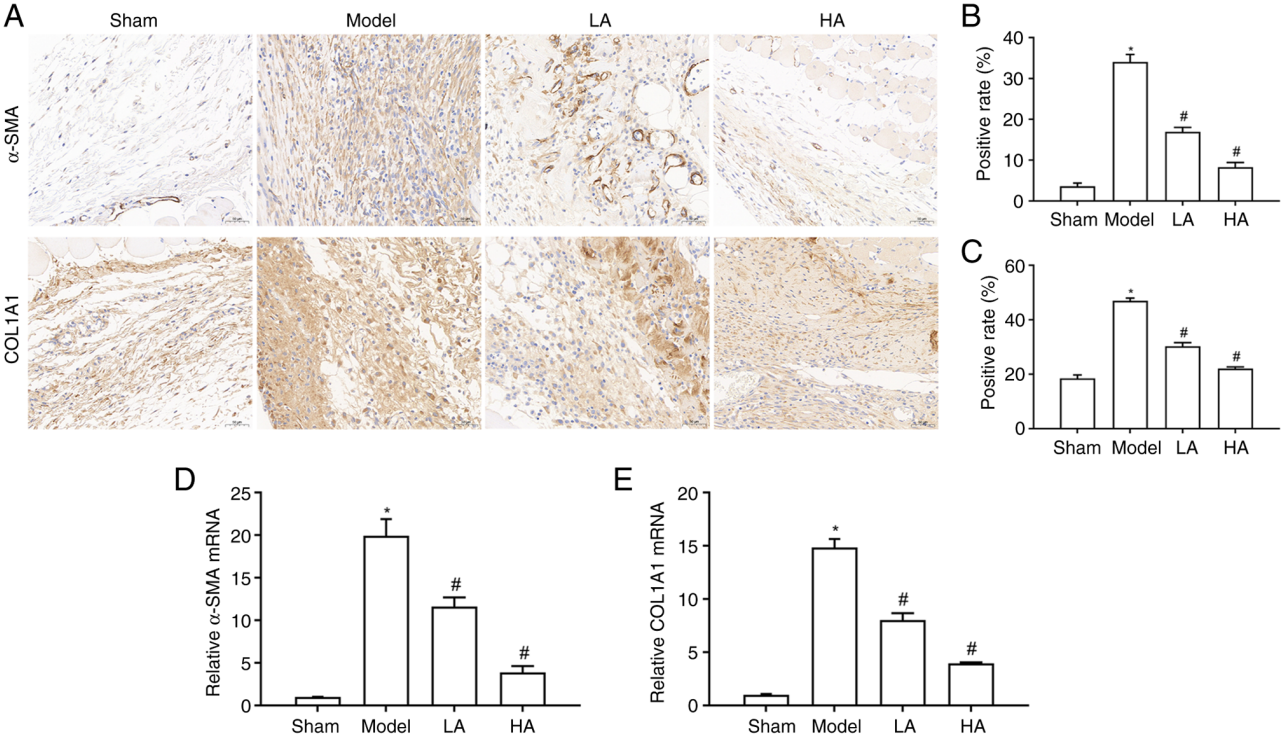

Figure 3. Aspirin downregulates the expression levels of α-SMA and COL1A1 in postoperative peritoneal adhesion. (A) Representative immunohistochemical staining of α-SMA and COL1A1 (magnification, x200; scale bar, 50 μm). (B and C) Quantitative analysis of positive staining areas for (B) α-SMA and (C) COL1A1 using ImageJ software (n=8). (D and E) Quantitative PCR analysis of mRNA expression levels for (D) α-SMA and (E) COL1A1. *P<0.05 vs. the sham group; $^{\#}$P<0.05 vs. the model group. α-SMA, α-smooth muscle actin; COL1A1, collagen type I α1 chain; HA, high-dose aspirin; low-dose aspirin.

results indicated that the mRNA and protein expression levels of α-SMA and COL1A1 were significantly downregulated in the aspirin-treated groups compared with those of the model group, with the high-dose group again demonstrating a more potent effect (Figs. 3D, E and 4A-C). Collectively, these results indicate that aspirin effectively attenuates PPA fibrosis in rats.

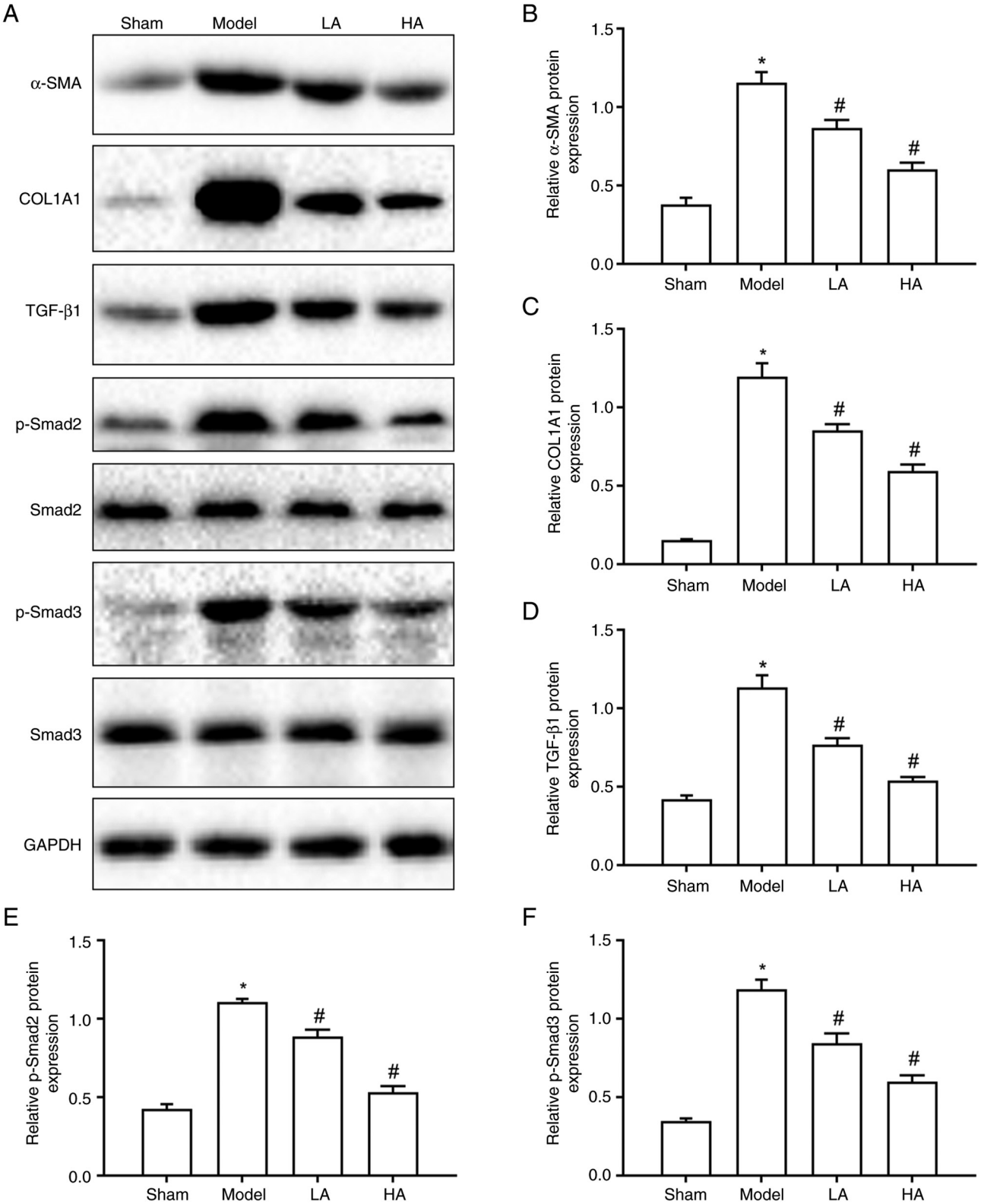

Figure 4. Aspirin downregulates the protein expression of α-SMA, COL1A1 and the TGF-β1-Smad2/Smad3 signaling pathway in postoperative peritoneal adhesion. (A) The protein expression levels of α-SMA, COL1A1, TGF-β1, p-Smad2, p-Smad3, Smad2 and Smad3 in adhesion tissues were detected by western blot analysis. (B-F) The relative protein expression levels of (B) α-SMA, (C) COL1A1, (D) TGF-β1, (E) p-Smad2 and (F) p-Smad3 were quantified (n=8). *P<0.05 vs. the sham group; #P<0.05 vs. the model group. α-SMA, α-smooth muscle actin; COL1A1, collagen type I α1 chain; HA, high-dose aspirin; LA, low-dose aspirin; p-, phosphorylated; LA, low-dose aspirin; TGF-β1, transforming growth factor-β1.

Aspirin attenuates PPA fibrosis by inhibiting the TGF-β1/Smad signaling pathway. To explore the underlying molecular mechanism by which aspirin alleviates peritoneal adhesion fibrosis, the expression levels of key proteins in the TGF-β1/Smad signaling pathway was examined. Western blotting and IHC analyses revealed that the protein expression

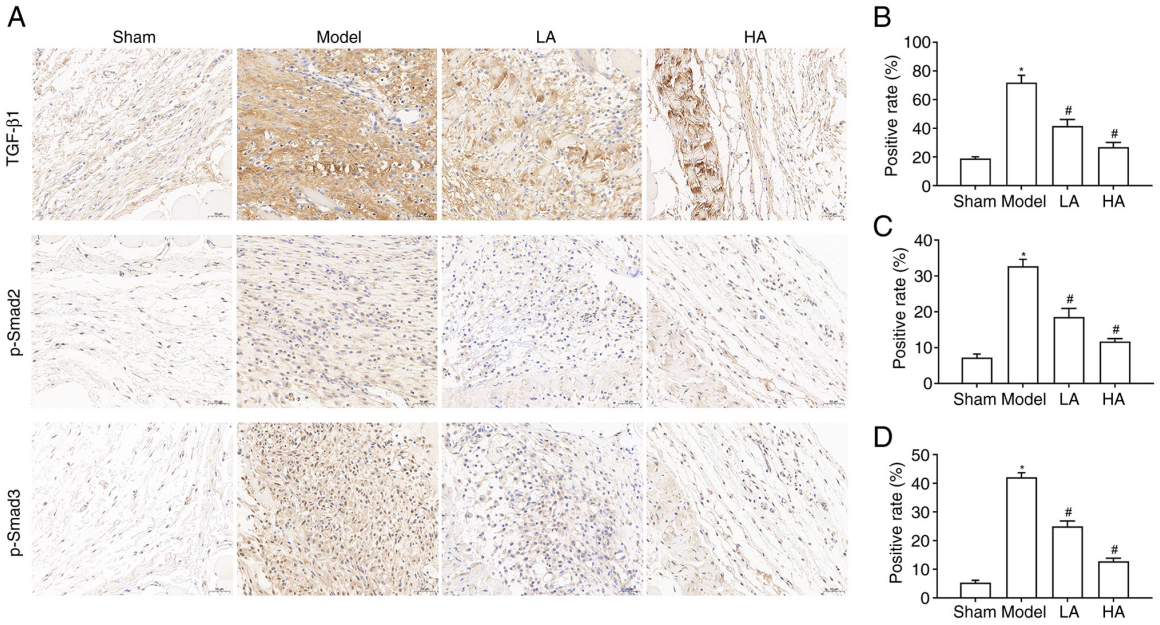

Figure 5. Aspirin downregulates the expression levels of the TGF-β1-Smad2/Smad3 pathway-related proteins in postoperative peritoneal adhesion. (A) Representative immunohistochemical staining of TGF-β1, p-Smad2, p-Smad3, Smad2 and Smad3 (magnification, x200; scale bar, 50 μm). (B-D) Quantitative analysis of positive staining areas for (B) TGF-β1, (C) p-Smad2 and (D) p-Smad3, performed using ImageJ software (n=8). *P<0.05 vs. sham group; #P<0.05 vs. model group. HA, high-dose aspirin; LA, low-dose aspirin; p-, phosphorylated; TGF-β1, transforming growth factor-β1.

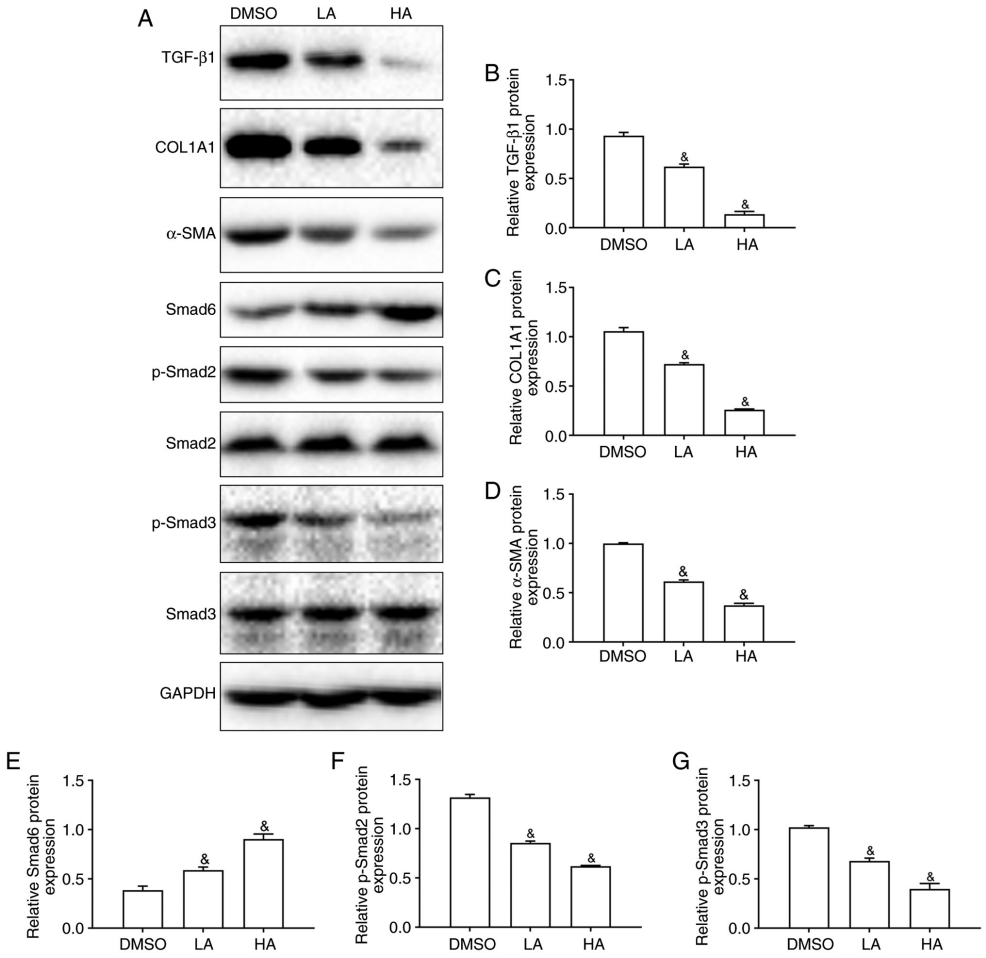

Figure 6. Impact of varying aspirin concentrations on α-SMA, COL1A1, TGF-β1, Smad6, p-Smad2 and p-Smad3 protein expressions in RPMCs. (A) Western blotting was performed to assess the protein levels of TGF-β1, COL1A1, α-SMA, Smad6, p-Smad2, p-Smad3, Smad2 and Smad3 in RPMCs. (B-G) Quantified relative protein expression of (B) TGF-β1, (C) COL1A1, (D) α-SMA, (E) Smad6, (F) p-Smad2 and (G) p-Smad3. &P<0.05 compared with the DMSO group. α-SMA, α-smooth muscle actin; COL1A1, collagen type I α1 chain; DMSO, dimethyl sulfoxide; HA, high-dose aspirin; LA, low-dose aspirin; p-, phosphorylated; RPMCs, rat primary peritoneal mesothelial cells; TGF-β1, transforming growth factor-β1.

levels of TGF-β1, phosphorylated (p-) Smad2 and p-Smad3 were significantly elevated in the model group. Aspirin treatment effectively suppressed the expression levels of these proteins, with the high-dose group indicating a more significant inhibitory effect. However, no notable changes were observed in the total protein levels of Smad2 and Smad3 across the groups (Figs. 4A, D-F and 5A-D). These findings suggest that the antifibrotic effect of aspirin may be associated with the inhibition of the TGF-β1/Smad signaling pathway activation.

In vitro effects of aspirin on RPMCs. To further investigate whether aspirin exerts its effects via regulation of the TGF-β1/Smad signaling pathway at the cellular level, RPMCs were treated with varying concentrations of aspirin. The results demonstrated that aspirin dose-dependently downregulated the protein expression of α-SMA, COL1A1, TGF-β1, p-Smad2 and p-Smad3, while upregulating the expression levels of Smad6, an inhibitory regulator of the pathway (Fig. 6A-G). These *in vitro* findings are consistent with the observations from the *in vivo* experiments. Taken collectively, the data confirm that aspirin can suppress the expression of fibrosis-related proteins by modulating the TGF-β1/Smad signaling pathway, thereby exerting an antifibrotic effect on postoperative peritoneal fibrosis.

Discussion

PPA is a common complication following abdominal surgery, with an incidence as high as 90-95% (29). It is widely accepted that its formation initiates from an inflammatory response triggered by peritoneal injury, leading to excessive collagen deposition and adhesion development (14). Consequently, interventions capable of inhibiting collagen production may aid in preventing or reducing PPA. However, there are currently no safe and effective pharmacological agents or measures to prevent PPA, underscoring the requirement for further research and development of novel anti-adhesive drugs. The present study demonstrated that aspirin effectively prevented PPA in rats. Notably, the anti-adhesion effect observed with high-dose aspirin treatment was comparable to that of sodium hyaluronate (HA), a well-established anti-adhesive agent reported in the literature (30,31). This finding strongly supports the potential of aspirin as a candidate drug for the prevention of PPA. Mechanistically, the protective effect of aspirin against PPA was primarily mediated by regulating the key profibrotic signaling pathway (TGF-β1/Smad pathway), thereby attenuating excessive ECM deposition and the fibrotic process.

The pathogenesis of PPA is closely associated with peritoneal fibrosis, which is characterized by excessive accumulation of ECM (8,32). TGF-β1 serves as a central driver of this fibrotic process (33,34). In the present study, it was found that TGF-β1 expression was significantly upregulated in the adhesion model. Notably, aspirin treatment dose-dependently reduced the expression levels of TGF-β1, type I collagen and α-SMA both *in vivo* and *in vitro*. These findings extend the known pharmacological actions of aspirin from anticoagulation and anti-inflammation to anti-fibrosis, which is consistent with its previously reported anti-fibrotic effects in hepatic fibrosis and endometrial fibrosis (35,36), suggesting that its antifibrotic action may be organ-non-specific.

The profibrotic effect of TGF-β1 is primarily mediated via the canonical Smad signaling pathway (17). TGF-β1 induces the phosphorylation of Smad2 and Smad3. p-Smad2/3 subsequently forms a transcriptional complex with Smad4, which translocates into the nucleus and activates the transcription of downstream target genes, including those encoding fibronectin, α-SMA and type I collagen, ultimately leading to excessive ECM synthesis and abnormal deposition (37). In the present study, it was found that aspirin not only reduced the expression levels of TGF-β1 but also decreased the levels of p-Smad2/3, thereby suppressing the transcription of fibrosis-related target genes, such as COL1A1 and α-SMA. These findings provide a clear molecular mechanistic explanation with regard to the ability of aspirin to alleviate ECM deposition and inhibit fibrosis. The high consistency between the *in vivo* and *in vitro* results strengthens the reliability of this conclusion. In addition, it was observed that aspirin treatment increased the expression levels of Smad6, which is consistent with a previous study (38). Smad6 is an inhibitory Smad; however, whether it interferes with the phosphorylation and nuclear translocation of Smad2/3 in the context of peritoneal fibrosis warrants further investigation.

The findings of the present study exhibit important translational implications. Aspirin is a classic non-steroidal anti-inflammatory drug with a long history of use, a well-established safety profile and low cost (39). If its efficacy in preventing adhesions is confirmed in subsequent clinical studies, it has the potential to be rapidly translated into a widely applicable clinical preventive strategy. This can reduce the difficulty of reoperation and related complications and also aid the improvement of patient long-term prognosis.

Nevertheless, the present study exhibits several limitations. Firstly, although the regulatory effect of aspirin was elucidated on the TGF-β1/Smad signaling pathway, the expression levels of the TGF-β receptors, the involvement of Smad4, or the nuclear translocation of p-Smad2/3 were not evaluated. Moreover, the precise upstream molecular targets of aspirin (for example, whether it acts directly on the TGF-β1 receptor or upstream kinases) remain unclear, resulting in an incomplete mechanistic chain that warrants further investigation. Secondly, the conclusions are derived from a rat model, and no positive control group such as HA was included. The optimal timing, dosage, treatment duration and safety of aspirin for preventing postoperative adhesions in humans still require validation via the conduct of well-designed clinical trials. Finally, the formation of peritoneal adhesions involves crosstalk among multiple signaling pathways such as the Wnt/β-catenin and the MAPK pathways. Whether aspirin also influences these pathways warrants further investigation.

In summary, the present study indicated that aspirin prevented PPA in rats by inhibiting postoperative peritoneal fibrosis. The underlying mechanism was likely associated with the suppression of the TGF-β1/Smad signaling pathway, leading to reduced expression of fibrosis-related proteins such as α-SMA and collagen. The present study provides

experimental evidence supporting the potential clinical application of aspirin as a preventive agent for PPA and offers a novel strategy for reducing PPA-related complications.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

YS and PX conceptualized and designed the study. SY and YR jointly performed the experiments, analyzed the data, curated the research data (including organizing, cleaning and maintaining the raw data to ensure its interpretability and reusability) and wrote the original draft. BL contributed to the interpretation of the experimental data, and together with PX, supervised the overall research process and managed project administration. YS reviewed and critically revised the manuscript. YS and PX confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The animal study protocol was approved by the Animal Ethics Committee of The First Affiliated Hospital of Yangtze University (approval no. KY20435; Jingzhou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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