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ORIGINAL ARTICLE

Basic Study

Effects of initiating time and dosage of *Panax notoginseng* on mucosal microvascular injury in experimental colitis

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Abstract

AIM

To investigate the effects of *Panax notoginseng* (PN) on microvascular injury in colitis, its mechanisms, initial administration time and dosage.

METHODS

Dextran sodium sulfate (DSS)- or iodoacetamide (IA)-induced rat colitis models were used to evaluate and investigate the effects of ethanol extract of PN on microvascular injuries and their related mechanisms. PN administration was initiated at 3 and 7 d after the model was established at doses of 0.5, 1.0 and 2.0

g/kg for 7 d. The severity of colitis was evaluated by disease activity index (DAI). The pathological lesions were observed under a microscope. Microvessel density (MVD) was evaluated by immunohistochemistry. Vascular permeability was evaluated using the Evans blue method. The serum concentrations of cytokines, including vascular endothelial growth factor (VEGF)A121, VEGFA165, interleukin (IL)-4, IL-6, IL-10 and tumor necrosis factor (TNF)-α, were detected by enzyme-linked immunosorbent assay. Myeloperoxidase (MPO) and superoxide dismutase (SOD) were measured to evaluate the level of oxidative stress. Expression of hypoxia-inducible factor (HIF)-1α protein was detected by western blotting.

RESULTS

Obvious colonic inflammation and injuries of mucosa and microvessels were observed in DSS- and IA-induced colitis groups. DAI scores, serum concentrations of VEGFA121, VEGFA165, VEGFA165/VEGFA121, IL-6 and TNF-α, and concentrations of MPO and HIF-1α in the colon were significantly higher while serum concentrations of IL-4 and IL-10 and MVD in colon were significantly lower in the colitis model groups than in the normal control group. PN promoted repair of injuries of colonic mucosa and microvessels, attenuated inflammation, and decreased DAI scores in rats with colitis. PN also decreased the serum concentrations of VEGFA121, VEGFA165, VEGFA165/VEGFA121, IL-6 and TNF-α, and concentrations of MPO and HIF-1α in the colon, and increased the serum concentrations of IL-4 and IL-10 as well as the concentration of SOD in the colon. The efficacy of PN was dosage dependent. In addition, DAI scores in the group administered PN on day 3 were significantly lower than in the group administered PN on day 7.

CONCLUSION

PN repairs vascular injury in experimental colitis *via* attenuating inflammation and oxidative stress in the colonic mucosa. Efficacy is related to initial administration time and dose.

Key words: Microvascular injury; *Panax notoginseng*; Ulcerative colitis; Oxidative stress

Core tip: *Panax notoginseng* (PN) is a traditional Chinese medicine used to treat ulcerative colitis, but its mechanisms are unclear. In our study, we found that PN promoted repair of injuries of colonic mucosa and microvessels in rat colitis. PN decreased concentrations of vascular endothelial growth factor, interleukin (IL)-6 and tumor necrosis factor-α while it increased the concentrations of IL-4 and IL-10 in serum. It also decreased concentrations of myeloperoxidase and hypoxia-inducible factor-1α while it increased the concentration of superoxide dismutase in colon. So it is concluded that PN repairs mucosal and vascular injuries in rat colitis *via* attenuating inflammation and oxidative stress in the colonic mucosa.

Wang SY, Tao P, Hu HY, Yuan JY, Zhao L, Sun BY, Zhang WJ, Lin J. Effects of initiating time and dosage of *Panax notoginseng* on mucosal microvascular injury in experimental colitis. *World J Gastroenterol* 2017; 23(47): 8308-8320 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i47/8308.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i47.8308

INTRODUCTION

Microvessels are a major component of the colonic mucosa that nourish the colonic tissue and clear metabolic waste *via* controlling the intestinal blood flow[1]. They also play an important role in maintaining normal intestinal permeability[2]. Recent studies have found that injury of the colonic microvessels precedes injury of the colonic mucosa in the development of experimental colitis[3]. The increased vascular permeability aggravates the early endothelial injury[4], which induces hypoxia of the colonic mucosa and further oxidative stress[5,6].

Previous studies have demonstrated that colonic mucosal hypoxia induced by mucosal vascular injury plays an important role in the pathogenesis of ulcerative colitis (UC)[7]. Our previous study found that colonic mucosal injury in rats with experimental colitis improved along with repair of the mucosal microvascular injury[8]. Therefore, microvascular injury is essential to the development of UC and could be a new therapeutic target. However, there are no drugs that can promote effective microvascular repair.

Panax notoginseng (PN) is a common Chinese herbal medicine that has long been used to treat vascular lesions[9]. Some studies have found that it promotes repair of vascular damage *via* proangiogenic and anti-apoptotic effects[10,11]. Animal experiments have shown that PN attenuates colonic mucosal injury and promotes mucosal repair in mouse models of colitis[12], but its mechanism of action is unclear. It is hypothesized that PN repair of mucosal injury could be related to its promotion of repair of microvascular injury. In this study, we investigated the mechanism of PN repair of colonic mucosal injury in rats with colitis from the aspect of promotion of vascular repair. We also investigated the relationship between dose and initial time of administration of PN and its efficacy.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats (120-140 g) were from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China). The Institutional Animal Care and Use Committee of Shanghai University of Traditional Chinese Medicine

approved all procedures. Rats were housed in a pathogen-free environment and allowed to acclimate to the environment for 7 d before inclusion in an experiment.

Reagents

Iodoacetamide (IA; purity > 99%), dextran sodium sulfate (DSS), formamide (purity > 99%) and Evans blue (dye content > 75%) were purchased from Sigma-Aldrich (St. Louis, MO, United States). Enzyme-linked immunosorbent assay (ELISA) kits for interleukin (IL)-4, IL-6, IL-10 and tumor necrosis factor (TNF)-α were purchased form R&D Systems (Minneapolis, MN, United States). ELISA kits for vascular endothelial growth factor (VEGF)A165 and VEGFA121 were purchased from Cloud-Clone Corp. (Katy, TX, United States). CD31 antibody (ab23680) and goat anti-mouse antibody were purchased from Abcam (Cambridge, United Kingdom). Myeloperoxidase (MPO) and superoxide dismutase (SOD) assay kits were purchased from Cell Signaling Technology (Danvers, MA, United States).

Preparation of PN

PN was purchased from Shanghai Huayu Traditional Chinese Medicine Co. Ltd. (Shanghai, China) and authenticated by Professor Yang Dong (Shanghai University of Traditional Chinese Medicine). PN (0.9 kg) was dissolved in tap water (9 L), boiled at 100 ℃ for 3 h, filtered through a sieve (150 μm), extracted with absolute ethanol, and dried in a freeze dryer. The brown extract powder of PN was stored at -20 ℃.

Animal models of colitis

Two rat models of colitis were used in this study. One was induced by 0.1 mL 6% IA dissolved in 1% methylcellulose given to rats once by enema (7 cm above the anus). The other was induced by DSS, in which rats were allowed free access to purified water containing 5% DSS (w/v) for 7 d. DSS solution was prepared daily.

PN intervention in vivo

Rats were divided into control, low-dose, medium-dose and high-dose groups (n = 6 each). For the intervention groups, PN was intragastrically administered to rats with IA-induced or DSS-induced colitis once daily for seven consecutive days at doses of 0.5, 1.0 or 2.0 g/kg. For the control groups, normal saline was given to the corresponding rats. The initial administration times of PN were 3 and 7 d after establishing the colitis models. Three independent experiments were performed in triplicate.

Disease activity index

Before, during and after treatment, the severity of colitis was evaluated with disease activity index (DAI), as described previously[13]. The parameters of DAI included weight loss (0, none; 1, 0%-5%; 2, 5%-10%; 3, 10%-20%; 4, > 20%), stool consistency (0, none; 2, loose stool; 4, watery), and bleeding (0, none; 1, trace; 2, mild occult blood; 3, obvious occult blood; 4, gross bleeding).

Histological assays

Segments of colon were fixed in formalin buffer and embedded in paraffin. Sections of 5 μm thick were deparaffinized and stained with hematoxylin and eosin (HE). The histological changes were assessed by a pathologist.

Microvessel density analysis

Five-micrometer-thick paraffin-embedded sections of colon were deparaffinized, subjected to heat-mediated antigen retrieval, and blocked with goat serum. The tissues were incubated with the primary anti-CD31 antibody (1:200, v/v) overnight at 4 ℃. After three 5-min washes, the horseradish peroxidase (HRP)-labeled secondary antibody (1: 300) was added and the samples were incubated at 37 ℃ for 1 h. The sections were counterstained with hematoxylin for 1 min at room temperature to visualize the endothelial cell nuclei. Three fields with CD31-positive cells in each section were chosen to assess microvessel density (MVD). Areas of highest vascularization were selected by scanning the sections at low magnification. Stained microvessels were counted in a single 200 × field within the selected field by three observers without previous knowledge of control groups. The following cellular structures were considered as countable microvessels: (1) stained lumen; (2) stained endothelial cell; and (3) stained endothelial cell cluster (1 and 2 were clearly separated from adjacent strained lumens, colonic mucosal cells and other connective tissue elements). The MVD value was calculated as the average vessel counts in three selected areas within a microscopic field.

Vascular permeability analysis

Vascular permeability (VP) of vessels in colonic mucosa was evaluated by the Evans blue method, as described previously[14]. Rats were anesthetized with intraperitoneal injection of sodium pentobarbital. Evans blue (1 mg/100 g) was injected intravenously 15 min before autopsy. Evans blue was extracted from the 1-cm segment of colonic tissue using formamide and measured by spectrophotometry at 610 nm. Results were expressed as OD value per milligram of colon.

ELISA

Blood samples were collected from the abdominal aorta. The serum concentrations of VEGFA165 (Cloud-Clone Corp.), VEGFA121 (Cloud-Clone Corp.), IL-4 (R&D Systems), IL-6 (R&D Systems), IL-10 (R&D

Systems) and TNF-α (R&D Systems) were detected using the appropriate ELISA kits.

SOD and MPO activity assays

SOD activity was measured using the SOD assay kit (Cell Signaling Technology). Tissue homogenate was prepared by vortex homogenizer, heated at 95 ℃ for 40 min and centrifuged at 178 × *g* at 4 ℃ for 10 min. Ethanol-chloroform mixture (5:3, v/v) was used to extract SOD in the homogenate for total SOD activity assay. MPO activity was measured using the MPO assay kit (Cell Signaling Technology). Tissue homogenate was prepared by vortex homogenizer, heated at 95 ℃ for 40 min and centrifuged at 714 × *g* at 4 ℃ for 10 min. The samples were added to phosphate buffer containing 30 mM H_2O_2 (pH 7.0) and incubated for 10 min. The enzymatic activity of SOD and MPO was expressed by the decrease of OD_{240}.

Western blotting

Hypoxia-inducible factor (HIF)-1α was measured by western blotting, as described previously[15]. Colonic tissue was cut into pieces and homogenized in 5-fold volumes of ice-cold homogenizing buffer (0.1 mmol/L NaCl, 0.1 mol/L Tris-HCl, and 0.001 mol/L EDTA) containing 1 mmol/L phenylmethylsulfonyl fluoride, 1 mg/mL aprotinin and 0.1 mmol/L leupeptin at 3000 × *g* and 4 ℃ for 1 h. Bovine serum albumin was used to estimate the protein content in supernatants. The protein samples (60 μg in each sample) were subjected to SDS-PAGE and transferred to polyvinylidene fluoride membranes using a transfer apparatus (Bio-Rad, Hercules, CA, United States). The membranes were blocked for 2 h, then the primary antibody anti-HIF-1α was added and incubated at 4 ℃ overnight, and the corresponding HRP-conjugated secondary antibody (Cell Signaling Technology) was added and incubated for 1 h. Protein-antibody complexes were detected by Clarity Western ECL Substrate (Bio-Rad), and results were authenticated with the ImageJ software (Gene Co. Ltd., China).

Statistical analysis

Data were presented as the mean ± SD. One-way analysis of variance or general linear model with repeated measures was used to analyze the data sets with three or more groups, and least significant difference *post hoc* test for multiple comparisons. Student's *t*-test was used to analyze data sets with two groups. $P < 0.05$ was considered significant.

RESULTS

Efficacy of PN on experimental colitis was dependent upon initial time of administration

After the colitis model was established, PN administration (1.0 g/kg) was initiated at days 3 and 7 for seven consecutive days (Figure 1A). Compared with the day 7 group, DAI scores, injuries of colonic mucosa and microvessels, serum concentrations of pro-inflammatory cytokines (IL-6 and TNF-α), and expression of HIF-1α and MPO were significantly lower (Figure 1B, C, E-G and I; Figure 2A, C and E; Figure 3A), and serum concentrations of anti-inflammatory cytokines (IL-4 and IL-10), MVD and SOD were significantly higher (Figure 1D, H and J; Figure 3C) in the day 3 group. The earlier PN was administered, the more effective it was in treating acute colitis.

PN repaired colonic mucosal injuries and microvessels

The rats in the IA- and DSS-induced experimental colitis groups had obvious injuries of the colonic mucosa and microvessels, as well as lower MVD. After being treated with PN (0.5, 1.0 and 2.0 g/kg) for seven consecutive days, colonic mucosal injuries and microvessels significantly improved and MVD increased compared with the model groups. The efficacy of PN in attenuating mucosal and microvascular injuries was dose dependent (Figure 4A-E). The effects of medium- and high-dose PN were superior to those of low-dose PN, but there were no significant differences between the medium- and high-dose groups (Figure 4A-E).

PN improved impaired VP

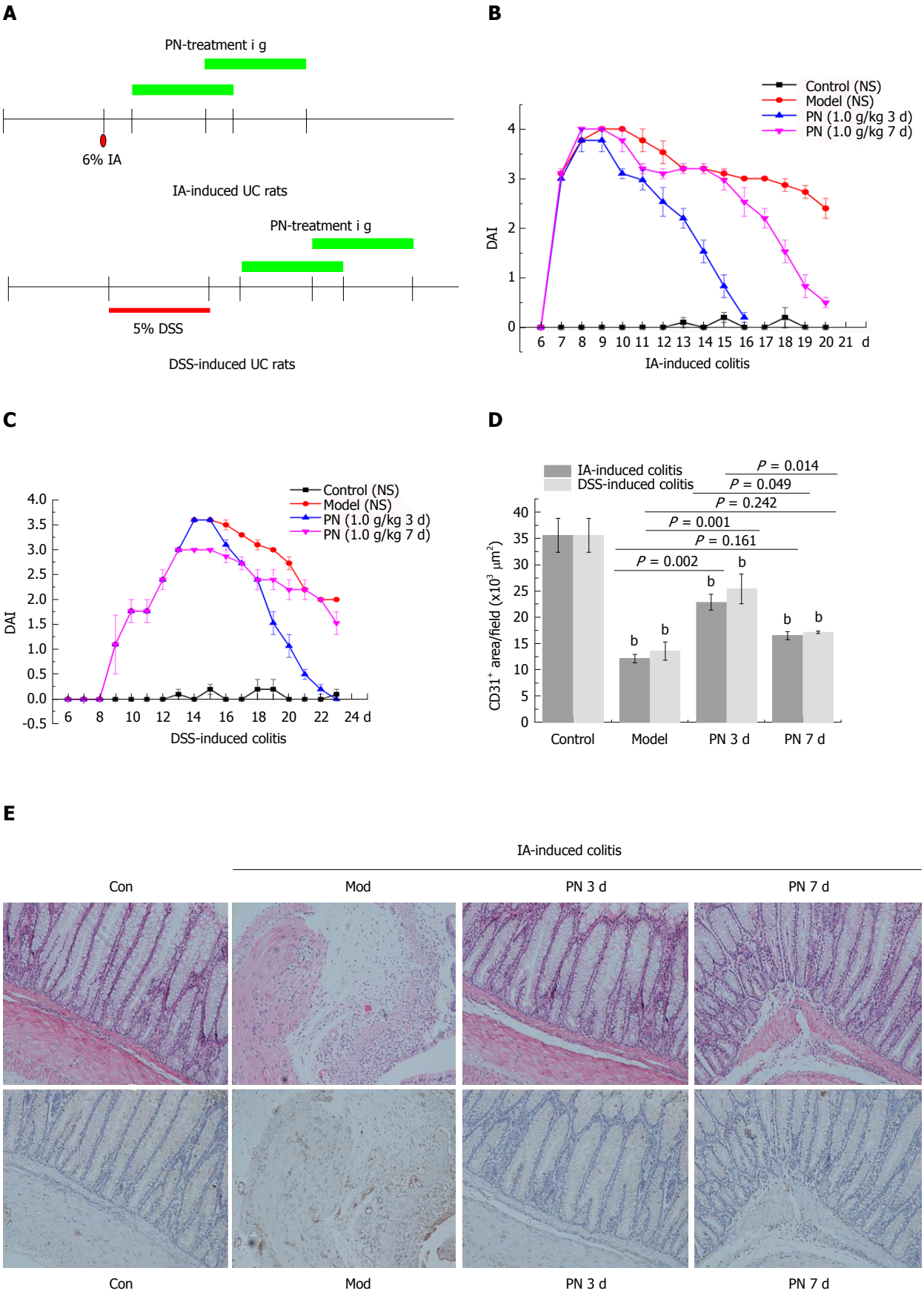
VP increased significantly in the groups with experimental colitis induced by DSS and IA compared with the normal control group. PN improved impaired VP in a dose-dependent manner. The effects of medium and high doses of PN were superior to those of low dose PN, but there were no significant differences between the medium- and high-dose groups (Figure 5).

PN reversed the disordered ratio of VEGFA165/VEGFA121

The serum concentrations of VEGFA165 and VEGFA121 and the ratio of VEGFA165/VEGFA121 increased significantly in the groups with experimental colitis induced by IA and DSS compared with the normal control group. PN significantly decreased elevated VEGFA165, VEGFA121 and VEGFA165/VEGFA121 ratio in a dose-dependent manner in rats with experimental colitis. The effects in the medium- and high-dose groups were superior to those of the low-dose group, but there were no significant differences between the medium- and high-dose groups (Figure 6A-C).

PN adjusted imbalance of pro-inflammatory and anti-inflammatory cytokines

The pro-inflammatory cytokines (IL-6 and TNF-α) increased significantly and the anti-inflammatory cytokines (IL-4 and IL-10) decreased significantly in the experimental colitis groups compared with the normal control group. PN down-regulated elevated IL-6 and TNF-α and up-regulated reduced IL-4 and IL-10 in a dose-dependent manner compared with the model control group (Figure 6D-G). The effects of medium


A
PN-treatment i g
6% IA
IA-induced UC rats
PN-treatment i g
5% DSS
DSS-induced UC rats
B
Control (NS)
Model (NS)
PN (1.0 g/kg 3 d)
PN (1.0 g/kg 7 d)
DAI
IA-induced colitis
C
Control (NS)
Model (NS)
PN (1.0 g/kg 3 d)
PN (1.0 g/kg 7 d)
DAI
DSS-induced colitis
D
IA-induced colitis
DSS-induced colitis
P = 0.014
P = 0.049
P = 0.242
P = 0.001
P = 0.161
P = 0.002
CD31+ area/field (x10³ μm²)
Control
Model
PN 3 d
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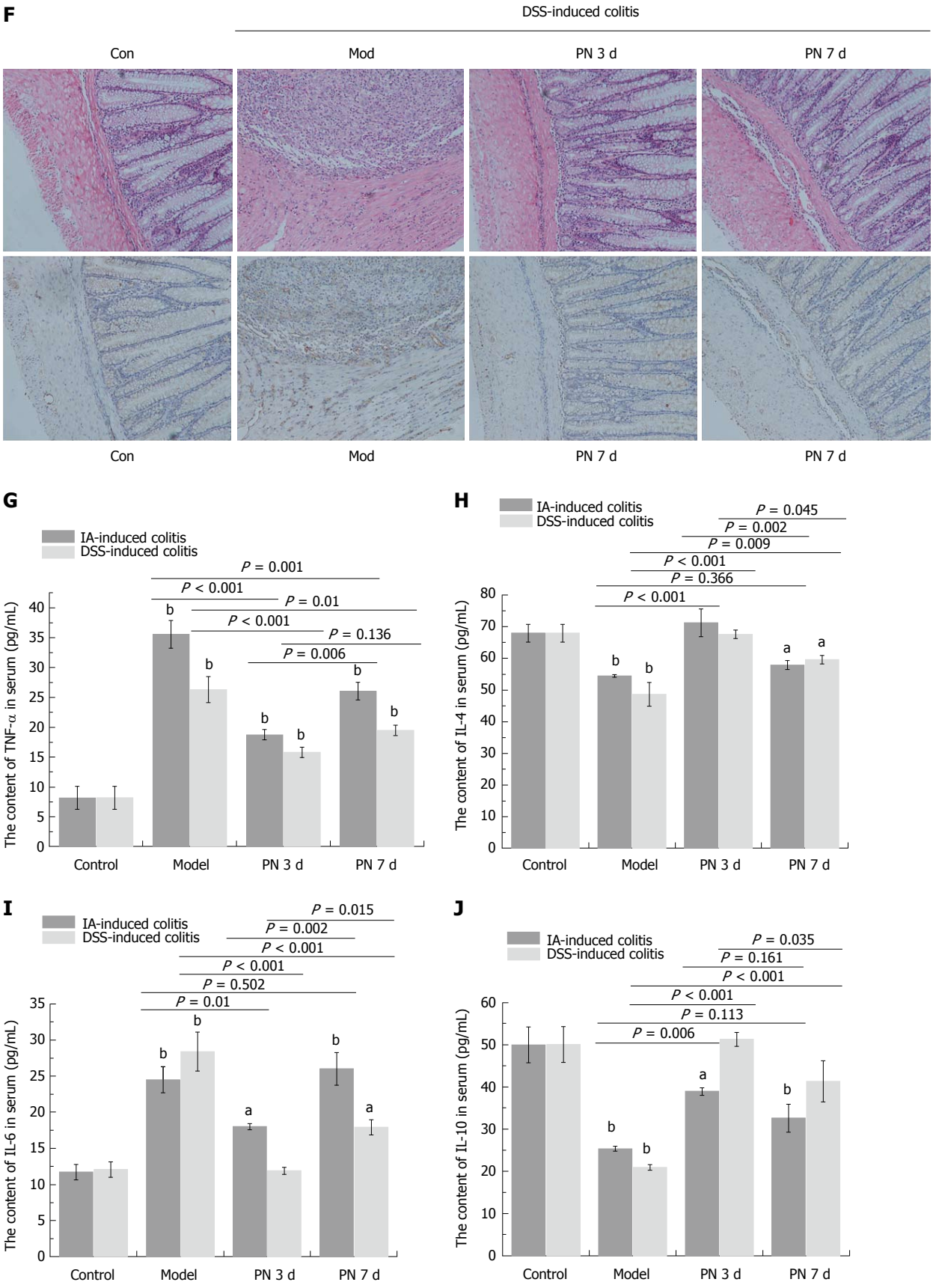

Figure 1 Efficacy of *Panax notoginseng* in experimental colitis was dependent on initial time of administration. A: PN administration (1.0 g/kg) was initiated at day 3 and 7 for seven consecutive days; B, C, G, J: DAI scores and serum concentrations of TNF-α and IL-6 in the day 3 group were significantly lower than those in the day 7 group; D, E: The pathological lesions of colonic mucosa and microvessels in the day 3 group were less than those in the day 7 group; F: MVD in the day 3 group was significantly higher than that in the day 7 group. Results are expressed as mean ± SD of three independent experiments performed in triplicate. [a]$P < 0.05$, [b]$P < 0.01$ *vs* normal control. DAI: Disease activity index; IL: Interleukin; MVD: Microvessel density; PN: *Panax notoginseng*; TNF: Tumor necrosis factor.

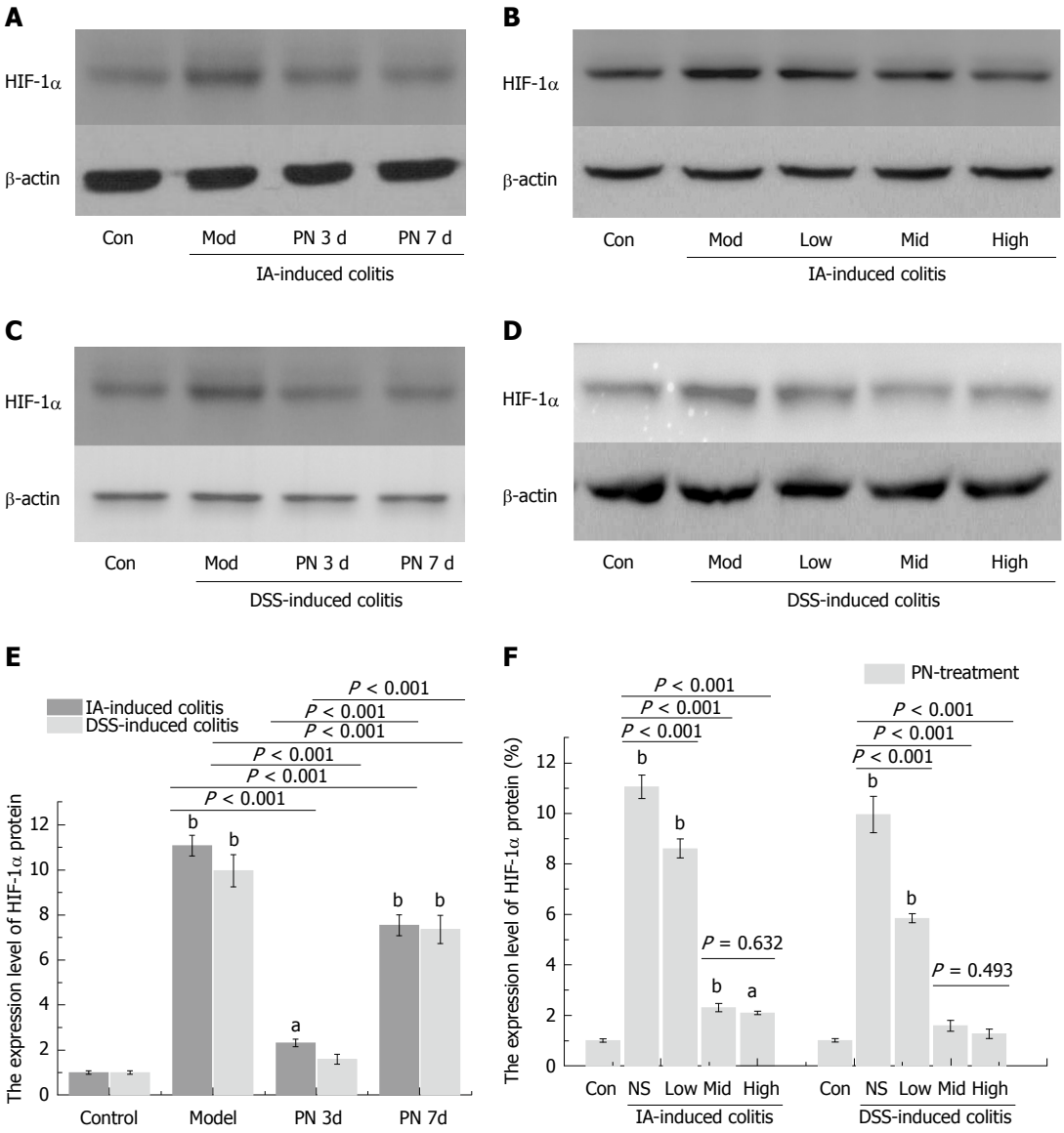

Figure 2 ***Panax notoginseng*** **improved hypoxia in colonic mucosa.** A-F: Increased expression of hypoxia-inducible factor-1α in colonic mucosa of the experimental colitis group was down-regulated by PN in a time- and dose-dependent manner. Results are expressed as mean ± SD of three independent experiments performed in triplicate. [a]$P < 0.05$, [b]$P < 0.01$ *vs* normal control. PN: *Panax notoginseng*.

and high dose PN were superior to those of low dose PN, but there were no significant differences between the medium- and high-dose groups.

PN improved hypoxia in colonic mucosa

Expression of HIF-1α in colonic mucosa was significantly up-regulated in the experimental colitis groups compared with the normal control group. PN down-regulated increased expression of HIF-1α in a dose-dependent manner compared with the model control group (Figure 2B, D and F). The effects of medium and high dose PN were superior to those of low dose PN, but there were no significant differences between the medium- and high-dose groups (Figure 2B, D and F).

PN blocked oxidative stress in colonic mucosa

The activity of MPO and SOD in colonic tissue was used to evaluate the anti-oxidative effect of PN. The activity of MPO increased and the activity of SOD decreased in the colon in the experimental colitis groups compared with the normal control group. PN down-regulated elevated MPO and up-regulated decreased SOD in a dose-dependent manner compared with the control group. The effects of medium and high dose PN were superior to those of low dose PN, but there were no significant differences between the medium- and high-dose groups (Figure 3B and D).

DISCUSSION

PN, also known as Sanqi or Tianqi, is a common Chinese herbal medicine with various activities and is used to treat cardiovascular diseases, pain, inflammation and hemorrhagic injury[16]. In recent

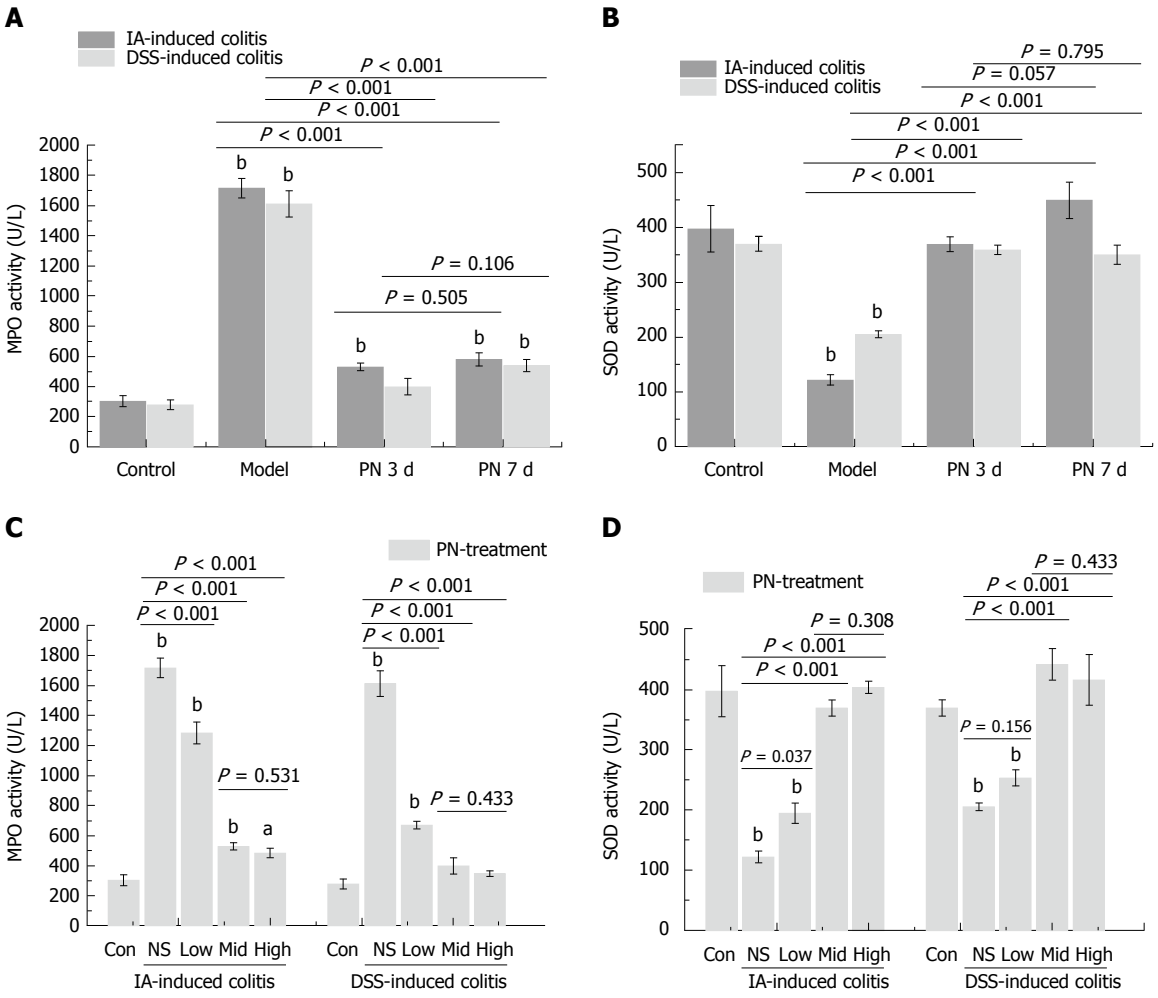

Figure 3 ***Panax notoginseng*** **blocked oxidative stress in colonic mucosa.** Activities of MPO and SOD in colonic tissue were used to evaluate the anti-oxidative effect of PN. A-D: The increased activity of MPO and decreased activity of SOD in the experimental colitis groups were reversed by PN in a time- and dose-dependent manner. Results are expressed as mean ± SD of three independent experiments performed in triplicate. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* normal control. MPO: Myeloperoxidase; PN: *Panax notoginseng*; SOD: Superoxide dismutase.

years, PN has been used to treat UC, with the effects of promoting repair of mucosal injury and attenuating inflammatory responses[17]. However, its mechanism of action is unclear.

It is well known that the efficacy of a drug is closely related to the initial administration time and its dose[18]. In previous studies, the initial time of PN administration was usually based on personal experience rather than experimental evidence, which affected the standardization and efficacy of PN treatment. We found that initiating PN at day 3 after establishing the colitis models was more effective than initiating at day 7, demonstrating improved mucosal injury, microvascular impairment, inflammatory response and hypoxia. In our other study that has not been published, we found mild mucosal injury and increased vessel permeability and concentrations of TNF-α, IL-6 and MPO on day 3 in a colitis model. This suggested that the changes of vessel permeability and inflammatory cytokines occurred in the early stage of colitis and preceded mucosal injury. That may be why early initiation of PN treatment (day 3) improved colitis more than initiating treatment on day 7. In addition, the efficacy of PN was dose dependent. The efficacy of medium and high doses was superior to that of the low dose, but there were no significant differences between the medium and high doses. This provided empirical evidence for using PN early and choosing the optimal dose in UC treatment.

Maintaining oxygen supply and metabolic clearance are the two crucial roles of colonic vessels. The severity of active UC is associated with mucosal hypoxia, which may result from increased oxygen consumption of inflammatory cells and decreased oxygen supply caused by vascular dysfunction[19,20]. The imbalance between oxygen consumption and supply, as well as excessive serum cytokines, leads to increased epithelial cell apoptosis and consequent impairment of mucosal barrier function[2,19]. Therefore, the levels of serum cytokines and expression of hypoxia- and oxidative stress-related proteins in colonic mucosa could reflect the status of hypoxia.

The serum concentrations of cytokines, including anti-inflammatory (IL-4 and IL-10) and pro-inflammatory (L-6

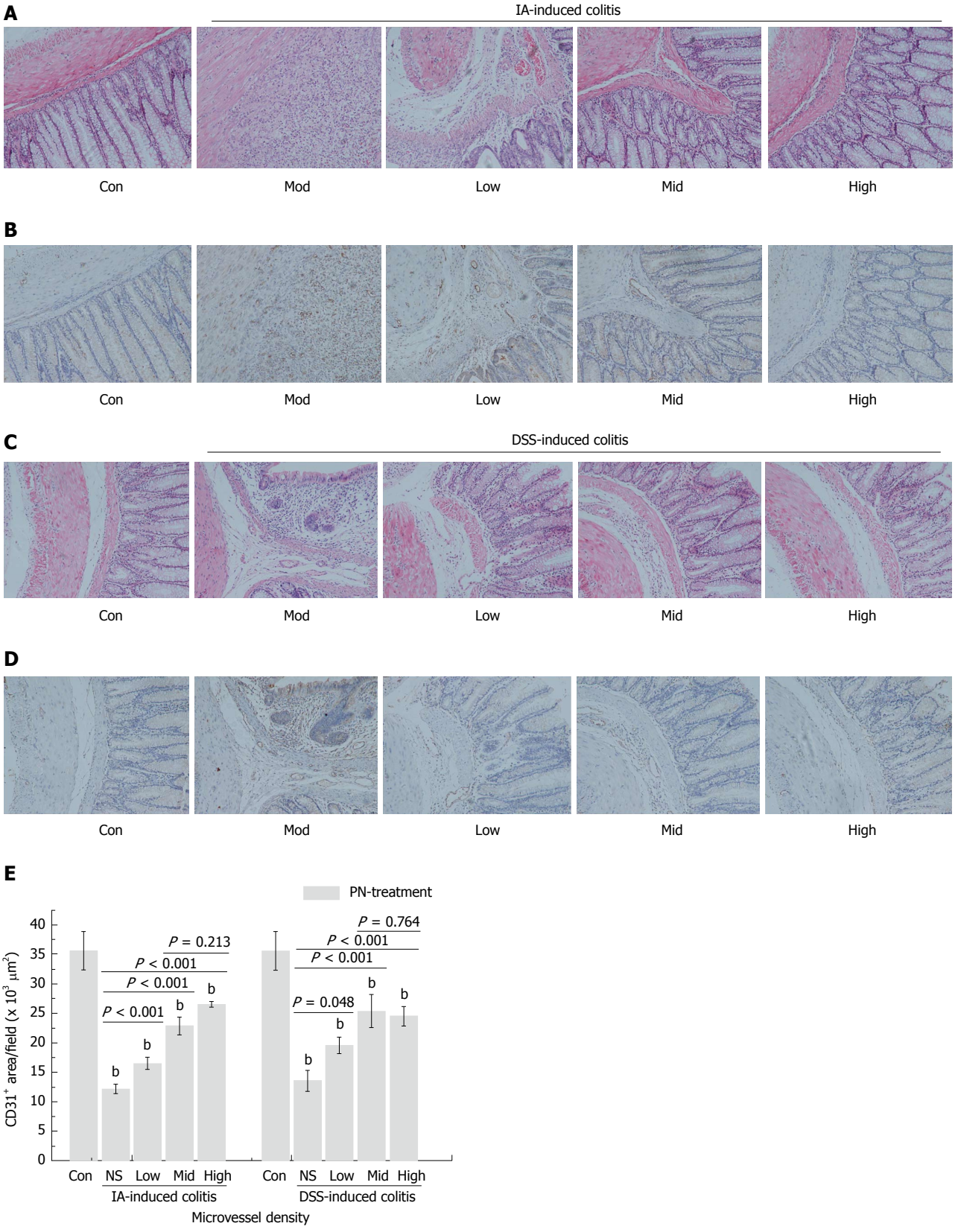

Figure 4 ***Panax notoginseng*** **repaired colonic mucosal injuries and microvessels.** IA- and DSS-induced experimental colitis groups were treated with PN (0.5, 1.0 and 2.0 g/kg) for seven consecutive days. A, C: Colonic mucosal injuries and microvessels significantly improved; B, D, E: Microvessel density increased compared with the control groups in a dose-dependent manner. Results are expressed as mean ± SD of three independent experiments performed in triplicate. $^{b}P < 0.01$ *vs* normal control. DSS: Dextran sodium sulfate; IA: Iodoacetamide; PN: *Panax notoginseng*.

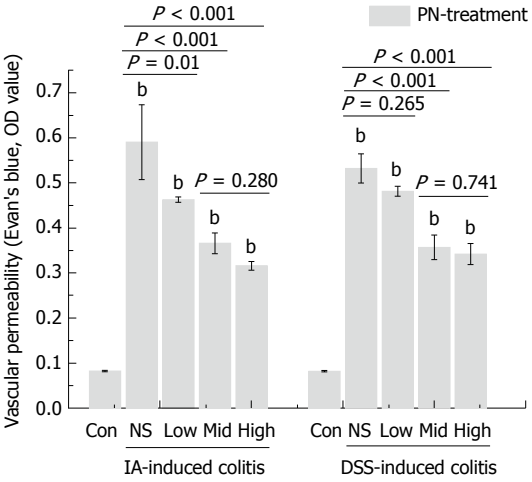

Figure 5 ***Panax notoginseng*** **improved impaired vascular permeability.** A and B: Increased VP in the IA- and DSS-induced experimental colitis groups was decreased by medium and high doses of PN. Results are expressed as mean ± SD of three independent experiments performed in triplicate. $^{b}P < 0.01$ *vs* normal control. DSS: Dextran sodium sulfate; IA: Iodoacetamide; PN: *Panax notoginseng*; VP: Vascular permeability.

and TNF-α) cytokines are indicators of inflammatory status[21,22]. In addition, HIF-1α is a crucial marker protein expressed under hypoxic conditions[23,24]. Its expression increases when hypoxia occurs in tissues and is usually used to assess the severity of hypoxia[25,26]. MPO and SOD, two kinds of oxidative enzymes, are crucial markers used to assess oxidative stress[27,28]. They could reflect the severity of oxidative stress and hypoxia in colonic mucosa[19,29]. VEGF, especially VEGFA, is an important cytokine implicated in angiogenesis[8]. VEGFA121 and VEGFA165, two isoforms of VEGF, are closely correlated to the angiogenesis of colonic microvessels[8,30].

Our previous study demonstrated that increased ratio of VEGFA165/VEGFA121 was in proportion to impairment of microvessels. In the present study, 7-d PN treatment reduced serum concentrations of VEGFA165 and VEGFA121 and the ratio of VEGFA165/VEGFA121, and attenuated impairment of microvessels compared with the colitis groups. PN down-regulated the expression of MPO and HIF-1α while up-regulating the expression of SOD in colonic mucosa. These findings demonstrate that PN attenuates hypoxia and oxidative stress in colonic mucosa. The changes in VEGFA isoforms, HIF-1α, MPO and SOD were dose dependent. The effects of medium and high doses were superior to those of low dose, but there were no significant differences between the medium and high doses. This suggested that the effects of PN reached a plateau when the dose was increased to a certain value. The optimal dose of PN is 1.0 g/kg for treating experimental colitis in rats.

In summary, PN is promising in UC treatment. It could improve hypoxia and relieve oxidative stress in the colon, attenuate vessel impairment and/or promote angiogenesis, and finally promote repair of colonic mucosa. Early use of PN at an optimal dose might yield better efficacy. However, there are still some unresolved problems in the present study that need further study. For example, what is the real effective component in PN for UC treatment? What are the mechanisms of PN attenuating oxidative stress and regulating angiogenesis? These are important questions for using PN for treatment of UC and warrant exploration in future studies.

ARTICLE HIGHLIGHTS

Research background

Panax notoginseng (PN) is a Chinese herbal medicine commonly used to treat ulcerative colitis (UC) and vascular diseases. Microvascular injury plays an important role in the pathogenesis of UC, but PN's effects on microvascular injury in UC are unclear. To clarify the effects of PN on microvascular injury is important for treating UC.

Research motivation

The effects of PN on microvascular injury in colitis, its initial administration time, its dosage and its related mechanisms were investigated. These are important questions for using PN for treatment of UC.

Research objectives

To clarify the effects of PN on microvascular injury and related affecting factors, as well as its mechanisms.

Research methods

Dextran sodium sulfate (DSS)- or iodoacetamide (IA)-induced rat colitis models were used. PN administration was initiated at 3 d and 7 d after the model was established at doses of 0.5, 1.0 and 2.0 g/kg for seven consecutive days. The severity of colitis was evaluated by disease activity index (DAI). The pathological lesions were observed under microscope. Microvessel density (MVD) was evaluated by immunohistochemistry. Vascular permeability was evaluated using the Evans blue method. The serum concentrations of vascular endothelial growth factor (VEGF)A121, VEGFA165, interleukin (IL)-4, IL-6, IL-10 and tumor necrosis factor (TNF)-α were detected by enzyme-linked immunosorbent assay. Myeloperoxidase (MPO) and superoxide dismutase (SOD) were measured to evaluate the level of oxidative stress. Expression of hypoxia-inducible factor (HIF)-1α protein was detected by western blotting. One-way ANOVA or general linear model with repeated measures was used to analyze the data sets with three or more groups, and least significant difference post hoc test for multiple comparisons. Student's *t*-test was used to analyze data sets with two groups. $P < 0.05$ was considered significant.

Research results

Obvious colonic inflammation and injuries of colonic mucosa and microvessels were observed in DSS- and IA-induced colitis in rats. DAI scores, the serum concentrations of VEGFA121, VEGFA165, VEGFA165/VEGFA121, IL-6 and TNF-α, and the concentrations of MPO and HIF-1α in the colon were significantly higher while the serum concentrations of IL-4 and IL-10 and MVD in colon were significantly lower in the colitis model groups than in the normal control group. PN promoted repair of the injuries of colonic mucosa and microvessels, attenuated inflammation and decreased DAI scores in rats with colitis. PN decreased the serum concentrations of VEGFA121, VEGFA165, VEGFA165/VEGFA121, IL-6 and TNF-α and the concentrations of MPO and HIF-1α in the colon. It also increased the serum concentrations of IL-4 and IL-10 as well as the concentration of SOD in the colon. The efficacy of PN was dosage dependent. In addition, DAI scores in the group initiating PN administration on day 3 were significantly lower than in the group initiating PN administration on day 7.

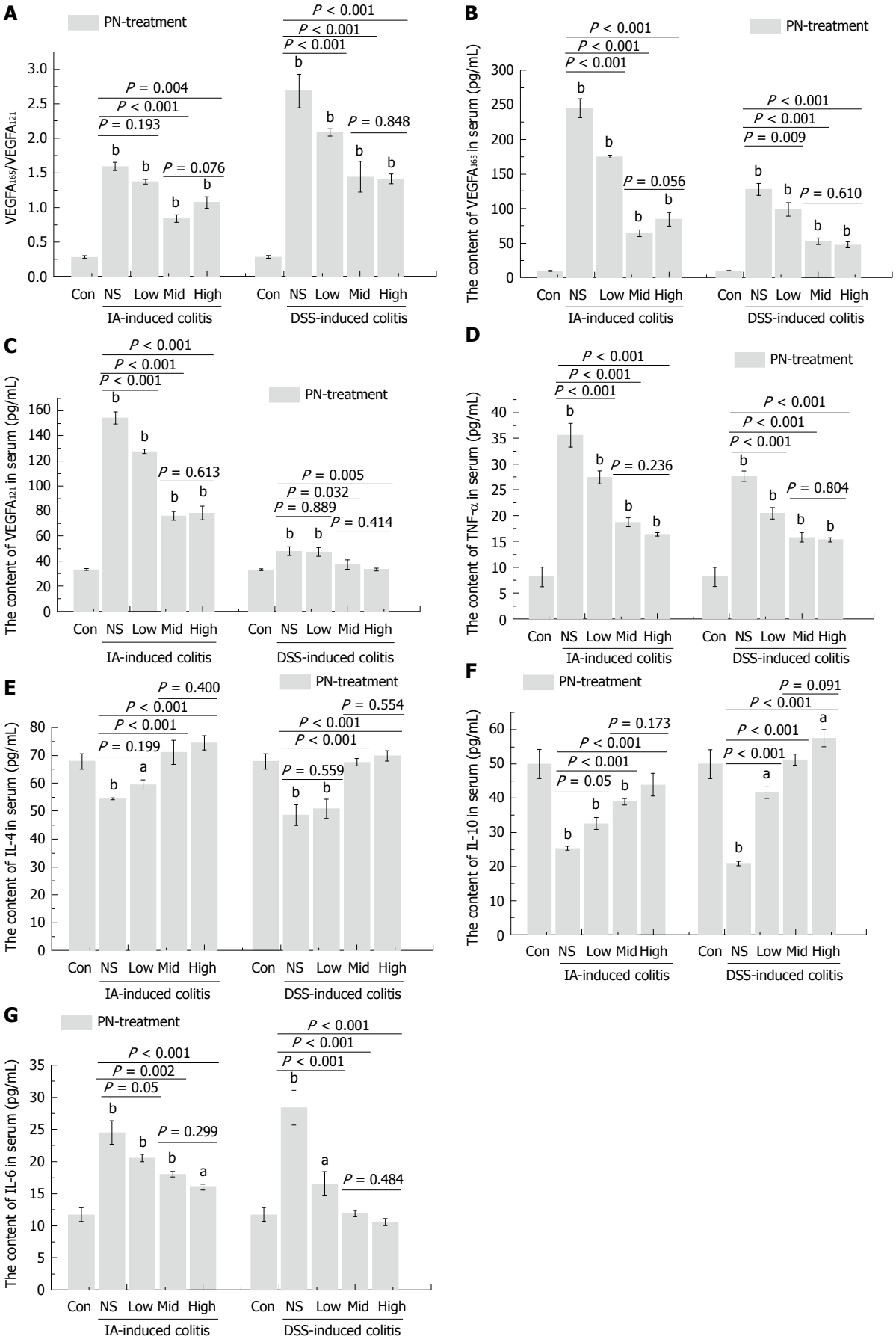

Figure 6 ***Panax notoginseng*** **reversed the disordered ratio of VEGFA165/VEGFA121 and adjusted the imbalance of pro-inflammatory and anti-inflammatory cytokines.** A-C: Increased serum concentrations of VEGF165 and VEGF121, as well as the increased ratio of VEGF165/VEGF121 in the experimental colitis groups were down-regulated by medium and high doses of PN; D-F: The increased serum concentrations of IL-6 and TNF-α and decreased serum concentrations of IL-4 and IL-10 in the experimental colitis groups were reversed by PN in a dose-dependent manner. Results are expressed as mean ± SD of three independent experiments performed in triplicate. [a]$P < 0.05$, [b]$P < 0.01$ *vs* normal control. IL: Interleukin; PN: *Panax notoginseng*; TNF: Tumor necrosis factor; VEGF: Vascular endothelial growth factor.

Research conclusions

PN repaired microvessel injury in experimental colitis *via* attenuating inflammation and oxidative stress in the colonic mucosa. The efficacy of PN was related to the initial administration time and the dose.

Research perspectives

Finding the real effective component in PN and clarifying the mechanisms of PN attenuating oxidative stress and regulating angiogenesis will be conducted in the future studies.

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