

Contrasting Effects of Pseudoephedrine and Papaverine in Dextran Sodium Sulfate-induced Colitis

Norman R. Harris, PhD, Robert D. Specian, PhD, Patsy R. Carter, BS, and Georgia A. Morgan, BS

Background: Dextran sodium sulfate (DSS) induces submucosal arteriolar constriction that reduces blood flow to the intestine, and the relevance of this decrease in flow needs further investigation. In the present study we examined the effects of a vasoconstrictor (pseudoephedrine) and a vasodilator (papaverine) on the outcome of DSS-induced colitis.

Methods: Mice were given DSS in drinking water for 6 days, with enemas on days 0, 1, 3, and 5 containing pseudoephedrine, papaverine, or no drug. At the conclusion of the 6-day protocol a disease activity index comprising weight loss, stool consistency, and rectal bleeding was evaluated, along with intravital microscopy observations of submucosal venular leukocyte and platelet adherence in the proximal colon and terminal ileum.

Results: Pseudoephedrine and papaverine had several contrasting effects on the outcome of DSS ingestion: pseudoephedrine induced the highest levels of weight loss, loose stools, venular platelet adherence, and overall disease activity index, while papaverine induced the highest levels of venular leukocyte adherence, but the lowest levels of rectal bleeding, loose stools, and overall disease activity index.

Conclusions: The results suggest that vasoconstriction worsens the pathological consequences of DSS in the mouse model of colitis.

(*Inflamm Bowel Dis* 2008;14:318–323)

Key Words: microcirculation, inflammatory bowel disease, leukocyte adhesion, platelet adhesion

Ingestion of dextran sodium sulfate (DSS) in experimental animals is used as a model of intestinal inflammation that mimics certain aspects of inflammatory bowel disease (IBD). Recent studies from our laboratory¹ and from Mori et al² have shown that DSS induces a reduction in flow through the submucosal arterioles and that the arteriolar vasoconstriction can be attenuated by an inhibitor of thromboxane synthase.¹ The thromboxane-induced constriction appeared to be influenced by the number of venular adherent platelets relative to the number of adherent leukocytes, with a high ratio of platelets to leukocytes associated with the largest thromboxane-induced constriction.¹

A decrease in intestinal blood flow has been noted in other chemical models of colitis, including rectal instillation of acetic acid^{3,4} or trinitrobenzenesulfonic acid (TNBS),⁵ and with systemic administration of indomethacin⁶ or mitomycin C.⁴ The relevance of a decrease in blood flow to human IBD has been discussed in a review by Hatoum et al,⁷ who observed dysfunctional endothelium-dependent vasodilation in submucosal arterioles obtained from IBD patients.⁸

Attenuating the vasoconstriction associated with colitis could prove to be advantageous. In animal models, experimental strategies aimed at blocking angiotensin II⁹ or endothelin-1^{10–12} have demonstrated the benefits of inhibiting the actions of vasoconstrictors. Additionally, the vasoconstrictor pseudoephedrine has been reported to induce human ischemic colitis.^{13–15}

In the current study we used the DSS mouse model of colitis to investigate the result of colonic administration of the vasoconstrictor pseudoephedrine versus the vasodilator papaverine. The results show that these 2 agents provide a contrast in several measured parameters including weight loss, stool consistency, rectal bleeding, and the microvascular accumulation of leukocytes and platelets.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice were purchased from Jackson Labs (Bar Harbor, ME) at an age of ≈2–3 months and weighing 25–30 g. The mice were placed on a 6-day protocol in which they were given water ad libitum that was filter-purified (Millipore, Bedford, MA) with or without addition of 3% (wt/vol) DSS (40 kD; ICN Biomedicals, Aurora, OH). On days 0, 1, 3, and 5 mice were given an intrarectal enema as described by Vallance et al¹⁶: following anesthesia with ket-

Received for publication September 4, 2007; Accepted September 7, 2007.

From the Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, Shreveport, Louisiana.

This study was performed with funding from the Crohn's and Colitis Foundation of America and the National Institute of Diabetes and Digestive and Kidney Diseases (P01DK043785). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Diabetes and Digestive and Kidney Diseases or the National Institutes of Health.

Reprints: Norman R. Harris, PhD, LSU Health Sciences Center, Department of Molecular and Cellular Physiology, 1501 Kings Highway, Shreveport, LA 71130 (e-mail: nharr6@lsuhsc.edu).

Copyright © 2007 Crohn's & Colitis Foundation of America, Inc.
DOI 10.1002/ibd.20303

Published online 16 October 2007 in Wiley InterScience (www.interscience.wiley.com).

amine (150 mg/kg) and xylazine (7.5 mg/kg), 100 μ L of a distilled water solution was injected through PE50 tubing attached to a syringe. The tubing was placed \approx 4 cm proximal to the anus and the mouse was held vertically for 30 seconds to help distribute the enema in the colon. The enema solution included pseudoephedrine, papaverine, or no drug. The desired amounts of pseudoephedrine and papaverine administered to the mice were 29 mg/kg and 12.5 mg/kg, respectively, as described previously.^{17,18} An unknown fraction of the enema might not be taken up by the colon, and therefore 10-fold concentrations were used to help attain the approximate desired drug administration. The 4 groups of mice included controls (no DSS; vehicle enema; $n = 40$), DSS mice given pseudoephedrine enemas ($n = 27$), DSS mice given papaverine enemas ($n = 29$), and untreated DSS mice (vehicle enema; $n = 43$). The procedures were approved by the Institutional Animal Care and Use Committee.

Disease Activity Index

At the end of 6 days on the DSS protocol, mice were evaluated by a disease activity index similar to that described by Cooper et al.¹⁹ Three parameters (weight loss, stool consistency, and occult/gross bleeding) were each evaluated on a 4-point scale and averaged for an overall disease activity index. Weight loss was attributed a value of 0 if body weight remained within 1% of baseline or higher, 1 for a 1%–5% loss, 2 for a 5%–10% loss, 3 for a 10%–15% loss, and 4 for a loss greater than 15%. Stool consistency was graded 0 for normal, 2 for loose stools that do not stick to the anus, and 4 for liquid stools that do stick to the anus. Bleeding was graded 0 for no blood, 2 for occult blood that was detectable by guaiac paper test (Helena Laboratories, Helena, TX), and 4 for gross bleeding. At the end of the 6-day protocol animals were killed with an overdose of pentobarbital.

Histology

For histology, tissue was recovered upon sacrifice. The entire large intestine, from the rectum to the cecum, was recovered and opened along the anti-mesenteric surface. The colon was rolled, starting with the rectum, and terminating at the proximal end of the colon. The tissue was pinned in this position and fixed in 4% neutral buffered formalin. After fixation the tissue was rinsed in phosphate buffer, dehydrated in ethanol, and cleared in xylene. The tissue was embedded in paraffin and sectioned at 5 μ m. The sections were deparaffinized and stained with hematoxylin and eosin. The entire length of the colon was observable in a single section.

Venular Leukocyte and Platelet Adherence

In a subset of animals (4–7 mice per enema group), leukocytes and platelets adhering to venular walls were monitored by intravital microscopy. As described previously by us,¹ mice were anesthetized with ketamine (150 mg/kg) and

xylazine (7.5 mg/kg) prior to cannulation of the jugular vein. The jugular cannula was used for injection of rhodamine 6G (0.6 mg/kg; Acros Organics, Morris Plains, NJ) to label leukocytes and for an injection of 1×10^8 platelets. The platelets were obtained from a protocol-matched donor mouse and labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE; Sigma, St. Louis, MO) as described previously in more detail.¹

With the animal placed on its side the ileum and proximal colon were gently exteriorized through an abdominal incision and positioned for microscopic observation. The microcirculation was viewed with a Nikon Diaphot microscope using a 20 \times objective and a dual filter cube (Chroma, Rockingham, VT) for simultaneous observation of rhodamine 6G-labeled leukocytes (red emission) and CFSE-labeled platelets (green emission). In each mouse \approx 10 venules having diameters in the range from 30–130 microns were video-recorded using a Sony DXC-990 camera connected to a DVD recorder (Panasonic DMRE100H). Playback analysis was performed by dividing the number of adherent leukocytes and platelets by the inner surface area of the venule: $= \pi \times \text{diameter} \times \text{length}$.

Statistics

The 4 groups were compared using analysis of variance (ANOVA) and the Student-Newman-Keuls multiple comparison test (GraphPad Instat Software, San Diego, CA). Minitab software (State College, PA) was used for linear regression. Values are presented as means \pm standard error.

RESULTS

All mice on the enema protocols lost body weight, although in the control group (no DSS) the drop in body weight was minimal, remaining within \approx 5% of baseline throughout the 6-day protocol. As shown in Figure 1, weight loss was most severe ($-14.3 \pm 0.8\%$) in the DSS mice given pseudoephedrine enemas ($P < 0.001$ versus the weight loss in untreated DSS mice, $-10.7 \pm 0.6\%$). Weight loss was not statistically different in the papaverine mice compared with the untreated DSS mice.

Papaverine had a noticeable inhibitory effect on rectal bleeding induced by DSS. With papaverine enemas, gross bleeding was present in only 7% of the papaverine mice (2 out of 29) versus 48%–49% in the pseudoephedrine and untreated DSS mice (Fig. 2). Additionally, with papaverine enemas no signs of occult blood (guaiac test) were found in 41% of mice versus 18% and 7% in the pseudoephedrine and untreated DSS mice, respectively. No gross bleeding or occult blood was found in any of the control mice (mice that did not receive DSS).

Examination of the histopathology slides (Fig. 3A–D) revealed a dramatic decrease in the mucosal injury after treatment with papaverine (Fig. 3C). The degree of inflam-

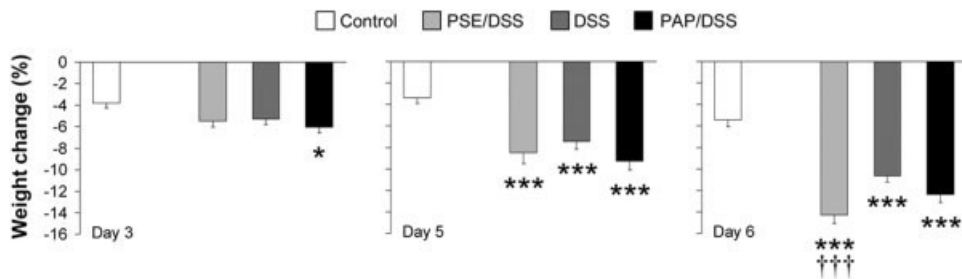


FIGURE 1. Weight change (%) on days 3, 5, and 6 of the 6-day DSS protocol in the 4 groups of mice: control, pseudoephedrine + DSS (PSE/DSS), untreated DSS, and papaverine + DSS (PAP/DSS). **P* < 0.05 versus control; ****P* < 0.001 versus control; †††*P* < 0.001 versus untreated DSS.

matory cell infiltrate was markedly reduced and there was a near absence of hemorrhage in the mucosa. Although crypt structure had not returned to control levels, the epithelial layer appeared to be intact. Some of the epithelial cells appeared squamous or cuboidal rather than columnar, but the barrier appeared intact. After treatment with pseudoephedrine the mucosal barrier still had patent breaks as seen with DSS alone and frank hemorrhage was apparent in the sections (Fig. 3D).

DSS induced only mild cases of loose stools (*P* < 0.05 versus controls), with an average score of 0.37 ± 0.12 on a 4-point scale. The score was highest in the mice given the pseudoephedrine enemas (0.59 ± 0.18 ; *P* < 0.01 versus controls), and was not statistically different from controls in the papaverine group (0.14 ± 0.10). No loose stools were observed in the control group (score = 0.0). Figure 4 shows these scores along with the 0–4 scale of the other 2 factors (bleeding, weight loss) that comprise the disease activity

index. As shown in Figure 4, the disease activity index was elevated in all groups of DSS mice, but was significantly attenuated in the papaverine group compared with the untreated DSS group (*P* < 0.001) due to the reduced frequency of loose stools and rectal bleeding.

Intravital microscopy was used to quantify endothelial adherence of leukocytes and platelets in venules ranging from 30–130 microns in diameter in the ileum and proximal colon. The surface area per unit length of vessel wall can vary by more than a factor of 4 over this range of diameters, and therefore adherence was normalized to surface area rather than length of venule. As shown in Figure 5, we subdivided the venules into 2 diameter ranges (30–80 microns and 80–130 microns) to determine whether the normalized values depended on the subset of venule diameter. The largest values of leukocyte adherence were found in the DSS mice given the papaverine enemas, with the values statistically different from controls (*P* < 0.01) in both small and large venules. In contrast, the largest values of venular platelet adherence occurred in the DSS mice given the pseudoephedrine enemas, with the values statistically greater than in both controls and untreated DSS mice. The most substantial normalized increases in platelet adherence in the pseudoephedrine mice occurred in the smaller subset of venules (*P* < 0.05).

As shown in Figure 5, the y-axis scale is twice as great for leukocyte adherence compared with platelet adherence, indicating the general trend for twice as many fluorescent leukocytes as platelets. However, it should be noted that the number of fluorescently labeled platelets was $\approx 1/10$ th of the total number of circulating platelets, and therefore the number of adherent platelets was likely to have exceeded the number of adherent leukocytes in all groups. In untreated DSS mice the platelet-to-leukocyte adherence ratio of fluorescent cells was found to be tightly correlated ($r^2 = 0.76$; *P* = 0.011) to the disease activity index (Fig. 6). Pseudoephedrine mice had the highest ratio of adherent platelets to adherent leukocytes.

DISCUSSION

DSS has been found to induce a significant decrease in submucosal flow,^{1,2} but the relevance of this decrease needs further investigation. In the present study we examined the

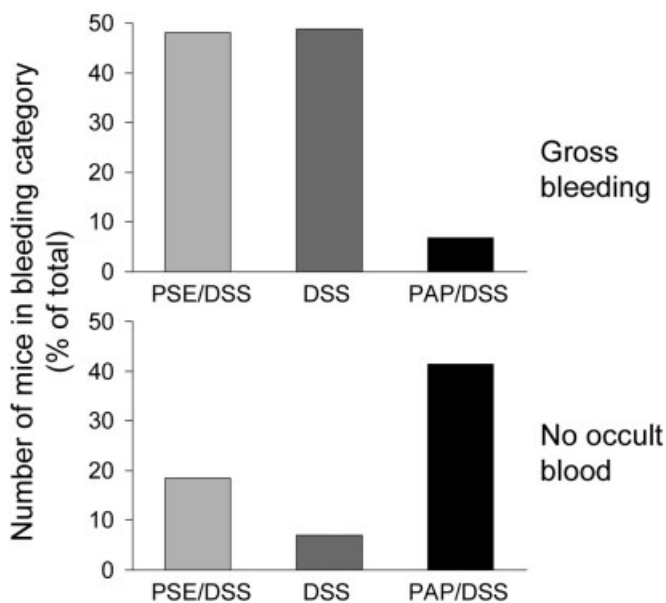


FIGURE 2. Percentage of mice with gross rectal bleeding (top panel) or no detectable blood (bottom panel) in the DSS groups: pseudoephedrine + DSS (PSE/DSS), untreated DSS, and papaverine + DSS (PAP/DSS).

effects of a vasoconstrictor (pseudoephedrine) and a vasodilator (papaverine) on the outcome of DSS-induced colitis. Pseudoephedrine and papaverine had several contrasting effects: pseudoephedrine induced the highest levels of weight loss, loose stools, venular platelet adherence, and overall

disease activity index, while papaverine induced the highest levels of venular leukocyte adherence, but the lowest levels of rectal bleeding, loose stools, and overall disease activity index. These results suggest that vasoconstriction could be detrimental in the DSS mouse model of colitis.

The presence and action of vasoconstrictors have been noted in several animal models of colitis, as well as in human IBD. Three such vasoconstrictors include angiotensin II, endothelin-1, and thromboxane. Elevated colonic mucosal levels of angiotensin II have been found in patients with Crohn's disease (CD),²⁰ a condition that has been reported to be perpetuated by chronic microvascular insufficiency, as reviewed by Thornton and Solomon.²¹ In the TNBS model of colitis the severity of the inflammation and the production of inflammatory cytokines were attenuated by the angiotensin II receptor antagonist Losartan and in angiotensinogen knock-out mice.⁹ Patients with ulcerative colitis and CD have elevated tissue^{22,23} and plasma²⁴ levels of the potent vasoconstrictor endothelin-1, the blockade of which attenuates TNBS and DSS-induced colitis.^{10–12} Finally, mucosal biopsies of patients with active IBD demonstrate a significant upregulation of thromboxane synthase that correlates with endoscopic and histologic scores.²⁵ Several animal models of colitis, including DSS, dinitrochlorobenzene, immune complex, and TNBS administration implicate thromboxane.^{1,26,27}

In a previous investigation from our laboratory¹ we found that DSS-induced arteriolar vasoconstriction could be attenuated by ozagrel, an inhibitor of thromboxane synthase. However, the arteriolar response to ozagrel depended on the relative amounts of leukocyte and platelet adhesion in the venules, which were located in close countercurrent pairing with the constricted arterioles. Ozagrel induced the highest amount of arteriolar relaxation when the ratio of platelet-to-leukocyte adherence was high, but induced little arteriolar relaxation when leukocytes outnumbered platelets. Since platelet adhesion was relatively similar in these vessels the possible interpretation was that the leukocytes could neutral-

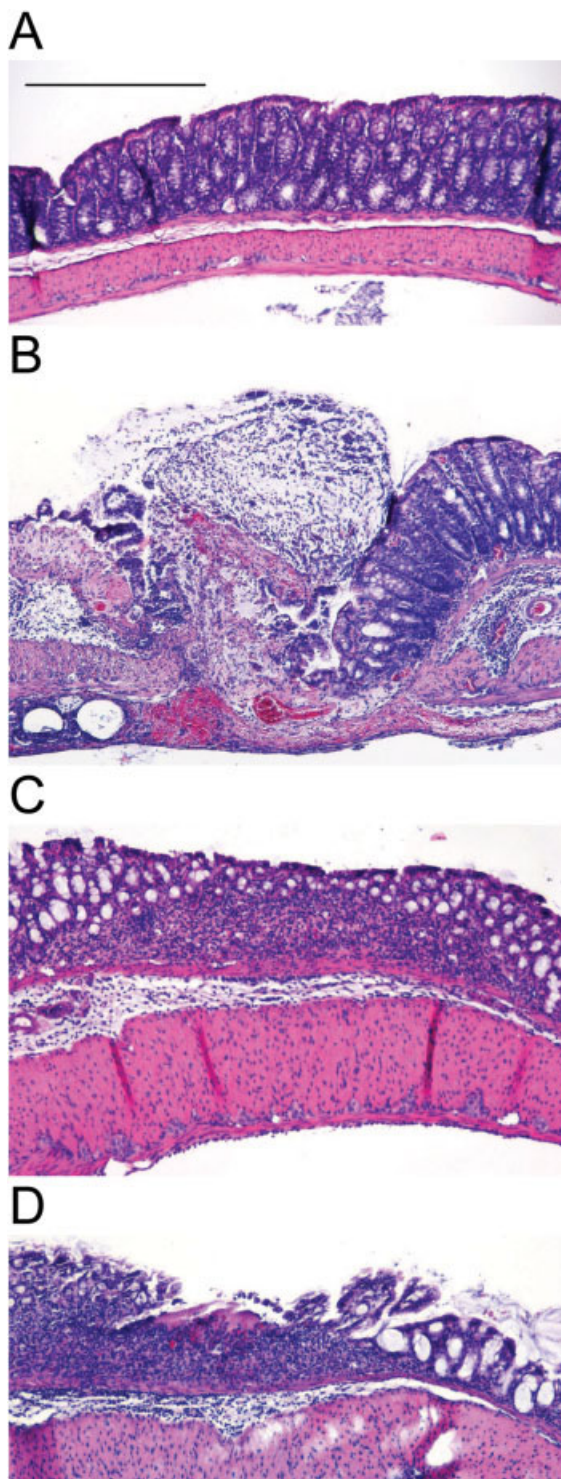


FIGURE 3. Histology of the mouse proximal colon. Scale bar = 400 μm in A; equal magnification in all panels. A: Control mouse proximal colon; note the regular arrangement of crypts. B: After exposure to DSS for 6 days the colon is markedly inflamed, the mucosal wall is thickened, and there is a transmural inflammatory cell infiltration. The mucosal barrier has been destroyed and frank hemorrhaging was histologically apparent. C: Papaverine effected a dramatic reduction of mucosal inflammation and the mucosal barrier has been repaired, although many of the cells have not regained their full columnar stature and are squamous-cuboidal in shape. There is little sign of hemorrhage. Although crypt architecture has not returned to control levels it has clearly improved over DSS treatment. D: Pseudoephedrine treatment resulted in modest improvements over DSS alone. Frank breaks in the epithelial barrier were prominent and massive inflammatory infiltration with frank hemorrhaging was histologically apparent.

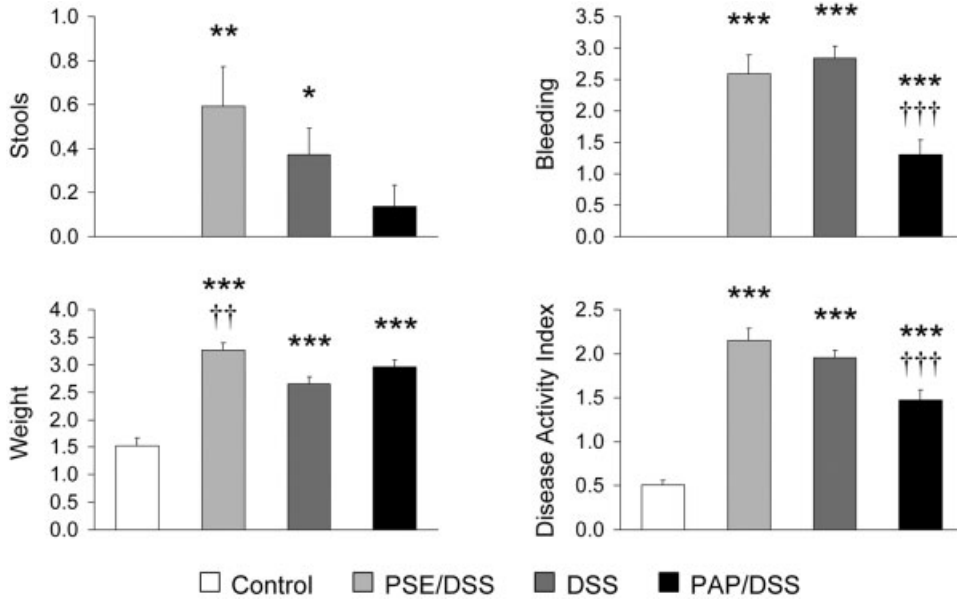


FIGURE 4. Components of the disease activity index (stools, bleeding, weight loss) in the 4 groups of mice: control, pseudoephedrine + DSS (PSE/DSS), untreated DSS, and papaverine + DSS (PAP/DSS). **P* < 0.05 versus control; ***P* < 0.01 versus control; ****P* < 0.001 versus control; ††*P* < 0.01 versus untreated DSS; †††*P* < 0.001 versus untreated DSS.

ize the vasoconstricting actions of platelet-derived thromboxane.

The ratio of platelet-to-leukocyte adherence was instructive in the current study as well. In DSS mice a tight correlation was found between the disease activity index and the platelet-to-leukocyte ratio. Moreover, there was a contrast

in the recruitment of platelets and leukocytes when the vasoactive molecules pseudoephedrine and papaverine were administered: the highest levels of platelet adherence were induced by the vasoconstrictor pseudoephedrine, and the highest levels of leukocyte adherence were induced by the vasodilator papaverine. A high level of platelet adherence

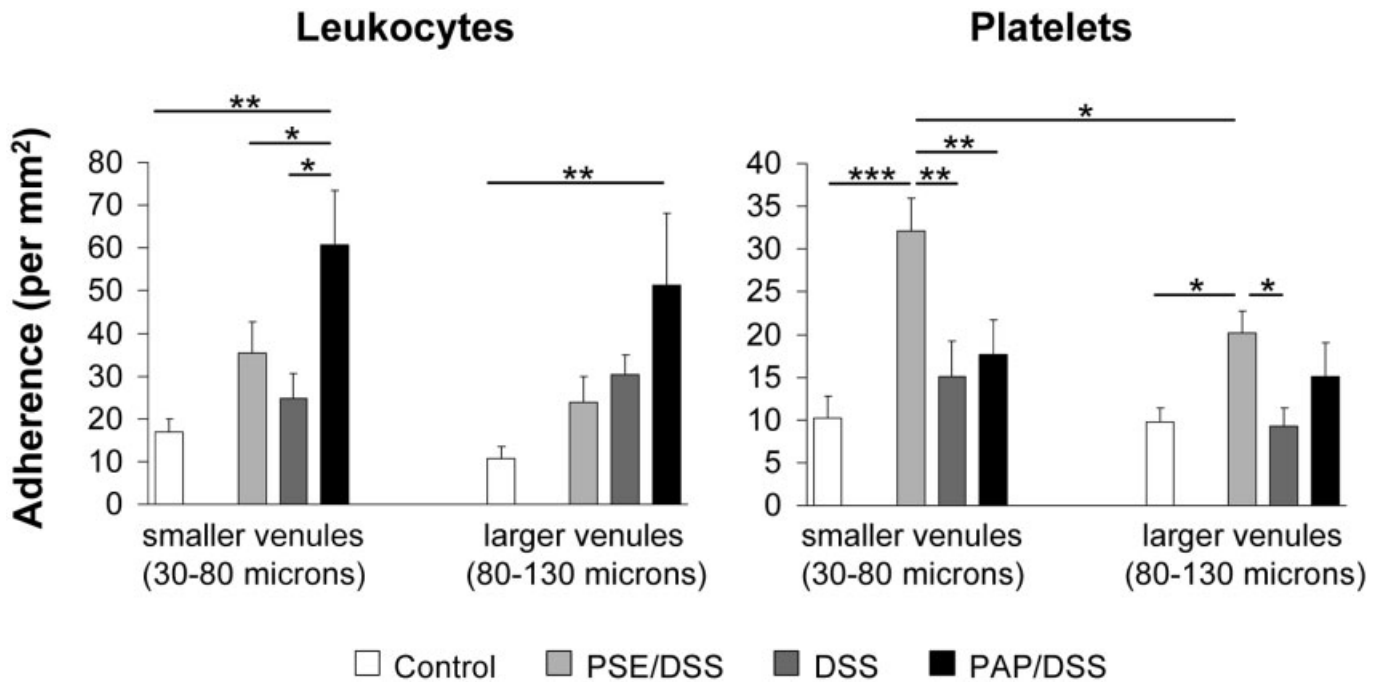


FIGURE 5. Venular leukocyte and platelet adherence in the proximal colon and terminal ileum in the 4 groups of mice: control, pseudoephedrine + DSS (PSE/DSS), untreated DSS, and papaverine + DSS (PAP/DSS). *N* = 18–43 venules per bar. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 between indicated groups.

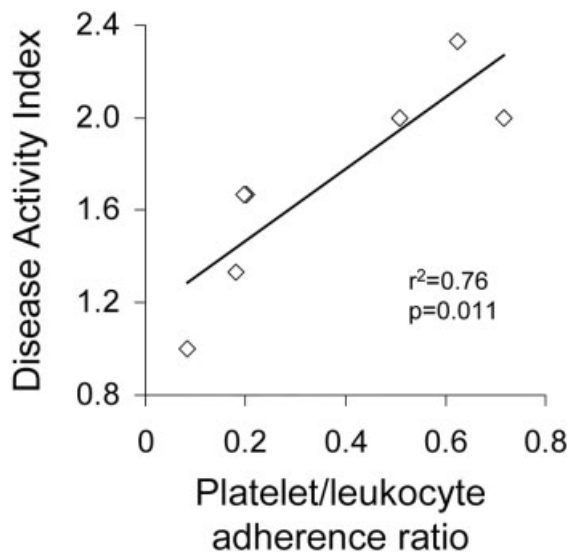


FIGURE 6. Linear regression between disease activity index and the ratio of venular platelet-to-leukocyte adherence in seven DSS mice (2 data points overlap at $x \approx 0.19$; $y = 1.67$).

could be indicative of the increased thrombogenic potential associated with DSS-induced colitis²⁸ and human IBD.²⁹ In contrast, the infiltration of leukocytes, while often correlated with inflammation, could have mixed consequences. Certain subsets of leukocytes may potentiate the inflammatory response, but other subsets may help resolve the inflammatory response. Further experiments may help determine whether the mucosal repair that we observed with papaverine administration might have been a consequence of the high levels of venular leukocyte adherence.

In summary, DSS-induced colitis was affected in contrasting directions by the vasoconstrictor pseudoephedrine and the vasodilator papaverine. In general, pseudoephedrine exacerbated the inflammatory consequences of DSS, while papaverine attenuated the disease activity index, in large part by significantly reducing rectal bleeding.

REFERENCES

- Harris NR, Whatley JR, Carter PR, et al. Venular constriction of submucosal arterioles induced by dextran sodium sulfate. *Inflamm Bowel Dis*. 2005;11:806–813.
- Mori M, Stokes KY, Vowinkel T, et al. Colonic blood flow responses in experimental colitis: time course and underlying mechanisms. *Am J Physiol Gastrointest Liver Physiol*. 2005;289:G1024–G1029.
- Fabia R, Ar'Rajab, Willen R, et al. The role of transient mucosal ischemia in acetic acid-induced colitis in the rat. *J Surg Res*. 1996;63:406–412.
- Kruschewski M, Foitzik T, Perez-Canto A, et al. Changes of colonic mucosal microcirculation and histology in two colitis models: an experimental study using intravital microscopy and a new histological scoring system. *Dig Dis Sci*. 2001;46:2336–2343.
- Fries W, Pagiario E, Canova E, et al. The effect of heparin on trinitrobenzene sulphonic acid-induced colitis in the rat. *Aliment Pharmacol Ther*. 1998;12:229–236.

- Ruh J, Schmidt E, Vogel F, et al. Indomethacin-induced disturbances in villous microcirculation in the rat ileum. *Microvasc Res*. 1999;58:137–143.
- Hatoum OA, Miura H, Binion DG. The vascular contribution in the pathogenesis of inflammatory bowel disease. *Am J Physiol Heart Circ Physiol*. 2003;285:H1791–H1796.
- Hatoum OA, Binion DG, Otterson MF, et al. Acquired microvascular dysfunction in inflammatory bowel disease: loss of nitric oxide-mediated vasodilation. *Gastroenterology*. 2003;125:58–69.
- Inokuchi Y, Morohashi T, Kawana I, et al. Amelioration of 2,4,6-trinitrobenzene sulphonic acid induced colitis in angiotensinogen gene knockout mice. *Gut*. 2005;54:349–356.
- Anthoni C, Mennigen RB, Rijcken EJ, et al. Bosentan, an endothelin receptor antagonist, reduces leucocyte adhesion and inflammation in a murine model of inflammatory bowel disease. *Int J Colorectal Dis*. 2006;21:409–418.
- Deniz M, Cetinel S, Kurtel H. Blood flow alterations in TNBS-induced colitis: role of endothelin receptors. *Inflamm Res*. 2004;53:329–336.
- Padol I, Huang JQ, Hogaboam CM, et al. Therapeutic effects of the endothelin receptor antagonist Ro 48-5695 in the TNBS/DNBS rat model of colitis. *Eur J Gastroenterol Hepatol*. 2000;12:257–265.
- Dowd J, Bailey D, Moussa K, et al. Ischemic colitis associated with pseudoephedrine: four cases. *Am J Gastroenterol*. 1999;94:2430–2434.
- Klestov A, Kubler P, Meulet J. Recurrent ischaemic colitis associated with pseudoephedrine use. *Intern Med J*. 2001;31:195–196.
- Traino AA, Buckley NA, Bassett ML. Probable ischemic colitis caused by pseudoephedrine with tramadol as a possible contributing factor. *Ann Pharmacother*. 2004;38:2068–2070.
- Vallance BA, Gunawan MI, Hewlett B, et al. TGF-beta1 gene transfer to the mouse colon leads to intestinal fibrosis. *Am J Physiol Gastrointest Liver Physiol*. 2005;289:G116–G128.
- Bhattacharjee AK, Kondoh T, Nagashima T, et al. Quantitative analysis of papaverine-mediated blood-brain barrier disruption in rats. *Biochem Biophys Res Commun*. 2001;289:548–552.
- NTP Toxicology and Carcinogenesis Studies of Ephedrine Sulfate (CAS No. 134-72-5) in F344/N Rats and B6C3F1 Mice (Feed Studies). *Natl Toxicol Program Tech Rep Ser*. 1986;307:1–186.
- Cooper HS, Murthy SN, Shah RS, et al. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest*. 1993;69:238–249.
- Jaszewski R, Tolia V, Ehrinpreis MN, et al. Increased colonic mucosal angiotensin I and II concentrations in Crohn's colitis. *Gastroenterology*. 1990;98:1543–1548.
- Thornton M, Solomon MJ. Crohn's disease: in defense of a microvascular aetiology. *Int J Colorectal Dis*. 2002;17:287–297.
- Kanazawa S, Tsunoda T, Onuma E, et al. VEGF, basic-FGF, and TGF-beta in Crohn's disease and ulcerative colitis: a novel mechanism of chronic intestinal inflammation. *Am J Gastroenterol*. 2001;96:822–828.
- Murch SH, Braegger CP, Sessa WC, et al. High endothelin-1 immunoreactivity in Crohn's disease and ulcerative colitis. *Lancet*. 1992;339:381–385.
- Letizia C, Boirivant M, De Toma G, et al. Plasma levels of endothelin-1 in patients with Crohn's disease and ulcerative colitis. *Ital J Gastroenterol Hepatol*. 1998;30:266–269.
- Carty E, Nickols C, Feakins RM, et al. Thromboxane synthase immunohistochemistry in inflammatory bowel disease. *J Clin Pathol*. 2002;55:367–370.
- Appleyard CB, Alvarez A, Percy WH. Temporal changes in colonic vascular architecture and inflammatory mediator levels in animal models of colitis. *Dig Dis Sci*. 2002;47:2007–2014.
- Zipser RD, Patterson JB, Kao HW, et al. Hypersensitive prostaglandin and thromboxane response to hormones in rabbit colitis. *Am J Physiol*. 1985;249:G457–G463.
- Anthoni C, Russell J, Wood KC, et al. Tissue factor: a mediator of inflammatory cell recruitment, tissue injury, and thrombus formation in experimental colitis. *J Exp Med*. 2007;204:1595–1601.
- Irving PM, Pasi KJ, Rampton DS. Thrombosis and inflammatory bowel disease. *Clin Gastroenterol Hepatol*. 2005;3:617–628.