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Decreased retinal blood flow in experimental colitis; improvement by eye drop administration of losartan

Megan N. Watts, BS, Randa S. Eshaq, MS, Patsy R. Carter, BS, and Norman R. Harris, PhD
Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, Shreveport, LA USA

Abstract

Patients with inflammatory bowel disease suffer not only from gut inflammation, but also from extraintestinal manifestations of the disease, including ocular pathology. The mechanisms causing ocular inflammation in these patients are unknown. The purpose of the current study was to investigate the possible vascular changes occurring in the retina using a mouse model of acute colitis, that is, ingestion of dextran sodium sulfate (DSS). Intravital microscopy of anesthetized mice revealed that DSS caused a significant 30–40% decrease in retinal red blood cell velocities, and a 45% decrease in total retinal blood flow, but no changes in intraocular pressure. To determine whether the decreases in retinal perfusion could be inhibited by an angiotensin II receptor antagonist, losartan was administered by eye drops in a subset of the mice prior to the intravital microscopy measurements. Topical losartan was able to largely attenuate the altered hemodynamics induced by DSS. We conclude that angiotensin II might be a possible target for reducing the vascular changes occurring distantly in the eye during colitis.

Keywords

colitis; retinal perfusion; angiotensin II; dextran sodium sulfate

1. Introduction

Ocular pathology is one of the most prevalent extraintestinal manifestations of the inflammatory bowel diseases (IBD) (Danese et al., 2005; Rothfuss et al., 2006; Larsen et al., 2010). Most tissues within the eye are vulnerable to the distant injury initiated by the inflamed gut (Strauss 1988; Felekis et al., 2009; Manganelli et al., 2009). In the anterior eye, IBD can cause inflammation in the eyelid, eyelash follicles, conjunctiva, sclera, episclera, and iris, and can also cause keratopathy and cataracts. In the posterior eye, choroiditis, retinal vasculitis, optic neuritis, retinal pigment epithelium disturbances, and serous retinal detachment can result. Anterior eye pathology is diagnosed in IBD patients more frequently than posterior eye pathology; however, some investigators have emphasized the need for more careful examination of the posterior eye due to potentially consequential inflammation that sometimes goes undiagnosed (Felekis et al., 2009).

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Corresponding author: Norman R. Harris, PhD, Professor, Department of Molecular and Cellular Physiology, LSU Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130, Phone: 318-675-6028, Fax: 318-675-6005, nharr6@lsuhsc.edu.

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In some cases, the ocular dysfunctions resolve by attenuating the underlying gut inflammation or by ocular administration of anti-inflammatory steroids. However, IBD has been found to be difficult to treat in a significant percentage of patients, and in addition, steroid treatments can induce undesirable ocular side effects such as cataracts and glaucoma (Mintz et al., 2004). Therefore, understanding the pathological mechanisms contributing to the ocular manifestations of IBD is imperative. Previous studies have indicated a possible vascular component to the ocular pathology induced by IBD, with occurrences of ischemia, hyperemia, neovascularization, hemorrhaging, and vasculitis (Manganelli et al., 2009).

Several inflammatory mediators having a role in IBD also have effects on the vasculature, including the vasoconstrictors endothelin-1, thromboxane, and angiotensin II. With respect to the latter, IBD patients have been found to produce higher than normal levels of angiotensin II (Jaszewski et al., 1990), with experimentally-induced colitis alleviated in angiotensinogen knockout mice (Inokuchi et al., 2005) and also attenuated by inhibition with the angiotensin II receptor antagonists losartan and candesartan (Inokuchi et al., 2005; Okawada et al., 2011).

Based on these considerations, the major goals of the current study were to investigate the potential altered vascular changes occurring in the retina in a commonly used animal model of IBD, and to determine the potential influence on these changes provided by the angiotensin receptor antagonist losartan.

2. Materials and methods

2.1 Animals

C57BL/6 mice (Jackson Laboratories), 2-3 months of age, were used for this study. The experimental protocols were approved by the Institutional Animal Care and Use Committee of LSUHSC-S and performed according to the criteria outlined by the National Institutes of Health and in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2 DSS induction of colitis

Acute colonic inflammation was induced in mice via administration of 5% dextran sodium sulfate (DSS; 40 kD; ICN Biomedicals; Aurora, OH) in drinking water for 6 days as we have performed previously (Lee et al., 2009; Carter et al., 2011). The DSS was added to filter-purified water (Millipore Corp., Bedford, MA), with untreated water administered for 6 days to age-matched control C57BL/6 mice. Body weights were measured on the first and final days of the protocol, and colon weight per unit length was used as an index of colonic inflammation.

2.3 Experimental procedure

Following 6 days of untreated or DSS-treated drinking water, mice were anesthetized with 150 mg/kg ketamine and 10 mg/kg xylazine. Under anesthesia, the left eye was moistened every 10 minutes with either saline or 0.4 mg/ml losartan in saline throughout the remainder of the experiment. At the 30-minute time point, measurements of retinal blood flow velocity and retinal vascular diameters were collected via intravital microscopy, as described in section 2.4. Following these measures, at the 45-minute time point, intraocular pressure (IOP) was determined by tonometry (Tonopen Vet; Reichert Inc.; Depew, NY) as described previously (Reitsamer et al., 2004; Lee and Harris 2008; Wright and Harris 2008). The tail was then clipped to obtain blood for a hematocrit reading, and following euthanasia (150 mg/kg pentobarbital), the colon was removed for measures of colon weight per unit length.

2.4 Retinal blood flow – intravital microscopy

Intravital microscopy was used to measure retinal hemodynamics as we have published previously (Wright et al., 2009; Wang et al., 2010; Wang et al., 2011; Yadav and Harris 2011; Wright et al., 2012). Under anesthesia (described in section 2.3), the mouse was placed on the stage of a Nikon Eclipse E600FN microscope (Nikon Instruments Inc.; Melville, NY), with the left eye under the objective at a position that allowed visualization of the retinal arterioles branching out of the central retinal artery, and venules draining into the central retinal vein. Through a femoral vein cannula, a bolus (~50 μ l) of fluorescein isothiocyanate (FITC)-dextran (5 mg/kg) was infused and the retinal vasculature was observed under 4 $\times$ magnification. The retinal arterioles were identified as the vessels filling first with the dye, with the venules filling subsequently. Approximately 2-4 minutes later, the diameters of the retinal arterioles and venules were captured with a CoolSNAP ES digital camera (Photometrics, Tucson, AZ) using a 10 $\times$ objective and fluorescein filter. Red blood cell (RBC) velocities were measured using fluorescently labeled (1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate; DiI; Invitrogen Molecular Probes, Eugene, OR) red blood cells obtained from donor C57BL/6 mice as we have described previously in greater detail (Wright et al., 2009; Wang et al., 2010; Wang et al., 2011; Yadav and Harris 2011; Wright et al., 2012). The DiI-labeled RBCs were seen as fluorescent streaks in the vessels, with the length of the streak proportional to RBC velocity, which was calculated as the streak length divided by the camera exposure time (10 ms). Measurements of the diameters (D) and RBC velocities were obtained using NIS Elements Basic Research software (Nikon Instruments, Melville, NY). Retinal blood flow in each arteriole and venule was calculated as $0.25V \cdot D^2$, with V being the mean RBC velocity obtained from ~10 fluorescent RBC streaks per vessel. Vascular wall shear rates were calculated as $8V/D$, with this calculation assuming laminar flow. Total retinal blood flow was obtained by summing the flows in each of the arterioles (and separately, venules) and averaging the total arteriolar and venular flows. Each retina had 4-7 arterioles and 4-7 venules.

2.5 Western blot measures of plasma angiotensin

Blood from control and DSS mice was obtained by femoral artery cannulation. Plasma was collected by centrifuging the blood at 10,000g for 10 min at 4°C, and stored at -80°C until used for the Western blot measures. 50 μ g of protein of each sample were loaded into a 4-15% polyacrylamide gel and subjected to electrophoresis and transferred into a nitrocellulose membrane. The membrane was incubated first in a blocking buffer for one hour at room temperature then probed with a goat anti-angiotensin I/II antibody from SantaCruz BioTechnology (Dallas, TX) in a 1:1000 dilution overnight at 4°C. The blot was then incubated with a horseradish peroxidase-conjugated anti-Goat antibody (GE Healthcare; Waukesha, WI) for one hour at room temperature. The Optiblot ECL detection kit (Abcam; Cambridge, MA) was used to detect the protein bands. Results of western blot analysis were quantified using ImageJ software available from NIH (version 1.40g).

2.6 Statistics

Statistical comparisons were performed using analysis of variance (ANOVA) with Student-Newman-Keuls post-hoc corrections (GraphPad software, San Diego, CA) with $p < 0.05$ used as the criterion to interpret significance. Data are presented as means $\pm$ standard error.

3. Results

The data provided in Table 1 show the DSS-induced decrease in body weight (~17%; $p < 0.001$ vs controls), increase in colonic inflammation (~50% increase in colon weight-to-length ratio; $p < 0.001$ vs controls), and decrease in hematocrit (~35%; $p < 0.001$ vs controls), with the latter being due to iron-deficient anemia (Carter et al., 2013). As expected with only

localized administration of losartan, the systemic and colonic data in Table 1 were similar between the losartan-treated groups and their respective untreated counterparts.

Intravital microscopy was used to measure hemodynamic values for the retinal circulation, focusing on the 4-7 arterioles per retina branching from the central retinal artery, and the 4-7 venules draining into the central retinal vein. Figure 1 shows the substantial 30-40% drop in retinal RBC velocities in the arterioles (Fig 1A) and venules (Fig 1B) induced by DSS. Acute eye drop administration of losartan did not alter RBC velocities in the control mice, but was able to attenuate the decrease in RBC velocities in the DSS mice.

No statistically significant DSS-induced changes were observed in the diameters of the retinal arterioles (Fig 2A); however, the venules of the DSS mice were ~10-15% smaller than in the untreated controls ($p<0.05$; Fig 2B).

Given that vascular wall shear rates are directly proportional to velocity and inversely proportional to diameter, the changes in arteriolar shear rates (Fig 3A) mirror those of arteriolar RBC velocities since there was little change in arteriolar diameters. DSS induced an approximate 30% decrease in shear rate in the arterioles, but only a 20-25% decrease in the venules (Fig 3B) due to the DSS-induced decrease in venular diameters (Fig 2B). Acute administration of losartan eye drops maintained shear rates near control levels in both arterioles and venules.

Total retinal blood flow rates were calculated from the RBC velocities and diameters of the individual arterioles and venules, with the results shown in Figure 4. Inasmuch as DSS induced significant decreases in RBC velocity and also tended to decrease retinal vascular diameters (primarily on the venular side), the decrease in retinal blood flow rate in the DSS mice was substantial, decreasing ~45% compared with the untreated controls ($p<0.001$). Losartan eye drops provided an approximate 60% attenuation of the DSS-induced decrease in retinal blood flow ($p<0.05$), and interestingly, slightly decreased retinal blood flow in the non-colitic controls ($p<0.05$). Measurements of intraocular pressures were similar between groups (Fig 5).

Angiotensin (I/II) levels in the plasma were found to be elevated by 67% in DSS mice compared to controls (Figure 6).

4. Discussion

Posterior eye complications caused by IBD can include choroiditis, retinal vasculitis, optic neuritis, retinal pigment epithelium disturbances, and serous retinal detachment, with a recommendation for careful fundus examination in these patients (Felekis et al., 2009). The mechanisms of distant ocular inflammation in IBD have not been determined, but animal models of the disease may be useful for this purpose. To our knowledge, this is the first report investigating the possible ocular vascular deficiency in the acute DSS model of IBD; however, we have recently observed a similar percentage decrease in retinal blood flow in a chronic colitis mouse model induced by T-lymphocyte reconstitution of immune-deficient mice (Watts et al., 2013). However, in the previous study, we did not investigate the possible mediators of the retinal vascular deficiency. Our data in the current DSS study suggest the possibility that angiotensin II could be involved, although our results do not establish a definitive cause-and-effect role of the vasoconstrictor.

The percentage drop in retinal blood flow occurring with DSS-induced colitis was 45%. Moreover, hematocrit values dropped by ~35% in these mice (Table 1), and this level of anemia, coupled with the reduced flow, could be expected to render the retina vulnerable to hypoxia and hypoxia-related pathology. The presence of hypoxic tissue is not likely to be

limited to the eye, as we have previously found that plasma erythropoietin levels increase by more than a factor of 10 in the DSS model (Carter et al., 2013). The anemia induced by DSS is consistent with an iron deficiency (the most common cause of anemia in IBD patients) (Goodhand et al., 2012), with decreased levels of serum iron and transferrin saturation occurring in these mice in the presence of an elevated iron-binding capacity (Carter et al., 2013).

The DSS-induced drop in hematocrit could be expected to reduce viscosity, which would reduce resistance to flow; however, flow was decreased rather than increased in the DSS mice. The observed decrease in retinal blood flow induced by DSS could have been caused one or more mechanisms, including 1) vasoconstriction, 2) a decrease in ocular perfusion pressure, 3) loss of the number of perfused capillaries, or 4) a combination of these. Our measurements of diameters in our intravital microscopy system were limited to the primary superficial layer arterioles and venules, which demonstrated a tendency for vasoconstriction, although this reached statistical significance only in the venules. It is possible that more severe constriction and/or capillary dropout could occur deeper in the retinal microvasculature, although capillary densities were not measured. Considering that acute losartan treatment in the DSS mice was able to recover more than half of the lost perfusion, it is possible, although speculative, to suggest that vasoconstriction may play a significant role in the altered perfusion.

With regard to perfusion pressure, previous measurements of cardiac output (Mori et al., 2005) and arterial blood pressure (Lee et al., 2009) in DSS models have not shown statistical differences from non-colitic controls, although the published tendencies for decreases in both of these parameters prevent us from excluding altered perfusion pressure as a contributor to the observed decrease in retinal blood flow. However, no changes in intraocular pressure were found in the current study (Figure 5), due to either DSS or losartan. The improvement in retinal blood flow provided by acute application of losartan would be unlikely to be attributed to changes in perfusion pressure, as there should not have been any affect on systemic hemodynamics.

In a final consideration, with losartan eye drops improving retinal flow, it appears possible that the drug reached the retina in an effective pharmacological concentration. However, since retinal losartan measurements were not performed, the mechanism of action remains speculative.

In summary, retinal blood flow is reduced substantially by 45% in an acute model of IBD, concomitant with a 35% decrease in circulating hematocrit. The decrease in retinal blood flow was attenuated substantially by the angiotensin II receptor antagonist losartan. Future studies may address the possibility that improvements in retinal blood flow may alleviate posterior eye pathology occurring as a result of gut inflammation.

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Ocular pathology occurs in a subset of IBD patients.
Ocular manifestations of IBD include vascular inflammation and dysfunction.
We find decreased retinal blood flow in a mouse model of colitis.
Inhibiting angiotensin II can attenuate the decrease in flow.

Figure 1. Red blood cell (RBC) velocity in arterioles (A) and venules (B) of the mouse retina. The four groups include untreated control (non-colitic) mice (N=8), control mice administered losartan eye drops beginning 30 minutes earlier (N=7), untreated DSS mice (N=10), and

DSS mice administered losartan eye drops (N=9). *** $p < 0.001$ vs untreated controls; ++ $p < 0.001$ vs untreated DSS mice. Data are presented as means $\pm$ SE.

Figure 2. Diameters of arterioles (A) and venules (B) in the mouse retina. The four groups include untreated control (non-colitic) mice (N=8), control mice administered losartan eye drops beginning 30 minutes earlier (N=7), untreated DSS mice (N=10), and DSS mice

administered losartan eye drops (N=9). * $p < 0.05$ vs untreated controls. Data are presented as means $\pm$ SE.

Figure 3. Vascular wall shear rate of arterioles (A) and venules (B) in the mouse retina. The four groups include untreated control (non-colitic) mice (N=8), control mice administered losartan eye drops beginning 30 minutes earlier (N=7), untreated DSS mice (N=10), and

DSS mice administered losartan eye drops (N=9). ** $p < 0.01$ and *** $p < 0.001$ vs untreated controls; + $p < 0.05$ and +++ $p < 0.001$ vs untreated DSS mice. Data are presented as means $\pm$ SE.

Figure 4.

Total blood flow rate of the mouse retina. The four groups include untreated control (non-colitic) mice (N=8), control mice administered losartan eye drops beginning 30 minutes earlier (N=7), untreated DSS mice (N=10), and DSS mice administered losartan eye drops (N=9). * $p < 0.05$ and *** $p < 0.001$ vs untreated controls; +++ $p < 0.001$ vs untreated DSS mice. Data are presented as means $\pm$ SE.

Figure 5.

Intraocular pressure in the four groups of mice. The four groups included untreated control (non-colitic) mice (N=8), control mice administered losartan eye drops beginning 30 minutes earlier (N=10), untreated DSS mice (N=11), and DSS mice administered losartan eye drops (N=7). No statistical differences were found among the four groups. Data are presented as means $\pm$ SE.

Figure 6.

Representative Western blots of angiotensin from control (Con) and DSS plasma, where similar loading was confirmed by Ponceau staining of plasma protein. B. Densitometric analysis of plasma Western blots from control mice (N=10) and DSS mice (N=10), normalized to protein levels (Ponceau staining). *** $p < 0.001$ vs controls.

Table 1

Colitis-induced changes in body weight, colon weight/length, and hematocrit.

	Untreated controls	Untreated DSS mice	Losartan-treated controls	Losartan-treated DSS mice
Body weight (g) on day 0	27.3 ± 0.8 (N=10)	27.3 ± 0.9 (N=12)	27.4 ± 0.5 (N=11)	26.0 ± 0.6 (N=10)
Body weight (g) on day 6	27.6 ± 0.7 (N=10)	22.9 ± 0.5 ^{***} (N=12)	27.2 ± 0.4 (N=11)	21.4 ± 0.6 ^{***} (N=10)
Colon weight per length (mg/cm)	22.8 ± 0.9 (N=10)	33.3 ± 1.1 ^{***} (N=12)	22.8 ± 0.9 (N=11)	34.2 ± 1.4 ^{***} (N=10)
Hematocrit (%)	46.2 ± 1.0 (N=10)	31.6 ± 1.6 ^{***} (N=11)	46.6 ± 0.8 (N=11)	28.4 ± 2.0 ^{***} (N=10)

Data are presented as means ± SE.

p<0.001 vs non-colitic controls