Detection of Nitric Oxide in Horses with Large Colon Volvulus

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Nitric oxide is present at basal concentrations in the large colon of normal horses and increases subsequent to volvulus as a result of an increased iNOS expression. Increased plasma and abdominal fluid nitric oxide concentrations in horses with colonic volvulus also likely reflect an increased iNOS expression. Authors’ address: School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803. © 1997 AAEP.

1. Introduction

The purpose of this study was to determine whether nitric oxide (NO) is present under basal conditions and if it increases subsequent to large colon volvulus in horses. NO is a labile gas produced from L-arginine through NO synthase (NOS); it has both cytoprotective and cytotoxic effects, depending on the timing and magnitude of its production. There are two main isoforms of NOS. Constitutive NOS (cNOS) is present under basal conditions predominantly in endothelium (eNOS). The eNOS-derived NO potentiates vasodilation, inhibits platelet adhesion and aggregation, and decreases neutrophil adhesion. An inducible isoform (iNOS) is expressed in response to cytokines or other stimuli (ischemia), which leads to sustained release of large quantities of NO. NO reacts with superoxide radicals to produce a potent oxidant (peroxynitrite) that can cause appreciable tissue damage. Because peroxynitrite promotes the formation of nitrotyrosine, its presence can be determined by staining for nitrotyrosine; nitrotyrosine staining colocalizes with the expression of iNOS.

2. Materials and Methods

Heparinized jugular plasma, peritoneal fluid, and urine were collected from both control (n = 10) and affected (n = 13) horses and were centrifuged, and the supernatant was frozen (−80°C) until analyzed for [NO], using a chemiluminescent method. Biopsy specimens were obtained from the pelvic flexure at surgery or necropsy; a portion of the specimens were snap frozen in ornithine carbamoyl transferase gel for NADPH diaphorase staining. The remaining tissue was fixed in zinc formalin for 24 h, embedded in paraffin, and stained with H&E, for iNOS and nitrotyrosine immunohistochemistry. Slides were scored from 0 to 3, depending on the intensity of stain for iNOS and nitrotyrosine; NADPH diaphorase stained slides were evaluated for the presence or absence of stain; different cells in different regions were evaluated separately. All data were statistically analyzed, using a Mann–Whitney U test; p < 0.05 was considered significant.

3. Results

There was no difference in urine [NO] between affected and control horses, but [NO] was signifi-
cantly higher in the plasma \((p = 0.005)\) and peritoneal fluid \((p = 0.0065)\) of affected horses. Surface epithelium was completely denuded in the majority of horses with colon volvulus. There was also extensive necrosis of the crypt epithelium, and marked hemorrhage and edema in the lamina propria and submucosa of affected horses. There was significantly greater submucosal \((p = 0.0013)\) and mucosal \((p = 0.0066)\) leukocyte iNOS intensity scores in affected horses. There was a trend \((p = 0.06)\) for a greater nitrotyrosine score in the crypt epithelium of affected horses. There were no significant differences observed in nitrotyrosine scores for the surface mucosal epithelium, or submucosal or mucosal leukocytes between control and affected horses.

There was positive NADPH diaphorase staining in the surface epithelium, mucosal vasculature, submucosal arteriolar endothelium (marked), submucosal venular endothelium (moderate), myenteric plexus (marked), submucosal plexus (moderate), muscularis vasculature (minimal), and mucosal (minimal) and submucosal (minimal) leukocytes (predominantly eosinophils and macrophages). There were no surface epithelial cells remaining to stain with NADPH in affected horses. Mucosal and submucosal leukocytes stained positive, which was similar to control horses. Generally, there was less intense staining of the submucosal arteriolar endothelium and myenteric plexus compared with controls. The mucosal vasculature, submucosal venular endothelium, muscularis vasculature, and submucosal plexus did not stain for NADPH diaphorase in affected horses.

4. Discussion

This study demonstrated that NO is present in the large colon of horses under basal conditions; it is localized predominantly to the enteric nervous system plexuses and the endothelium. Additionally, NO increases subsequent to iNOS expression predominantly in mucosal and submucosal leukocytes secondary to large colon volvulus. Lack of an increase in nitrotyrosine staining intensity in affected horses may reflect the time required for peroxynitrite formation. Decreased or absent NADPH staining in mucosal, submucosal, and muscularis vasculature of affected horses likely reflects loss of endothelium, caused by ischemic injury. We conclude that the iNOS becomes expressed in leukocytes in the large colon of horses subsequent to volvulus.

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References