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Equine Endotoxemia: Pathomorphological Aspects of Endotoxin-induced Damage in Equine Mesenteric Arteries

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Summary

To evaluate the effects of endotoxin on the morphology of the equine mesenteric vasculature, each of two thoroughbred horses were given two intravenous injections (24 h apart) of a sublethal dose of endotoxin (10 µg/kg). Each injection produced results similar to those of clinical cases of equine colic with obstructive nature of the loop of bowel: diarrhoea within 2 h after administration, followed by cessation of both faecal excretion and sounds of intestinal peristalsis. The most prominent morphological change was the development of moniliform appearance of small mesenteric arteries, in which there were contracted and dilated segments of the small mesenteric arteries. This was accompanied by parietal hyalinization and intramural and extramural haemorrhage. These mesenteric vascular changes appear to reflect dynamic vasoconstriction in the living animal, resulting in reduction of mesenteric and intestinal blood flow and possibly inducing alterations of gastrointestinal function such as cessation of intestinal peristalsis.

Introduction

Endotoxemia is suspected to be a consequence of naturally occurring gastrointestinal problems and Gram-negative infections in the horse (Burrows, 1981; Moore et al., 1981; Fessler et al., 1989). Endotoxins have been detected in the plasma in clinical and experimental cases of colic (Myers et al., 1982; King and Gerring, 1988; Fessler et al., 1989). The majority of the types of colic in clinical equine subjects were dislocation colic such as intussusception, strangulation, incarceration and volvulus (Myers et al., 1982; King and Gerring, 1988; Fessler et al., 1989; King and Gerring, 1991). There appears to be a significant correlation between the onset of clinical signs of colic with a severe obstructive nature and the release of endotoxin from the obstructed loop of bowel into the bloodstream (King and Gerring, 1988). Consistent findings in clinical equine colic include alterations in faecal consistency and cessation of intestinal sounds. The mechanism by which alterations of gastrointestinal function leading to disturbances of intestinal motility and blood flow are involved in the

pathogenesis of equine colic is poorly understood (Hackett, 1987; Messer and Beeman, 1987). The effects of endotoxin on intestinal motility and blood flow are therefore of interest to researchers. Physiological studies have provided evidence that infusion of endotoxin causes a prolonged reduction in intestinal blood flow (Clark and Moore, 1989) and disruption of intestinal motility in conscious horses (Clark and Moore, 1989; King and Gerring, 1991). These reports speculated that the reduction in intestinal blood flow may have been related to an increase in mesenteric vascular resistance, possibly caused by mesenteric vasoconstriction, thereby reducing blood flow. Similarly, it has been reported that endotoxin administration in cats induces mesenteric vasoconstriction, resulting in reduced mesenteric blood flow (Cohen et al., 1973). However, endotoxin-induced morphological changes in the mesenteric vasculature of the horse have not been investigated.

It has been hypothesized that endotoxin administration in the horse would cause mesenteric vasoconstriction, thereby leading to morphological changes in the mesenteric vasculature. The purpose of this study was therefore to evaluate the effects of two intravenous (i.v.) injections (24 h apart) of sublethal doses of endotoxin on the morphology of the equine mesenteric vasculature.

Materials and Methods

Two male thoroughbred horses aged 5 years (horse 1 and horse 2) were used as the test animals. These horses were withdrawn from training because of locomotor disorders.

Toxin of *Escherichia coli* origin, i.e. *E. coli* O26: lipopolysaccharide (Difco Laboratories, Detroit, MI, USA), was used as the test toxin. The species and serotype of the endotoxin were the same as those of the endotoxin used by Burrows (1970). The endotoxin (10 µg/kg body weight) was dissolved in 20 ml of sterile isotonic saline and administered by bolus i.v. injection into a jugular vein on two occasions 24 h apart. The 10 µg/kg dose was applied with each injection. Pilot studies had shown that this dose and route of administration gave a realistic clinical picture of clinical cases of colic.

As controls for evaluating the significance of any morphological changes seen in the test animals, two male thoroughbred horses aged 3 and 4 years (horse 3 and horse 4, respectively), which had been withdrawn from racing because of poor performance, were injected with saline solution on two occasions 24 h apart.

Clinical examination was undertaken seven times for each injection: just before the injection, and 1, 2, 3, 4, 5 and 6 h after injection. After necropsy and macroscopic inspection, samples for histological examination were collected from the anterior and posterior mesentery and from major visceral organs. Specimens were fixed in 10% neutral buffered formalin, and tissues were embedded in paraffin wax. Sections 4 μm thick were prepared and stained with haematoxylin and eosin (HE). Other stains used were Mallory's phosphotungstic acid-haematoxylin (PTAH) and periodic acid-Schiff (PAS). To help evaluate the significance of the abnormalities seen in the mesenteric arteries, cut sections of the mesenteric arteries were removed from the mounted and covered sections and excised. The specimens were then post-fixed in 2.5% glutaraldehyde and embedded in Epon. The ultrathin sections obtained from the mesenteric arteries were double-stained with uranyl acetate and lead citrate, and examined under a Hitachi H-600 transmission electron microscope (Hitachi Koki Co. Ltd., Tokyo, Japan).

Results

Clinical findings

The general response following the first and second endotoxin administrations in horse 1 and horse 2 were one of gradual onset of depression. The period of depression lasted for 4 to 6 h and then gradually subsided. The horses had recovered by the following day. Horse 1 had recovered to clinically near normal by 24 h after the second endotoxin injection. In contrast, horse 2 showed marked depression leading with drooping head and unresponsiveness to most environmental stimuli after the second injection and then remained recumbent, and was still in this state 24 h after the second injection. The control animals showed no abnormal signs throughout the 48 h observation period. At this point, all four horses were euthanized by intravenous injection of an overdose of barbiturates, and immediately necropsied.

In both horses 1 and 2, the sounds of intestinal peristalsis became very weak immediately after the first injection of endotoxin and could not be detected at all 1, 2 or 3 h after injection. Very weak borborygmi then appeared and persisted until 6 h after injection. Intestinal sounds returned to normal thereafter. Immediately following the second injection of endotoxin, the borborygmi completely ceased and were not heard again until 24 h after injection; that is at the end of the observation period. For 2 h immediately following the first injection, excretion of soft faeces or watery diarrhoea were observed at irregular intervals, 11 times in horse 1 and seven in horse 2. No excretion of faeces was observed from 2 h after the first injection of endotoxin. Both horses 1 and 2 excreted normal faeces just before the second injection of endotoxin. However, no excretion of faeces was observed following the second injection of endotoxin. The detailed clinical, haematological and biochemical findings in these horses will be published in another paper.

Pathological, histopathological and electron microscopic findings

At necropsy, the most conspicuous gastrointestinal changes in the horses injected with endotoxin were found in the mesenteric vasculature. Multiple petechiae and ecchymoses were observed grossly (Fig. 1). Histologically, these bleeding spots appeared as intramural and extramural haemorrhages in the vascular walls of small arteries (Figs 2 and 3). Ultramicroscopy revealed severe thickening due to the formations of multiple vacuoles in the internal elastic layers of the small arteries (Fig. 4). Almost all of the intramural regions of the arteries were occupied by red blood cells and plasma components. The affected mesentery was fixed in formalin, dehydrated with alcohol, and soaked in methyl salicylate to prepare transparent specimens. On observation of specimens with transmission light to examine the morphology of the blood vessels distributed in the mesentery, many small arteries appeared moniliform; that is, there were many small arteries that were contracted or dilated in segments (Fig. 5). The small veins were also dilated. On examination of serial sections of the small arteries the contracted segments had oedematous and loosened walls and partial parietal hyalinization. The lumina were very

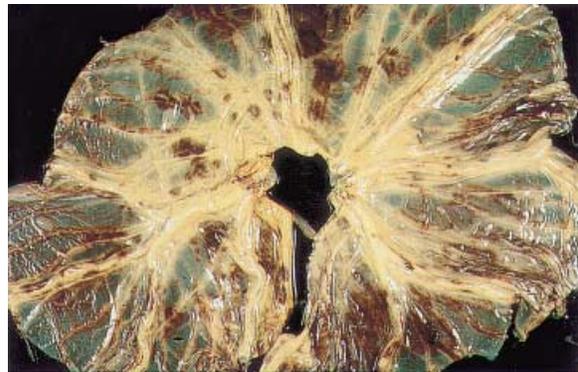


Fig. 1. Multiple petechiae and ecchymoses in the anterior mesentery of horse 1 after two injections of endotoxin.

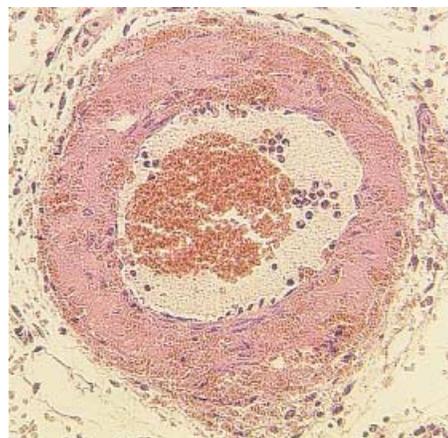


Fig. 2. Cross-section of a small artery in the anterior mesentery (horse 2). The artery shows intramural and extramural haemorrhages. The lumen is dilated and filled with red blood cells. HE stain. Magnification: $\times 40$.

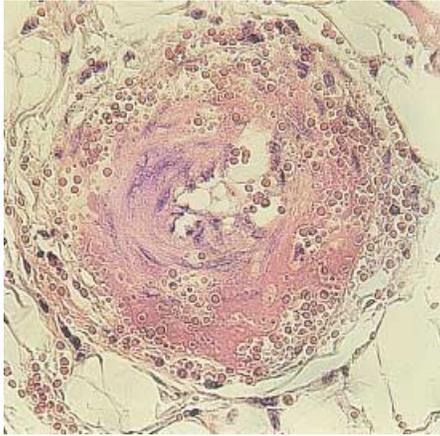


Fig. 3. Cross-section of a small artery in the anterior mesentery (horse 1). The artery shows oedema and loosening of the walls, accompanied by partial parietal hyalinization and luminal narrowing. HE stain. Magnification: $\times 348$.

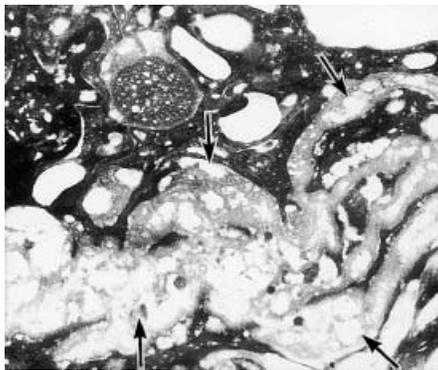


Fig. 4. Ultramicroscopy indicated severe thickening due to the formation of multiple vacuoles in the internal elastic layers of small arteries in the anterior mesentery (horse 2). Arrows indicate the thickened internal elastic layer. Magnification: $\times 10\ 000$.

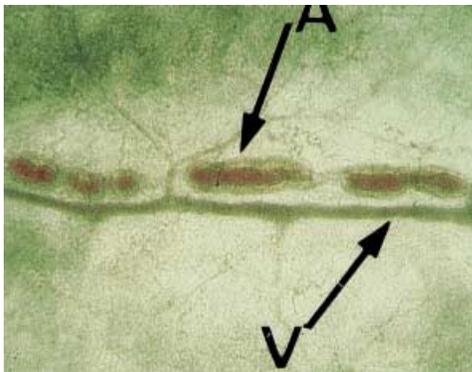


Fig. 5. Transparent specimen of the blood vessels distributed in the mesentery of Figs 2 and 3. The small artery had a moniliform morphology; that is, segments that were either contracted or dilated were observed frequently. (a) Small artery that appears to be in arteriospasm. (b) Small vein. Low power magnification.

narrow (Fig. 3). In the dilated segments there were intramural and extramural haemorrhages in all layers of the arterial walls (Fig. 2). The vascular lumina were dilated and filled with red blood cells. Oedema in the nerve fascicles distributed in the

mesentery, or swelling of axons, was occasionally observed. Parietal hyalinization and intramural haemorrhage were observed in the arteries running inside the anterior and posterior ganglia. Oedema was also occasionally found in the nerve bundles in these areas.

Mild (horse 1) to severe (horse 2) congestion and mild parietal oedematous thickening with petechiae and ecchymotic haemorrhages were observed grossly in the stomach and the small and large intestines. The small intestine contained a moderate quantity of yellow viscous liquid. The contents of the large intestine were slightly dry. Histologically, severe congestion and haemorrhage were detected in the lamina propria of the large intestine. Furthermore, parietal oedema, loosening or hyalinization was occasionally observed in the submucosal small arteries of the small and large intestines. Edematous degeneration of smooth muscle cells was seen occasionally in the muscular tunics, especially in the longitudinal layers of the muscular tunics, of the small and large intestines. The myocytes contained fine vacuoles and showed apyknomorphous changes. Spherical swelling of smooth muscle fibres was found. There were no significant findings in the mesenteric vasculature and the gastrointestinal tracts of the control horses.

Severe congestion, massive haemorrhagic necrosis and diffuse acute proximal tubular necrosis were seen in the renal cortices of horses 1 and 2. Scattered bleeding foci were observed in the cardiac musculature of both horses. These foci centred on the small arteries, which showed hyalinization and intramural bleeding. There were no abnormal findings in the visceral of the control horses.

Discussion

The clinical abdominal response to the administration of endotoxin included diarrhoea within 2 h after the first injection followed by cessation of faecal excretion and a decrease in the number of borborygmi followed by their entire cessation. The diarrhoea could be an acute effect of endotoxin mediated mainly by prostaglandin $F_{2\alpha}$ on bowel motility (King and Gerring, 1991). Alterations of gastrointestinal function such as cessation of intestinal peristalsis might be induced by decreases in intestinal blood flow (Clark and Moore (1989); King and Gerring, 1991) and mesenteric vascular damage as follows. From a pathomorphological viewpoint, the most prominent changes were abnormalities in the mesenteric small arteries (oedema and loosening of the vessel walls, as well as parietal hyalinization accompanied by intramural and extramural haemorrhage). The mesenteric small arteries had a moniliform morphology that strikingly resembled the features of the arteriospasm, angiospasm or vasospasm observed by arteriography in various organs in living animals and humans. One can assume that these arteriospasm-like changes resulted from disturbances in vascular motility (possibly vasoconstriction), which in turn arose from the aforementioned parietal abnormalities in the small arteries. The possibility that these spasm-like changes were produced by causes other than arteriospasm as an essential state of the disease can not be ruled out. However, it must be considered that these changes reflect dynamic phenomena in the living animal, since the small arteries in which arteriospasm-like changes were detected presented hyalinization and parietal oedematous swelling and loosening. Ultrastructural changes similar to those observed in the present study – especially a marked thickening of the

internal elastic lamina of mesenteric arteries – have been reported in rabbits injected with endotoxin (Stewart and Anderson, 1971). The fact that the small mesenteric arteries showed marked changes suggests that the mesenteric arteries might respond differently to endotoxin than might the arteries in other tissues and organs. A recent study in rats in which it was found that relaxation and contraction are physiologically and morphologically more specific in the mesenteric arteries than in arteries elsewhere, may support this hypothesis (Kobayashi et al., 2000).

The aforementioned lesions in the small mesenteric arteries might be caused by mesenteric vasoconstriction, resulting in a reduction of mesenteric and intestinal blood flow, and possibly inducing disturbances of gastrointestinal function such as cessation of intestinal peristalsis. Furthermore, as a high incidence of degeneration of smooth muscle cells was observed, especially in the outer longitudinal layer of the muscular tunics where the mesenteric blood vessels run, it seems that the formation of lesions in the myocytes was closely associated with the above abnormalities in the mesenteric blood vessels. Degeneration of the smooth muscle cells may also have contributed to the alterations of gastrointestinal function observed clinically in these cases.

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