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SHORT PAPER

Arterionecrosis of the Equine Mesentery in Naturally Occurring Endotoxaemia

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Summary

This report describes the mesenteric arteriolar lesions in a Thoroughbred racehorse with endotoxaemia due to colic. The vascular lesions consisted of a striking loss of medial smooth muscle cells, associated with granular cell debris derived from necrosed muscle cells, plasma insudation, erythrocyte infiltration and the deposition of a fibrinoid substance (fibrinoid degeneration) in the entire arterial wall, possibly produced by the infiltration of blood components through endothelial cell junctions into the arterial wall. The morphology of the mesenteric arteriolar necrosis closely resembled that seen in experimental equine endotoxaemia and in horses that died from colic; it also resembled that of Shiga toxin-induced arteriolar lesions in oedema disease of swine and of the arterionecrosis in human cerebral arteries that may lead to hypertensive intracerebral haemorrhage.

Keywords: arterionecrosis; bacterial infection; endotoxaemia; horse; man; mesentery

Equine endotoxaemia occurs in at least 25 % of horses admitted with colic (King and Gerring, 1988). An experimental study by Oikawa and Shiga (2002) suggested that endotoxin-induced damage to the equine mesenteric arteries resulted in a disturbance of intestinal blood flow and motility. The mesenteric circulation system has one of the body's largest vascular beds and is thus capable of affecting the systemic circulation, especially the gastrointestinal circulation, in horses. However, there appears to be no comprehensive description of the pathology of the mesenteric arteries in naturally occurring equine endotoxaemia. This report describes the mesenteric vascular lesions observed in a Thoroughbred racehorse that died of endotoxaemia resulting from colic.

A 3-year-old female Thoroughbred racehorse with a history of sudden onset of colic was brought to our equine referral hospital. On presentation the following signs of colic and shock were observed: turning to look at the abdomen, an absence of gut sounds, dull behaviour, an increase in pulse to 60/min, a decrease in rectal temperature to 37.0 °C, cyanotic mucous membranes, cold body surface, sweating, and cold extremities and ears. Haematological examination revealed leucopenia ($2400/mm^3$), haemoconcentration characterized by increased packed cell volume (66%) and total serum protein value 9.4 g/dl, metabolic acidosis characterized by increased blood lactate concentration (7.2 mmol/litre) and decreased blood pH (7.085), and elevated blood glucose (136 mg/dl). Peritoneal fluid collected by abdominocentesis revealed a turbid, dirty red fluid with large numbers of erythrocytes, a protein concentration of 6 g/dl, and a pH of 7.5. On laparotomy, the peritoneal cavity was found to contain a large volume of the turbid red-brown fluid contaminated with gastrointestinal contents. The horse died during laparotomy.

A post-mortem examination was carried out immediately after death. Samples for histological

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examination were collected from the anterior and posterior mesentery. The mesentery was fixed in formalin, dehydrated with alcohol, and soaked in methyl salicylate to prepare transparent specimens. Histological examination was performed on serial paraffin wax sections of the arterial segments in which a moniliform morphology was observed under a dissecting microscope (Fig. 1; Oikawa and Shiga, 2002). The stains used were haematoxylin and eosin (HE), elastica van Gieson (EVG), colloidal iron, Mallory's stain, phosphotungstic acid–haematoxylin (PTAH), Congo red and alcian blue (pH 2.5) —periodic acid-Schiff (ABPAS). The external diameters of arteries were determined with an ocular micrometer (OMSD-1; Olympus, Tokyo, Japan) by measuring the outer limit of the mural smooth muscle. For ultrastructural examination, arterial segments in which a moniliform morphology had been observed in the transparent specimens were washed with phosphate buffer, post-fixed with 2.5% glutaraldehyde, and embedded in Epon. The ultrathin sections obtained from the mesenteric vasculature were double-stained with uranyl acetate and lead citrate, and examined by transmission electron microscopy (TEM) (Hitachi H-600; Hitachi Koki Co., Tokyo, Japan).

For the detection of endotoxin, blood samples were taken aseptically from the jugular vein into a heparin-coated, pyrogen-free plastic syringe (EG tube; Seikagaku Kogyo Ltd, Tokyo, Japan) at laparotomy. Platelet-rich plasma (PRP) was prepared by centrifugation at 150 ***g*** for 10 min at 4 °C. Interfering factors were removed by exposing 0.1 ml of PRP to 0.2 ml of 0.32 M perchloric acid at 37 °C for 20 min. The supernate obtained by centrifugation at 1000 ***g*** for 15 min was used for assay after being neutralized with an equal volume of 0.18 M NaOH (Obayashi, 1984). Samples of peritoneal fluid were also taken with a sterile syringe at laparotomy and processed similarly by centrifugation at 150 ***g*** for 10 min at 4 °C. The supernate was filtered through a pyrogen-free 0.45-μm filter assembly and used for assay. Endotoxin in the plasma and peritoneal fluid samples was measured by chromogenic endotoxin-specific assay (Obayashi *et al.*, 1985, 1986) with an endotoxin-specific chromogenic test (ES test; Seikagaku Kogyo). Blood and peritoneal fluid were also taken from 20 clinically healthy Thoroughbred racehorses and assayed to provide reference values.

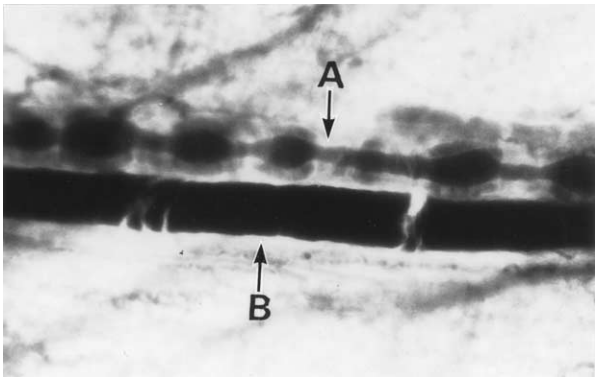

Fig. 1. Transparent specimen of the mesenteric vasculature. The arteriole (A) has a moniliform morphology, i.e., frequently showing segments that are either contracted or dilated. A small vein (B) shows dilatation due to congestion. ×24.

Post-mortem examination revealed fibrinous haemorrhagic peritonitis due to rupture of the caecum near the caecocolic fold, multiple petechiae and ecchymoses throughout the anterior and posterior mesentery, slight to mild congestion in the stomach and the small and large intestines, massive haemorrhage in the adrenal cortices, and a swollen and cloudy appearance of the liver and kidneys. No abnormal positioning of the intestinal tract (e.g., displacement, herniation or torsion) was found.

Microscopically, the external diameters of the moniliform arterioles were seen to range from approximately 100 to 250 μm. Fig. 2a shows (1) dense restiform masses, stained red with HE, in the arteriolar walls, (2) intramural and perivascular infiltration by erythrocytes, and (3) disappearance of the majority of myocytes in the tunica media; in the nuclei of the remaining myocytes, however, karyorrhexis and pyknosis were frequently observed. The restiform masses observed in the arterial walls were stained deep red with Mallory and ABPAS stains, yellowish brown with EVG, and deep blue with PTAH (Fig. 2b), but no staining appeared with the colloidal iron or Congo red method. The staining indicated that fibrinoid substances had been deposited (fibrinoid degeneration) and that plasma glycoprotein was present. Partial disruption was found in the internal elastic lamina. Under a dissecting microscope, the findings described above were more obvious in the dilated arteries (Fig. 2a and b) than in the stenosed arteries (Fig. 2c and d). The surrounding minute blood vessels (mainly capillaries) sometimes contained fibrinous thrombi. Moderate haemorrhage was seen in the lamina propria and submucosa of the small and large intestinal mucosa. Oedematous degeneration, spherical swelling and loss of smooth muscle cells were seen in the muscular tunics (especially in the longitudinal layers) of the small and large intestines.

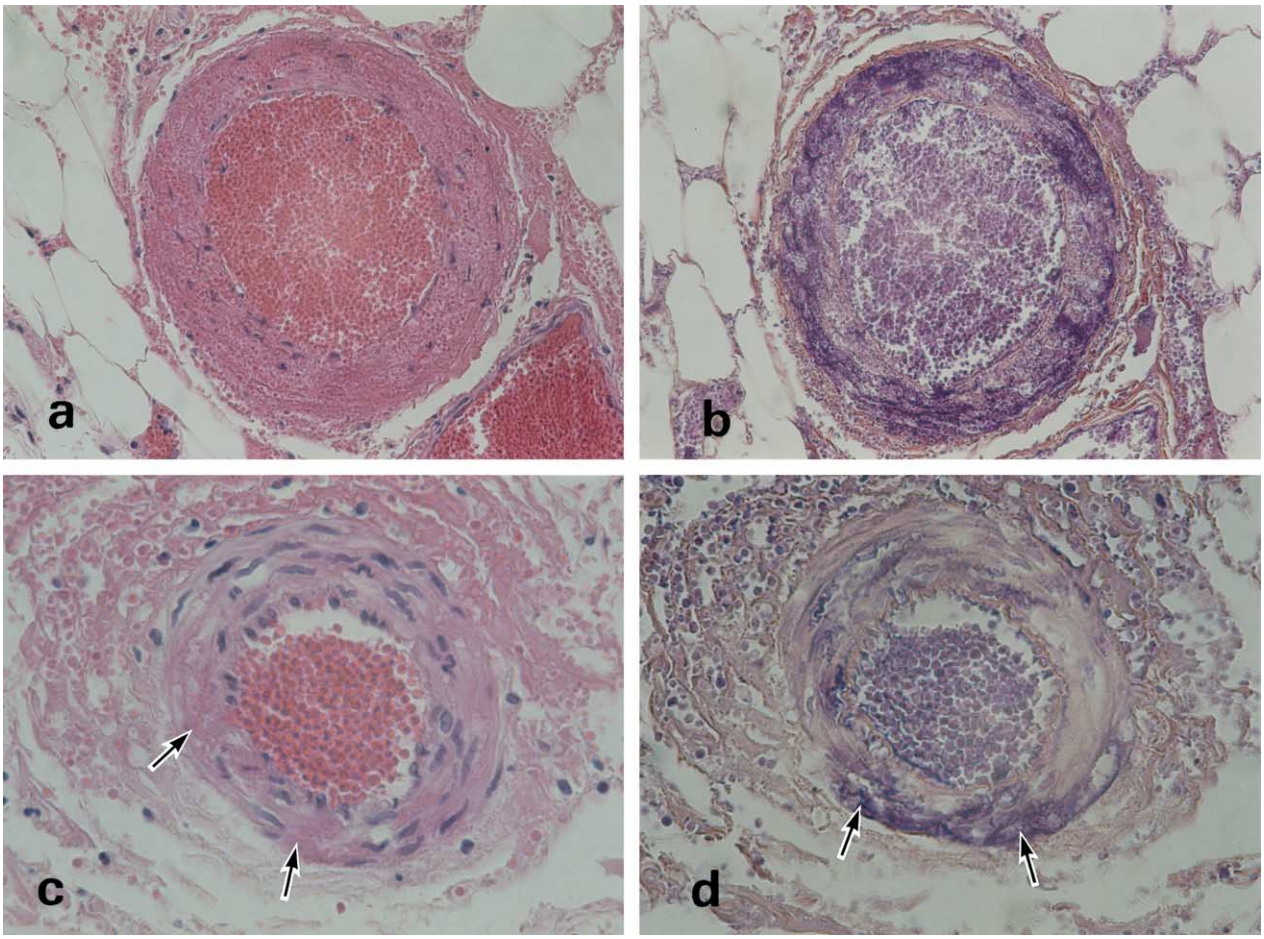

Fig. 2a–d. Cross-section of arterioles in the mesentery. (a) An arteriole with luminal dilatation shows severe vascular changes, consisting of intramural infiltration of erythrocytes, parietal deposition of fibrinoid material, medial smooth muscle cell loss and necrosis. HE. × 240. (b) An arteriole with luminal dilatation shows marked parietal deposition of fibrinoid substance. PTAH. × 240. (c) and (d) Arrows indicate partial deposition of fibrinoid material in an arteriole with a narrowed lumen. (c) HE. × 422. (d) PTAH. × 422.

TEM showed that the endothelial cells of the affected arterioles were swollen, with cytoplasm of low electron density and enlarged clear cytoplasmic vacuoles that sometimes contained a homogeneous substance of high electron density (Figs. 3 and 4). Some endothelial cells had dark, condensed cytoplasm indicative of coagulation necrosis. Marked swelling and partial disruption of the internal elastic lamina were seen (Fig. 5). Electron-dense granular material resembling blood plasma protein had accumulated just beneath the endothelial cells and at the endothelial cell junctions (Fig. 5). Highly electron-dense fibrillar material indicative of a fibrinoid substance had been deposited beneath the internal elastic lamina (Fig. 5). In the media, the smooth muscle cells had almost disappeared, and the cellular spaces remaining were vacant or contained necrotic smooth muscle cells or granular cell debris, or both, derived from the necrosed muscle cells (Figs. 3 and 4). Fibrinoid deposits, insudation of blood plasma constituents, and infiltration of erythrocytes were seen in the entire arterial wall (Fig. 3). The smooth muscle cell myofilaments in the media were dispersed and frayed, forming a fibrillar structure in which fibrinoid substances were deposited (Fig. 6). Platelets mixed with fibrillar material, neutrophils and erythrocytes were present, either adherent to the intima or free in the lumen.

The mean peripheral plasma and peritoneal fluid endotoxin concentrations in the 20 clinically healthy Thoroughbred racehorses were 6.4 ± 3.4 and 0.5 ± 0.4 pg/ml, respectively. Plasma and peritoneal fluid samples obtained from the horse with colic had endotoxin values of 797.7 and 13 844.0 pg/ml, respectively.

The horse's death was considered to be caused by acute endotoxaemia associated with absorption of endotoxin from peritoneal fluid contaminated

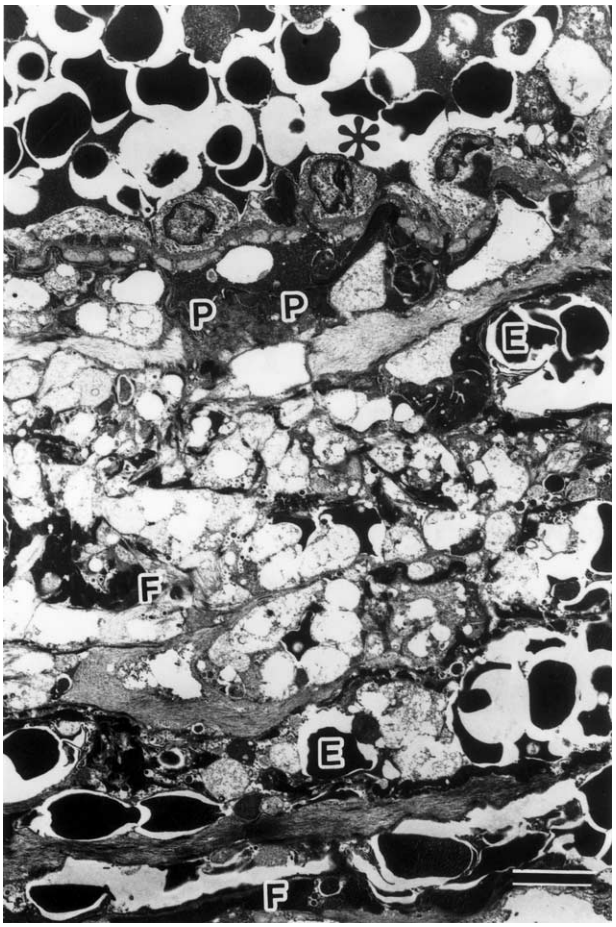

Fig. 3. Ultrastructure of a mesenteric arteriole. Marked medial smooth muscle cell loss with granular cell debris, infiltration of erythrocytes (E) and blood plasma constituents (P), and deposition of fibrinoid substance (F) are evident in the media. The lumen is indicated by an asterisk. TEM. Bar, 2.5 μm.

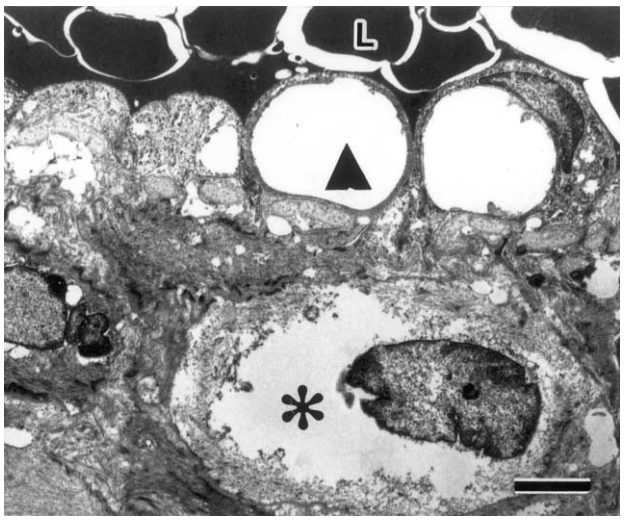

Fig. 4. Endothelial cells (arrowhead) are distended by clear cytoplasmic vacuoles. Myocytes (asterisk) are degenerative. Lumen, L. TEM. Bar, 2.5 μm.

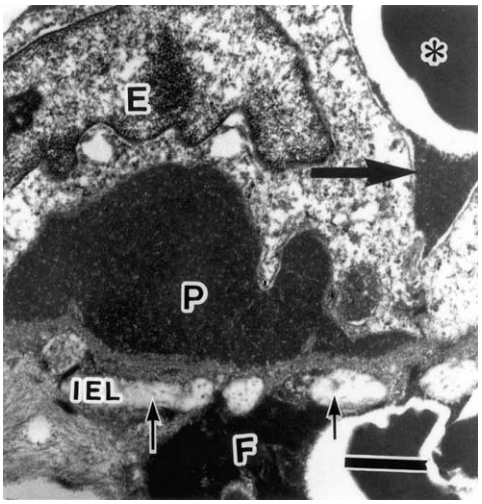

Fig. 5. Blood plasma proteins (P) beneath an endothelial cell (E) and between endothelial cell junctions (large arrow). Marked thickening and disruption of the internal elastic lamina (IEL) are also prominent (small arrows). Fibrinoid material (F) is deposited beneath the IEL. The lumen is indicated by an asterisk. TEM. Bar, 0.7 μm.

with gastro-intestinal contents. This assumption was based on the high endotoxin concentrations and the clinical and pathological resemblance to experimental equine endotoxaemia (Oikawa and Shiga, 2002; Oikawa and Yamaoka, 2003). The endotoxin concentrations were similar to those reported previously in horses that died of endotoxaemia with colic (King and Gerring, 1988; Fessler *et al.*, 1989).

Although proof is lacking, the lesions observed in the mesenteric arterioles may well have resulted from a hypercytokinaemia cascade elicited by endotoxin (MacKay, 2000). We believe that the pathomorphogenesis of the arterial necrosis was based on endothelial injury, leading to enlargement

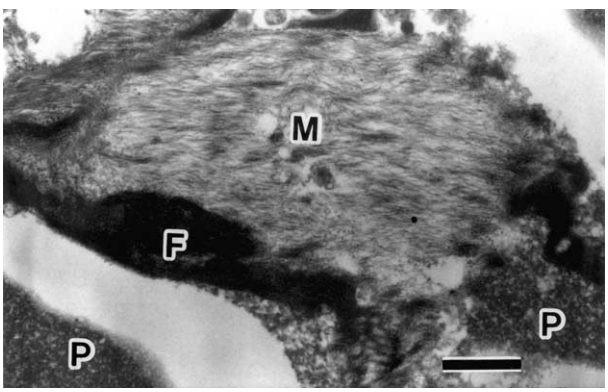

Fig. 6. Dispersed and frayed smooth muscle cell myofilaments (M) in the media show deposits of fibrinoid substance (F) and infiltration by blood plasma constituents (P). TEM. Bar, 0.7 μm.

of the spaces at intercellular junctions and an increase in endothelial permeability, followed by infiltration of blood components into the subendothelial tissues. Finally, enzymes in blood components, such as elastase, collagenase and protease, damaged the internal elastic lamina and caused histolysis of arterial walls, resulting in necrosis and loss of the medial smooth muscle cells (Ooneda *et al.*, 1973). In addition, plasma components that had infiltrated into the arterial walls, including coagulation proteins, were presumably deposited as fibrinoid substances, leading to arterionecrosis. The moniliform changes in the arterioles seen in the cleared mesenteric specimens reflected the severity of the vascular damage, i.e., the morphological damage to the walls was greater in the dilated arterioles than in the contracted and stenosed arterioles. In other words, owing to the decrease of medial smooth muscle cells, the lumen became dilated.

The vascular lesions in the mesentery may have resulted in disturbance of mesenteric and intestinal blood flow, inducing mucosal haemorrhage and degeneration of smooth muscle cells in the muscular tunics of the small and large intestine.

The morphology of the mesenteric arteriolar lesions was closely similar to that found in experimental endotoxaemia in Thoroughbred horses (Oikawa and Shiga, 2002) and in spontaneous cases that died of colic (Oikawa, 2003); it also resembled that of Shiga toxin-induced arteriolar lesions in oedema disease of swine (Matise *et al.*, 2000) and of the arterionecrosis in human cerebral arteries that may lead to hypertensive intracerebral haemorrhage (Ooneda *et al.*, 1973).

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