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Mesenteric Arterionecrosis in Natural and Experimental Equine Endotoxaemia

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Summary

To test the hypothesis that mesenteric arterionecrosis (MA) occurs in horses with naturally occurring endotoxaemia (ET) and in those with experimentally induced ET, the mesentery and gastrointestinal tract of 21 Thoroughbred racehorses (15 with spontaneous colic suspected to be due to ET, and six with experimentally induced ET) were examined. MA, which occurred in 13 of the 15 horses with spontaneous colic and in all six of the cases of experimental ET, was morphologically similar in the two groups of animals. This suggested that the pathogenesis of the MA was fundamentally similar in the two groups, and that MA is a pathognomonic feature of equine ET. In addition to histolysis of the arterial walls associated with infiltration of blood components, changes were noted in the medial smooth muscle including formation of many intracellular vacuoles within single smooth muscle cells, cytoplasmolysis, necrosis with granules and vacuoles, and coagulation necrosis; similar changes have been observed in cases of prolonged angiospasm or vasoconstriction. It is suggested that the effects of sustained arterial contraction leading to intimal and medial damage influence the pathomorphogenesis of MA.

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Introduction

There is a growing body of evidence to suggest that endotoxins play an important role in the pathogenesis of a number of colics (Moore and Barton, 2003). Previously, we reported that mesenteric arterionecrosis (MA), in which the arterial lesions were characterized by medial necrosis accompanied by intramural and transmural haemorrhage (erythrodiapedesis) and the deposition of fibrinoid substances within the media of the mesentery, in two Thoroughbred horses with experimentally induced endotoxaemia (ET) (Oikawa and Shiga, 2002) was closely similar to that seen in a Thoroughbred horse with naturally occurring ET associated with spontaneous colic (Oikawa *et al.*, 2004). The luminal diameter of affected arteries ranged from approximately 50 to 300 μm (arteriole to small artery). We hypothesized that MA occurs in most cases of naturally occurring equine endotoxaemia with colic. To test this hypothesis, the present study was designed to compare the morphology of the mesenteric vasculature in 15 Thoroughbred horses with spontaneous colic suspected to be due to ET with that of six Thoroughbred horses with experimentally induced ET, including two horses (nos 5 and 6) that had been studied previously (Oikawa and Shiga, 2002; Oikawa and Yamaoka, 2003).

Materials and Methods

Experimental ET

Horses. Table 1 gives an overview of the experiment. Six Thoroughbred horses (nos 1–6) were

Table 1
Production of experimental endotoxaemia by LPS administered intravenously

Horse no.	Age (years)	Sex	Experimental group	Endotoxin (LPS) dosage (μg/kg)	Fate of animal
1	4	Male	A	10	Killed[†]
2	4	Male	A	10	Killed[†]
3	4	Male	B	20	Died[‡]
4	4	Female	B	20	Died[‡]
5	4	Male	C	10+10[*]	Killed[¶]
6	5	Male	C	10+10[*]	Killed[¶]
7	4	Male	Control	None	Killed[§]
8	4	Male	Control	None	Killed[§]

[*]Two doses administered, 24 h apart.
[†]At 24 h after LPS injection.
[‡]Horses 3 and 4 died 13 and 20 h after LPS injection, respectively.
[¶]At 24 h after the second LPS injection.
[§]Killed 24 h after a second intravenous injection of saline.

used for the experimental induction of ET. These horses had been withdrawn from training because of locomotor disorders, and despite repeated treatment had failed to return to good health. It was, therefore, decided that they had no chance of recovery. As controls for evaluating the significance of any morphological changes seen in the test animals, two healthy age-matched Thoroughbred horses (7 and 8), which had been withdrawn from racing because of poor performance, were used.

Experimental design. An endotoxin derived from *Escherichia coli* O26 lipopolysaccharide (LPS; Difco Laboratories, Detroit, Michigan, USA) was injected into a jugular vein in each of horses 1–6 (Table 1). The LPS was given as an abrupt bolus infusion (i.e., an intravenous injection lasting no more than 20 sec of a dose high enough to obtain noticeable effects) at 10 μg/kg to two horses (Group A; 1 and 2) and at 20 μg/kg to two other horses (Group B; 3 and 4). Two horses (Group C; 5 and 6) used in our previous studies (Oikawa and Shiga, 2002; Oikawa and Yamaoka, 2003) had also received an abrupt bolus infusion (10 μg/kg on two occasions 24 h apart). Two control horses (7 and 8) were given an injection of 20 ml of pyrogen-free sterile isotonic saline into the left jugular vein on two occasions 24 h apart. All animals were given food and water *ad libitum* during the entire experimental period, and after the injections were allowed unrestrained movement. Clinical, haematological and blood biochemical examinations (see below) were carried out frequently during the experiment. The animals were killed (Table 1) by the intravenous injection of sodium pentobarbital (30 mg/kg) as follows: 24 h after a single injection of LPS (horses 1 and 2); 24 h

after the second of two injections of LPS (horses 5 and 6); 24 h after the second of two injections of saline (horses 7 and 8 [controls]). Horses 3 and 4 died 13 and 20 h after a single LPS injection, respectively. The experiments conformed to the guidelines for care and use of research animals of the Japanese Association for Experimental Animals and were approved by the Animal Care and Use Committee of the Equine Research Institute.

Spontaneous Colic Suspected to be Due to ET

Horses. Fifteen Thoroughbred horses (nos 9–23) with spontaneous colic (Table 2) consisted of seven (12–14, 16, 18, 20, 23) with acute gastrointestinal tract rupture (GTR), four (17, 19, 21, 22) with small-intestinal strangulating obstruction (SIO), three (9–11) with colitis associated with acute abdominal pain, and one (15) with intussusception of the small intestine (IS), as discovered during laparotomy or at necropsy. Nine horses (12, 14–21) died naturally and six (9–11, 13, 22, 23) were subjected to euthanasia.

Clinical, haematological and blood biochemical examination. As in the animals with experimental ET (see above), to evaluate the degree of disease severity (Thoefner *et al.*, 2000; Moore and Barton, 2003), heart rate, rectal temperature and total white blood cell count (WBC) were measured at multiple points throughout the disease process.

Determination of blood endotoxin concentration. At the time of necropsy, this was measured in horses 14, 18 and 21 by an endotoxin-specific chromogenic test (ES test; Seikagaku Kogyo, Tokyo), described previously (Oikawa *et al.*, 2004).

Table 2
Details of 15 horses with spontaneous colic

Horse no.	Age (years)	Sex	Duration of colic (h)	Heart rate (/min)	Rectal tempera-ture (°C)	WBC count (/μl)	Endotoxin in plasma (pg/ml)	Killed (K) or died (D)	Diagnosis	Peritoneal fluid
9	3	F	29	90	40.5	2100	–	K	Colitis	Yellow
10[*]	3	M	48	86	40.0	15800	–	K	Colitis	Turbid
11[*]	3	M	58	41	38.2	3500	–	K	Colitis	Turbid
12	3	M	36	52	37.6	5500	–	D	GTR	GC
13	7	M	34	78	37.4	2800	–	K	GTR	GC
14	3	F	8	60	37.0	2400	797.7	D	GTR	GC
15	17	F	10	80	37.6	4900	–	D	IS	S
16	14	G	12	120	40.3	–	–	D	GTR	GC
17	5	F	50	136	39.6	18500	–	D	SIO	S
18	16	G	15	70	39.8	–	93.9	D	GTR	GC
19	11	G	48	120	36.5	–	–	D	SIO	S
20	4	M	8	–	–	–	–	D	GTR	GC
21[†]	1	F	24	90	40.3	7300	24.4	D	SIO	Turbid
22	12	F	52	112	36.4	2200	–	K	SIO	Turbid
23[†]	1	M	24	–	–	26800	–	K	GTR	GC

F, female; M, male; G, gelding; GTR, acute gastrointestinal rupture; SIO, small-intestinal strangulating obstruction; IS, intussusception of the small intestine; GC, leakage of gut contents into peritoneal cavity; S, serosanguinous; –, not done.
[*] Horse with septicaemia.
[†] Translocation of endogeneous bacterial endotoxin into the systemic circulation was suspected.

Pathological and Ultrastructural Examination

Post-mortem examinations were carried out immediately after death or euthanasia. After macroscopical inspection, samples for histology were collected from the mesentery and intestinal tissue of all horses (1–23). The specimens were immersion-fixed in 10% phosphate-buffered formalin, embedded in paraffin wax, sectioned (4 μm), and stained with haematoxylin and eosin (HE). Other stains used on selected tissue sections included elastica van Gieson (EVG), Mallory, phosphotungstic acid-haematoxylin (PTAH), periodic acid-Schiff (PAS), colloidal iron, Congo red, alcian blue, and alcian blue (pH 2.5)-periodic acid-Schiff (ABPAS). In addition, transparent specimens of the mesentery were prepared from all horses (1–23) to examine the morphological distribution of vascular lesions under a dissecting microscope, as described previously (Oikawa and Shiga, 2002; Oikawa et al., 2004). Serial sections of the mesenteric vasculature for light microscopy were made when considered necessary (horses 1, 2, 4, 5, 7, 14, 18, 21). For transmission electron microscopy (TEM), mesenteric arterial tissues (horses 1, 2, 4, 5, 7, 8, 14, 18, 21), fixed initially in 10% phosphate-buffered formalin, were post-fixed in 2.5% glutaraldehyde, and embedded in Epon. The tissue blocks to be examined were taken from mesenteric areas seen under the dissecting microscope to contain small arteries and arterioles with microaneurysm-like or moniliform morphology. Semi-thin sections of each block (horses 1, 2, 4, 5, 7, 8, 14, 18, 21) were stained with 1% toluidine blue and examined by light microscopy. For TEM, ultrathin sections (60–70 nm) of the block were cut and stained with uranyl acetate and lead citrate, and viewed with a Hitachi H-600 electron microscope.

Bacteriological Examination

Specimens from gastrointestinal lesions, mesenteric lymph nodes, peripheral blood, peritoneal fluid and major visceral organs were collected aseptically from horses 9–11 with colitis, horse 21 with SIO, and horse 23 with GTR (Table 3). Bacterial culture under aerobic and anaerobic conditions was performed by routine methods as follows. Briefly, pieces of tissue were emulsified and diluted decimally (up to 1 in 10^9). Each dilution was stored anaerobically (AneroPack; Mitsubishi Gas Chemical, Tokyo) at 4 °C until cultured at 37 °C for 48 h, aerobically and anaerobically on Columbia Agar (Nippon Becton Dickinson, Tokyo) supplemented with heparinized horse blood 5%, and aerobically on MacConkey Agar (Nissui Seiyaku, Tokyo). The methods for counting organisms in tissues and fluids and for identification of isolates were those previously described (Kreig and Holt, 1984; Barrow and Feltham, 1993).

Table 3
Bacteriological examination of five horses with spontaneous colic

| Horse no. | Tissues | Colony-forming units (per g or ml) | | | | |
		E. coli	GNSAB	GNB	GPSAB	Miscellaneous bacteria[*]
9	Colonic lesions	3×10^7	–	–	–	2.4×10^7
10	Colonic lesions	3×10^4	7×10^5	–	–	2×10^4
	Liver	–	–	–	–	2×10^2
	Spleen	1×10^2	–	–	–	7×10^2
11	Blood	–	–	–	–	2×10^6
	Lung	–	–	–	–	2×10^6
21	Peritoneal fluid	–	1×10^4	–	–	2×10^2
	Strangulated intestine	–	1×10^8	2×10^7	–	5×10^6
	Mesenteric lymph node	5×10^3	2×10^5	–	5×10^5	11×10^4
23	Mesenteric lymph node	5×10^5	–	–	–	3×10^4

E. coli, *Escherichia coli*; GNSAB, Gram-negative strictly anaerobic bacilli; GNB, Gram-negative bacilli; GPSAB, Gram-positive strictly anaerobic bacilli.

[*] *Corynebacterium* sp.; *Bacteroides fragilis*; *Gemella morbillorum*; *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*; *Pasteurella pneumotropica*; *Enterobacter cloacae*; *Staphylococcus aureus*; *Enterobacter aerogenes*; *Proteus vulgaris*; *Rhodococcus equi*; *Citrobacter* sp. –, Negative.

Results

Clinical Findings

Control group. There were no abnormal findings in the two control horses (7, 8) throughout the experiments.

Experimentally induced ET. All three groups (A, B, C) exhibited qualitatively similar biological responses. After the infusion of LPS, transient diarrhoea was followed by diminution or disappearance of the sounds of intestinal peristalsis, increase in heart rate and respiratory rate, a decrease followed by an increase in rectal temperature, and a significant decrease in WBC and blood glucose (hypoglycaemia), as compared with the values obtained before the injection of LPS.

Spontaneous colic suspected to be due to ET. All 15 horses with spontaneous colic were suspected to have ET on the basis of clinical, microbiological and necropsy findings and peripheral plasma endotoxin concentrations. On admission, leucopaenia or leucocytosis and an increase or decrease in rectal temperature, as well as tachycardia, were evident in most of the horses when they were brought to our equine referral hospital (Table 2).

Blood endotoxin concentration in spontaneous colic cases. The maximum plasma endotoxin concentrations in peripheral blood during the period between the first visit to the equine referral hospital and death were 797.7 pg/ml and 93.9 pg/ml in horses 14 and 18 with GTR, and 24.4 pg/ml in horse 21 with SIO (Table 2).

Microbiological Findings

Spontaneous colic suspected to be due to ET. The results of bacterial culture suggested that two horses with colitis (10, 11) had septicaemia, and in two horses with SIO and GTR (21, 23), bacteria were isolated in significant numbers from the mesenteric lymph nodes (Table 3). In these two cases, translocation of endogenous bacterial endotoxin into the systemic circulation (Berg and Garlington, 1979) was suspected.

Necropsy Findings

Control group. No gross lesions were observed in the mesentery.

Experimentally induced ET and spontaneous colic suspected to be due to ET. Multiple petechiae and ecchymoses were evident macroscopically in the mesentery of the four horses (3–6) in Groups B and C, and of 13 (10–14, 16–23) of the 15 horses affected by colic, even though no intestinal malpositioning was apparent.

Microscopical Findings

Control group. Light microscopy showed that the lumen of the mesenteric arteries was distended, and the one-layered endothelium was closely attached to the internal elastic lamina. In the
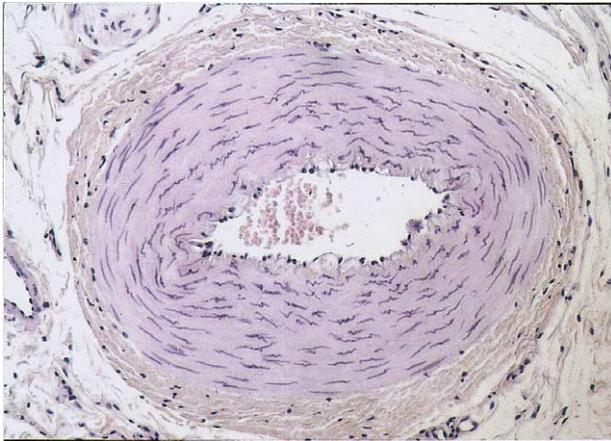
Fig. 1. Cross-section of a small artery in the mesentery of a control horse (no. 7). The mesenteric artery is distended and the medial smooth muscle cells are uniformly stained with eosin but do not contain intracellular vacuoles. HE. ×50.

Fig. 3. Transparent specimen of the mesenteric vasculature observed under a dissecting microscope (horse 18). Arteriole (A) showing microaneurysm-like morphology associated with transmural haemorrhage. Lymphatics (L) show dilatation due to accumulation of lymph fluid. The specimen was prepared by fixation in formalin, dehydration with alcohol, and soaking in methyl salicylate. ×30.

media, the cytoplasm of the smooth muscle cells was uniformly stained with eosin and did not contain vacuoles (Fig. 1). Electron microscopical examination showed that the medial smooth muscle cells were spindle-shaped with a central nucleus of similar shape. Intracellular vacuole formation was rarely seen (Fig. 2).

Experimentally induced ET and spontaneous colic suspected to be due to ET. Examination of the transparent mesentery with multiple haemorrhage in experimental and spontaneous cases (see above) showed that many small arteries and arterioles had a microaneurysm-like or moniliform appearance, i.e. alternating segments of constriction and

dilatation (Fig. 3) (Oikawa and Shiga, 2002; Oikawa *et al.*, 2004). The multiple petechiae and ecchymoses evident grossly in the mesentery were derived from transmural haemorrhage (adventitial erythrodiapedesis) of dilated segments in the small arteries or arterioles (Figs 3 and 4). The vascular damage was more severe in the dilated segments of the artery (Fig. 4) than in the constricted segments.
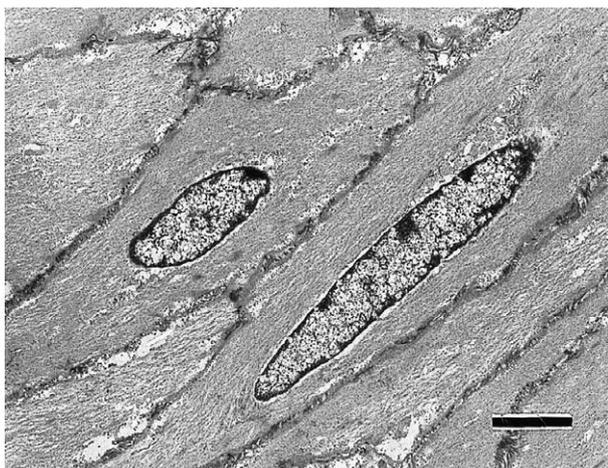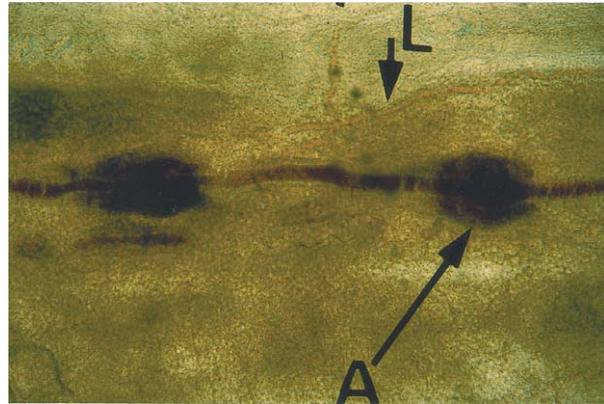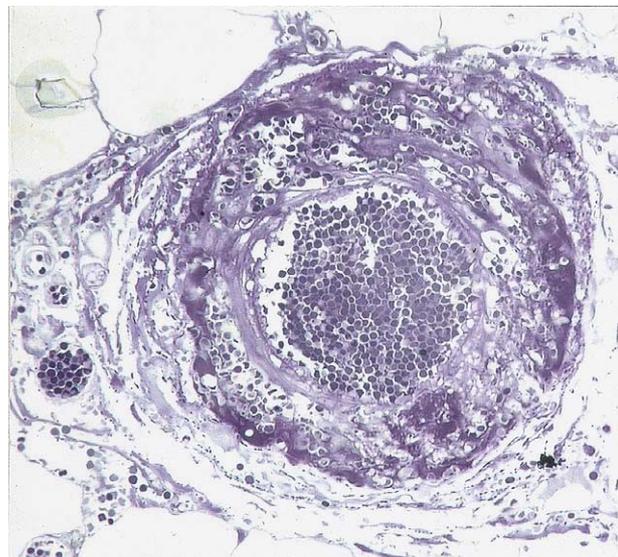

Fig. 2. Electron micrograph of the media of the same artery as that shown in Fig. 1. The medial smooth muscle cells are spindle-shaped with a central nucleus of similar shape. Intracellular vacuoles are not evident. TEM. Bar, 3.4 μm.

Fig. 4. Cross-section of a small artery in the mesentery (horse 18). Intramural and transmural haemorrhage and disappearance of the medial smooth muscle cells are evident in dilated segments of the artery. These vascular lesions are more marked in the outer than in the inner media. Semi-thin section obtained from tissue embedded in Epon. Toluidin blue stain. ×290.
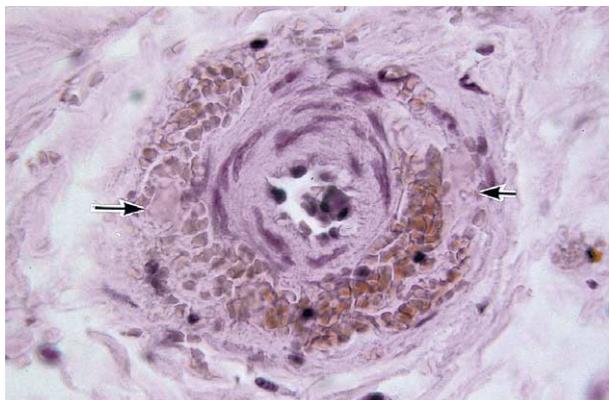
Fig. 5. Cross-section of an arteriole in the mesentery (horse 14). Intramural and transmural haemorrhage, deposition of fibrinoid substances (arrows) and loss of the medial smooth muscle cells are more marked in the outer than in the inner media. HE. ×445.

The common morphology of the mesenteric artery in experimentally induced ET and spontaneous colic suspected to be due to ET included signs of endothelial injury (enlargement of the spaces at intercellular junctions), infiltration of blood components into the subendothelial tissues with deposition of fibrinoid substances, intramural and transmural haemorrhage, and disappearance of the majority of the medial smooth muscle cells. The mesenteric arterial walls were stained yellowish brown with EVG, reddish-purple with PAS, deep red with Mallory and ABPAS stains, and deep blue with PTAH, but there was no apparent staining with colloidal iron, alcian blue or Congo red indicative of deposition of fibrinoid substances or the presence of plasma glycoprotein. MA tended to be more marked in the outer than in the inner media (Figs 4 and 5). MA, albeit varying in severity, was found in all of the experimental horses (1–6) and in 13 horses (10–14, 16–23) of the 15 affected by spontaneous colic. In the experimental cases, the severity and frequency of occurrence of MA were more marked in the four horses of Groups B and C than in the two horses of Group A (Table 4). In the Group A horses, a few areas of slight MA (partial deposition of fibrinoid material in the arteries or arterioles) were found, but most of the arteries and arterioles possessed narrowed or occluded lumina and numerous intracellular vacuoles close to the nucleus in the medial smooth muscle cells (Fig. 6). TEM of specimens from the Group A horses revealed intracellular vacuoles of various sizes in the medial smooth muscle cells (Fig. 7). In the tunica media of vessels affected by MA in Groups B and C and in horses with spontaneous colic, TEM revealed various

Table 4
Distribution of mesenteric arterionecrosis in cases of spontaneous colic and experimental endotoxaemia (ET)

Cases	Number of cases examined	Number of horses with arterionecrosis in	
		Mesenteric artery	Blood vessel in intestinal wall
Spontaneous colic	15(3)	13(3)	7(1)
GTR	7(2)	7(2)	2(1)
Colitis	3	2	2
SIO	4(1)	4(1)	2
IS	1	0	1
Experimental ET	6	6	4
Group A	2	2*	0
Group B	2	2	2*
Group C	2	2	2*
Controls	2	0	0

GTR, acute gastrointestinal rupture; SIO, small-intestinal strangulating obstruction; IS, intussusception of the small intestine. Numbers in parenthesis are numbers of horses in which plasma endotoxin concentrations were significantly elevated.
*Mild lesions.

manifestations of medial smooth muscle cell injury, including highly electron-dense cytoplasm with peripheral nuclear chromatin aggregation (obviously necrotic), and cytoplasmolysis (cytoplasmic necrosis). Also observed were vacuole formation resulting from lysis of cytoplasmic components (Fig. 8), coagulation necrosis with intracellular vacuoles, and necrotic cells with
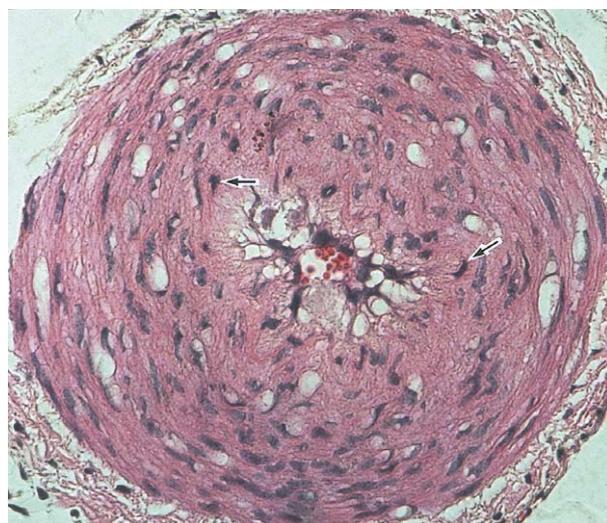

Fig. 6. Cross-section of a small artery in the mesentery in horse 1 from Group A. The artery has a narrowed lumen and many intracellular vacuoles within the arterial media. There is pyknosis of the nuclei of the medial myocytes (arrows) just beneath the internal elastic lamina. HE. ×330.
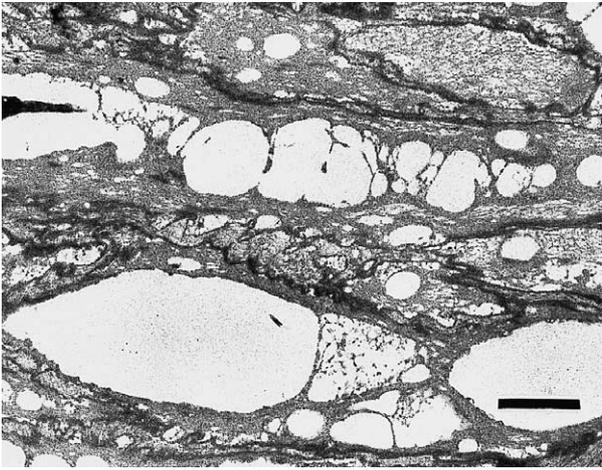
Fig. 7. Electron micrograph of the media of the same artery as that shown in Fig. 6. The medial smooth muscle cells have many vacuoles of various sizes in their cytoplasm. TEM. Bar, 2.4 μm.

granules and vacuoles. Histological examination of serial sections of the arterial and arteriolar segments revealed that MA was present segmentally to varying degrees in some animals. The external diameters of the affected arterial segments ranged from approximately 50 to 500 μm, i.e. from the size of arterioles to that of small arteries. Arterionecrosis was occasionally found in the parietal vessels of the intestinal walls as well as in the mesentery in the four experimental cases of Groups B and C, and in two GTR (14 and 23), two colitis (10 and 11) and two SIO (21 and 22) cases (Table 4). All three cases of spontaneous colic that were endotoxin-positive (14, 18, 21) also had marked MA (Table 4).

Discussion

The clinical manifestations of systemic inflammatory response syndrome (SIRS; hypercytokinaemia), as described by Bone (1996), include high or low rectal temperature, increased cardiac and respiratory rate, and leucocytosis or leucopaenia. Such signs, which are elicited by LPS, indicated that SIRS may have been occurring in all the horses in the present study except the two control animals. Oikawa et al. (2004) reported that the normal reference value for peripheral plasma endotoxin concentration, obtained from 20 healthy horses, was 6.4 ± 3.4 pg/ml. The three animals (14, 18, 21) with spontaneous colic tested in the present study
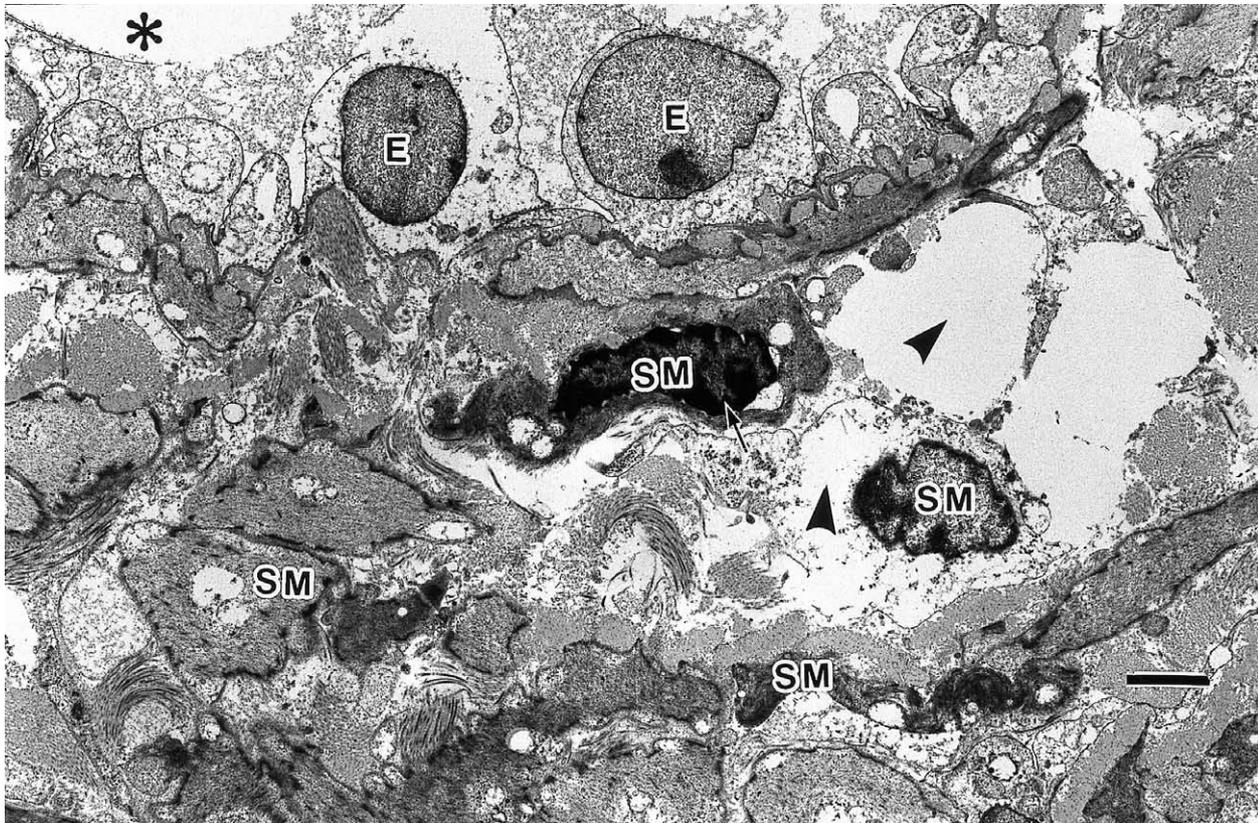

Fig. 8. Electron micrograph of a small artery in the mesentery (horse 14). Irregularly distorted and extended cytoplasm of medial smooth muscle cells (SM) with highly electron-dense cytoplasm and peripheral nuclear chromatin aggregation (arrow) and cytoplasmolysis (arrowheads). Endothelial cells (E) are swollen. The lumen is indicated with an asterisk. TEM. Bar, 11.8 μm.

were, therefore, clearly endotoxin-positive (Table 2). However, horses 14 and 18 represented two of the seven cases of GTR in the study, and in such cases it is possible that endotoxin leaks from the gut to the peritoneal cavity, subsequently entering the systemic circulation. The microbiological findings suggested that four animals (10, 11, 21, 23) were cases of ET.

The morphological and histochemical findings were identical with those seen in three cases of ET, two experimental and one natural, with MA (Oikawa and Shiga, 2002; Oikawa *et al.*, 2004). The histopathological similarity of the MA in horses with experimental ET and spontaneous colic in this study suggests that the pathogenesis of MA was fundamentally the same in each case. The fact that MA was present in the majority of cases of spontaneous colic suspected to be due to ET suggests that MA is a pathognomonic feature of equine ET. The microscopical appearance of the arterial lesions suggested that the medial smooth muscle cells were the major target of injury. Joris and Majno (1977, 1981b) suggested that the occurrence of many vacuoles in the cytoplasm of medial smooth muscle cells of small arteries was the result of sustained arterial spasm or vasoconstriction, due to herniation of one smooth muscle cell into another (cell-to-cell herniation). Furthermore, the cytoplasmic necrosis (cytoplasmolysis) and coagulation necrosis of the medial smooth muscle cells observed in the present study have been noted frequently in experimental angiospasm in monkeys (Alksne and Greenhoot, 1974; Fein *et al.*, 1974), dogs (Tanabe *et al.*, 1978; Ohkuma and Suzuki, 1999) and rats (Kobori *et al.*, 1979; Joris and Majno, 1981a,b; Wisniewski *et al.*, 1995; Ono *et al.*, 1997), and in spontaneous arterial spasm and contraction in man (Takebayashi, 1985; Masawa *et al.*, 1993; Zubkov *et al.*, 2000). Our finding that MA was more severe in the outer than in the inner media might be explained by the Frank–Laplace formula ($T = PR/H$, where T is wall tensile stress, P is pressure, R is radius, and H is wall thickness), i.e. more mechanical stress may be exerted in the outer than in the inner media due to the difference in their radii. In addition, because there are few vasa vasorum at the adventitia of the small arteries and arterioles, the nutritional state of the outer media may be poor as compared with that of the inner media. In vasospasm, the arteries initially develop many vacuoles in the medial smooth muscle, and the myocytes then become necrotic (Kobori *et al.*, 1979; Masawa *et al.*, 1993). There is still a possibility that infiltration of blood components into the arterial wall might induce necrosis and disappearance of medial smooth muscle cells through enzymes that play a role in cytolysis or histolysis, such as elastase and collagenase, which are present in circulating blood and are released from macrophages or neutrophils (Ooneda *et al.*, 1973; Oikawa *et al.*, 2004). However, we take the view that the pathomorphogenesis of MA observed in both our experimental and spontaneous cases was based not only on endothelial injury, possibly from hypercytokinaemia elicited by LPS (Oikawa *et al.*, 2004), but also on the effects of prolonged mesenteric arterial contraction or vasospasm, leading to intimal (Joris and Majno, 1981a; Ono *et al.*, 1997) and medial (Joris and Majno, 1981b; Takebayashi, 1985; Masawa *et al.*, 1993; Wisniewski *et al.*, 1995) damage.

The degree of severity and the frequency of occurrence of MA were more marked in groups B and C than in group A. Pilot studies (unpublished) showed that MA was not present in experimental horses that died 2 h after an abrupt bolus infusion of LPS (20 μg/kg). The present study suggests that the differences in the degree of severity and the frequency of occurrence of MA reflected differences in the duration and intensity of vasospasm-associated responses in the vessels (which are dependent on dose and time) rather than a direct effect of LPS.

Acknowledgments

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