Vascular Changes in Duodenal Mucosa in Shigellosis and Cholera

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Vascular endothelial cells are highly specialized cells with numerous sensory and modulator functions. Our previous studies show extensive microvascular changes in rectal mucosal vasculature of patients with acute infective diarrhea (Mathan and Mathan 1985a, Gut 26:710–717). We looked for changes in the duodenal mucosal vasculature in two naturally occurring diarrheal infections: shigellosis and cholera. Duodenal mucosal biopsies from 14 patients with shigellosis, 12 patients with cholera, and 10 healthy volunteers were examined under the electron microscope. There were extensive microvascular changes in the duodenum in shigellosis and cholera. Congestion and dilatation of capillaries and venules, stagnation of blood, thinning of the endothelial lining, and platelet clumping were commonly seen in both conditions. Endothelial damage was also common to both conditions but was mild to moderate in cholera and severe in shigellosis with frank hemorrhage, frequent formation of stress fibers, widening of intercellular spaces, cytoplasmic blebbing, cell fragmentation, and intravascular thrombosis. Erythrocyte aggregates, platelet aggregates, and leucocyte plugging lead to capillary obstruction. The arterioles were severely constricted. These changes in the endothelial lining of the microvasculature could contribute to the pathogenesis of the disease resulting in peripheral vascular insufficiency, inadequate oxygen delivery to intestine, and organ dysfunction. The factors influencing these changes, their implications, and possible therapeutic interventions are discussed. Clin. Anat. 16:317–327, 2003.

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Key words: endothelial cell; capillaries; venules; arterioles; diarrhea; bacterial infection

INTRODUCTION

Vascular endothelial cells are highly specialized cells with numerous sensory and modulator functions. The most obvious and well-known function of endothelial cells is the provision of a protective, non-adherent surface for transport of substances from and into the blood stream. In addition, due to their strategic position between the circulating blood and the rest of the vessel wall, endothelial cells regulate vascular tone, initiate immune and inflammatory responses, and facilitate white blood cell activation and migration (Cotran, 1987; Pober, 1988). The multifunctional and dynamic nature of the endothelial cell makes it a prime candidate for study of pathomechanisms in disease processes.

Our previous studies of rectal mucosal biopsies from healthy South Indians, prone to repeated enteric infections, have shown evidence of vascular injury with reduplication of the basal lamina and occlusive thrombi (Mathan and Mathan, 1985b). This indicates damage to endothelial cells that probably occurred during previous episodes of diarrhea. In addition to these changes in healthy individuals, we have found microvascular changes in the rectal mucosa of patients with acute infective diarrhea (Mathan and Mathan, 1985a). In a murine model of endotoxin-induced diarrhea, vascular changes were seen both in the small and large intestines (Mathan et al., 1988; Koshi et al., 1993). These findings indicate that microvascular en-
Dothelial cells are targeted in diarrheal diseases. There is not much information, however, on the state of the small intestinal microvasculature during episodes of diarrhea. To determine if small intestinal vasculature is affected during episodes, we looked for electron microscopic changes in the duodenal mucosal vasculature in patients with two naturally occurring bacterial infections: shigellosis, the classical diarrhea featuring invasive colitis (Labrec et al., 1964) and cholera, the classic toxigenic diarrhea primarily affecting the small intestine (Banwell and Sherr, 1973).

**MATERIALS AND METHODS**

Duodenal mucosal biopsies were available in the Department of Gastrointestinal Sciences from 14 patients with shigellosis and 12 patients with cholera, confirmed by bacteriological studies, and 10 healthy volunteers. Informed consent was obtained from all subjects and the Institutional Research Committee gave ethical approval. All patients were initially seen at the hospital emergency services. After stabilization and intravenous rehydration, 2–3 mucosal biopsy samples were obtained from the junction of the second and third part of the duodenum or the proximal third part through a forward viewing Olympus fibre-optic upper gastrointestinal endoscope (Tokyo, Japan). Tissue samples were taken within 72 hr of the onset of diarrhea and before specific treatment was begun. All biopsy samples were immediately retrieved, fixed in 2.5% glutaraldehyde in sodium cacodylate buffer (pH 7.4; 400 mOsm), postfixed in osmium tetroxide in veronal acetate buffer (pH 7.4), and embedded in araldite. Ultrathin sections were cut on a diamond knife (Diatome, Switzerland), stained with uranyl acetate and lead citrate, and examined with a Philips EM201C electron microscope (Eindhoven, The Netherlands). The final analysis was carried out on coded sections with the observer unaware of the clinical and laboratory diagnosis. From each patient and control, 2–4 blocks were studied. In each study section all blood vessels in the lamina propria, that is all arterioles and venules at the crypt base and capillaries among the intestinal glands and in the villi, were photographed. A minimum of 20 vascular cross sections were examined in each block. The vessels were identified as capillaries, venules, and arterioles using the criteria set by Rhodin (1967, 1968). Capillaries had a small diameter, and a single layer of endothelial cells with pericytes. Venules had thin endothelium and an incomplete layer of pericytes, fibroblasts, or smooth muscle. Arterioles had a well-defined elastic lamina and one or two layers of smooth muscle cells.

We were looking for morphological changes in the endothelial cells, basement membrane, pericytes, and vascular smooth muscle. We also looked for signs of vascular constriction, alterations in erythrocytes, increased platelet and leucocyte activity, and blood cell migration. In particular, we sought evidence of endothelial damage and activation.

**RESULTS**

**Vasculature of Controls Subjects**

**Capillaries.** The wall of the capillaries consisted of flattened endothelial cells on the basement membrane that was surrounded by scattered pericytes with long, thin processes extending along the circumference. The nucleus was ovoid in shape. Cytoplasmic organelles, intercellular junctions between endothelial cells, and fenestrae in the cytoplasm were normal (Fig. 1a).

**Venules.** Endothelial cells lining the venules were elongated, flattened, and thicker than those lining the capillaries. The nucleus was ovoid. A small Golgi body, a few mitochondria, and endoplasmic reticulum were found in the juxtanuclear position (Fig. 1b). Occasionally, there was mild ruffling of the surface membrane usually not described in normal endothelial cells.

**Arterioles.** Endothelial cells of arterioles were thick. The nucleus was large and almost circular in profile. Internal elastic lamina and one or two layers of closely placed smooth muscle surrounded the endothelial cells.

**Patients With Shigellosis and Cholera**

Changes were seen in capillaries, venules, and arterioles of patients with shigellosis and cholera and were more pronounced in those with shigellosis.

**Vascular Changes in Shigellosis**

**Capillaries.** Light microscopy. One-micron thick survey sections stained with toluidine blue showed striking alteration in capillaries of the lamina propria. There was capillary dilatation and sludging of erythrocytes (Fig. 2a). Free hemorrhage into the lamina propria occurred from congested capillaries where the endothelial lining was thinned out and disrupted (Fig. 2b). Platelet aggregation was prominent in many areas. Some vessels contained polymorphonuclear neutrophils (PMNs), usually margined, few eosinophils and basophils. The vascular lesions were patchy in distribution.

**Electron microscopy.** Ultrastructurally, there was widespread damage to endothelial cells with swelling and protrusion of cytoplasmic blebs from the endothelium...
Vascular Changes in Shigellosis and Cholera

Cellular organelles were damaged with dilatation of endoplasmic reticulum, swelling of mitochondria, and disorganization of cristae (Fig. 3), formation of myelin figures, and increased electron lucency of cytoplasm. Degenerative changes in the endothelial cells were accompanied by similar changes in the pericytes. When endothelial damage was severe, the cell membrane was indistinct, and the cytoplasm filled with numerous membrane-bound vacuoles representing damaged organelles. Nuclear material was pyknotic and crenated. Cellular debris and stagnant erythrocytes filled the capillary lumen. Endothelial damage also led to denudation of vessel wall, but aggregates of actin filaments at the periphery of endothelial cells (Fig. 4) formed stress fibers illustrating the cells attempt to maintain structural integrity.

There were signs of increased platelet activity and platelet aggregates in the vessel (Fig. 5). Not all capillary profiles showed evidence of damage. In some capillaries the only change observed was hypertrophy of the endothelial cells. Such hypertrophy suggests

Fig. 1. a: Duodenal mucosal capillary from a healthy volunteer. Normal, flattened endothelial cells (E) line the blood vessel. Three RBCs are seen in the lumen. Scale bar = 2 μm. b: Post capillary venule from duodenal mucosa of a healthy volunteer showing normal endothelial lining. Scale bar = 2 μm.

Fig. 2. a: Light micrograph of duodenal mucosal microvasculature near the crypt base (CB) of a patient with shigellosis. The vessels are dilated and congested with stagnant blood cells (V). Scale bar = 8 μm. b: Light micrograph of duodenal mucosal vasculature adjacent to the surface epithelium (Epi) in a patient with shigellosis. Capillaries (arrowheads) are congested. There are areas of vascular dehiscence with hemorrhage into the adjacent tissue. Intercellular spaces between enterocytes are markedly widened (asterisk) due to disease. R, RBCs in the perivascular space. Scale bar = 8 μm.
cell activation in response to bacterial endotoxin, cytokines or as part of regenerative activity after injury. Prominent Weibel-Palade bodies and increase in micropinocytic vesicles were also seen occasionally (Fig. 6). Other capillaries were normal except for perivascular edema.

**Venules. Light microscopy.** Venules adjacent to the bases of intestinal crypts were dilated and congested. Stagnation of erythrocytes in the lumen suggested slowing of blood flow (Fig. 7). Collapsed and thrombosed venules were seen occasionally in the deeper regions of the mucosa. There were focal areas of endothelial thinning and denudation. Platelet aggregates were seen in the lumen.

**Electron microscopy.** Venules were markedly dilated and contained large numbers of erythrocytes that showed alteration in shape, fragmentation, reduced hemoglobin and diapedesis through the vessel walls (Fig. 8a). Sometimes the characteristic blurred appearance of the cytoplasm and cell membrane at sites of erythrocyte-endothelial interaction were noted (Fig. 8b). Degenerative changes in the endothelial cells were more severe and widespread than those in the capillaries. Numerous irregular blebs from the cell membrane protruded into the vascular lumen or into the perivenular space (Fig. 9). These blebs were filled with rarefied cytoplasm, and were at times large enough to compromise the vascular lumen. There were organelle damage and degenerative changes in the nucleus leading to extrusion of the nuclear material (Fig. 10). Often there was marked condensation of cytoplasmic and nuclear material and fragmentation of endothelial cells. There was an increase in number and size of Weibel-Palade bodies and lysosomal granules, frequent formation of stress fibers along the basal surface of the cells and widening of intercellular junctions (Fig. 11). Due to extensive endothelial damage there was complete breakdown of vessel wall in places. These were sites of hemorrhage into tissue and served as foci for formation of platelet thrombi. At times thrombi were large enough to occlude the lumen (Fig. 12). Individual platelets showed signs of activation as evidenced by degranulation and pseudopod formation.

In scattered vessels there was accumulation of PMNs. Concurrent with the degenerative changes there were signs of endothelial activation and repair as seen by the reduplication of the basement membrane.

**Arterioles.** There was constriction of the arterioles and endothelial damage with ruffling of the surface membrane, swelling of mitochondria, and accumulation of lipid bodies and lysosomal granules in the cytoplasm (Fig. 13). Actin filaments were aggregated to form stress fibers within the cell. Mitochondrial swelling with distortion of cristae was evident in the surrounding smooth muscle.
Vascular Changes in Shigellois and Cholera

Capillaries. Light microscopy. Capillaries were dilated and congested with stagnant erythrocytes and clumps of platelets.

Electron microscopy. Erythrocytes were distorted, fragmented, and dehemoglobinized and some were in diapedesis through the endothelial cytoplasm. Remnants of dehemoglobinized erythrocytes were present as ghost cells in the lumen (Fig. 14). In the endothelial cells, pinocytic vesicles were more numerous than in control material. Endothelial cells showed moderate degree of damage with swelling, formation of microvilli at the luminal surface, dilated rough endoplasmic reticulum and swelling of mitochondria. In scattered vessels there was evidence of more severe endothelial injury with accumulation of lipid bodies and multivesicular bodies in the cytoplasm, disruption of cell membrane, and cell fragmentation. Though the severity of changes was less when compared to that in shigellosis, there was more marked signs of intravascular coagulation with aggregation of platelets and fibrin within the lumen, and platelet adherence to the basal lamina in areas of endothelial separation (Fig. 15).

Venules. Light microscopy. Survey sections showed venules that were thin-walled and dilated. Margination of PMN was a frequent finding.

Electron microscopy. There was dilatation and congestion of venules. Degenerative changes in the endothelial cells led to formation of gaps in the endothelial lining. Polymorphonuclear neutrophils were marginated and adherent to the endothelial surface and in diapedesis into the surrounding tissue. Though
PMNs usually leave the circulation by passing through the intercellular spaces, an occasional PMN was seen migrating through the endothelial cell (Fig. 16). PMNs frequently exhibited degenerative changes with cytoplasmic swelling, discontinuous surface membranes, and loss of cytoplasmic granules. Aggregates of activated platelets were present at sites of endothelial injury and in the vascular lumen. In addition to these degenerative changes, there were other endothelial cells showing regenerative activity with prominent Golgi and numerous Weibel-Palade bodies. Interendothelial junctions were normal but in markedly dilated vessels, there was separation of the junction with platelet adhesion.

**Arterioles.** Changes in the arterioles were similar to those seen in shigellosis with constriction of the lumen and mild to moderate damage to endothelial cells and smooth muscle cells.

**DISCUSSION**

This study reports acute changes in the duodenal mucosal microvasculature of patients with two types of infective diarrhea, shigellosis and cholera. Shigellosis or bacillary dysentery is caused by *Shigella dysenteriae* and is the paradigm of invasive dysentery primarily affecting the colon (Lebrec et al., 1964). Even though dysentery is characteristic of shigellosis; it may be preceded by watery diarrhea, probably due to a combination of increased jejunal secretion and poor colonic absorption (Rout et al., 1975). In contrast, cholera, caused by *Vibrio cholerae*, is the paradigm of toxigenic secretory diarrhea primarily affecting the small intestine (Banwell and Sherr, 1973). Each of
these pathogens has a distinctive mechanism by which they cause disease. Shigella locally invades gut epithelium via M cells and causes intense mucosal inflammation and destruction of the colonic and rectal epithelium. *Vibrio cholerae* is a noninvasive pathogen that colonizes the luminal surface of epithelial cells where it secretes cholera toxin (CT), a potent enterotoxin that induces a voluminous diarrhea (Bloom and Boedeker, 1996).

The small intestinal mucosa is richly supplied by blood vessels. During recent years, it has been recognised that the physiological functions of the vascular endothelium extend well beyond its primary barrier function. It is now seen as an active organ that plays an important regulatory role in a variety of physiological homeostatic processes such as inflammation, coagulation, and regulation of vascular tone (Kirkpatrick et al., 1996).

Our study shows extensive microvascular changes in the duodenal vasculature in shigellosis and cholera. Congestion and dilatation of capillaries and venules, stagnation of blood, thinning of the endothelial lining, and platelet clumping was common in both conditions. Endothelial damage was also common to both conditions but was mild to moderate in cholera and severe in shigellosis with frank hemorrhage, frequent formation of stress fibers and widening of intercellular spaces, cytoplasmic blebbing, cell fragmentation, and

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**Fig. 10.** Venule from patients with shigellosis. Cell fragments (F) from injured endothelial cells float free in the lumen. V, vessel wall. Scale bar = 1.5 μm.

**Fig. 11.** Venule from a patient with shigellosis, showing wide separation at the intercellular junction between endothelial cells (asterisk). E, endothelial cells. Scale bar = 1.5 μm.

**Fig. 12.** Venule from a patient with shigellosis. A large thrombus of adherent, activated platelets (P) are plugging a defect in the vessel wall (arrow) and obstructing the lumen. Extravasated RBCs (R) are seen in the tissue. Scale bar = 3 μm.
intravascular thrombosis. Erythrocyte aggregates, platelet aggregates, and leukocyte plugging lead to capillary obstruction.

An important function of the vascular endothelium is to provide a permeable barrier between the intravascular compartment and surrounding structures, and increased vascular permeability is a hallmark of inflammation (Cotran et al., 1994). Such increase in permeability is due to adaptations of the endothelial lining (Kaley and Altura, 1977; Cotran et al., 1994; Dejana et al., 1995; Tedgui, 1996). In shigellosis and cholera, the barrier function of the endothelial lining is deranged at various levels. The endothelial cytoplasm becomes permeable to erythrocytes. There is also widening of intercellular spaces and aggregation of cytoplasmic filaments to form stress fibers within endothelial cells. The size of intercellular junctions is regulated by contraction of actin filaments found in endothelial cells (Gotlieb and Koo, 1990; Dejana et al., 1995). The widening of the intercellular spaces and formation of stress fibers in shigellosis are indicators of considerable changes in the endothelial stability and junctional integrity. Extreme endothelial contraction as seen in cholera may widen intercellular junctions to a dimension that permits contact between platelets and subendothelial tissue. This in turn paves the way to platelet adhesion and aggregation that initiates hemostasis as platelet endothelial cell adhesion molecule (PECAM-1) and platelet activation-dependent granule external membrane (PADGEM) ad-
hesive molecules for platelets are concentrated at the
interendothelial cleft (Dejana et al., 1995; Burns et al.,
2000). Increased numbers of pinocytic vesicles in the
capillary cytoplasm as seen in cholera have been re-
ported earlier in animal models of the disease (Chen
etal., 1971; Asakura et al., 1974) and are thought to
play a role in the increased secretion.

Changes in the microvasculature found in shigello-
sis and cholera taken together suggest some degree of
endothelial activation. Activated endothelial cells syn-
thesize and release mediators of inflammation, espe-
cially IL-1 and TNFα. They also regulate the expres-
sion of cell adhesion molecules on the luminal surface
of endothelial cells (Cotran et al., 1994) and stimulate
endothelial cell production of substances such as tis-
sue factor, plasminogen activator inhibitor, and plate-
let activating factor that increase the procoagulant
activity (Cotran, 1987).

In our study of blood vessels in shigellosis and
cholera, we found ultrastructural features of activation
in many endothelial cells and evidence of proinflam-
matory and procoagulant changes in the blood vessels.
Both diseases are caused by gram negative bacteria
that secrete bacterial lipopolysaccharide, LPS or en-
dotoxin, which is a known stimulus for endothelial cell
activation (Pugin et al., 1993).

Blood platelets have a unique relationship with
vascular surfaces. In contrast to lymphocytes and neu-
trophils, which migrate through intact blood vessels,
platelets do not interact with normal vasculature but
attach to sites of endothelial injury or disease. In the
initial stages, platelets put out pseudopodia onto the
exposed subendothelium, thereby preventing extrav-
asation of blood. In more extensive damage large
aggregates form and obstruct the vascular lumen
(Roth, 1992). Cooling et al. (1998) have shown that
Shiga toxin may bind platelets via specific glycosphin-
golipid receptors. Such binding may contribute to the
platelet activation and microthrombi formation in bac-
cillary dysentery.

Our studies show increased polymorphonuclear
neutrophil activity, heightened PMN-endothelial cell
interaction, and PMN migration in duodenal vascula-
ture in shigellosis and cholera. Shigellosis is a proto-
type of inflammatory diarrhea with pronounced PMN
reaction. PMN interactions in the colonic mucosa of
patients with shigellosis have been reported by earlier
workers (Speelman et al., 1984; Mathan and Mathan,
1986; Anand et al., 1986). PMNs play an important
role in controlling dissemination of shigellae in human
intestine (Zhang et al., 2001). Cholera was previously
thought to be a form of secretory diarrhea unaccom-
panied by inflammatory changes but a report from our
lab has described significant infiltration of PMNs into
the lamina propria and crypt epithelium of duodenal
mucosa (Mathan et al., 1995).

The adhesion of leukocytes and emigration from
the vessel wall is a physiological phenomenon, which
can be dramatically increased in inflammation and
vascular disease (Wautier et al., 1992; Cotran et al.,
1994; Granger and Kubes, 1994). In inflammatory vas-
cular injury, both endothelial cells and leucocytes un-
dergo rapid change in the milieu of inflammatory
mediators. Such activation of endothelial cells and
PMNs leads to sequestration of PMNs in the micro-
circulation, adhesion to endothelium, and migration
into the inflamed tissue. Unchecked PMN activation
leads to massive granule protease release, oxygen rad-
ical generation, and other activation events that exac-
terbate endothelial damage (Carlos and Harlan, 1990).
An unexpected finding was the migration of an occa-
sional PMN through the endothelial cytoplasm rather
than through the more commonly recognised pathway
between the endothelial cells (Burns et al., 2000).

Endothelial cell death was common in cholera and
frequent in shigellosis. Two modes of cell death pre-
dominate in pathological processes, necrosis and apo-
ptosis or programmed cell death with distinct morpho-
logical differences between the two. In our study,
necrotic changes of irregular clumping of nuclear chro-

Fig. 16. Capillary from a patient with cholera. A polymorphonu-
clear neutrophil (N) is seen in the cytoplasm of an endothelial cell (E).
Scale bar = 1 μm.
matin, gross swelling of intracytoplasmic organelles, discontinuities in the membranes, and disintegration of intracytoplasmic components as described in necrosis (Cotran et al., 1994) were commonly seen in both conditions. Apoptosis is identified by compaction and peripheral margination of nuclear chromatin, condensation of cytoplasm, followed by budding of cell into discrete membrane-bound fragments (apoptotic bodies) and extrusion of apoptotic fragments into the vascular lumen (Gobe et al., 1997). Many studies indicate that apoptosis may play a crucial role in the pathogenesis of shigellosis (Zychlinsky et al., 1996; Zychlinsky and Sansonetti, 1997; Monack and Falkow, 2000). Apoptosis of vascular endothelial cells in shigellosis has not been reported and further studies, using specific markers of apoptosis need to be done.

Many factors probably contribute to the vascular injury in shigellosis and cholera. Both *Shigella dysenteriae* and *Vibrio cholerae* are Gram negative organisms that produce bacterial endotoxin, chemically characterized as lipopolysaccharide (LPS). Endotoxin possesses a wide spectrum of biological activities (Morrisson and Ulevitch, 1978; Moncada et al., 1991; Montgomery et al., 1991). With regards to vascular endothelium, LPS causes direct damage to endothelial cells (Harlan et al., 1983); brings about activation (Pugin et al., 1993); stimulates secretion of inflammatory mediators (Moldow et al., 1993); upregulates the expression of inducible cell adhesion molecules (ELAM-1 and ICAM-1) on the luminal surface (Osborn, 1990); and causes vascular damage in vivo (McKay et al., 1966; Gaynor, 1970; Richman et al., 1980; Reidy and Schwartz, 1983; Meyrick and Brigham, 1983; Koshi et al., 1993). These earlier studies suggest that bacterial endotoxin could account for many of the vascular changes of shigellosis and cholera. In addition, *Shigella dysenteriae* produces an exotoxin, Shiga toxin, that is also known to injure vascular endothelial cells (Fontaine et al., 1988; Tesh and O’Brien, 1991).

The extensive microvascular damage found in the duodenal mucosal lamina propria in patients with shigellosis and cholera is a relatively new observation. A recent report on the ultrastructural changes in the duodenal mucosa of patients with cholera (Mathan et al., 1995) describes some of these changes, but they have not been reported previously in patients with shigellosis. More work is required in this field. The role of stress fibers in arteriolar and venular endothelium needs to be investigated further. Endothelial activation and cell death by apoptosis needs to be confirmed using immunocytochemistry and in situ hybridization. Adhesion molecules specific for neutrophil recruitment expressed on the endothelial surface during these diseases need to be identified.

Diarrhea and dysentery are still major causes of morbidity and mortality in many tropical and subtropical countries. It is clear from this study that the microvascular endothelium in shigellosis and cholera responds to the infection, not simply as a target for injury, but by undergoing specific alterations in structure and function. The increased vasoconstrictive responses, interaction of leucocytes with the blood vessel wall, and procoagulatory influences contributes to impairment of tissue perfusion. Recent work shows that vascular inflammation can be limited by anti-inflammatory mechanisms that maintain the integrity and homeostasis of the vascular wall (Tedgui and Mallat, 2001) and such therapeutic intervention may have a role in limiting the course of these diseases.

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