CONCISE REPORT

Experimental Thrombocytopenia and Capillary Ultrastructure

By David Shepro, H. E. Sweetman, and H. B. Hechtman

Reduced circulating platelets or thrombocytopenia is associated frequently with a propensity for petechial and purpuric hemorrhages. This observation has led to the concept that platelets support vascular endothelium and maintain vascular integrity by a mechanism separate from hemostasis or vascular repair. Experimental thrombocytopenic animal models have been used to investigate vessel wall defects that might result from low platelet counts. However, electron microscopic studies of tissues from thrombocytopenic animals have not shown any apparent ultrastructural changes in the microvascular endothelium or microvessel wall, with one exception: tongue skeletal muscle capillaries that are normally nonfenestrated and continuous were reported to be fenestrated and attenuated in experimental thrombocytopenic rabbits.

The purpose of the present investigation was to examine tongue skeletal muscle capillaries in control and thrombocytopenic mice and hamsters to determine if attenuation and fenestration of skeletal muscle capillaries are characteristic of a thrombocytopenic state in these animals. In addition to skeletal muscle capillaries, several other tissues were examined for ultrastructural changes in capillary endothelium associated with thrombocytopenia. Our data show no ultrastructural differences in capillaries in any of the tissues examined between control and thrombocytopenic hamsters and rats.

MATERIALS AND METHODS

Animals

All hamsters were outbred Lak/LVG(SYR) males weighing 101-110 g, and mice were CD-1 males and females (Charles River Labs, Wilmington, Mass.). Rabbits were adult males (3-4 kg) New Zealand Whites (Thomas Fazio Labs, Assonet, Mass.).

Antiplatelet Serum Production and Induction of Thrombocytopenia

Antiplatelet sera were raised in rabbits to both hamster and mouse platelets according to the method described by Dale and Hurley. Heat-inactivated sera were also incubated with respective species of erythrocytes. Intraperitoneal injection of antiplatelet serum induced severe thrombocytopenia (platelet counts <10,000/cu mm) by 3 hr, and platelet counts did not start to increase significantly until 36 hr. All animals remained thrombocytopenic for 24 hr prior to experiments.

Electron Microscopy

Tissue biopsies were taken from anesthetized animals and fixed in Karnovsky’s fixative at room temperature overnight and embedded in Epon by standard methods. Thin sections were stained and examined on a JEOL JEM 100B electron microscope.

RESULTS

Control and Thrombocytopenic Hamster Tissue

Biopsies from the tongue and cremaster muscles, cheek pouch and abdominal skin of normal and thrombocytopenic hamsters showed normal capillary endothelium ultrastructure. Attenuated or fenestrated capillary endothelia were never observed in the muscular regions of tongues from control or thrombocytopenic hamsters (Fig. 1A). When capillaries of the connective tissue or lamina propria region of the lingual mucosa were compared with the underlying internal skeletal muscle fibers, approximately half of the lamina propria capillaries were attenuated or fenestrated (Fig. 1B).

Control and Thrombocytopenic Mouse Tissue

Biopsies from the tongues of control and thrombocytopenic mice did not show any significant change in capillary ultrastructure. Capillaries from the muscular region of the tongue from both groups of mice showed a continuous nonfenestrated endothelium; only the capillaries of the lamina propria showed attenuation and fenestration. These results parallel those observed for hamster tissues.

DISCUSSION

In the present investigation, the ultrastructure of capillary endothelium from normal and thrombocytopenic animals was compared and no structural differences were observed in any of the tissues examined, including tongue muscle capillaries. Fenestrated or attenuated endothelial cells were not seen in tongue muscle capillaries from thrombocytopenic hamsters and mice when care was taken to trim away the lamina.

From the Departments of Biology and Surgery, Boston University, and the Department of Surgery, Peter Bent Brigham Hospital, Boston, Mass.

Supported in part by NIH Grants HL 16714 and GM 24891; U.S. Army Medical Research and Development Command, DAMD 17-78-C-0026; and the Brigham Surgical Group.

Submitted July 14, 1980; accepted July 21, 1980.

Address reprint requests to Dr. David Shepro, Microvascular Research, 2 Cummingston Street, Boston, Mass. 02215.

© 1980 by Grune & Stratton, Inc.

0006-4971/80/5605-0031$01.00/0
propia prior to sectioning. In contrast, fenestrated or attenuated regions of endothelial cells were found in capillaries in the lamina propria of the lingual mucosa of all tissues sampled, both normal and thrombocytopenic. These observations are similar to observations for the lamina propia previously reported.\textsuperscript{14}

The reported changes in rabbit tongue muscle capillary endothelium associated with thrombocytopenia\textsuperscript{12} may be species characteristic or be due to fortuitous sampling. Capillary fenestrae occur in 1 of approximately 60 cross-sectioned blood capillaries in normal adult rat skeletal muscle.\textsuperscript{15} That the method of biopsy

Fig. 1. (A) Cross-section of a capillary from the muscular region of a thrombocytopenic hamster tongue. An erythrocyte is shown in the vessel lumen (L) and a muscle fiber (MF) is in close proximity. Endothelial cell cytoplasm (arrows) has a normal thickness of 550 nm ($\times$ 9000; bar, 1 $\mu$m). (B) Cross-section of a capillary from the lamina propria of lingual mucosa of a thrombocytopenic hamster. An erythrocyte is shown in the vessel lumen (L) and the endothelial cell has regions of attenuated cytoplasm (arrows) ($\times$ 14,000; bar, 1 $\mu$m).
Fig. 2. (A) Cross-section of a capillary from the tongue muscle of a normal rabbit. The lumen (L) is partially collapsed, and the capillary is surrounded by muscle fibers (MF). The endothelial cytoplasm (arrows) has a normal thickness of 500 nm (X 10,750; bar. 1 μm). (B and C) Cross-sectioned capillaries from the lamina propria of lingular mucosa biopsied from normal rabbits. The endothelial cytoplasm is attenuated to 50-200 nm (arrows), and the vessel lumen is shown (X 10,750; bar. 1 μm).

REFERENCES

Experimental thrombocytopenia and capillary ultrastructure

D Shepro, HE Sweetman and HB Hechtman