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KEYWORDS: microvasculature, peripheral nerve, injection replica SEM

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STEREOSCOPIC OBSERVATION OF THE MICROVASCULATURE OF PERIPHERAL NERVES

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Abstract. The microvasculature of peripheral nerves in dog and human samples was studied by an injection replica scanning electron microscope (SEM) method. The three-dimensional relationship of epineural and intraneural vessels was well demonstrated by this method. Epineural vessels were made of both longitudinal arterioles and venules and also a close meshwork of capillaries. In the intraneural microvasculature, longitudinal arterioles and venules made up the perineural vessels, and grouped capillaries corresponded to the fine vasculature of intrafascicular vessels. Between epineural and intraneural vessels, there was little anastomosis but nutrient arteries ran through the epineural vasculature into the intraneural vessels. There was little interconnection of vessels among the intraneural vasculature. It was postulated that the close meshwork of the epineural layer can resist pressure from outside the peripheral nerves but that longitudinal venules seemed to be affected by pressure and tension at the localized area.

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The microcirculation in peripheral nerves has been observed by light microscopy of sectioned (1) or injected preparations (2-5) and vital dissecting microscopy (6). The blood supply of the peripheral nerve has been studied especially in regard to degeneration after ischaemia or traumatic injury (7) and repair after neurorrhaphy (8). According to recent work by Sunderland (2-5) and Lundborg (1, 6), the vascular system of the peripheral nerves consists of extraneural and intraneural vessels.

The extraneural vessels comprise nutrient and epineural vessels; the intraneural vessels make up a vascular plexus in the nerve trunks. Both inosculate themselves. This research, however, was conducted by light microscopy of sectioned samples (2-5), microangiography (7-9) and vital dissecting microscopy (4) and does not describe the microcirculation of the peripheral nerves well enough. The three-dimensional architecture of the peripheral nerve vessels has not been demonstrated by previous methods. The present study was made to survey the stereoscopic architecture of the microcirculation of peripheral nerves in dogs and human amputated legs by an injection replica scanning electron microscope (SEM) method (10).
MATERIALS AND METHODS

Seven adult hybrid dogs and two amputated human legs were used. The dogs were anesthetized and the abdominal aorta was dissected and cannulated at the level of the renal artery. Both lower extremities were irrigated with normal saline solution containing heparin. After the vessels had been completely irrigated, barium solution (Micropaque) was injected through the catheter in two dogs. Microangiography of the nerves dissected was made under soft X-ray apparatus (CBM type, Japan Softex Co.).

Methylmethacrylate containing catalyst (Mercox) was injected into the abdominal aorta of five dogs. The resin was polymerized at room temperature for one night, then the sciatic nerve and the posterior tibial nerve and adjacent perineural tissue were dissected out under a binocular light microscope. Maceration in sodium hydroxide solution and washing in hot water was repeated to remove the corroded mass of nerves attached to the vessels, and the casts were then dried. In the same way, human amputated lower legs were irrigated and injected with resin through the posterior tibial artery. The dissected posterior tibial nerve was macerated after polymerization. Dried casts of the nerve vessel were cut into pieces under the microscope, and examined with a SEM (Japan Electron Optics Laboratory Co., Ltd.) after coating with gold palladium in a vacuum evaporator. After observation of the epineural vascular system, micro-dissection was made under a binocular light microscopy to survey the vasculature of the intraneural system. Dissected samples were coated with metal and examined by SEM.

RESULTS

Microangiography of the Sciatic Nerve in Dogs

The microangiographic picture of the sciatic nerve injected with Micropaque showed a communicating vascular system in the perineurium as well as in the endoneurium. There was definite straight arrangement of the arterioles and venules and, to a certain degree, a close network of capillaries (Fig. 1). Microangiography was useful for observing the general vascular pattern of peripheral nerves, but had marked limitations when identifying individual vessels and studying the relationship of extraneural to intraneural vasculature. In comparison with the injection replica SEM method described later, it was very difficult to study the vascular architecture at the level of the capillary network.

Observation of the Peripheral Nerve Vasculature by the Injection Replica SEM Method

Peripheral Nerve Vasculature in Dogs

SEM examination of the resin cast of peripheral nerve vessels injected with methylmethacrylate finely demonstrated microvasculature in the sciatic (Fig. 2A) and the posterior tibial nerves (Fig. 2B).

The general view of the resin cast showed extraneural vasculature composed of two kinds of vascular pattern; longitudinally aligned vessels over 100 \( \mu \) in diameter and a close network of capillaries, some 10 \( \mu \) in diameter. The former vessels were probably arterioles and venules in the extraneurium, and anastomoses were found to some distance. Slightly smaller vessels with ridges and grooves corresponded to arterioles and larger vessels with smooth surface to venules. Usually, two or three pairs of arterioles and venules were oriented.
Microvasculature of Peripheral Nerves

longitudinally and no side to side shunt formation was seen between them (Fig. 2). A capillary network of the longitudinal vessels covered the surface of the peripheral nerves. The vascular architecture was similar in both the sciatic nerve and the posterior tibial nerve but the capillary network was thinner in the posterior tibial nerve than in the sciatic nerve (Fig. 2). Intraneural vascular architecture was examined after removing the surface network of epineural vasculature by a micro-dissecting method.

Epineural microvasculature. Closer views of the vascular system of the sciatic nerve clearly showed a thick network as well as longitudinal vessels (Fig. 3A).

Fig. 1. Microangiography of the sciatic nerve showing microvasculature, but fine architecture is unclear.

Fig. 2. Scanning electron micrograph of the resin cast of peripheral nerves in dogs. A: Microvasculature of the sciatic nerve showing longitudinally aligned arterioles and venules and the close network of the capillary. × 30. B: Microvasculature of the posterior tibial nerve. × 25.

Fig. 3. Network of extraneural capillaries. A: In the sciatic nerve, this close network is connected with the longitudinal vessels and anastomotic vessels. × 60. B: Similar vascular architecture was found in the posterior tibial nerve. × 60.

Fig. 4. Intraneural microvasculature of the peripheral nerves in dogs. A: Intraneural capillary was observed after dissecting away the extraneural vessels. × 60. B: Closer view showing longitudinal capillaries and intrafascicular grouped capillaries. × 120.

Fig. 5. Little anastomosing vessels were seen among the longitudinal arterioles and venules. × 120.

Fig. 6. Peripheral nerve vasculature in human. A: Extraneural network of the capillaries and longitudinal vessels in the posterior tibial nerve. × 84. B: Capillary network was composed of the fine vascular interconnection. × 120.

Fig. 7. After extraneural network of the capillaries (A, × 60) was removed, the intraneural vessels were apparent (B, × 60).
Fig. 3.
Fig. 4.
The network was made up of capillaries which form anastomoses between the arterioles and venules, and formed complicated anastomoses. Such capillary network was randomly connected with the longitudinal and anastomotic vessels. In the posterior tibial nerve, a similar vascular pattern was seen (Fig. 3B). Intraneural vascular structures were partly seen through the epineural network as shown in Fig. 2B.

**Intraneural microvasculature.** The epineural vasculature was easily dissected away as there were few connecting vessels between epi- and intraneural layers. The intraneural vascular system was the visible. It was mainly composed of larger vessels (separate or duplicated) running longitudinally. These vessels were probably arterioles and venules; the former had an uneven surface and were about 100 μ in diameter, the latter had a smooth surface and were larger. Grouped capillaries of fine vessels which probably corresponded to the intrafascicular meshwork were also arranged in parallel with the larger vessels. The
Fig. 7.
capillaries were 20-30 μ in size and formed a coil-like pattern. The intraneural vascular system of the posterior tibial nerves was very like the vasculature in the sciatic nerve.

Capillary groups of small vessels were randomly mixed among larger arterioles and venules. Anastomotic vessels or shunts between the larger vessels and capillary groups were rare (Fig. 4). The arterioles with shrunken ridges and the venules with smooth surfaces rarely branched on the way to the peripheral circulation.

Peripheral Nerve Vasculature in Humans

SEM observation of injection replica of the posterior tibial nerve in human clearly demonstrated a vascular system similar to that in the canine peripheral nerves. In the general views, the longitudinal vessels and the small capillary network were recognized. The capillaries made a very close network on the deeper vasculature anastomosed with the longitudinal vessels (Fig. 6). Through the network of capillary vessels, intraneural vessels could be seen in the higher magnification. Smooth surfaced vessels corresponded to venules whereas uneven ones were arterioles. Fig. 7 showed both epineurial capillary network (A) and intraneural vessels (B) after taking off the extraneural network. Intraneural vessels were composed of the arterioles and venules running longitudinally and the capillary groups arranged with them but showing a wavy pattern. Among the vessels and grouped capillaries, anastomoses were rare.

DISCUSSION

In peripheral nerve trunks, a very good blood supply is maintained by numerous small nutrient blood vessels. The arteriae nervorum develops well to enter the trunks and does not leave it, terminating intraneurally. The size and shape of these arteries varies from subject to subject. The capillary vessels of peripheral nerves have been studied by observation of vessels perfused with non-biological substances such as contrast media (7, 8), dyes (1-6) and silicone rubber (11). Detailed description of the vascular architecture of the peripheral nerves has been presented by Sunderland (2-5). More recently, the intravital microscopic technique (1, 6) has been utilized to analyze intraneural microvascular flow in vivo under various experimental conditions. However, there have been considerable limitations in investigating the stereoscopic architecture of the vasculature and in elucidating the relationship between the epineural and intraneural vascular system. The technique of injection replica SEM observation (10) as used in this work has made it possible to study the three-dimensional vasculature of peripheral nerves and other organs.

In general, peripheral nerve trunks are supplied by segmental vessels branching through the mesoneurium (2-5, 11, 12). However, a nerve often does not receive a nutrient artery for a considerable distance in some regions of trunks.
such as the median and sciatic nerves. As previous investigators (1-6, 11, 12, 13) have pointed out, each peripheral nerve is abundantly vascularized by the repeated division and anastomosis within the nerve.

According to Kuczyński (13), the vascular supply of peripheral nerves consists of four longitudinal systems; 1) surface chains, 2) interfunicular chains, 3) peripheral channels and 4) an interfunicular capillary net. This is a detailed concept of the peripheral nerve vasculature, but functionally is not adequate.

Recently, two main vascular systems of the intrinsic and extrinsic vessels have been documented by Lundborg (1, 6). When the local nutrient vessels reach the epineurium, they divide into ascending and descending branches and anastomoses with the intraneural, “intrinsic system”. This is composed of the epineural, perineural, and endoneural plexuses and their communicating vessels. In the epineural plexus, there is a great number of arterioles and venules, most running longitudinally. The venules seem to predominate, forming numerous anastomoses in all directions and arteriovenular shunts. The vessels in the epineural plexus also have numerous communications with plexuses in the perineurium and endoneurium. The well developed perineural plexus has a close relationship with the interfascicular endoneural vascular bed which extends the whole length of the nerve and consists mainly of capillaries. These vessels are mostly arranged parallel to the axis of the nerve, but sometimes they are obliquely oriented or perpendicular to the axis. According to the studies by Lundborg (1, 6), characteristic u-shaped loop anastomoses are frequent. Interfascicular vessels communicate with interfascicular vessels by numerous anastomoses, which often pierce the perineural layer obliquely.

Stereoscopic views of the resin casts of peripheral nerves showed the intrinsic vascular system of epineural, perineural and endoneural layers and also its communications with the vascular architecture. In particular, the microvascular network was well demonstrated by the injection replica SEM method. The main arterioles and venules ran longitudinally but their anastomosis was rare in the sciatic and posterior tibial nerves. However, the capillary network was very close and had many communications with in the epineural layer. These findings are different from previous studies which confirmed a number of anastomoses and shunts in epineural vessels. The difference may be due to the method used in the previous works; heaping of the vessels can be observed by microangiography and light microscopic techniques. On the other hand, the close capillary meshwork with a very thick interconnection among the epineural arterioles and venules can have resistance to regional compression and tension. However, there is little anastomosis of vessels between the epineural and endoneural layers, although longitudinal vessels in the endoneurium are abundant. In the interfascicular vascular system, a number of capillaries with a wavy pattern were grouped longitudinally and made a capillary bed. They were independent and made individual vascular systems. Among such vascular systems,
there were few anastomotic vessels. This capillary architecture is different from
the description by Kuczynski (13) and Lundborg (1, 6), which describes mesh-
work communicating very closely.

The epineural meshwork of the capillary is a strong structure with consid-
erable resistance to mechanical trauma (14). Concerning the mechanical is-
chaemia, Sunderland (15) suggested that an increase in the pressure of intra-
neural venules secondary to increased tissue pressure such as in the carpal
tunnel syndrome constitutes the initial pathological change. Intraneural lon-
titudinal venules can be affected by the pressure increase at the localized area;
however, intrafascicular vasculature made of a group of numerous capillaries
seem to resist pressure and tension. According to the findings, the develop-
ment of vascular architecture is influenced very little by compression and stretching.
This results in an unbroken intraneural vascular network, peculiar to the per-
ipheral nerves.

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Microvasculature of Peripheral Nerves
