Effects of Arterial Ischemia And Venous Congestion on The Lumbar Nerve Root in Dogs

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ABSTRACT

The development of radiculopathy in patients with lumbar canal stenosis is thought to be closely related to intraradicular edema resulting from compression. However, there is little agreement as to which is more essential for intermittent claudication, ischemia or congestion. The aim of the present experimental investigation was to examine the effect of ischemia and congestion on the nerve root using dogs. The aorta was clamped as an ischemia model of the nerve root and the inferior vena cava was clamped as a congestion model at the 6th costal level for 30 minutes using forceps transpleurally. Measurements of blood flow, partial oxygen pressure, and conduction velocity in the nerve root were repeated over a period of one hour after release of clamping. Finally, we examined the status of intraradicular blood-nerve barrier under fluorescence and transmission electron microscope. Immediately after clamping of the inferior vena cava, the central venous pressure increased by about 4 times and marked extravasation of protein tracers was induced in the lumbar nerve root. Blood flow, partial oxygen pressure and conduction velocity of the nerve root were more severely affected by aorta clamp but this ischemia model did not show any intraradicular edema. The blood-nerve barrier in the nerve root was more easily broken by venous congestion than by arterial ischemia. In conclusion, venous congestion may be an essential factor precipitating circulatory disturbance in compressed nerve roots and inducing neurogenic intermittent claudication.
INTRODUCTION

It is well known that symptom of lumbar canal stenosis, neurogenic intermittent claudication, occurs when patients are standing or walking.\(^1\)\(^-\)\(^4\) Although there have been many reports about neurogenic intermittent claudication associated with lumbar canal stenosis, the pathogenesis of claudication has not yet been completely clarified. Blau and Logue\(^5\) postulated that neurogenic intermittent claudication might be evoked with ischemic neuritis of the cauda equina. Evans\(^6\) advocated exercise-induced ischemia, as the cause of intermittent claudication which is the characteristic syndrome of this disease. They supposed that reduced blood flow in the spinal nerve roots has been demonstrated during exercise, and this might contribute to the pathogenesis of neurogenic claudication. Ehni et al.\(^7\) stressed the postural changes in the lordotic position, but flexion permitted the contrast medium to pass. Yamada et al.\(^8\) reported the importance of intermittent constriction of the cauda equina associated with postural change. They thought that the ligamentous fluvum had a significant role in dynamic narrowing of the canal. Kavanaugh et al.\(^9\) reported that the increase of cerebrospinal fluid pressure below the blocked area might obstruct venous return and be cause of anoxia of the cauda equina. Verbiest thought that this theory deserved further consideration, since he commonly found enlargement of the epidural venous plexus during decompression of spinal canal stenosis.\(^10\)

Basic research investigations in this aspect, however, are quite few and details of circulatory problems in the nerve roots are not so well understood as in the peripheral nerve. The development of neurogenic intermittent claudication in patients with lumbar canal stenosis is thought to be closely related to intraradicular edema resulting from compression.\(^11\)\(^-\)\(^13\) However, the basic pathophysiology of circulatory disturbance induced by ischemia and congestion is not fully understood. The aim of the present experimental investigation was to examine the effect of ischemia and congestion on the nerve root.

MATERIALS AND METHODS

The experiment was carried out under the control of the local animal ethics committee in accordance with the guidelines on animal experiments in our university, Japanese government animal protection and management law, and Japanese government notification on feeding and safekeeping of animals. Forty adult dogs, weighing 7 to 15kg, were anesthetized with intramuscular injection of 3 ml of Ketalar (Ketamine 50mg/ml; Warner-Lumbert, Morris Plains, NJ) and ventilated on a respirator under general anesthesia (O\(_2\): 3ml/min, N\(_2\)O: 3ml/min, Halothane: 1.5ml/min). The femoral artery and vein were canulated, and arterial blood pressure and central venous pressure (CVP) were monitored in all animals throughout the experiment. A tip of the arterial catheter was placed in the femoral artery and a tip of the venous catheter was inserted into the abdominal vena cava through the femoral vein. Animals were maintained at constant physiologic levels during experiment. Each animal was placed in the prone position on a flame. At first, the sixth and seventh lumbar laminae were removed, and the seventh lumbar nerve root was exposed widely. Next, the sixth rib was removed and the transpleural approach was used to access the Aorta and inferior vena cava. Aorta was clamped as an ischemia model of the nerve root (Fig.1A) and inferior vena cava was clamped as a congestion model (Fig.1B) at the 6\(^{th}\) costal level for 30 minutes using forceps transpleurally. Measurements of blood flow, partial oxygen pressure (PO\(_2\)), and conduction velocity in the nerve root were repeated over a period of thirty minute after release of clamping.

**Measurement of intraradicular blood flow**

To measure the intraradicular blood flow in 10 animals, a tissue blood flow meter
(DHM-3001, M.T. Giken Co., Tokyo, Japan) was employed. A small electrode with a diameter of 200 μm (MHD-60: M.T. Giken Co., Tokyo, Japan) was inserted into the dorsal root at midpoint between the dural sac and the dorsal root ganglion.\textsuperscript{14,15} We used a polarized voltage of 600 mV and a direct current of 20 A for 25 seconds to electrochemically generate hydrogen in the dorsal root. We measured the blood flow twice before clamping the Aorta (n=5) or vena cava (n=5), in order to establish the average intraradicular blood flow in animals.

**Measurement of intraradicular PO\textsubscript{2}**

PO\textsubscript{2} in the nerve root was measured by the polarographical method (Model POG-5000S PO\textsubscript{2} Meter: M.T. Giken Co., Tokyo, Japan).\textsuperscript{16,17} After calibration, PO\textsubscript{2} needle type electrode with a diameter of 10 μm (POE-10N: M.T. Giken Co., Tokyo, Japan) was inserted into the dorsal root at midpoint between the dural sac and the dorsal root ganglion. After intraradicular PO\textsubscript{2} stabilized, we recorded it twice and calculated the average value of the normal state before clamping the Aorta (n=5) or vena cava (n=5).

**Electrophysiological study**

Nerve root conduction studies were carried out using electro-myometer (Neuromatic 2000 DISA electromyography unit, Dantec Electronics, Bristol, U.K.) using 10 animals. The sciatic nerve was exposed at the thigh and stimulated with 0.1-msec square-wave voltage pulses at a rate of 2/s using a bipolar electrode. The stimulus intensity was adjusted to 1.5-2 times of the motor threshold and 20 responses were summated. The evoked potentials were recorded directly from the dorsal root at the entry site of the dural sac and proximal to the dorsal root ganglion after clamping the Aorta (n=5) or vena cava (n=5), and then the sensory nerve conduction velocity was calculated by “the distance between 2 site (m) / the time lag between 2 site (S).”

**Preparation for fluorescence and transmission electron microscopic study**

Finally, we examine the status of the intraradicular blood-nerve barrier under fluorescence and transmission electron microscope after injection of Evans blue albumin (EBA) and Horseradish peroxidase (HRP) into the cephalic vein to find out what sort of circulatory disturbance occurred in the nerve root.\textsuperscript{11} After clamping of the Aorta (n=5) or the vena cava (n=5) for 30 minutes, EBA (10ml/kg, molecular weight approximately 59,000) and HRP (type-II, molecular weight approximately 43,000 Sigma Co., St.Louis, MO.) were injected intravenously and allowed to circulate for 30 minutes. EBA was prepared by mixing 5% bovine albumin (Wako Chemical Co., Osaka, Japan) with 1% Evans blue (Sigma Chemical Co., St.Louis, MO.). The animals were fixed by intraraortal perfusion with 4% paraformaldehyde and 1% glutaraldehyde in 0.15 M cacodylate buffer, pH 7.2 at 20°C. The nerve root sections were resected at the midpoint between the dural sac and the dorsal root ganglion, and at the outlet of dural sleeve of 7\textsuperscript{th} lumbar nerve root bilaterally. These sections were divided into 2 groups. After the nerve root sections fixed with 4% paraformaldehyde for 24 hours, twenty-micron thick sections were mounted with 50% aqueous glycerin to be examined under the fluorescence microscope at 380 m\textmu W (BX-51, Olympus, Tokyo, Japan). The other sections were rinsed in 0.05M tris-HCl buffer, postfixed at room temperature for 3 hours in 2% OsO\textsubscript{4} in 0.1 M sodium cacodylate buffer, impregnated with 2% uranyl acetate, dehydrated in graded ethanol, and embedded in epoxy resin. For light microscopy, 1-3 μm thick toluidin blue stained sections were used. For electron microscopy, ultrathin sections contrasted with uranyl acetate and lead citrate were examined a under electron microscope (JSM2000, Nippon Denshi, Tokyo, Japan).
Statistical analysis

From the record of each animal in both groups, arterial blood flow, CVP, intraradicular blood flow, PO2 and conduction velocity during experiment were determined. Unless otherwise stated, data are presented as the mean ± the standard error of the mean (SEM) of at least five separate experiments. And also, the averaged data were expressed as percentage of the average value before clamping of Aorta or inferior vena cava. Comparison values were performed using a repeated-measures analysis of variance and post hoc (Scheffe) compared before and after clamping of Aorta or inferior vena cava. Date were entered into a database and analyzed by using SPSS statistical soft-ware, version 14.0.J (SPSS Inc, Chicago, IL). A probability of 5% was considered statistically significant.

RESULTS

Immediately after Aorta clamping, blood pressure in the femoral artery dropped to 26-40 mmHg and meantime (p<0.05) (Fig.2A), central venous pressure was slightly elevated (Fig.2B). When the vena cava was clamped, central venous pressure increased to about 4 times of the pressure before clamping (p<0.05) (Fig.2B) and blood pressure in the femoral artery was reduced by half (p<0.05) (Fig.2A). After release of clamping, both arterial and venous pressures quickly returned to the pressure before clamping.

The blood flow in the seventh posterior nerve root due to Aorta and vena cava clamping fall to 50 to 60% of the blood flow before clamping in the ischemic model (P<0.05) and to about 20% in the congestion model (p<0.05). When the clamp was released, the intraradicular blood flow in the congestion model was restored with in 1 hour. The intraradicular blood flow in the ischemic model, however, did not recover and stayed at the reduced level (P<0.05) (Fig.3A) (Table 1). The changes of PO2 in the nerve root indicated a similar tendency to blood flow, 50 to 60% drop in the ischemic model (p<0.05) and 20 to 40% drop in the congestion model. After release of clamping, intraradicular PO2 recovered completely in both models (Fig.3B) (Table 1). Conduction velocity of the nerve root diminished by 40 to 50 % in the ischemia model (p<0.05) and 10 to 20% in the congestion model. This drop of conduction velocity returned almost completely within one hour after release of clamping (Fig.3C) (Table 1).

After intravenous injection of protein tracers, marked extravasation of the tracers in the 7th lumbar nerve root bilaterally was induced after 30 minutes clamping of the vena cava (Fig.4B,5C,D) but not by Aorta clamping (Fig.4A,5A,B). There was no extravasation of EBA and the blood-nerve barrier was maintained in the nerve root after clamping of the Aorta (Fig.4A). After clamping of the vena cava, however, the red fluorescence of EBA was seen outside the endoneurial microvessels and diffusely through out the endoneurial space of the nerve root (Fig.4B). HRP had the same distribution as the EBA tracer. By electron microscopy, capillaries of the nerve root were shown to have an inner diameter of approximately 5 to 10 μm. After clamping of the Aorta, HRP product did not appear in the endoneurial space when it was injected intravenously (Fig.5A) and their endothelial cells are linked by tight junctions (zonulae occludens) showing the existence of a blood-nerve barrier (Fig.5B). Some HRP-labeled vesicles related to paracellular transport were detected in the endothelial cells. However, these vesicles didn’t appear to carry the product away from capillary to the endoneurial space. After clamping of the vena cava, the extravasation of HRP tracer was observed in endoneurial spaces between the nerve fibers, especially the perivascular spaces (Fig.5C). The tight junction between the endothelial cells appeared to intact. However, the pinocytotic vesicles in the endothelium appeared to carry the dark-stained HRP product away from the capillay lumen and the product extravasated into the endoneurial space (Fig.5D). This increased trancellular transport of the tracer indicates breakdown of the blood-nerve
barrier, leading to edema formation in the nerve root.

DISCUSSION

Ischemic nerve root injury is a ubiquitous insult that can lead to a wide range of neuropathologic consequences, depending on the severity and duration of the ischemic event. Ischemic injury to nerve roots predominantly causes demyelination, although prolonged ischemia can also interfere with axonal transport, leading to axonal damage and Wallerian degeneration of the nerve fiber. Vascular damage and fibrosis are common findings within the spinal canal and intervertebral foramina, and such vascular damage is significantly related to the severity of degenerative disc disease. Disc protrusion may lead to compression of epidural veins and dilation of non-compressed veins. Cooper et al. noted a significant relationship between evidence of venous obstruction, intraneurial and perineurial fibrosis, and neural atrophy. Fibrosis may further impede nutrient transfer to endoneurial fibers, as well as predisposing to nerve stretch injury. The present study assessed the influence of arterial ischemia and venous congestion resulting from obstruction of blood flow without nerve root compression on intraradicular blood flow and radicular function. As a result, it was confirmed that nerve root ischemia had a more serious influence on blood flow, PO2, and conduction velocity than nerve root congestion. After 30 min of nerve root ischemia, recovery occurred with reperfusion, but longer ischemic periods will cause a permanent effect on radicular function due to oxygen deficiency. When changes of the femoral arterial and central venous pressures were monitored after obstruction of blood flow, both the arterial and venous pressures decreased after aortic blockade and the arterial pressure increased slightly after obstruction of the inferior vena cava. However, the central venous pressure showed an approximately 4-fold increase immediately after obstruction of the inferior vena cava, and this sudden increase in venous pressure could have a marked influence on the capillary pressure in the nerve roots. Usubiaga et al. demonstrated that clamping of the vena cava can be used experimentally to increase the systemic venous pressure. The same maneuver also produces congestion of the epidural veins and increases the epidural pressure, but they did not describe the changes of nerve root circulation.

The arachnoid membrane acts as a diffusion barrier for the nerve root and the blood-nerve barrier is also created by the vascular endothelial cells of the endoneurial microvessels. These nerve root barriers protect and maintain the nerve fibers in a constant environment. The capillary vessels of the nerve roots are lined by endothelial cells that contain only a few pinocytic vesicles and are bound by tight junctions to form the blood-nerve barrier. Protein tracers that are injected intravenously do not normally leak out of the vessels due to this barrier. When arterial ischemia was induced, protein tracers remained in the blood vessels, indicating maintenance of the integrity of the blood-nerve barrier (Fig.4A, 5A,B). On the other hand, venous congestion disrupted the blood-nerve barrier and there was extravasation and edema in the nerve roots (Fig.4B, 5C,D). Thus, the blood-nerve barrier that regulates vascular permeability in the nerve root seems to be susceptible to congestion which raises the intra vascular pressure rather than to ischemia which decreases the pressure.

An experiment performed by Olmarker demonstrated that the capillaries and venules of the nerve root could be occluded by mild compression of around 30-40 mmHg. Takahashi et al. found that the epidural pressure is only 15 to 18 mmHg during lumbar flexion in LSCS patients, but reaches 80 to 100 mm Hg during lumbar extension. The epidural pressure increases with walking and the patient then stops walking because of leg pain and/or claudication. The pressure decreases immediately after walking is stopped and leg pain then subsides. There is a repeated pattern of increasing and decreasing pressure during walking. Although, these pressure changes are not great enough to disturb arterial blood flow, the
epidural venous system may become congested if the pressure is higher than 10 to 30 mmHg. Accordingly, in patients with symptomatic stenosis, the subarachnoid space is obstructed and the cauda equina nerve roots undergo strangulation by the diffusion barrier in the arachnoid membrane, while the nutritional pathway to the nerve roots from the CSF is blocked and intermittent increases of compression may damage the nerve tissue.

It is known that sites of nerve root compression by spinal canal stenosis frequently show gadolinium enhancement on MR images, suggesting that there is breakdown of the blood-nerve barrier and edema of the nerve root. In lumbar spinal stenosis associated with intermittent claudication, Jinkins et al. first reported gadolinium enhancement of the cauda equina above the level of stenosis. Our previous experimental study showed that the blood-nerve barrier of the nerve root is disrupted and intraradicular edema is produced by acute compression with a microsurgical clip at more than 15 g of force for one hour or by chronic compression due to wrapping the nerve root for at least one month with a silastic tube slightly larger than the nerve root diameter. In patients with compression radiculopathy, total circumferential compression of the cauda equina associated with closure of the subarachnoid space is assumed to block all routes for the supply of nourishment and removal of waste via the CSF, thereby triggering various disorders in combination with chemical factors released by inflammatory cells. Elevation of the capillary pressure induced by venous stasis is thought to cause intraradicular edema and the inflammatory response produced by compression, as well as mechanical damage to the blood-nerve barrier, because venous blood flow is stopped by compression at a very low pressure. As a result, the subarachnoid space is occluded, and congestion as well as nerve fiber degeneration occurs in the cauda equina. Efflux of excess fluid into the subarachnoid space is impaired by breakdown of the blood-nerve barrier, leading to an increase of endoneurial pressure. Although this pressure increase is reversible, a compartment syndrome may occur in the cauda equina at the site of stenosis that disturbs blood flow and axonal flow, provoking ectopic discharges or conduction disturbance that is essentially responsible for NIC in nerve fibers with chronic damage. Ikawa et al. demonstrated that ectopic firing was elicited by venous stasis in a rat model of lumbar canal stenosis. Thus, venous congestion may be an essential factor precipitating circulatory disturbance in compressed nerve roots and inducing neurogenic intermittent claudication.

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LEGENDS

Figure 1. Aorta was clamped as an ischemia model of the nerve root (A) and inferior venacava was clamped as a congestion model (B) at the 6'th costal level for 30 minutes using forceps transpleurally.

Figure 2. Changes of blood pressure after Aorta or inferior vena cava clamp. (A) Changes of blood pressure in femoral artery.(B) Changes of central venous pressure (CVP) (Scheffe, *, #<0.05)

Figure 3. Changes of intraradicular blood flow (A), PO2 (B) and conduction velocity (C) after Aorta or inferior vena cava clamp. The averaged data were expressed as percentage of the average value before clamping. (Scheffe, *, #<0.05)

Figure 4. Transverse sections of the nerve root seen under a fluorescence microscope. (A) Ischemia model. EBA emits a bright red fluorescence in clear contrast to the green fluorescence of the nerve tissue. After intravenous injection of EBA, EBA was limited inside the blood vessels, and the blood-nerve barrier was maintained. (B) Congestion model. EBA emits a bright red fluorescence, which leaked outside the blood vessels, and intraradicular edema was seen under a fluorescent microscope.
Figure 5. Transverse sections of capillary in the nerve root seen under an electron microscope. (A,B) Ischemia model. After intravenous injection of HRP, the tracer was blocked at the tight junction to extravasate. The pinocytotic vesicles contain the reaction product seen in the endothelial cells. However, HRP is not observed in the endoneurial space. (C,D) Congestion model. After intravenous injection of HRP, the electron-dense reaction product of HRP passed through endothelial cells and entered to the endoneurial space. In the endothelial cell of the capillary, pinocytotic vesicles containing the reaction product, about 50-70 nm in diameter, are thought to move toward the perivascular (endoneurial) space by vesicular transport. However, HRP was blocked at the tight junction to extravasate. A: arachnoid membrane, D: dura mater, E: endothelial cell, En: endoneurial space, L: capillary lumen, P: pinocytotic vesicle, T: tight junction.

Table 1. Changes of intraradicular blood flow, PO2 and conduction velocity after Aorta (A) or inferior vena cava clamp (B).