Circulatory disturbance of the spinal cord with epidural neoplasm in rats

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An experimental model of spinal epidural neoplasm was produced in rats by injecting Walker 256 carcinoma cell suspension anterior to the T12-13 vertebral body. With this model, spinal cord blood flow (SCBF) and its response to CO₂ inhalation were estimated by the carbon-14-antipyrine autoradiography and the hydrogen clearance methods. In the early stages after tumor implantation, weakness, axonal swelling, and edema of the white matter were observed, while both SCBF and its response to CO₂ inhalation remained normal. In the next stage, the tumor invaded the spinal canal and compressed the spinal cord epidurally. The edema of the white matter progressed, while the gray matter was morphologically intact. The SCBF and its response to CO₂ inhalation were altered at both the compression area and caudally in the spinal cord. Changes in response to CO₂ inhalation appeared earlier than the SCBF decrease. In the last stage, the SCBF decreased rapidly to the critical level, producing irreversible nervous tissue damage. Microangiographic studies revealed extensive obliteration of the spinal epidural venous plexus and patency of the larger nutritional vessels. From the data obtained, the progressive vascular pathophysiology related to spinal epidural neoplasm is as follows: 1) the vertebral venous plexus is compressed and obliterated in the early stages of the disease, and vasogenic edema appears in the spinal cord; 2) as the tumor grows, mechanical compression of the spinal cord is added and the circulatory disturbance increases; and 3) in the last stage, SCBF decreases rapidly to a critical flow level, and the loss of cord function becomes irreversible.

KEY WORDS • spinal cord compression • spinal tumor • spinal cord blood flow • paraplegia

Spinal cord compression by epidural metastasis is a serious neurological complication of systemic cancer. Barron, et al., reported that up to 5% of patients with malignancy suffered from this complication. This figure has been increasing recently because of progress in cancer treatment with prolonged patient life and increased opportunity for metastasis.

To investigate the pathophysiology, Ushio and his colleagues developed an experimental model of spinal epidural neoplasm that mimics human disease. Subsequent studies using this model revealed the importance of vasogenic edema and blood-spinal cord barrier dysfunction as a cause of neurological deficit.

In this investigation, changes in spinal cord blood flow (SCBF) and its response to CO₂ inhalation were studied using the rat spinal epidural neoplasm model. The relationship of regional circulatory disturbances to progression in neurological deficits was also investigated.

Materials and Methods

Experimental Model

Details of the experimental model have been given. A Walker 256 carcinoma maintained by serial subcutaneous transplantation was placed into cell suspension, with a concentration of 1 × 10⁶ viable tumor cells per milliliter of Earle’s basic medium. A dose of 0.1 ml tumor cell suspension was injected percutaneously anterior to the T-12 or T-13 vertebral body of female Sprague-Dawley rats weighing 150 to 180 gm each. After inoculation, the animals were observed daily and graded with respect to hindlimb function (Table 1).

Blood Flow Study

Thirty rats harboring the epidural neoplasm and displaying hindlimb paresis of various grades and six control rats were used to study SCBF and its response
Circulation in spinal epidural tumor model

TABLE 1

<table>
<thead>
<tr>
<th>Grade</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>normal</td>
</tr>
<tr>
<td>1</td>
<td>slight weakness: hip instability observed when animal runs or jumps</td>
</tr>
<tr>
<td>2</td>
<td>mild weakness: animal is able to run</td>
</tr>
<tr>
<td>3</td>
<td>moderate weakness: animal is able to walk but unable to run</td>
</tr>
<tr>
<td>4</td>
<td>marked weakness: animal can stand but is unable to walk</td>
</tr>
<tr>
<td>5</td>
<td>severe weakness: animal cannot stand and there is only a slight movement of the legs</td>
</tr>
<tr>
<td>6</td>
<td>paraplegia: no movement is observed</td>
</tr>
</tbody>
</table>

to CO₂ inhalation by the hydrogen clearance method¹ as shown in Fig. 1. The rats were anesthetized with intraperitoneal ketamine hydrochloride (50 mg/kg), and a femoral artery was cannulated. A midline dorsal skin incision was made from T-3 to T-9. Three spinous processes were removed at the level of maximum cord compression (T12-13), and at two segments rostral (T10-11) and two segments caudal (L1-2) to that level. Tiny burr holes (about 0.5 mm in diameter) were made in each lamina. Hydrogen electrodes were prepared using a fine platinum wire (100 μm in diameter) with a 1.5-mm exposure at the tip. The electrodes were implanted under a microscope through the burr holes into the spinal cord slightly to the right of the midline, usually adjacent to the median spinal vein. The electrode was fixed to the spine with Oxycel cotton and adhesive. The electrode was allowed to float with a flexible copper lead in order to prevent electrode dislocation by respiratory movement. An Ag/AgCl reference electrode was placed subcutaneously. The head of the rat was covered with a plastic chamber for H₂ or CO₂ inhalation. After 30 minutes of electrode stabilization, the animal was saturated with a 7% H₂ and air mixture. Then the H₂ supply was discontinued and clearance curves were obtained. Regional SCBF was calculated by the initial-slope method. Spinal cord blood flow response to CO₂ was estimated by the hydrogen clearance method with the inhalation of a 3%, 5%, 7%, and 10% mixture of CO₂ and air. Autopsies were performed in all animals for identification of electrode position and histopathological examination.

Changes in SCBF were also investigated topographically by the carbon-14 (¹⁴C)-antipyrine autoradiographic method¹⁸ in 17 rats showing variously graded neurological deficits. Details of the procedure have been described elsewhere.²⁷ Preparation of the animals was similar to that for the hydrogen clearance method, and 75 μCi/kg of ¹⁴C-antipyrine was infused intravenously for 60 seconds. Arterial blood samples were withdrawn at 5- to 8-second intervals, and animals were decapitated at the end of the tracer infusion. The spinal cord was removed immediately, frozen with dry ice, cut into 40-μm serial longitudinal sections, and prepared for autoradiography. The tissue-blood partition coefficient for antipyrine was measured using four rats with Grade 6 weakness by injecting 30 μCi of ¹⁴C-antipyrine intravenously. After 60 minutes the animals were decapitated and the spinal cords prepared for autoradiography. The partition coefficient was calculated for both gray matter and white matter using the blood tracer concentration. A densitometer and a microcomputer were employed for analysis.

Miscellaneous physiological parameters, including arterial blood pressure, arterial blood gas, and body temperature, were monitored periodically during the blood flow study. Animals with abnormal parameters were omitted from the study.

Microangiography

Six animals with Grade 5 to 6 weakness were used for microangiographic study by a method described previously.¹¹ The animals were anesthetized and heparinized, and cannulas were placed into the left femoral vein and ascending aorta. The right atrium was opened for drainage. The animals were placed in a warm bath, perfused initially with 150 ml of saline with 5% sucrose added, and then with 100 ml of fixative (2.5% glutaraldehyde and 5% sucrose mixture) via the ascending aorta.

Three or 4 ml of india ink with 5% gelatin added was injected slowly into the femoral vein to stain the venous system after clipping of the inferior vena cava. The inferior vena cava was then reopened and Micropaque solution (40% barium sulphate and 5% gelatin mixture) was injected via the ascending aorta to stain the arterial system. At the end of the procedure, the inferior and superior venae cavae, ascending aorta, and left femoral vein were ligated. All infused liquids were warmed to 37°C, and infusion pressure was monitored to maintain a level of 120 to 130 mm Hg. The animals were skinned,

FIG. 2. A coronal section of the spine in a rat with Grade 6 weakness. The tumor grows in the retroperitoneal space, invading the spinal canal and compressing the spinal cord.

immersed in chilled formalin for a week, decalcified, dehydrated, and placed in 98% methylsalicylate to render the tissue transparent.

Results

Physical Observation

Most of the rats developed hindlimb weakness (Grade 1, see Table 1) about 10 to 15 days after tumor inoculation, and became paraplegic within 3 days after they reached Grade 3 weakness. Bladder and bowel incontinence accompanied paraplegia.

Autopsy Findings

The tumor situated at the thoracolumbar junction protruded into both the thoracic and abdominal cavities, surrounded the vertebral bodies, extended through the intervertebral foramina into the epidural space, and compressed the spinal cord. The compression was usually ventrolateral or dorsolateral; however, in some animals the cord was squeezed by circumferential tumor (Fig. 2). The large vessels (anterior and posterior spinal arteries and median spinal veins) were compressed and stretched. However, they were patent even in the advanced stage of compression.

Edema of white matter and axonal swelling were observed at the early stage of compression. Both became more prominent as the compression progressed. In comparison to the white matter, the neuronal areas of gray matter were well preserved until the end stage of compression. Hemorrhages in the gray matter were rarely observed.

Fig. 3. Changes in gray matter spinal cord blood flow (SCBF, solid lines), and its response to CO2 inhalation (dashed lines) at T10–11 (rostral), T12–13 (compression site), and L1–2 (caudal) cord levels measured by the hydrogen clearance method. The reduction of SCBF is shown only at the compression site and the rostral level. The CO2 response effect is more sensitive than is SCBF. For a definition of grade of weakness see Table 1.
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Hydrogen Clearance Study

The position of the electrodes was verified histopathologically. Most of the electrode tracks were on a line that connects the dorsal horn and ventral horn, and the electrodes' recording surface was assumed to be in the gray matter. Hemorrhage at the electrode tracks was rarely observed.

The normal control SCBF values (mean ± standard error of the mean (SEM), in ml/100 gm/min) were: 35.9 ± 3.2 at T10-11, 45.0 ± 3.5 at T12-13, and 46.0 ± 5.2 at L1-2. There was a tendency for the SCBF at T10-11 to be slightly lower than at the T12-13 (p < 0.1) or L1-2 (p < 0.2) levels. The SCBF values remained within the normal range in the early stage of the weakness (Grades 1 and 2); however, they decreased gradually with further progression of the weakness not only at the compression site but also caudal to it. When the animal became completely paraplegic (Grade 6), SCBF values decreased severely: 14.9 ± 0.6 ml/100 gm/min at the compression site (T12-13) and 20.3 ± 0.6 ml/100 gm/min at the caudal level (L1-2). The SCBF remained unchanged at the T10-11 rostral level: 27.5 ± 3.8 ml/100 gm/min (Fig. 3).

Inhalation of CO₂ made the animal respire at a higher rate than normal, with slightly increased PaO₂ and slightly decreased pH. The PaCO₂ increased to 105 mm Hg; however, only a minimal change in blood pressure was observed. There was a straight-line relationship between PaCO₂ and SCBF as far as a PaCO₂ of 80 to 90 mm Hg. The SCBF response to CO₂ inhalation was expressed as [ΔSCBF/ΔPaCO₂/SCBF (at PaCO₂ = 35 mm Hg) × 100].

In the normal control animals, mean (± SEM) SCBF response to CO₂ inhalation was: 6.0 ± 0.9 mm Hg at T10-11, 5.9 ± 1.1 mm Hg at T12-13, and 7.0 ± 0.4 mm Hg at L1-2. There was no significant difference among these values. The SCBF response was disturbed at the compression and caudal levels of the spinal cord commensurate with the SCBF decrease; however, it appeared to be more susceptible to change than was SCBF. In the animals with Grade 6 weakness, SCBF response to CO₂ inhalation was 4.1 ± 0.9 mm Hg at T10-11, 0.48 ± 0.17 mm Hg at T12-13, and 1.28 ± 0.47 mm Hg at L1-2. The SCBF response at rostral levels was not affected throughout the extent of the compression (Fig. 3).

14C-Antipyrine Study

The antipyrine partition coefficients (mean ± SEM) were 1.08 ± 0.10 for gray matter, 0.77 ± 0.04 for white matter, 1.02 ± 0.18 for peritumoral gray matter, and 0.71 ± 0.09 for peritumoral white matter. There was no significant difference between values of normal and peritumoral cord tissue. These values were used for calculation of SCBF by 14C-antipyrine autoradiography. In the normal control rats, the lower thoracic SCBF was slightly below lumbar SCBF. As the grade of compression progressed, the SCBF decreased at the compression site and in caudal levels similarly to the results of the hydrogen clearance method (Fig. 4).

Microangiography Findings

For microangiographic study, the arteries were stained white and veins were stained black (Fig. 5). At the level of tumor compression, the spinal arteries branching from the lower part of the intercostal arteries, subcostal artery, and the upper part of the lumbar arteries were stretched; however, they remained patent even when surrounded by tumor. The spinal root arteries were compressed laterally, and the number of ventral root arteries was not decreased. In three animals, the great ventral artery of Adamkiewicz was identified at the T-12 or T-13 vertebral level and was patent.

The epidural venous plexus was observed angiographically to form an arcuate pattern at the upper thoracic and lower lumbar levels. The staining of the epidural venous plexus was interrupted around the compression site, and the radicular veins were not observed. The dorsal median vein was the only draining vein at the level of cord compression except for an occasional patent ventral median vein.

Discussion

The animal model used mimics human spinal epidural metastasis both clinically and histopathologically. The animals deteriorated rapidly once neurological symptoms started. The gray matter of the spinal cord was well preserved morphologically, while the white matter tissue showed a remarkable edematous change. The histological change was essentially similar to the "edematous type of malacia" seen in human disease.

The SCBF at the compression site did not remarkably decrease in the early and mid stages of the disease. This observation may support the hypothesis that vasogenic edema caused by obliteration of the spinal venous plexus by tumor plays a more important role in causing symptoms than does blood flow reduction or direct cord compression. There are several reports of spinal cord deficits caused by disturbances in venous drainage. For example, Hughes reported a case of "spinal venous infarction." The patient was a 67-year-old man with pancreatic cancer, who became paraplegic 1 week after the initial paresis. Autopsy revealed an extensive spinal cord infarction associated with thrombosis of the inferior vena cava. Vasogenic edema, demyelination, and swelling of the veins on the cord surface are frequently seen in patients with spinal epidural neoplasm. These observations are identical to those described in the clinical report of "venous infarction" mentioned above. In our study, rats developed neurological symptoms (Grade 1 to 2 weakness) without any mass in the spinal canal. This may indicate that the initial weakness was not due to direct compression by the tumor but to the disturbance of venous drainage caused by obstruction of the spinal venous plexus by the tumor.
Venous congestion by itself cannot explain all the pathophysiology of the spinal epidural neoplasm. Venous congestion usually produces hemorrhages in the spinal cord, while spinal epidural neoplasms do not. Shin demonstrated experimentally in dogs that screw compression obliterating the spinal venous plexus caused the spinal demyelination in venous congestion to be more severe and extensive without marked changes in the gray matter. The histological changes he obtained resemble those seen in rats with Grade 3 to 4 weakness. Thus, two factors may be involved in the mechanism promoting the middle stages of spinal epidural neoplasm development: venous congestion and direct compression by tumor.

In the terminal stage of spinal epidural neoplasm (Grades 5 and 6), SCBF decreased rapidly at the compression site and caudally. As shown in a previous microangiographic study, the larger vessels are relatively well preserved, while the smaller arterioles or capillaries are narrowed and obstructed. Therefore, the reduction of SCBF would not be caused by the obliteration of extramedullary nutritional vessels, such as radicular arteries, which might explain why spinal cord hemorrhage was rare. Spinal cord ischemia should be considered to be a third factor in the pathophysiology of spinal epidural neoplasm (Fig. 6).

From a therapeutic point of view, SCBF is the most important factor determining whether cord function is preserved or not. The blood flow observed at the compression site in rats with Grade 6 weakness (14.5 to 16.6 ml/100 gm/min) is considered to be a critically low level that will cause irreversible damage to the nervous tissue. Permanent damage will occur unless immediate treatment is undertaken at this stage.

In this study, the disturbance of SCBF and its response to CO₂ inhalation developed not only at the compression site but also at caudal levels. The blood flow from the spinal cord empties into the epidural venous plexus and drains partly into the azygous system and partly into the inferior vena cava through the intercostal and intervertebral veins. The lower part of the azygous system and channels to the inferior vena cava can easily be obstructed by the tumor. As a result, venous congestion seems to have developed in the caudal part rather than in the rostral part. This view is also supported by the evidence that the SCBF response to CO₂ inhalation was disturbed earlier than the SCBF. The back pressure due to the venous congestion may prevent flow increase by hypercapnic vasodilatation. A schematic diagram of this hypothetical pathophysiology is shown in Fig. 6.

Treatments such as decompressive surgery, radiation therapy, chemotherapy, and administration of high-dose steroids have been used for patients with spinal epidural metastasis. The optimum treatment modality is not yet established. As shown in this study, venous congestion due to obstruction of the spinal epidural venous plexus may be an important factor in the pathophysiology of spinal epidural neoplasm.
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Fig. 6. Schematic diagram showing a proposed pathophysiology in the spinal cord with epidural tumor. SCBF = spinal cord blood flow.

factor not only in understanding the pathophysiology but also in planning the therapy of this disease.

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References


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