Edema and circulatory disturbance in the spinal cord compressed by epidural neoplasms in rabbits

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An experimental model of spinal cord compression by epidural neoplasms was produced in rabbits by injecting a VX2 tumor-cell suspension anterior to the T-13 vertebral body. With this experimental model, edema and circulatory disturbance of the spinal cord compressed by epidural tumors were studied. The characteristic histopathological findings in the compressed spinal cord were edema and axonal swelling in the white matter. Water content and uptake of intravenously injected 99mTc pertechnetate in the compressed spinal cord were significantly greater than in the spinal cord distant from the tumor, and increased in proportion to the degree of neurological loss. Microangiography and fluorescein angiography demonstrated stenosis or obstruction of the epidural venous plexus and impairment of venous drainage in the compressed spinal cord at the early stage of neurological symptoms. It is suggested that venous stasis and subsequent vasogenic edema in the spinal cord play an important role in the symptomatology of metastatic epidural spinal cord compression.

KEY WORDS □ spinal epidural neoplasm □ spinal cord compression □ spinal cord edema □ blood-spinal cord barrier □ spinal cord circulation

Epidural metastasis compressing the spinal cord is a frequent and serious complication of systemic cancer. About 5% of patients with systemic cancer develop spinal cord compression at some time during the course of their disease. The pathogenesis of the neurological loss produced by spinal cord compression is incompletely understood and treatment has been unsatisfactory.

We developed an experimental model of epidural spinal cord metastasis in rabbits, following the method described by Ushio, et al., and investigated edema and circulatory disturbance of the spinal cord compressed by epidural neoplasms.

Materials and Methods

A VX2 tumor (virus-induced epidermoid carcinoma) was transplanted into the thigh muscle of 5-month-old rabbits, and removed 21 days after inoculation. Under aseptic conditions, the tumor was minced with fine scissors in Earle's basic medium, and passed through 40-mesh and then 80-mesh stainless steel screens. A concentration of $1 \times 10^6$ viable cells per 0.5 ml was obtained by means of trypan blue exclusion test of viability.

We anesthetized 151 female rabbits, weighing 2.5 to 3 kg, intravenously with 20 mg/kg sodium pentobarbital. The VX2 tumor-cell suspension ($1 \times 10^6$ viable cells) was injected percutaneously anterior to the T-13 vertebral body with a No. 22 needle. After inoculation, the animals were graded daily with respect to function of the hind limbs according to the following scale:

- Grade 0: normal
- Grade 1: unstable running
- Grade 2: weak, but able to run
- Grade 3: attempts to walk
- Grade 4: moves hind limbs only on pinprick
- Grade 5: paraplegia.

All animals were autopsied upon death and histopathological examinations were performed as described previously.

Water Content of Spinal Cord

The water content of the spinal cord underlying the tumor, and of the cord distant from the tumor, was...
measured by the wet/dry weight method* in 22 tumor-bearing rabbits at various grades of weakness, and in seven normal control rabbits. The animals were anesthetized with sodium pentobarbital, heparinized, and sacrificed by exsanguination from both femoral arteries. The spinal cord with dura was quickly removed en bloc from the lower thoracic to the lower lumbar segments by laminectomy. The spinal cord was cut into approximately 7 mm lengths at the compressed site and at distant, presumably normal, regions (T-8 and L-4 segments). After removal of the dura, each block was immediately weighed in previously tared bottles. The samples were dried at 100°C and reweighed to obtain a constant dry weight. The water content was expressed as percent of wet weight.

Uptake of 99mTc Pertechnetate in Spinal Cord

Alterations of the blood-brain (spinal cord) barrier in the compressed spinal cord were investigated in 21 tumor-bearing rabbits at various grades of weakness by measuring spinal cord uptake of intravenously injected technetium-99m (99mTc) pertechnetate. Six animals served as controls. The animals were sacrificed by exsanguination 15 minutes after intravenous injection of 1 mCi of 99mTc pertechnetate. Laminectomy was performed, and the cord with intact dura was removed en bloc from the segments between the upper thoracic to the lower lumbar regions. The spinal cord was cut into seven blocks; one was from the area underlying the tumor and three successive blocks, approximately 7 mm in length each, were cut cephalad and caudad to the compressed site. After removal of the dura, each block was weighed in previously tared bottles. Radioactivity of 99mTc pertechnetate in the spinal cord was counted 60 minutes after it was administered in a scintillation counter. For each block, counts per minute per 1 mg of wet weight of tissue (cpm/mg) were calculated and the data were expressed as percent of the cpm/mg of the most cephalad block. The radioactivity of a small fragment of paravertebral muscle was also counted as a control.

Microangiography

Animals at various grades of weakness were anesthetized and heparinized. A midline incision was made in the sternum to expose the heart, a polyethylene catheter was introduced into the ascending aorta through the left ventricle, and the right atrium was opened to serve as a drain. The animals were perfused with 10% formalin in saline until all of the visceral blood volume was flushed out, and the perfusate drained from the atrium was free of blood. The infusion pressure of 140 mm Hg was monitored on a pressure recorder. After perfusion, 150 ml of silicone rubber was infused through a catheter at room temperature into three animals and they were placed overnight in a refrigerator. The spinal cord was removed and immersed in 10% formalin for 1 week. After fixation, the spinal cord was dehydrated in alcohol, immersed in 100% chloroform for 5 minutes, then placed in 98% synthetic methyl salicylate which rendered the tissue transparent. The epidural vertebral venous plexus was examined after removal of the spinal cord. In 17 animals, after perfusion with 10% formalin in saline, approximately 150 ml of micropaque solution (40% barium sulfate and 5% gelatin in hot water at 40°C) was infused. The animals were placed overnight in a refrigerator, the vertebrae and spinal cord were then removed en bloc and immersed in 10% formalin. The blocks were decalcified in 40% formic acid and 4-mm transverse sections of the vertebrae and spinal cord were made in five of 17 animals. In the other 12 animals, the spinal cord was removed by laminectomy from the lower thoracic to the lower lumbar segments. Contact microangiograms of all specimens were taken by a supersoft x-ray apparatus with a focus-film distance of 70 cm; secondary voltage, 12 kV; electric current, 17 mA; and exposure time, 30 seconds.

Fluorescein Angiography

Seven animals at various grades of symptomatic spinal cord compression were premedicated with 0.1 mg of atropin sulfate. Under sodium pentobarbital anesthesia, tracheostomy and catheterization into the femoral artery and vein were performed. An arterial catheter was introduced into the aorta up to the level of the sternum and used for continuous blood pressure monitoring, periodic gas determination, and injection of fluorescein dye. The animals were fixed in a prone position by a stereotaxic apparatus,† and immobilized with pancuronium bromide. A volume respirator was used to maintain pO2 between 90 and 140 mm Hg and pCO2 between 30 and 35 mm Hg. Normal blood pressure was maintained by infusing Ringer's solution into the femoral vein. Laminitectomy of four vertebrae centered at the level of tumor invasion was performed. After a single injection of 1 ml of 1% fluorescein sodium into the aorta through the catheter, serial fluorescein angiograms of the exposed spinal cord were taken without opening the dura.

Results

Experimental Model

Most of the animals developed weakness of the hindlimbs (Grade 1) about 18 days after tumor in-

*Well-type scintillation counter manufactured by Tracor Analysis Co., Chicago, Illinois.

†Microfil MV-122 manufactured by Canton Biomedical Products, Inc., Boulder, Colorado.

‡Todai Noken stereotaxic apparatus manufactured by Takahashi Shoten, Tokyo, Japan.
occlusion, and became completely paraplegic (Grade 5) about 8 days later. Figure 1 shows a Grade 3 rabbit. Bladder and bowel incontinence was observed in almost all animals from Grade 4. At autopsy, lung metastasis was found in 45% of the animals. The implanted tumor grew into the epidural space either directly through the intervertebral foramina or by invading the vertebral bodies. Usually one segment of the spinal cord was compressed by the epidural tumor located on the posterolateral or anterolateral aspects (Fig. 2). In some animals, the spinal cord was squeezed by the tumor surrounding the cord (Fig. 3).

Histological examination disclosed edema and axonal swelling in the white matter of the compressed cord at the early stage of compression (from Grade 2). In the late stage of compression (Grades 4 and 5), these changes of the white matter became more marked, and were accompanied by necrosis and hemorrhage. Compared to the white matter, the gray matter was relatively well preserved.

Fig. 1. A Grade 3 rabbit. The animal cannot maintain its hip in position; however, it moves its hindlimbs voluntarily in an attempt to walk.

Fig. 2. Transverse section through the spine and spinal cord in a Grade 5 rabbit. The vertebral body is infiltrated by the tumor and the spinal cord is severely compressed by the posterolaterally located epidural tumor. H & E, × 1.3.

Fig. 3. Spinal cord of a Grade 5 rabbit. The laminae were removed to show the epidural tumor (T) squeezing the spinal cord.
TABLE 1

<table>
<thead>
<tr>
<th>Grade of Weakness</th>
<th>No. of Animals</th>
<th>Water Content (% ± SD)</th>
<th>Distant Cord (D)</th>
<th>Compressed Cord (C)</th>
<th>C/D × 100 ± SD</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>66.4 ± 0.5</td>
<td>66.3 ± 0.5</td>
<td>100 ± 0.8</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>65.5 ± 1.0</td>
<td>66.4 ± 1.3</td>
<td>102 ± 0.6</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>65.1 ± 0.9</td>
<td>68.0 ± 2.0</td>
<td>105 ± 3.8</td>
<td>p &lt; 0.02</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>65.7 ± 0.4</td>
<td>67.4 ± 1.1</td>
<td>103 ± 2.1</td>
<td>p &lt; 0.025</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>65.8 ± 0.7</td>
<td>67.7 ± 0.9</td>
<td>103 ± 1.8</td>
<td>p &lt; 0.025</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>22</td>
<td>65.7 ± 0.7</td>
<td>67.2 ± 1.2</td>
<td>103 ± 2.0</td>
<td>p &lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

*P values compare the water content of compressed and distant cord. NS = not significant.

Water Content of Spinal Cord

In normal rabbits, there was no difference in water content of the spinal cord between the T-8 (65.7 ± 0.5%), T-13 (65.9 ± 0.6%), and L-4 (65.6 ± 0.3%) segments. Table 1 shows the water content of the distant (normal) and compressed spinal cord in rabbits at the five different grades of weakness. The water content of the spinal cord compressed by the epidural tumor (67.2 ± 1.2%) was significantly greater than that distant from the tumor (65.7 ± 0.7%) (p < 0.001). The increase of the water content in the compressed spinal cord was proportional to the degree of neurological loss, and it became statistically significant in Grade 3 of weakness.

Uptake of 99mTc Pertechnetate in Spinal Cord

In normal animals there was no difference in uptake of 99mTc pertechnetate in the spinal cord from the lower thoracic to the lower lumbar segments. The uptake of 99mTc pertechnetate in the normal spinal cord was approximately one-tenth of that in the paravertebral muscle. Figure 4 shows the uptake of 99mTc pertechnetate in the spinal cord of tumor-bearing animals at the five different grades of weakness. The uptake of 99mTc pertechnetate in the spinal cord compressed by the epidural tumor (Block IV) was significantly higher than that distant from the tumor (Block I), even in Grade 1 animals (1.2 times greater than that of the distant normal cord, p < 0.001). Uptake increased in proportion to the degree of neurological loss and in animals with complete paraplegia (Grade 5), the uptake of 99mTc pertechnetate in the spinal cord compressed by the epidural tumor was 3.7 times greater than that of the distant cord (p < 0.005). The uptake of 99mTc pertechnetate was higher in the caudal (Blocks V, VI, and VII) than in the cephalad segments (Blocks I, II, and III).

Microangiography

The central arteries of the spinal cord at the level of tumor invasion were displaced, stretched, and decreased in number. These changes were more marked in animals with a high grade of neurological symptoms. The anterior and posterior spinal arteries remained patent even in the far advanced stage of compression. At the early stage, dilated and tortuous veins were seen at the surface of the spinal cord. In the normal portion, the anterior internal vertebral venous plexus situated at the posterior surface of the vertebral body showed the arcuate pattern of the plexus with right- and left-sided arcs meeting in the middle of each vertebral body. Each radicular vein segmentally drained into this plexus. These vascular patterns are similar to those seen in humans. In the area of tumor invasion, the vertebral venous plexus was infiltrated by the tumor or narrowed due to compression by the epidural tumor. Stenosis or obstruction of the epidural venous plexus was observed in animals at the early stage of neurological symptoms (Figs. 5 and 6).

Fluorescein Angiography

Fluorescein angiography revealed a circulatory disturbance of the compressed spinal cord which was evidenced by delayed filling of the arteries, poor visualization of vessels, and retention of the dye in the veins. Figure 7 left shows the fluorescein angiogram of the early arterial phase. The normal portion (upper half) shows good filling of the arteries. The arteries at the level of tumor invasion (lower half) remain dark. Figure 7 right shows the venous phase. The arteries are visualized poorly at the compressed portion (lower half). Dye retention in the veins on the surface of the spinal cord was observed at the compressed portion.

Discussion

In 1977, an animal model of spinal cord compression by epidural neoplasms was developed by percutaneously injecting rats with Walker 256 tumor anterior to the T-12 or T-13 vertebral body, and an experimental approach to the disease was initiated. In the present study, we have developed a similar experimental model in rabbits in order to circumvent the disadvantages of the rat model. The rabbit model is superior to the rat model for several reasons. First, since the spinal cord of rabbits is larger than that of rats, experimental procedures and observations are
Experimental spinal cord compression by epidural tumor

FIG. 4. Uptake of $^{99m}$Tc pertechnetate in consecutive blocks of the spinal cord. The uptake in the compressed spinal cord (Block IV) is significantly higher than that in the adjacent cord, and increases as the grade of weakness progresses. The uptake is higher in the caudal than in the cephalad (oral) blocks.

much easier. Second, the spinal cord vasculature of the rabbit is similar to that of humans. Third, the VX2 tumor used in the new model is highly invasive, and vertebral bodies are often invaded in a manner frequently seen in humans. Finally, the VX2 tumor is resistant to chemotherapy, as are most of the tumors which in humans metastasize to the epidural spaces. This new model in the rabbit, however, has a few disadvantages. The animals are relatively expensive. Survival time of tumor-bearing animals is short; usually they die approximately 2 weeks after the onset of neurological symptoms, and some die of lung metastasis before paraplegia develops.

The histopathological findings of the compressed spinal cord in rabbits were similar to those in rats, and consisted of edema and axonal swelling and well preserved neurons until the late stage of compression. Ushio, et al., demonstrated that in rats, vasogenic...
edema develops in the spinal cord underlying epidural neoplasms. The present study not only confirms their findings, but also demonstrates that the water content of the compressed spinal cord increases in proportion to the degree of neurological loss. This finding corresponds with the results of our quantitative investigation of the blood-spinal cord barrier which showed that, in the compressed spinal cord, $^{99m}$Tc pertechnetate uptake is significantly higher than in areas distant from the tumor, and that it increases as the degree of spinal cord compression progresses.

Regarding the pathogenesis of this type of spinal cord edema, the significance of venous compression and stasis has been emphasized from autopsy findings and clinical observations. Barron, et al., reported the characteristic findings of the spinal cord compressed by epidural metastases at the time of autopsy to be an "edematous type of malacia" of the white matter. They suggested that the pathogenesis of the signs and symptoms produced by epidural metastasis was neither simple mechanical compression nor anoxia or asphyxia of the compressed spinal cord, but rather the disturbance of venous drainage. Nagashima, et al., reported the autopsy of a paraplegic patient with leukemia which diffusely invaded the paravertebral soft tissue, including the external vertebral venous plexus and intervertebral vein, and proposed that the spinal cord softening may have been due to the failure of venous return. Austin and Auld and Buerman observed that edema of the spinal cord in epidural metastases was more marked than that seen in other types of spinal cord compression (such as benign epidural tumor and disc disease), suggesting that edema was produced by the disturbance of venous drainage. The above hypotheses, however, have not

**Fig. 5.** Anterior internal vertebral venous plexus of a Grade 3 rabbit after injection of Microfil. **Left:** The plexus of the normal portion (arrow), showing the arcuate pattern of the plexus with right- and left-sided arcs meeting in the middle of each vertebral body. **Right:** The portion at the level of tumor invasion, showing the anterior internal vertebral venous plexus (arrows) completely obliterated by the epidural tumor (T).

**Fig. 6.** Contact microangiograms of a transverse section of the spinal column and spinal cord in a Grade 3 rabbit after injection of Micropaque. **Left:** Normal portion, showing the spinal cord (SC) encased by the vertebral body (VB) and lamina (L). Arteries, veins, and venous plexus are normal in configuration. **Right:** The section at the level of tumor invasion, showing the epidural tumor (T) compressing the spinal cord. The anterior internal vertebral venous plexus (VP, left) is completely obliterated. Radicular artery (RA) and central artery (CA) are markedly stretched, but are patent. AS = anterior spinal artery; PA = perforating artery; PS = posterior spinal artery; DSV = dorsal spinal vein.
Experimental spinal cord compression by epidural tumor

been verified either clinically or experimentally. The present experiment clearly demonstrated that the initial circulatory disturbance in the spinal cord compressed by epidural neoplasms is caused by stenosis and occlusion of the epidural venous plexus. The disturbance of venous drainage was already seen at the early stage of cord compression when the breakdown of the blood-spinal cord barrier and edema started to develop. The anterior and posterior spinal arteries, however, remained patent until the late stage of compression.

From the data collected in the present study, we conclude that 1) breakdown of the blood-spinal cord barrier and vasogenic edema develop in the spinal cord compressed by the epidural tumor, and that they progress in proportion to the grade of neurological loss; and 2) venous stasis of the spinal cord due to stenosis or obstruction of the vertebral venous plexus by the epidural tumor may play an important role in the development of spinal cord edema, and in the symptomatology of metastatic epidural spinal cord compression.

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References


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Fig. 7. Fluorescein angiograms of the spinal cord in a Grade 4 rabbit. Left: Arterial phase. Normal portion (upper half) shows normal filling of the arteries. The arteries at the site of the tumor invasion (lower half) are still dark and unfilled with fluorescein dye except for the posterior spinal artery. Right: Late venous phase showing delayed filling of the vessels at the compressed portion (lower half).