Spinal cord blood flow patterns in experimental traumatic paraplegia

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Alterations in spinal cord blood flow patterns in experimental traumatic paraplegia were studied by using thioflavine S, a fluorescent dye that stains the endothelium of blood vessels. Feline spinal cords, exposed by laminectomy, were traumatized with a 400 gm-cm contusion, and a rapid intravenous injection of thioflavine S administered. The spinal cords were excised within one circulation time after the injection, and the resulting spinal cord sections were examined under ultraviolet light. As thioflavine S is a fluorescent substance that binds to the blood vessel walls and as the spinal cord was excised within one circulation time, it was possible to determine in which vessels blood was flowing at the time of injection. Control animals showed blood flow in all parts of the spinal cord. At 15 min postcontusion there was a marked decrease in the number of vessels perfused in the white matter; however, at 30 min many of the vessels had evidence of renewed blood flow. By 1 hr postcontusion the entire gray matter showed hemorrhagic infarction. The number of fluorescing vessels in the white matter decreased considerably between 1 and 8 hrs post-contusion. At 8 hrs the thioflavine S-stained vessels were limited to the peripheral half of the white matter; however, by 24 hrs most of the vessels again fluoresced, but the gray matter remained without perfusion. In this experimentally-produced lesion, the gray matter of the spinal cord was infarcted by 1 hr postcontusion; the white matter was ischemic between 1 and 8 hrs with resumed blood flow by 24 hrs.

Key Words: spinal cord blood flow patterns, spinal cord contusion, experimental paraplegia, intramedullary microvasculature

Studies of the early alterations in the traumatized spinal cord have included examination of the histopathology, the pial circulation, and fine structure of the myelinated nerve fibers and the microvasculature; however, little is known of the circulatory changes occurring within the intrinsic vasculature of the spinal cord following injury. The importance of the first hours postcontusion apropos development of spinal cord pathology and the degree of neurological recovery following treatment has been stressed by various investigators. Recently, a historical review of experimental spinal cord trauma has been published. The purpose of the present study is to describe changes in blood flow patterns in the intrinsic vessels of the injured spinal cord during the first 24 hrs after a contusion sufficient to produce paraplegia.

Materials and Methods

Adult cats of both sexes were anesthetized with sodium pentobarbital (30 mg/kg), and
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a laminectomy was performed. The spinal cord was exposed at the T-10 level with the dura mater left intact. Using the model designed by Allen and modified by Albin, et al., we produced spinal cord contusions by dropping a known weight through a plastic tube onto an impounder resting on the intact dura mater. Vertical orientation of the tube was checked with a small bubble balance mounted on top of the tube. The contusion was designated as the product of the weight (grams) and the distance (centimeters) through which the weight had fallen. Eight cats were traumatized with contusions varying between 140 and 400 gm-cm to determine the injury necessary to produce a permanent paraplegia.* The animals' sensory and motor functions were tested twice each week for a period of 4 months. Those cats with a contusion of at least 340 gm-cm remained permanently paraplegic.

Thirty-two cats were divided into groups of four as follows: control, 5 min postcontusion, 15 min, 30 min, 1 hr, 4 hrs, 8 hrs, 24 hrs. Experimental animals were traumatized with a 400 gm-cm contusion. At one of the specified time intervals after contusion, each animal was given a rapid injection of 4% thioflavine S (1 cc/kg) into the femoral vein, and the T-10 level of the spinal cord was excised within one circulation time (10 sec) after the injection. According to the methods described by Dohrmann and Wick, the tissue was frozen by immersion in liquid nitrogen, 150 μ transverse sections were cut and the sections were photographed under ultraviolet light. As controls, four of the cats were operated and injected with thioflavine S in the same manner as the experimental animals; however, the spinal cords were not contused. The sections of spinal cord were then defrosted, photographed, placed in 10% formalin, and examined by light microscopy.

**Results**

In all cases the spinal cord substance had

* A paraplegia from which no return of sensory or motor function occurs by 4 months is termed "permanent paraplegia" in this study.
† Thioflavine S is a fluorescent dye which stains the endothelium of blood vessels without damaging them. When injected intravenously it will selectively stain vessels through which it has passed. (See refs. 29 and 30.)

**Control Group**

At all time intervals the vascular pattern of the spinal cord appeared normal in the control group (Fig. 1). An abundance of fluorescing vessels was noted in both the gray and white matter. Vessels of the white matter radiated from the periphery toward the center of the cord. The gray matter showed a greater concentration of vessels which were less regularly arranged than those in the white matter.

**Five-Minute Group**

In the 5-min group the vasculature of the white matter appeared normal, having both the quantity and the arrangement of fluorescing vessels identical to those described in the controls (Fig. 2). Hemorrhagic areas were apparent within the gray matter and no staining of vessels was seen in these areas. The pattern of fluorescence in the nonhemorrhagic regions of the gray matter appeared normal.

**Fifteen-Minute Group**

A significant decrease in the number of fluorescent vessels within the white matter was noted at 15 min after trauma (Fig. 3);
within more vessels of the white matter at 30 min than at 15 min (Fig. 4). Petechial hemorrhages within the white matter were more pronounced than at earlier intervals with decreased staining of vessels in the area surrounding them. A large portion of the gray matter was hemorrhagic. Only a few vessels of the gray matter fluoresced and extravasation of dye from some of them was noted.

**One-Hour Group**

Very few vessels in the white matter near the gray-white interface fluoresced at 1 hr (Fig. 5); however, the larger vessels near the periphery of the spinal cord were perfused with thioflavine S. The entire gray matter was hemorrhagic and contained no vascular fluorescence.

**Four-Hour Group**

The number of vessels stained with thioflavine S was reduced further by 4 hrs following contusion (Fig. 6). Fluorescent vessels were limited to the peripheral half of the white matter of the spinal cord where they were present in small patches. There was no fluorescence in the gray matter which remained completely hemorrhagic.

**Eight-Hour Group**

Extravasation of dye from several vessels

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Figure 2. Fluorescence photomicrograph showing fluorescent vessels and a central hemorrhagic zone (arrows) with no evidence of thioflavine S perfusion at 5 min postcontusion. ×11.

Only about 25% of the vessels showed staining with thioflavine S. Fluorescence in the vasculature of the gray matter was also considerably reduced and hemorrhagic areas, containing nonfluorescing vessels, were larger than at 5 min.

**Thirty-Minute Group**

Intraluminal thioflavine S was present

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Figure 3. Injured spinal cord at 15 min posttrauma. *Left:* Fluorescence photomicrograph showing hemorrhages (arrows) primarily in the gray matter. No thioflavine S-stained vessels are present in the region of hemorrhage and few thioflavine S-stained vessels can be seen in either gray or white matter. ×11. *Right:* Photomicrograph of same spinal cord without ultraviolet light to illustrate how the hemorrhagic areas compared to the regions of nonfluorescence in left photomicrograph. ×12.
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Fig. 4. Fluorescence photomicrograph of spinal cord at 30 min after contusion. Hemorrhagic areas are present in both gray and white matter. Extravasation of thioflavine S from several fluorescent vessels can be seen (arrows) at the periphery of the hemorrhagic region. × 11.

Vascular fluorescence within the white matter was noted at 8 hrs (Fig. 7); however, the number and arrangement of fluorescing vessels in the white matter appeared as in the 4-hr group. The gray matter showed no fluorescing vessels. Hemorrhagic areas of the cord appeared more diffuse than at earlier intervals and considerable swelling was noted.

Twenty-Four-Hour Group

Vascular fluorescence within the white matter was restored to a nearly normal pattern by 24 hrs after trauma (Fig. 8). Staining of vessels was absent only in the immediate area of the hemorrhages. The gray matter remained hemorrhagic and without evidence of intravascular thioflavine S.

Fig. 5. Fluorescence photomicrograph at 1 hr posttrauma showing infarcted gray matter and a few fluorescing vessels in the white matter. × 11.

Fig. 6. Fluorescence photomicrograph showing thioflavine S-stained vessels only in peripheral half of white matter at 4 hrs postcontusion. × 11.

Fig. 7. Fluorescence photomicrograph at 8 hrs postcontusion with evidence of per fused vessels mainly in the periphery of the white matter. Gray matter shows no evidence of blood flow. × 11.
sation of apparent blood flow by one hour after contusion. Dohrmann, et al.,8,10,12 have reported tears in the muscular venules of the gray matter after experimental spinal cord contusion. This phenomenon of tearing may account for the extravasation of dye from vessels, most evident at the 30-min interval.

A generalized decrease in the number of

Discussion

Because thioflavine S stains the endothelium of the vessels through which it passes,9,10 it is possible to qualitatively determine in which portions of the spinal cord blood is flowing at the time of injection providing that the segment of spinal cord is excised within one circulation time (10 sec.)11 following the injection.15 In contrast to microperfusion studies, which illustrate vascular patency at a given perfusion pressure with a given perfusate, this technique delineates the in vivo blood flow patterns in the intramedullary vasculature.

The pattern of fluorescing vessels seen with thioflavine S in the control animals corresponded to the normal pattern of blood vessels in the spinal cord. The presence of dye in all of these vessels indicated that there was blood flow in virtually all vessels of the gray and white matter. This pattern and those at subsequent time intervals are summarized by the line drawings in Fig. 9.

The first major changes in the spinal cord blood flow patterns began at about 15 minutes postcontusion. At that time the number of vessels stained with thioflavine S in both the gray and white matter was markedly reduced.15 It is possible that vasospasm and/or stasis and/or thrombosis of vessels of the gray matter might be responsible for the ces-

Fig. 8. Fluorescence photomicrograph at 24 hrs posttrauma. Gray matter remains infarcted; however, there are fluorescing vessels in abundance throughout the white matter. ×11.

Fig. 9. Line drawings summarizing patterns of thioflavine S-stained vessels and areas of hemorrhagic nonperfusion (blackened areas) within the injured spinal cord at specified time intervals post-contusion. The microvasculature of the white matter shows a decrease in perfusion at 15 min postcontusion, resumption of some blood flow from 30 min through 8 hrs, and restoration of most blood flow by 24 hrs.
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vessels in the white matter with evidence of blood flow was seen at 15 min but by 30 min the number had increased again. This transient decrease could have been related to vasocostriction. Conversely, the decreased perfusion seen in the white matter peripheral to the gray-white interface at 1, 4, and 8 hrs may have been due to stasis. The studies of Wagner, et al.,25 and Green, et al.,28 on feline spinal cord edema following trauma showed progression of the edema fluid from the gray matter and central white matter at 1 hr, extending through half of the white matter by 4 hrs and encompassing the entire spinal cord at 8 hrs. The regions of edema at 1 and 4 hrs corresponded with the areas of decreased blood flow in the white matter described in the present study.

Our findings support the experimental results of Kelly, et al.,24 and Ducker and Perot.19 Kelly, et al.,24 reported a rapid decrease in spinal cord $pO_2$ over the first 30 min to 1 hr posttrauma and a persistent state of hypoxia for 7 hrs, at which time recordings were stopped. Ducker and Perot19 described a decrease in spinal cord blood flow, as measured with xenon 133, during the first 2 hrs following contusion with a decrease in tissue oxygen shortly thereafter. The progressively diminishing blood flow in the first hour post-contusion and ischemia for at least the following 8 hrs noted in the present study correlates well with the reports mentioned above.

In studies of time curves of the pial circulation of the canine spinal cord following contusion, Wagner, et al.,26 described a long venous phase which was attributed to stasis. Microperfusion of the spinal cords of dogs and cats postcontusion utilizing barium sulfate was used by Fairholm and Turnbull20,33 who reported extravasation of barium from the vessels of the gray matter up to 48 hrs after injury and a perfusion blockage in the posteriorcentral spinal cord vessels by 4 hrs following trauma. Fried and Goodkin23 confirmed this decrease in barium sulfate perfusion in primate spinal cords after contusion and noted the decrease to be most marked at 24 hrs after the injury.

Blood flow patterns in the feline spinal cord in transitory traumatic paraplegia were studied by Dohrmann and Wick,34 who noted that the alterations in the perfusion of the white matter disappeared and perfusion returned to normal much sooner than in permanent traumatic paraplegia. As shown in this study, blood flow patterns in the spinal cord were greatly altered after contusion. Much of the neuronal damage characteristically seen in spinal cord injury may well be secondary to ischemia. Within the 24-hr period investigated, the gray matter appeared infarcted after 1 hr, and the white matter showed ischemia between 1 and 8 hrs with a return of blood flow by 24 hrs.

The fact that the changes in blood flow in the white matter were not apparent immediately after injury, as were those in the gray matter, indicates that the pathological mechanisms involved in spinal cord injury include functional as well as structural components.

References


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