Spinal Cord Infarction: A Highly Reproducible Stroke Model

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SUMMARY The study of focal central nervous system ischemia has been impeded by the lack of animal models that are both reproducible and simulate human ischemic strokes. To circumvent these problems, we have developed a rabbit spinal cord infarction model. Infarction of the caudal lumbar cord is produced by temporary occlusion of the abdominal aorta below the renal arteries. One hour of ischemia causes all of the animals to develop spinal cord infarctions in a highly reproducible pattern. These animals can be maintained alive for at least 2 weeks post-operatively. We believe that the tissue changes in spinal cord infarction are qualitatively similar to infarction of most areas of the central nervous system. This model has numerous features which make it particularly useful for neurochemical, neuropathological, and neurophysiological studies.

FUNDAMENTAL UNDERSTANDING of the pathophysiology of focal occlusive cerebrovascular disease in man has been impeded by the lack of adequate experimental animal models which are both reproducible and closely simulate human ischemic strokes. Many animal models have been used, but regardless of species, when single cerebral vessels are occluded, the infarcts produced vary considerably in size and distribution.1,2 Surgical isolation of a given cerebral artery, in an effort to limit or eliminate collateral flow to its territory of distribution, requires complex procedures that may alter the usual tissue responses to an experimentally induced ischemic insult. Further understanding of focal ischemia requires a method of determining the precise anatomical location of the injury before morphological evidence of irreversible damage is manifest. It was, therefore, apparent that a new approach to the problem of focal CNS ischemia was needed. We have developed a highly reproducible stroke model by re-examining a 300-year-old spinal cord infarction experiment.

Materials and Methods

New Zealand male albino rabbits weighing 2 to 3 kg were anesthetized with ketamine, 20 mg/kg, and ether inhalation. An abdominal incision was made and the aorta was isolated at the level of the renal arteries. A Scoville-Lewis aneurysm clip was placed on the aorta just caudal to the left (lower) renal artery, and the incision 'was temporarily closed. The animals were allowed to awaken, and after one hour they were reanesthetized with ether alone. The incision was reopened, the clip removed, and the incision was then permanently closed with deep sutures and skin clips. Postoperatively the animals were given daily subcutaneous injection of cephaloridine, 0.25 mg/kg, and Credé maneuvers were performed twice daily. After either one or two weeks the animals were sacrificed for histopathological studies.

The lumbar spinal cords were either removed by multiple laminectomies or extruded from the spinal canal with the plunger from a 1 ml syringe.3 The spinal cord was either frozen or placed in 10% phosphate buffered formalin. Formalin-fixed sections taken serially through the dorsal root ganglia were embedded in paraffin, celloidin, or plastic. Cross or longitudinal sections were cut and stained with hematoxylin-eosin (H&E) and oil red O (frozen sections), toluidine blue (plastic embedded sections) or H&E, cresyl violet, and Woelke/Loyez (paraffin and celloidin).

Results

One hour ischemic lesions have been induced in 40 animals, and without exception they have become paraplegic, incontinent, and without sensibility to noxious stimuli in the hind quarters. Eighteen animals were given antibiotics and 17 of them survived until they were sacrificed (13 were allowed to live 1 week and 4 were kept alive for 2 weeks).

Figure 1 shows the appearance of a paraplegic rabbit one week after the lesion was produced. Figures 2 and 3 compare a control and experimental myelin stained cross section of the spinal cord at the caudal end of the lumbar region one week after one hour of ischemia. In the experimental animal there is a total loss of the myelinated fibers in the gray matter and little evidence of damage to the long white matter tracts as evidenced by cellular infiltration or myelin breakdown on fat stains. Figure 4 is a corresponding cresyl violet stained section of the lumbar cord of the experimental animal after a one week survival. It demonstrates total neuronal loss, neovascularization, reactive gliosis, and macrophage infiltration in the gray matter with relative sparing of the substantia gelatinosa.

Similar damage is seen at all levels from approximately the mid-lumbar level to the conus medullaris. The progression of post-infarction inflammatory cellular infiltration in CNS tissue at one and two weeks was observed.4 The distribution and extent of the lesions was identical in all animals at both one and two weeks after the lesion was produced.
SPINAL CORD INFARCTION AS STROKE MODEL/Zivin & DeGirolami

FIGURE 1. A photograph of a rabbit paraplegic for one week. The posture of the hind legs is never assumed by a normal animal. The duration of spinal cord ischemia was one hour.

FIGURE 2. The lumbar spinal cord from a control rabbit. The cord is normal. (Loyez, × 22)

FIGURE 3. The lumbar spinal cord from a paraplegic rabbit. The duration of spinal cord ischemia was one hour. The animal was sacrificed one week after the insult. The gray matter is uniformly destroyed, the long white matter tracts are generally intact. (Loyez, × 22)

FIGURE 4. High magnification of the lesion produced in the lumbar region one week after one hour of ischemia. There is generalized complete gray matter necrosis with reactive gliosis and round cell infiltration. Arrows indicate the approximate outline of the gray matter. (Cresyl violet, × 35)

Discussion

We have demonstrated that it is possible to induce a reproducible infarction in the lower lumbar and sacral regions of the rabbit spinal cord by one hour of occlusion of the abdominal aorta. With appropriate postoperative care these animals can be maintained for at least 2 weeks. Therefore, we can identify regions of the spinal cord which will inevitably undergo irreversible damage after an ischemic insult of known duration.

In 1667, Niels Stensen (Stenonis, Steno) described a procedure he performed on dogs: "... on making a ligature in the descending aorta, without previous cutting, that voluntary movement of all the parts below ceased in proportion to the tightness of the band, while it was restored similarly as the knot was loosened." During the latter part of the nineteenth century a number of complete histopathologic descriptions established that ligation of the rabbit abdominal aorta resulted in spinal cord infarction (reviewed by DeBuck and DeMoor). More recent histological and physiological studies have indicated that abdominal aortic occlusion for 20-25 minutes results in irreversible flaccid paralysis of the hind limbs in most animals. This phenomenon is due to ischemic necrosis of the spinal neurons and glia. The reason that rabbit spinal cord is especially susceptible to infarction is that its main source of blood in the caudal portions is segmental supply from the abdominal aorta.

There have been a substantial number of investigators who have used other spinal cord infarction models in recent years (reviewed in part by Fosburg and Brewer). The animals most frequently used for...
these studies are cats, dogs, and monkeys. As is true of humans, the caudal portions of the spinal cords of these animals are predominantly supplied by the thoracic aorta, and production of spinal cord infarctions in these animals requires occlusion of the aorta in the thoracic region. Consequently, all of the abdominal viscera, and especially the anoxia sensitive kidneys, are simultaneously rendered ischemic.

The rabbit spinal cord infarction model would appear to have several advantages over other models of focal central nervous system ischemia.

1. The anatomy is simplified. The spinal cord has a relatively uniform segmental organization. Lesions which vary in size in the rostrocaudal direction would affect functionally similar structures at any level. Analysis of the consequences of the lesion are, therefore, not substantially influenced by the size of the lesion within fairly broad limits.

2. The lesion is highly reproducible. It is uniform in the caudal lumbar region with total involvement of gray matter.

3. Animals can be followed clinically for lengthy periods. They do not suffer as many secondary problems as do animals with large cerebral lesions (e.g., no herniation, cardiac arrhythmias, or respiratory problems develop).

4. Functional deficits are stereotyped. Injury to even small amounts of tissue is readily apparent clinically.

5. Seizures do not occur. Repeated generalized convulsions are observed in several small animal cerebral infarction models although such seizures are uncommon in ischemic strokes in humans.15

6. Surgical procedures are relatively simple and remote from infarcted tissue.

7. Post-operative complications are minimal.

8. Control tissue rostral to the lesion is present in each animal.

9. The spinal cord is readily accessible and has a relatively small diameter. Consequently, rapid removal, fixation, or freezing are facilitated relative to similar procedures in cerebral models.

We believe it is reasonable to assume that the fundamental tissue changes that occur in spinal cord infarction are similar qualitatively if not quantitatively to the changes that occur in most regions of the brain during ischemia. No single animal model can perfectly represent all aspects of human central nervous system infarction; however, rabbit spinal cord infarction is a good facsimile of cerebral injury and apparently is the most reproducible CNS focal ischemia model yet devised.

We are currently pursuing correlative neurochemical, neuropathological, and neurophysiological studies with this model.

References


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Stroke. 1980;11:200-202
doi: 10.1161/01.STR.11.2.200

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1980 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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