Histopathology of experimental hematomyelia

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The pathology of hematomyelia was examined in 35 rats following the stereotactic injection of 2 μl blood into the dorsal columns of the thoracic spinal cord. This experimental model produced a small ball-hemorrhage without associated neurological deficits or significant tissue injury. Histological sections of the whole spinal cord were studied at intervals ranging from 2 hours to 4 months after injection. In acute experiments (2 to 6 hours postinjection), blood was sometimes seen within the lumen of the central canal extending rostrally to the level of the fourth ventricle. Between 24 hours and 3 days, the parenchymal hematoma became consolidated and there was an intense proliferation of microglial cells at the perimeter of the lesion. The cells invaded the hematoma, infiltrated its core, and removed erythrocytes by phagocytosis. Rostral to the lesion, the lumen of the central canal was found to contain varying amounts of fibrin, proteinaceous material, and cellular debris for up to 15 days. These findings were much less prominent in the segments of the canal caudal to the lesion.

Healing of the parenchymal hematoma was usually complete within 4 to 6 weeks except for residual hemosiderin-laden microglial cells and focal gliosis at the lesion site. It is concluded that the clearance of atraumatic hematomyelia probably involves two primary mechanisms: 1) phagocytosis of the focal hemorrhage by microglial cells; and 2) drainage of blood products in a rostral direction through the central canal of the spinal cord.

Key Words: hematomyelia · intramedullary hemorrhage · microglial cell · astrogliosis · spinal cord · rat

The pathology of hematomyelia is incompletely understood. Although intramedullary hemorrhage is a common complication of spinal cord trauma and can occur spontaneously in association with intraspinal tumors, arteriovenous malformations, thrombocytopenia, arteriosclerosis, meningovascular syphilis, and syringomyelia, most information is derived from autopsy material and little is known about the acute and subacute pathological findings. In experimental models of spinal cord trauma, central core hemorrhages have been studied in some detail, but the pathological findings are invariably subordinate to those of intramedullary pulping caused by hyperemia, edema, and the disruption of axons. An experimental model of atraumatic hematomyelia has not been reported before.

To examine the fate of hemorrhage within the parenchymal tissue of the spinal cord, a measured volume of fresh whole blood was injected stereotactically into the dorsal columns of the thoracic spinal cord in rats. The experimental model produced a small ball-hemorrhage in the white matter that was associated with minimal tissue damage and no discernible neurological deficits. At intervals ranging from 2 hours to 4 months postinjection, serial sections of the whole spinal cord were examined by light microscopy using standard histological techniques. In this contribution, the pathological findings are summarized and discussed.

Materials and Methods

Following review and approval of the protocol by an institutional Animal Care and Use Committee, 56 adult Sprague-Dawley rats (weighing 300 to 350 gm each) were anesthetized briefly with a mixture of halothane, 70% nitrous oxide, and 30% oxygen. Twenty-one rats had femoral lines established and were used solely as blood donors. In 35 rats, a laminectomy was performed at T-5 or T-6 under the operating microscope. Attention was given to preserving epidural veins, and hemostasis was achieved without using electrocautery. A Kopf stereotactic frame mounted with a 10-μ Hamilton sy-
T. H. Milhorat, et al.

Fig. 1. Photomicrographs of experimental hematomyelia at 3 hours after injection of blood. A: Axial section through a lesion at T5–6 showing parenchymal hematoma in the ventral midline of the dorsal columns at the junction with the central gray matter (arrow). H & E, × 150. B: Same section at higher magnification showing proteinaceous material and erythrocytes within the central canal lumen. H & E, × 150. C: Axial section of the spinal cord at C-2 showing clumps of erythrocytes within the central canal lumen. H & E, × 150. D: Axial section of the spinal cord at T-10 showing occasional erythrocytes within the central canal. H & E, × 150.

rings and a No. 30 needle with a short bevel was brought into the operative field. Under microscopic guidance, the needle was passed through the translucent dura and advanced between the arteries and veins into the dorsal columns of the spinal cord to a depth of 1 mm. Fresh whole blood was withdrawn rapidly from a donor rat and transferred immediately to the stereotactic syringe. A total of 2 μl blood was injected into the dorsal columns over an interval of 20 seconds and the needle was removed. The wounds were closed and the animals were allowed to awaken from anesthesia. Neurological assessments were carried out on all animals.

Animals with experimentally induced hematomyelia were given a lethal dose of pentobarbital and perfused through the heart with normal saline for 10 minutes and a buffered solution of 10% formalin for 20 minutes at the following postinjection times: 2 hours (two animals), 3 hours (six animals), 6 hours (one animal), 24 hours (two animals), 2 days (two animals), 3 days (three animals), 4 days (one animal), 5 days (three animals), 7 days (three animals), 10 days (three animals), 15 days (two animals), 17 days (one animal), 21 days (one animal), 23 days (one animal), 30 days (one animal), 42 days (one animal), 92 days (one animal), and 117 days (one animal). The brains and spinal cords were removed in one specimen, divided into 5-mm blocks from the pontomedullary junction to the conus medullaris, and dehydrated in a tissue processor prior to embedding in paraffin. The blocks were cut serially with a microtome to a thickness of 6 μm; the sections were mounted on glass slides and stained with hematoxylin and eosin and one or more of the following stains: phosphotungstic acid hematoxylin, Prussian blue, silver carbonate, Gram-Weigert, Mallory, and Masson's trichrome. The sections were viewed through a microscope at magnifications ranging from × 4 to × 1000.

Results

There were no discernible differences in the ability to run, climb, jump, or stand, or in the responses to light touch and painful stimuli 1 hour after intramedullary injection in any of the 35 rats described in this report, as compared to normal controls. Delayed neurological deficits, including bladder and bowel disturbances, were not observed in animals followed for up to 4 months.

Two to 6 Hours Post-Hemorrhage

Histological sections through the lesion site revealed a small ball-hemorrhage formed by densely packed erythrocytes and occasional mononuclear cells. The lesion produced only minimal mass effect and was not associated with distortion or compression of the central canal. In four of the nine animals, studied 2 to 6 hours postinjection, blood was seen within the lumen of the central canal (Fig. 1). The number of erythrocytes varied from one to five cells per cross-sectional area to clumps of 20 or more cells that extended rostrally from the level of injection to the fourth ventricle. Caudal to the lesion, the lumen of the central canal contained one to five cells per cross-sectional area or was empty. Hematomas associated with the extension of whole blood into the central canal were located in the ventrolateral quadrant of the dorsal columns (two animals) and the ventral midline (two animals). In none of these cases was blood injected directly into the central gray

J. Neurosurg. / Volume 75 / December, 1991
Histopathology of experimental hematomyelia

One to 3 Days Post-Hemorrhage

After 24 hours, the clot became consolidated and there was an intense proliferation of microglial cells at the perimeter of the lesion (Fig. 2). Resting cells with stellate prolongations were transformed into ameboid and pseudopodic forms which invaded the hematoma, infiltrated its core, and removed erythrocytes by phagocytosis. The lumen of the central canal was found to contain varying amounts of fibrin, proteinaceous fluid, and crenated erythrocytes that were more numerous rostral to the level of the hemorrhage.

Four to 15 Days Post-Hemorrhage

Histological sections through the lesion site revealed continuing infiltration of the parenchymal hemorrhage by microglial cells. Cells containing ingested erythrocytes stained positively for iron after the 5th postinjection day, reflecting the splitting of the hemoglobin molecule to form hemosiderin. By 15 days, most erythrocytes had been replaced by iron granule bodies (hemosiderin-laden microglial cells) which filled the core of the hemorrhage (Fig. 3). A few reactive astrocytes were usually seen at the circumference of the lesion by 10 days.

In eight of the 12 animals examined 4 to 15 days post-hemorrhage, serial sections of the whole spinal cord revealed varying amounts of debris within the lumen of the central canal. The most commonly encountered substances were fibrin casts or webs, cell membranes, and amorphous material which stained positively for protein. Other types of debris included ghost cells and occasional iron granule bodies that were sometimes seen between ependymal cells at the level of the lesion (Fig. 4 left). Intraluminal debris was observed at all levels of the spinal cord, but was present in much greater amounts and for longer periods of time (10 to 15 days) in the segments of the canal between T5-6 and the obex (Fig. 4 right). Subependymal glial nodules that were insufficiently developed to describe as epen-
Fig. 5. Photomicrographs of experimental hematomyelia at 42 days after injection of blood. H & E, x 150. Left: Axial section through the lesion at T5-6 showing fine glial scar in the midline of the dorsal columns (arrow). Right: Same section at higher magnification showing tuberal gliosis and hemosiderin-laden microglial cells (arrow).

dymal granulations were encountered one to five levels above the lesion in three animals at 10 days, 15 days, and 23 days, respectively. There was no evidence of central canal occlusion or syrinx formation in this experimental model.

Sixteen Days to 4 Months Post-Hemorrhage

After 15 days, there was a marked contraction of the parenchymal hematoma (Fig. 5). Clearance of the hemorrhage was essentially complete within 30 days except for residual iron granule bodies and focal gliosis that were present with little change at 117 days. Collagen formation was not observed in preparations stained with Masson's trichrome.

Discussion

Detailed information concerning the histopathology of hematomyelia is of potential value in the interpretation of magnetic resonance (MR) images, the design of treatment strategies for intramedullary hemorrhage, and the study of mechanisms involved in the development of posttraumatic syringomyelia. The experimental model employed in this study is not representative of lesions encountered in clinical practice. However, it provides a means for examining the consequences of intramedullary hemorrhage using lesions that are controlled in terms of size, shape, and location, and is distinguished from models of spinal cord trauma by the absence of significant tissue damage. The pathological findings in atraumatic hematomyelia have not been reported previously.

In the current study, the injection of fresh whole blood into the dorsal columns of the spinal cord was found to have two specific effects: 1) the induction of a local cellular response characterized by the acute proliferation of microglial cells and the subacute proliferation of astrocytes, and 2) the drainage of varying amounts of blood or blood products into the central canal of the spinal cord.

Local Cellular Response

The microglial response was found to occur in the following sequence: transition of resting cells at the perimeter of the hemorrhage into ameboid types within 24 hours, invasion of the hematoma with infiltration of its core after 24 hours, and phagocytosis of erythrocytes with conversion of microglial cells into iron granule bodies between 5 days and 4 weeks. This sequence of events is similar to that known to occur in response to penetrating injuries, inflammations, and necrosis of nervous tissue.2-6 In the latter cases, however, the ingestion of destroyed axons and nerve cells leads to the formation of fat granule bodies which greatly outnumber iron granule bodies unless the lesion has produced significant intramedullary hemorrhage.

The ability of microglial cells to proliferate rapidly and to remove substances by phagocytosis is consistent with their function as scavenger cells. In the broadest sense, the microglia serve as the reticuloendothelial system of the brain and spinal cord, and are extremely motile cells that not only invade injured areas, but are believed to carry digested substances to regional capillaries for transport across the endothelium.2-6 The observation in the current study that iron granule cells are sometimes seen between ependymal cells and within the lumen of the central canal suggests an alternative route for removing digested substances.

In the subacute stages of healing, a proliferation of astrocytes was usually evident at the perimeter of the lesion. Glial fibers were laid down between 3 and 6 weeks after injection of blood, and the hematoma was replaced by a fine glial scar without evidence of collagen formation. On the basis of these findings, it seems reasonable to conclude that intramedullary hemorrhage in the absence of significant tissue injury provokes a comparatively minor astrogliotic response.

Sink Action of Central Canal

In this experimental model, the extension of blood or blood products from parenchymal hemorrhages into the central canal of the spinal cord was similar to the clearance of periventricular hemorrhages that extend from the subependymal white matter of the basal ganglia into the lateral ventricles. Deep intramedullary hemorrhages, which were situated in the ventral midline and ventrolateral quadrant of the dorsal columns, were associated with the drainage of varying amounts of whole blood into the lumen of the central canal during the acute stages of hemorrhage (2 to 6 hours), whereas more superficial hemmorhages were associated with the intraluminal drainage of secondary blood products such as fibrin and cellular debris after 24 hours. These differences presumably reflect the distance of parenchymal
Histopathology of experimental hematomyelia

hemorrhages from the ependymal lining of the central canal.

Intraluminal filling appeared to be more pronounced and to persist for longer periods in the segments of the canal rostral to the level of the hemorrhage. Drainage extended as far rostrally as the fourth ventricle, suggesting that some substances may have been cleared into the cerebrospinal fluid (CSF) in a manner resembling the clearance of periventricular hemorrhages. The drainage of blood products in a rostral direction is not surprising when it is remembered that the lumen of the central canal is closed at its caudal end and open rostrally where it is anatomically continuous with the fourth ventricle through a well-defined aperture. Like the cerebral ventricles, the central canal of the spinal cord contains CSF and is lined by ciliated ependymal cells. Whether ciliary movement contributes to the clearance of the central canal similar to the function proposed for the ventricular ependyma3,13 remains to be established. Overall, the foregoing findings are consistent with a sink action of the central canal that is capable of clearing substances as large as erythrocytes from the parenchymal tissues of the spinal cord into the CSF.

It is important to emphasize that the findings in experimental hematomyelia may be very different from those encountered clinically. In man, the lumbar and thoracic segments of the spinal cord are frequently obliterated during the middle years of adult life by a proliferation of ependymal cells3,16 This phenomenon is unique to man; in other mammalian species the lumen of the central canal remains patent throughout life. Although any comparison of these results with human disorders is therefore speculative, it is interesting to observe that in patients with posttraumatic syringomyelia, the syrinx cavity is typically found extending rostrally from the level of the injury3,12,13,14,17. The possibility that syrinx formation is attributed in part to the rostral drainage of necrotic tissue or blood products through the lumen of the central canal is currently under investigation.

References


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