Noncommunicating syringomyelia following occlusion of central canal in rats

Experimental model and histological findings

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This report describes a new and reliable technique for producing experimental noncommunicating syringomyelia. In 30 rats, 1.2 to 1.6 μl of kaolin was microinjected into the dorsal columns and central gray matter of the spinal cord at C-6. The inoculations caused transient neurological deficits in four animals and no deficits in 26 animals. Within 24 hours, kaolin and polymorphonuclear leukocytes entered the central canal and drained rostrally. The clearance of inflammatory products induced a proliferation of ependymal cells and periependymal fibrous astrocytes, which formed synechiae and obstructed the central canal at the level of injection and at one or more levels up to C-1. In 22 animals followed for 48 hours or longer, the upper end of the central canal became acutely dilated and formed an ependyma-lined syrinx that enlarged to massive dimensions within 6 weeks. The rostral syrinxes did not communicate with the fourth ventricle and were not associated with hydrocephalus.

The histological findings in acute noncommunicating syringomyelia were characterized by progressive stretching and thinning of the ependyma, elongation of intracanalicular septae, and the formation of periependymal edema. After 3 weeks, there was progressive compression of the periependymal tissues associated with stretching of axons, fragmentation of myelin sheaths, and the formation of myelin droplets. These findings and the sequence in which they evolved were identical in most respects to those occurring in acute and subacute noncommunicating hydrocephalus.

KEY WORDS • syringomyelia • hydromyelia • ependyma • kaolin • edema • hydrocephalus • cerebrospinal fluid • demyelinating disease

Cavitary enlargement of the spinal cord (syringomyelia) has attracted increasing attention in recent years as the availability of magnetic resonance (MR) imaging has contributed to early diagnosis and the detection of cases that were either not recognized or classified erroneously as something else.14,21,23-26 Although syringomyelia is associated with a wide variety of congenital and acquired disorders, its pathogenesis is poorly understood. The study of syrinx formation has been limited until now by the availability of reliable animal models.12 The most effective experimental technique is to inject sclerosing agents such as kaolin into the cisterna magna,4,12,13,28 which produces basilar arachnoiditis and leads to symmetrical enlargement of the cavities proximal to the block, including all four cerebral ventricles (communicating hydrocephalus) and the central canal of the spinal cord (hydro-
myelia has not been available previously, the current model may prove useful in the study of pathogenetic mechanisms.

Materials and Methods

Following review and approval of the experimental protocol by our institution's Animal Care and Use Committee, adult male Sprague-Dawley rats, weighing 300 to 350 gm each, were anesthetized briefly with a mixture of halothane, 70% nitrous oxide, and 30% oxygen. Preliminary experiments were carried out in approximately 25 rats to establish dose parameters for the intramedullary injection of kaolin.

In 34 animals, a single-level laminectomy was performed at C-6 under the operating microscope. Hemostasis was achieved without the use of electrocautery. A Kopf frame, mounted with a No. 30 needle and a 10-μl Hamilton syringe, was brought into the operating field. With a previously described stereotactic technique, the needle was advanced under microscopic guidance through the translucent dura into the dorsal midline of the spinal cord to a depth of 1.3 mm. The bevel of the needle was arranged to point caudally or laterally along the longitudinal axis of the spinal cord. In 30 animals, 1.2 to 1.6 μl kaolin was injected into the dorsal columns and central gray matter of the spinal cord during a period of 30 seconds. Four animals served as controls and received an injection of 1.6 μl normal saline. After closure of the wounds, the animals were returned to their cages and neurological assessments were made on a daily basis.

Animals receiving injections of kaolin were killed with a lethal dose of intraperitoneal 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: 24 hours (eight animals); 48 hours (six animals); 72 hours (four animals); 7 days (four animals); 21 days (four animals); or 42 days (four animals). The four rats in the control group were killed and perfused at 1, 7, 21, or 42 days, respectively. The brain and spinal cord were removed as one specimen, divided into blocks, and dehydrated in a tissue processor prior to embedding in paraffin. The cerebral hemispheres were sectioned coronally. The lower brain stem and upper cervical spinal cord were sectioned axially or sagittally. Caudal to C-6, the spinal cord was sectioned axially. A microtome was used to cut thin serial sections to a uniform thickness of 7 μm. The sections were mounted on glass slides, stained either with hematoxylin and eosin or luxol fast blue, and viewed through a microscope at magnifications ranging from ×20 to ×1000.

Results

Experimental Model

There were no discernible differences in the ability to run, stand, and jump, or in the responses to light touch and painful stimuli in the four saline-injected rats and 26 kaolin-injected rats 1 hour after injection. Four kaolin-injected rats exhibited slight weakness in one or both lower extremities that cleared within 24 hours. Animals that were followed for 1 to 14 days did not exhibit neurological deficits. In six of eight kaolin-injected animals followed for 14 to 42 days, there was evidence of slowly progressive impairment in the ability to stand and jump.

Axial sections through the spinal cord revealed kaolin crystals and polymorphonuclear leukocytes at the injection site (Fig. 1) which entered the central canal within 24 hours and drained rostrally toward the fourth ventricle (Fig. 2 left). Caudal to the level of injection, the central canal contained occasional cells and crystals of kaolin, or was empty. The drainage of inflammatory products through the upper end of the canal appeared to induce an ascending proliferation of septae or synechiae that obstructed the lumen at C-6 and at one or two levels lower.
more levels up to C-1 (Fig. 2 right). The obstructed segments of the canal became acutely dilated and produced a rostral, ependyma-lined syrinx that enlarged to massive proportions within 6 weeks (Fig. 3). Caudal to the syrinx, there was gradual dilatation of the central canal associated with granuloma formation at the injection site. The syrinxes in this experimental model did not communicate with the fourth ventricle and there was no histological evidence of basilar arachnoiditis, ventricular ependymitis, or hydrocephalus.

**Histological Findings**

Within 24 hours after kaolin injection, there was evidence of marked germinall activity in the basal layer of the ependymal epithelium and the subependymal glial sheath rostral to the level of injection. Newly formed ependymal cells and fibrous astrocytes produced invaginations or buds that obstructed the lumen of the canal at one or more levels up to C-1 (Fig. 4). Intracanalicular synechiae were well developed within 48 to 72 hours and contributed to the formation of monolocular or segmented cavities that dilated acutely. After 72 hours, the obstructed segments of the canal enlarged slowly and produced a large ependyma-lined syrinx rostral to the level of the injection that did not communicate with the fourth ventricle.

With continuing enlargement of syrinx cavities, there was stretching and thinning of the ependymal epithelium, elongation of intracanalicular synechiae, and formation of spongy changes within the periependymal tissues. Elongated synechiae were composed of a single layer of ependymal cells (Fig. 5 left) or a complex band of fibrous astrocytes (Fig. 5 right), and appeared as irregular, transverse septae within the main body of some syrinx cavities. In subacute syrinxes (21 to 42 days after kaolin injection), the ependymal epithelium was markedly thinned or denuded (Fig. 6), and very large cavities were often lined predominantly by fibrous astrocytes derived from the subependymal glial sheath. There was increasing vacuolization of the periependymal tissues associated with wide separation of the cellular elements characteristic of edema.
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After 3 weeks, there was increasing compression of the periependymal tissues associated with stretching of axons, fragmentation of myelin sheaths, and the formation of myelin droplets (Fig. 7). These changes appeared to be confined to the white matter, especially within the dorsal columns, and the gray matter surrounding the syrinxes was selectively spared. There was no evidence of cell loss or phagocytosis of myelin during the period of this study.

Analysis of findings in four saline-injected rats revealed no evidence of local injury at the injection site or changes in size of the central canal lumen. There was no histological evidence of ependymitis, germinal activity in the basal layer of the ependymal epithelium, or subependymal gliosis at 1, 7, 21, or 42 days following injection.

Discussion

Experimental Model

Previously reported techniques for producing experimental syringomyelia have consisted of injecting sclerosing agents into the cisterna magna or basilar cisterns.5,8,13,28 These models yield a communicating type of syringomyelia (hydromyelia) in association with hydrocephalus, which resembles the pattern of cavity enlargement in man following meningeal infections, subarachnoid hemorrhage, and other causes of basilar arachnoiditis.13 In patients with all types of syringomyelia, however, MR imaging has demonstrated that less than 10% of cavities communicate directly with the fourth ventricle,23 although the compressed or distorted segment of the central canal may be anatomically patent at the time of necropsy.4 The availability of experimental models for both noncommunicating and communicating types of syringomyelia may be helpful in examining differences in pathogenesis.

The experimental technique developed for this study would appear to provide the first reproducible model of noncommunicating syringomyelia. In 22 rats followed for 48 hours or longer after induction, there was a sizable syrinx present rostral to the level of injection that enlarged progressively and did not communicate with the fourth ventricle. Since the cavities were lined by ependyma, they were presumably formed by segmental dilatation of the central canal. A particular advantage of the current model is that the microinjection technique produces minimal or no neurological deficits. This makes it possible to follow animals for prolonged periods of time and provides an opportunity to study the sequential pathological and pathophysiological consequences of progressive canaliculat enlargement.

The mechanism by which noncommunicating syrinxes enlarge is poorly understood. In the current experimental model, the intramedullary injection of kaolin produced complete occlusion of the central canal below the level of the obex, so that syrinx formation cannot be ascribed to the "water hammer" effect of the CSF pulse wave12 or to the establishment of a pressure gradient between the intracranial and intraspinal compartments that draws fluid from the fourth ventricle into the central canal.27 Nevertheless, the development of syrinxes caudal to blocks does not invalidate the influence of hydrodynamic factors or necessarily conflict with the proposition that CSF enters the cavity from the subarachnoid space through enlarged Virchow-Robin spaces or the dorsal roots.2 Other possible sources of a net increase in syrinx fluid include inflam-
matory products, secretion by the ependyma, and interstitial fluid derived from water during metabolism.

The observation that syringomyelia can be produced experimentally by the intramedullary injection of kaolin is consistent with the recently described "sink function" of the central canal. The observation that syringomyelia can be produced experimentally by the intramedullary injection of kaolin is consistent with the recently described "sink function" of the central canal. Following the micro-injection of vital dyes, horseradish peroxidase, and blood into the dorsal columns of the rat spinal cord, these substances are cleared rapidly into the central canal and drain rostrally to the fourth ventricle. It has been suggested that, in patients with postratamatic syringomyelia, the location of syrinxes which are typically found above the level of injury may be attributable in part to the rostral drainage of necrotic tissue or blood products through the central canal. The findings in the current study provide further evidence that the central canal can function as a sink that is capable of removing substances as large as cellular elements from the parenchymal tissues of the spinal cord.

In the current model of noncommunicating syringomyelia, occlusion of the upper end of the central canal was found to produce acute dilatation of the lumen caudal to the block. A similar relationship has been observed in patients with Chiari malformation, basilar impression, cervical disc herniation, and various extradural cysts and tumors, in which the syrinx cavity is often found immediately caudal to the constricted segment of the spinal cord. It is interesting to speculate, based on evidence that the central canal serves as a sink and drains substances in a rostral direction to the fourth ventricle, that obstruction of this outflow pathway may play a role in the pathogenesis of syringomyelia. The gradual enlargement of the central canal caudal to the level of injection in these experiments suggests that canalicular enlargement may be influenced by a variety of factors such as luminal length and regional differences in CSF dynamics.

Histological Findings

The pathology of kaolin-induced noncommunicating syringomyelia appears to involve several steps: 1) induction of a localized inflammatory reaction; 2) drainage of inflammatory products in a rostral direction through the central canal; 3) occlusion of the canalicular lumen by proliferating ependymal cells and fibrous astrocytes; and 4) dilatation of the obstructed segments of the canal. Within 24 hours, there was evidence of intense gerinal activity in the basal layer of the ependymal epithelium and the subependymal glial sheath rostral to the level of injection. Although it is likely that the proliferation of gerinal cells was induced wholly or in part by the ascending drainage of inflammatory products, a similar type of response has been reported in experimental hydrocephalus as a consequence of acute stretching of the ventricular ependyma. For example, following obstruction of the fourth ventricle in primates, gerinal activity is seen within 2-3 hours at the angles of the lateral ventricles involving undifferentiated cells immediately beneath the ependymal epithelium. The germinating cells produce new ependymal cells and fibrous astrocytes which form synchial adhesions between the ventricular walls. Stretching and tearing forces appear to be the stimuli for synchial formation in this experimental model, but germinant cells are known to mobilize rapidly and produce new ependymal cells and fibrous astrocytes in response to a wide variety of stressful stimuli including intraventricular hemorrhage, air or positive contrast ventriculography, and acute ventricular infections.

It is obvious that caution must be exercised when comparing the findings in this study with those encountered in patients with syringomyelia. However, it is not uncommon for routine MR imaging to demonstrate loculated syrinx cavities that are enlarged segmentally or divided by transverse trabeculae. Such findings are of diagnostic interest and may influence surgical decisions such as the optimal site for shunt placement. Based on the results of the current study, it seems reasonable to suggest that segmental dilatation or trabeculation of syrinx may reflect one or more of the following events: acute syrinx formation, recent dissection of a pre-existing syrinx, or irritation of the syrinx walls by substances such as blood.

In the current experiments, the rate of canalicular dilatation was especially rapid during the first 48 to 72 hours, and slowed thereafter to produce gradual but progressive enlargement of the syrinx cavity. This time sequence is similar to the biphasic rate of cavity enlargement occurring in acute noncommunicating hydrocephalus which has been attributed to the development of compensatory changes such as transependymal drainage of CSF, reduced intracavitary pressure as influenced by Poiseuille's law, and atrophy of the white matter.

The histological findings in acute noncommunicating syringomyelia were characterized by progressive stretching and thinning of the ependyma, elongation of intracanalicular septae, and the formation of perisyrineal edema. After 3 weeks, there was progressive compression of the perisyrineal tissues associated with stretching of axons, fragmentation of myelin sheaths, and the formation of myelin droplets. There was no evidence of cell loss or phagocytosis of myelin up to 6 weeks following syrinx formation. These findings and the sequence in which they evolved were identical in most respects to those occurring in acute and subacute noncommunicating hydrocephalus.

References

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