Reconstitution of shunted mantle in experimental hydrocephalus

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The morphological mechanism of the reconstitution of shunted mantle was studied histopathologically in 22 kaolin-treated hydrocephalic puppies. A remarkable attenuation of cerebral mantle to less than 1 cm in thickness was seen on computerized tomography (CT) scans of four animals sacrificed 1 to 2 months after kaolin treatment (preshunt group). Ventricular shunting resulted in successful recovery of the mantle on repeated CT scans obtained 1 to 2 months after shunting in seven animals (postshunt group). In the remaining 11 animals the cerebral mantle, which had been reduced to 4 mm in thickness prior to shunting, failed to recover even 2 months after the procedure (shunt-refractory group).

On gross inspection, the preshunt specimens showed marked thinning of the white matter, with the cortical ribbon well preserved, while the postshunt specimens consisted predominantly of thickened white matter. Histopathological examination of the attenuated white matter of the preshunt specimens showed decreased nerve-fiber density, myelin destruction with myelin regeneration and/or repair of myelin sheaths, and reactive astrocytosis, which were prominent especially in the periventricular white matter. The main findings in the reconstituted white matter of the postshunt specimens were extensive myelin regeneration of residual axons and remarkable astroglial proliferation with mesenchymal reaction, particularly at capillaries. No clear evidence of increased numbers of nerve fibers or axonal regeneration was observed. The shunt-refractory specimens showed remarkable attenuation of cortex, in which reduced numbers of neurons and loss of cortical lamination were noted, with vestigial white matter.

The results indicate that astroglial proliferation with mesenchymal reaction and myelin regeneration contribute to the reconstitution of the cerebral mantle volume following ventricular shunting in this model. It is suggested that the critical factor for mantle reconstitution in chronic hydrocephalus is whether cortex is preserved.

Key Words • hydrocephalus • cerebral mantle • shunt • dog

The extremely reduced cerebral mantle seen in infantile hydrocephalus is often observed to be completely reconstituted when examined months to years after ventricular shunting. The morphological mechanism of gradual mantle reconstitution, which is thought to involve a structural increase in brain tissue, has not been established. In experimental models of acute and subacute hydrocephalus, prompt reduction of ventricular size and rapid gross recovery of the cerebral mantle have been reported by many investigators. Hochwald, et al., and Epstein, et al., whose experimental results showed no histopathological differences between pre- and postshunt specimens, considered rapid mantle recovery to be due to a mechanical shortening of fibers or a rearrangement of pre-existing elements. On the other hand, increased vascular beds or brain elasticity have also been assumed to contribute to the rapid recovery of the shunted mantle.

In chronic experimental hydrocephalus using adult animals, many investigators have failed to observe the gradual reconstitution of the mantle probably because of the difficulty in producing the extreme mantle attenuation seen in clinical cases of infantile hydrocephalus. Therefore, little information is available to describe adequately the histopathological changes in the cortex and adjoining white matter during this event. We have successfully produced a suitable model for chronic infantile hydrocephalus using 1-month-old mongrel puppies, in which the cerebral mantle is reduced to 4 mm minimum thickness. The present morphological investigation was conducted to determine...
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Fig. 1. Computerized tomography scans of a representative animal from each group. A: The preshunt group scan shows remarkable dilatation of ventricles with cerebral mantle reduced to 1 cm in thickness. B: The postshunt group scan shows almost complete recovery of the cerebral mantle 2 months after ventricular shunting. C: The shunt-refractory group scan shows extreme attenuation of the cerebral mantle, which failed to recover after ventricular shunting.

the elements of the cerebral mantle that are reconstituted after ventricular shunting and to clarify whether neuronal regeneration contributes to the reconstitution.

Materials and Methods

Kaolin solution (200 mg/ml/kg) was injected percutaneously into the cisterna magna of mongrel puppies weighing 1 to 1.5 kg about 1 month after birth. One or 2 months after treatment, 22 puppies showed severe hydrocephalus with a mantle thickness of less than 1 cm on computerized tomography (CT). Eighteen animals underwent ventriculoperitoneal shunting using a one-piece catheter with a distal slit valve of low pressure (20 to 30 mm H2O) via a right parietal burr hole following full-thickness mantle biopsy. The other four puppies were sacrificed as a "preshunt group" (Fig. 1A). About 1 to 2 months later, seven of the 18 shunted animals showed successful recovery of the mantle on repeat CT scan and served as a "postshunt group" (Fig. 1B). In the remaining 11 animals the shunt failed to recover after ventricular shunting; these served as a "shunt-refractory group" (Fig. 1C). Another age-matched six animals that did not receive kaolin treatment were sacrificed as normal controls.

In the following morphological studies, the cerebral mantle of the postshunt group was compared with that of the preshunt group, the shunt-refractory group, and the normal controls. The mantle biopsy specimen taken prior to shunting was compared with the cerebral mantle of the preshunt group.

Puppies of both groups were perfused with 2.5% glutaraldehyde and 1.5% paraformaldehyde solution buffered with 0.1 M sodium cacodylate. Coronal brain sections were embedded in paraffin following dehydration and stained for light microscopy with hematoxylin and eosin, cresyl-violet, and Luxol fast blue. For the ultrastructural study, the cortex and white matter of coronal brain sections at the chiasmal level were cut and fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide, then embedded in Epon. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined under an electron microscope.

Results

In the postshunt group the cerebral mantle, which had been about 1 cm thick prior to shunting, showed complete recovery of ventricular size and gross configuration in all seven animals. The cerebral mantle in the

Fig. 2. Macroscopic photographs of representative animals from each group and a normal control. A: The cerebral mantle of the preshunt group consisted of extremely attenuated white matter and spared cortical ribbon. B: The postshunt group showed an increased volume of white matter in comparison with the preshunt group. The cortical ribbon of the recovered mantle was slightly thicker than that of the preshunt specimens. C: The cerebral mantle of the shunt-refractory group showed attenuated cortical ribbon and vestigial white matter that was indistinguishable from the cortex. D: A normal control matched in age with the postshunt group. H & E, Luxol fast blue × 2.
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FIG. 3. Photomicrographs of periventricular white matter (A, B, and C) and deep white matter (D, E, and F). In the preshunt specimens (A and D), the number of myelinated nerve fibers is reduced in comparison with normal controls (C and F), and a number of myelin globules and reactive astrogliosis are seen more prominently in the periventricular white matter (A). Interstitial edema is not present in either the periventricular (A) or deep white matter (D). In the postshunt specimens (B and E), there is remarkable proliferation of astroglial cells and their processes, which seemed to replace nerve fibers, particularly adjacent to capillaries. The number of myelin globules in postshunt specimens is decreased in comparison with preshunt specimens. Increased staining of myelin is also noted in the periventricular white matter of postshunt specimens (B). Toluidine blue, × 200.

shunt-refractory group, which had decreased to 4 mm thick prior to shunting, remained at the same thickness, although a shunt revision was performed for shunt malfunction in four animals.

Preshunt Group Specimens

Macroscopic findings of the preshunt cerebral mantle (1 cm thick) consisted of extremely attenuated white matter and spared cortical ribbon (Fig. 2A). The cortex showed no definite cytoarchitectural changes, although several pyramidal neurons appeared atrophic under light and electron microscopy. Light microscopic examination of the periventricular white matter near the anterior horn of the lateral ventricles showed a number of myelin globules and fragmented myelin sheaths, a few ballooned axons, and reactive astrocystosis (Fig. 3A). The number of myelinated nerve fibers was reduced in comparison with age-matched normal controls (Fig. 3C). The ependymal lining was disrupted and almost completely lost in some portions. Extracellular edema was present, but its extent was minimal and limited to the periventricular region. In the deep white matter, myelin globules and myelin fragments were also noted, but their number was reduced (Fig. 3D) in comparison with that in the periventricular region. Under electron microscopy, a few ballooned axons containing numerous dense bodies, myelin destruction, and increased numbers of glial filaments in astrocytic cell processes
were seen in the periventricular white matter. A number of repaired myelin sheaths and/or remyelinated nerve fibers were also observed in the same region. In the deep white matter, myelin destruction and increased numbers of astroglial cells were also seen, but these cells were not as numerous as in the periventricular white matter. Intracellular edema was not evident in the attenuated white matter.

**Postshunt Group Specimens**

On gross inspection, the shunted cerebral mantle was observed to have become thicker than in the preshunt specimens, mainly as a result of an increased volume of white matter (Fig. 2B). The cortical ribbon of the recovered mantle was slightly thicker than that of preshunt specimens. Microscopically, a few pyramidal neurons appeared atrophic in the cortex of the reconstituted cerebral mantle. However, under light and electron microscopy, the histopathological appearance of the cortex was similar in the two groups.

In the thickened white matter near the ventricular portion of the anterior horn, the most prominent histopathological findings were extensive proliferation of astroglial cells and their processes, which seemed to replace nerve fibers. Increased staining of myelin was noted in the periventricular white matter. The extent of myelin destruction was decreased, and extracellular edema was not present anywhere in the white matter. Astrocytic proliferation was recognized, particularly adjacent to capillaries, not only in the periventricular white matter but also in the deep white matter (Fig. 3B and E). The ependymal lining was almost completely restored and showed thickening of the subependymal glial layer in some of the seven animals. Electron microscopic study of the reconstituted white matter showed extensive regeneration and repair of myelin sheaths (Figs. 4A and 7). Proliferation of astroglial cells with numerous glial filaments in their perikaryon and foot processes was also noted throughout the white matter (Fig. 5A). Increased numbers of vascular feet with abundant glial filaments, collagen fibers, and mesenchymal cells were observed in the abluminal space around capillaries (Fig. 5B). Ependymal cells showed prominent interdigitation of upper and lateral cell surfaces and fully repaired junctional complexes (Fig. 6).

**Shunt-Refractory Group Specimens**

Gross inspection of the cerebral mantle, which had been reduced to 4 mm in thickness, revealed attenuated cortical ribbon and vestigial white matter. The vestigial white matter was indistinguishable from the cortex and was not stained by Luxol fast blue (Fig. 2C). Light microscopy of the attenuated cortex showed reduced numbers of neurons and loss of cortical lamination as well as many atrophic pyramidal neurons.

**Discussion**

The main histopathological changes in chronic experimental hydrocephalus are disruption of the ependymal lining, degeneration of nerve fibers and myelin sheath, reactive astrocytosis, and reduction of interstitial edema in the periventricular white matter. Little information is available with regard to myelin regeneration, however. Although the cortex had been considered to be spared in experimental hydrocephalus, varicostic change...
in dendrites and reduced dendritic spine densities have been found recently using Golgi's staining method.\textsuperscript{11} In our experiments, histopathological changes in preshunt white matter did not differ from those described in the above reports, except for remyelination. The preshunt cortex of our model showed only a few atrophic pyramidal neurons under light and electron microscopy.

The shunted mantle in our model was reconstituted 1 to 2 months after ventricular shunting, predominantly by the thickening of white matter. Light and electron microscopic study of the reconstituted (postshunt) white matter revealed extensive regeneration and/or repair of the myelin sheath and prominent astroglial proliferation with mesenchymal reaction throughout the white matter (Figs. 4, 5, and 7), in comparison with preshunt white matter (Fig. 3A and D). The ependymal lining was almost completely repaired by thickening of the subependymal glial layer. However, evidence of neuronal regeneration was not observed in our study. Taking these findings into consideration, the elements that appear to regenerate or repair in the reconstituted white matter are the myelin sheath, astroglia with mesenchymal reaction, and the ependyma.

While regeneration and repair of myelin sheath in the central nervous system (CNS) have been demon-

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**Fig. 5.** Electron micrographs of the reconstituted white matter in a postshunt specimen. A: Increased numbers of astroglial processes including abundant glial filaments are observed. B: Around capillaries, increased numbers of vascular feet with abundant glial filaments, collagen fibers, and a mesenchymal cell (asterisk) are observed in the abluminal space. L = capillary lumen. Bar = 1 μm.

**Fig. 6.** Electron micrograph of the restored ependymal lining in a postshunt specimen showing prominent interdigitation of upper and lateral cell surfaces and fully recovered junctional complexes. V = ventricle. Bar = 3 μm.

**Fig. 7.** Electron micrograph of the repair of myelin sheath in reconstituted white matter in a postshunt specimen. The myelin sheath surrounds an oligodendrocyte (open arrow). The process of myelin formation is also seen (arrowheads). Bar = 2 μm.
Reconstitution following nation phalic clear procedure? the are served sures. Mitotic animals suits significant region. In our study, definite evidence of regenerated myelin sheath was observed in the periventricular region of preshunt white matter and throughout the postshunt white matter. It is unclear whether myelination and/or myelin regeneration are accelerated following ventricular shunting. Some investigators reported that in man hydrocephalus controlled by shunting, unlike untreated hydrocephalus, was associated with normal myelination. Others reported no significant difference in myelination between shunted and nonshunted hydrocephalic infants. Considering the results of our experiment, it is conceivable that young animals may have a large capacity to remyelinate and that ventricular shunting may accelerate myelin regeneration (Figs. 4 and 7). It is well known that regenerated myelin sheath is one-third the thickness of normal myelin sheath. Therefore, it is inconceivable that myelin regeneration plays a major role in the voluminous recovery of shunt-reconstituted white matter.

Astroglia are known to have considerable ability to proliferate and repair various lesions in the CNS. Mitotic figures and astrocyte migration following tissue damage have actually been recognized in recent research. In experimental hydrocephalus, mitotic figures and proliferation of astroglia with time were observed in the periventricular white matter. They were considered to originate from pre-existing astroglia and/or vestigial germinal cells in the subependymal layer. In our experimental model, the rich increase in astroglia associated with mesenchymal reaction was observed throughout the reconstituted white matter and was regarded as a main contributor to the restoration of the shunted mantle.

Ependymal cells are considered to regenerate in experimental hydrocephalus possibly arising from subependymal precursors of vestigial germinal cells. Rubin, et al. reported that the ependymal lining appeared normal under light microscopy 1 week after shunting in experimental hydrocephalus. In our experiments, light microscopy revealed almost complete recovery of the ependymal lining in postshunt specimens. Electron microscopic study of the restored lining revealed complete recovery of intercellular junctional complexes and prominent interdigitation of ependymal cell surfaces. In the mature CNS, neuronal elements have been considered not to regenerate; however, recent electron microscopic studies have demonstrated clear evidence of axonal regeneration under experimental circumstances. Our observations showed no evidence of axonal regeneration in the reconstituted white matter, but proliferation of astroglia and associated mesenchymal elements (connective tissue) may have interfered with axonal regeneration.

While early shunting is considered to be of the utmost importance in the prevention of secondary tissue damage under the hydrocephalic condition, it is also important to determine the minimum width of cerebral mantle that can be reconstituted by ventricular shunting. In clinical cases of chronic infantile hydrocephalus, even cerebral mantle reduced to a few millimeters and in which one can distinguish the thin ribbon of cortex and subjacent white matter has been observed to be reconstituted following shunting. In the present study, the cerebral mantle of the shunt-refractory group was attenuated to around 4 mm, and definite evidence of cortical damage was common. In contrast, cerebral mantle that showed successful reconstitution after shunting (postshunt group) was reduced to about 1 cm in thickness, and evidence of cortical damage was not present. From our results and clinical evidence it is suggested that, once tissue damage extends over the cortex, the cerebral mantle is not likely to be reconstituted. Therefore, whether or not cortical damage is evident might be a critical factor in shunt reconstitution.

Conclusions

This experiment indicated that structural recovery in shunted hydrocephalus is derived from astroglial proliferation with mesenchymal reaction and repair and/or regeneration of myelin sheath. Further studies on the functional contribution of these reconstituted neuroglial elements will be required.

References


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