Altered formation and bulk absorption of cerebrospinal fluid in FGF-2-induced hydrocephalus


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Johanson, C. E. J. Szmydnger-Chodobska, A. Chodobski, A. Baird, P. McMillan, and E. G. Stopa. Altered formation and bulk absorption of cerebrospinal fluid in FGF-2-induced hydrocephalus. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R263–R271, 1999.—Up-regulation of certain growth factors in the central nervous system can alter brain fluid dynamics. Hydrocephalus was produced in adult Sprague-Dawley rats by infusion of recombinant basic fibroblast growth factor (FGF-2) at 1 µg/day into a lateral ventricle for 2, 3, 5, or 10–12 days. Lateral and third ventricular enlargement progressively increased from 2 to 10 days. Ventriculomegaly was also induced by a 75% reduced dose of FGF-2. At 10–12 days, there was a 29% attenuation in cerebrospinal fluid (CSF) formation rate, from 2.5 to 1.8 µl/min (P < 0.01). Choroid plexus, the main site of CSF secretion, had an augmented number of dark epithelial cells, which have previously been associated with decreased choroidal fluid formation. The twofold elevated resistance to CSF absorption, i.e., 0.8 to 1.7 mmHg·min⁻¹·µl⁻¹, was attributable, at least in part, to enhanced fibrosis and collagen deposits in the arachnoid villi, a major site for CSF absorption. Normal CSF pressure (2–3 mmHg) was consistent with a patent cerebral aqueduct and reduced CSF formation rate. The FGF-2-induced ventriculomegaly is interpreted as an exaggerated increase in intracranial pressure; growth factors; fluid dynamics; ventriculomegaly; normal-pressure hydrocephalus; extracellular matrix; neuroendocrine regulation; fibroblast growth factor and transforming growth factor-β (TGF-β) suggest their potential for altering tissues that mediate CSF turnover.

Neuropeptides and neurotransmitters regulate CSF production through changes in CP blood flow and epithelial ion transport (1, 3, 4, 11, 22, 23, 39). Because growth factors interact with other peptides to modify ion transport in the kidney (17), this raises the question of the ability of growth factors to control fluid balance in the brain. In fact, upregulation of TGF-β in CNS alters the extracellular milieu and causes hydrocephalus (16, 47). Moreover, the elevation of intraventricular FGF-2 (9, 42) and TGF-β (28, 45) leads to ventriculomegaly, i.e., enlargement of the ventricles. Still, there is limited mechanistic information on how growth factors in CSF alter the properties of the CP, ependyma, and arachnoid membrane.

Before this study, there has been scant attention paid to growth factor effects on CSF dynamics. Hydrocephalus induced by growth factors could be caused by overproduction of CSF by CP and/or by impairment of CSF drainage (7). Thus there is a need for further analyses of CSF flow as it relates to FGF-2 concentrations in the ventricular system. Moreover, in view of observations that FGF-2 confers neuroprotection in cerebral ischemia animal models (26) and therefore has potential application to therapeutic strategies for stroke, it seems important to systematically investigate the chronic effects of FGF-2 on the ultrastructure and function of the CSF system and adjacent brain tissue.

We used an adult rat model involving intracerebroventricular infusion of FGF-2 to create hydrocephalus for testing the hypothesis that growth factor-induced fluid accumulation in the ventricles is the result of an imbalance between formation and absorption of CSF. Evidence is presented that FGF-2 continuously infused into a lateral ventricle caused a marked increase in the resistance to CSF absorption, probably caused by fibrosis and collagen deposits at the arachnoid villi site of fluid egress into the superior sagittal sinus.

Another aspect of the investigation assessed the effect of intracerebroventricular FGF-2 infusion on intraventricular pressure (IVP) and ventriculomegaly. If FGF-2, for example, were to cause an obstruction in CSF flow through the cerebral aqueduct, then elevated IVP with subsequently enlarging lateral and third ventricles would result. On the other hand, if exogenous FGF-2 were not to cause blockage of the aqueduct and consequently a rise in IVP, then another explanation would be necessary for the ventriculomegaly. Our findings of an open aqueduct and stable...
IVP subsequent to FGF-2 infusion lead us to conclude that the observed ventricular enlargement was the result of an ex vacuo type of hydrocephalus.

MATERIALS AND METHODS

Animals. Experiments were performed on male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250–350 g. Animals were housed in a temperature-controlled room at 22°C with a 12:12-h light-dark cycle and maintained on standard pelleted rat chow and tap water ad libitum. Surgical procedures, as well as the associated experimental and recovery protocols, were approved by the Rhode Island Hospital Animal Welfare Committee.

Implantation of osmotic minipumps and FGF-2 administration. Osmotic minipump implantation was performed under sterile conditions. Rats were anesthetized with ketamine and xylazine (50 and 7.5 mg/kg im, respectively) and implanted with a stainless steel cannula in the right lateral ventricle. Positioning of the cannula was 1 mm caudal and 2 mm lateral to the bregma, and 2–3 mm below the level of the dura at a right angle to the skull surface. Stainless steel screws and dental acrylic resin fixed the cannula in place.

To induce hydrocephalus, we infused 18-kDa recombinant human FGF-2 (Ciblex, San Diego, CA) intracerebroventricu- larly by osmotic minipump (Alza, Palo Alto, CA). Minipumps were inserted into the subcutaneous pocket on the animal’s back in the midscapular region. Three doses of FGF-2 were used: 1, 0.5, and 0.25 µg/day icv. Sterile 0.9% NaCl, containing 0.1% rat serum albumin and 20 µg/ml of 3-kDa heparin from bovine intestinal mucosa (Sigma, St. Louis, MO), was used as the vehicle. FGF-2 was infused for 2, 3, 5, and 10–12 days. For control experiments, the vehicle was administered.

With an osmotic pump inflow of 0.5 µl/h and a CSF formation of 1.8 µl/min diluting the exogenous FGF-2 infused into the ventricle, it can be calculated for the lowest dose that the FGF-2 concentration in CSF was 190 ng/ml. The actual FGF-2 concentration would probably have been significantly less than this, to the extent that the peptide was taken up by cells lining the ventricle (including the CP) and was cleared from the ventricular and subarachnoid CSF into the brain interstitium (20).

Measurement of CSF formation rate. In rats administered FGF-2 for 10–12 days and in respective control animals, the CSF formation rate was determined after the measurement of resistance to CSF absorption (Rab; see Determination of Rab) and at a controlled IVP (4). We assumed that CSF absorption rate is independent of IVP within the range of 6.2–10.2 mmHg. In animals infused for 10–12 days with FGF-2 at 1 µg/day, the measured CSF formation rate was 1.78 ± 0.03 µl/min. This was a 29% reduction in the CSF production rate observed in controls (P < 0.01). CSF absorption rate (which is equal to CSF formation rate plus the constant rate of inflow of ACSF into the ventriculocisternal perfusion system) is plotted as a function of IVP during perfusion (Fig. 1). The slope of each regression in Fig. 1 is the conductance of CSF flow;
its mean value in FGF-2 treatment was 0.37, less than half that of the control value of 0.98 (P < 0.001).

IVP₀ in untreated control rats was generally ~3 mmHg (Fig. 2). There were no significant elevations in IVP₀ in any of the hydrocephalic animals. In fact, there was a statistically significant decrease in IVP₀ 3 days after the start of the 1 µg/day of FGF-2 infusion (P < 0.05). However, the IVP₀ returned to the control level by 5 days and remained at this level even after 10–12 days of FGF-2 infusion (Fig. 2).

In controls, the R_ab was usually about 0.8 mmHg·min⁻¹·µl⁻¹. By 2 days of infusion of FGF-2 at 1 µg/day, there was an 80% increase in the resistance to CSF outflow (Fig. 3). R_ab was also significantly elevated at 3 days and again at 10–12 days after the start of FGF-2 infusion (Fig. 3).

Ventriculomegaly. Enlargement of the lateral ventricles occurred 2 days after the daily infusion of 1 µg of FGF-2 into the ventricular system. However, even at 5 days the lateral ventricles were not expanded to the degree found at 10–12 days (Fig. 4). By 10–12 days, the ventricles on average were approximately doubled in size compared with controls. In all FGF-2 infusion regimens, the FGF-2-infused ventricle and the contra-lateral noninfused ventricle were enlarged to a comparable degree.

A dose-response analysis was done to further delineate the development of ventriculomegaly. When FGF-2 infusion was reduced by 50% and 75%, the ventricles at 10–12 days were still enlarged to the same degree as in the 1-µg/day infusions (see photographs in Fig. 5 for the effects of a 75% reduction in dose of FGF-2 for 2 and 12 days of infusion).

Structural analyses. The ultrastructure of the choroidal epithelium was generally intact at 2 and 3 days after the FGF-2 infusion at 1 µg/day. There was a marked increase in the number of dark epithelial cells in lateral ventricle CP exposed to exogenous FGF-2 for 10 days. Dark cells had intact organelles but were conspicuous for their shrunken nature (Fig. 6). Macrophages were also evident on the apical surface of the CP at 10 days of FGF-2 infusion.

FGF-2-treated rats (1 µg/day for 10 days) also displayed some pronounced fibrotic changes in the villi of the superior sagittal sinus (Fig. 8). The most conspicuous ultrastructural alteration was an enhanced depositi
tion of collagen fibrils in regions near the arachnoid villi.

**DISCUSSION**

Overview. CSF originates predominantly from the CP tissues, and it has a major impact on the brain extracellular environment (20). Normally CSF formation and absorption exist in a balance that prevents fluid and pressure buildup in the CNS. It is of interest how augmented levels of growth factors like FGF-2 and TGF-β can disrupt CSF homeostasis (9, 42, 45, 47) and the meninges (38). Fluid retention in CNS can occur by overproduced CSF, impaired interventricular flow of CSF, or compromised CSF reabsorption. Although we obtained evidence for impaired CSF reabsorption after FGF-2 infusion, we did not observe the aqueductal blockage or CSF hypersecretion that occurs in some other forms of hydrocephalus (7, 31, 37).

CSF formation rate and choroid plexus. Because FGF-2 promotes angiogenesis and mitosis in other epithelia (21), we expected that any FGF-2 modulation of CP secretory activity would be stimulatory (42). However, FGF-2 at 1 µg/day icv did not induce either vascularization or epithelial cell division in CP, even after 12 days of intracerebroventricular infusion of the peptide. Moreover, the nearly 30% reduction in net CSF formation rate after 10–12 days of FGF-2 treatment would more likely be associated with decreased rather than increased blood flow in CP. Because we did not observe augmented CSF pressure at any point in the time course analysis (Fig. 2), it seems safe to rule out elevated IVP as the cause of reduced CSF formation (31).

Dark epithelial cells in CP. An increased number of dark epithelial cells in CP (10, 33, 43) has been associated with diminished production of CSF; this
phenomenon may be a response to hydrocephalus. There were more dark cells in CP after FGF-2 (Fig. 6). Arginine vasopressin (AVP), which colocalizes with FGF-2 in CP epithelium (unpublished observations), also increases dark cell number (22) and decreases CSF formation rate (3). We propose that these peptide effects are a neuroendocrine type of modulation (39). Thus growth factors may interact with AVP to regulate ion transport in CP, as is the case in the thick ascending limb (17).

The reduction in net CSF formation rate by FGF-2 and AVP (3) may be in part attributable to increased absorption of ventricular CSF by the dark epithelium in CP. Accordingly, FGF-2 and AVP may stimulate the Na\(^+\)K\(^+\)-2Cl\(^-\) cotransporter (1, 23, 27) at the apical (CSF facing) membrane of the CP epithelium (46), thus removing these ions and water from the ventricles. Such transchoroidal clearance of CSF would effectively decrease net formation of CSF. The role of the CP dark epithelial cells in such putative reabsorptive cotransport (22, 33) and the alterations in bidirectional fluid movement across the CP in states of hydrocephalus and ventriculomegaly (37) are hypotheses that need further testing.

Ventriculomegaly and IVP. In this growth factor model of hydrocephalus, the lateral ventricles began to enlarge 2–5 days after the start of FGF-2 infusion. Ventricular volume augmentation occurred even in the absence of intracranial hypertension; in fact, there was a transient decrease in IVP\(_0\) at 3 days. The low-to-normal IVP\(_0\) (Fig. 2) is consistent with the observed patent cerebral aqueduct between the third and fourth ventricles.

The ventriculomegaly would presumably have been greater in the absence of the curtailed CSF formation rate, which was at least partial compensation for the enhanced resistance to CSF outflow caused by FGF-2 administration (Fig. 3). Further analysis is needed to ascertain whether the lowered CSF production in FGF-2 treatment was a homeostatic adjustment to the mechanical distension of the ventricles, or rather a direct effect of FGF-2 on the CP epithelium.

Ventriculomegaly in other hydrocephalus models, e.g., after kaolin injection into the cisterna magna, is accompanied by elevated IVP (31). It is unlikely in the FGF-2 hydrocephalus that the ventricular expansion was caused by an undetected rise in IVP. An alternative, more probable explanation is that the lateral ventricles were expanding passively in reaction to various changes in the subependymal brain parenchyma and interstitial space (see Ex vacuo expansion of the ventricles against the brain).

Structural alterations of the ependymal wall. The ependyma was changed at 5 days, but not in the manner observed in most hydrocephalus disorders. We did not find the flattening and stripping of the ependymal lining often reported for high-pressure hydrocephalus (37). In other hydrocephalus models, the augmented IVP that accompanies ventriculomegaly and ependymal damage can create an abnormal transependymal route for CSF absorption, promoted by the
elevated IVP and reduced resistance to the bulk flow of fluid (absorption) from ventricle to brain interstitium. In the absence of increased IVP in the FGF-2-induced hydrocephalus (Fig. 2), it is unlikely that the altered ependymal lining at 5 days (Fig. 7) allowed CSF flow into the brain.

The transience of the FGF-2-induced hypertrophy of the ependymal wall is noteworthy. This stimulated cell growth phenomenon was observed at 5 days, but was not evident at earlier (2 and 3 days) and later times (10–12 days). Transient or temporally specific effects caused by cytokines and growth factors have also been noticed in the adjacent subventricular zone (M. Del Bigio, personal communication), e.g., in regard to FGF-2 modulation of multipotential progenitor cells (18). This points to the need for delineating the time course of growth factor-inducing effects on both the ependyma and subependymal stem cells in the adult mammalian CSF system.

Ex vacuuo expansion of the ventricles against the brain. To explain the ventriculomegaly after FGF-2 infusion, we think that the ventricles “passively” expanded in response to changes in the underlying brain tissue, perhaps by more than one process: 1) viscoelasticity. Low- or normal-pressure hydrocephalic states with simultaneous ventriculomegaly have been associated with an altered viscoelastic modulus of the brain (40). Brain interstitial components, i.e., extracellular matrix proteins, can be significantly upregulated by growth factors (16, 47). The consequent viscosity changes in the extracellular milieu can thereby alter the resistance offered to fluid flow through the brain interstices. Increases in brain tissue elasticity could also lead to brain compression as the ventricles dilate outward. 2) Brain interstitial fluid volume. When elevated IVP occurs in hydrocephalus, ventricular CSF can flow by convection into the brain interstitium, distending the latter with an extracellular fluid edema (37). Conversely, in low- or normal-pressure hydrocephalus, extracellular water can be expelled from the brain parenchyma (40). Information is needed on how factors such as FGF-2 and TGF-β, by substantially altering the brain extracellular matrix properties (47), can effect significant changes in the water content of the interstitial spaces. Loss of interstitial water (8) would decrease brain volume and also alter the viscoelasticity of the cerebral tissue (40); such factors could favor an ex vacuuo type of dilation of the ventricular cavities. 3) Brain parenchymal cell volume. Growth factors have neurotrophic activities (13, 21, 26, 30, 36, 41), but they can also promote cell death (14). Microglia-derived nerve growth factor causes cell death in the developing retina (14). Using the same FGF-2 delivery regimen as in the present study, we found neuronal death in the caudate-putamen (2). This FGF-2 effect may be indirect, i.e., by way of stimulated microglia that have FGF receptors (34); excessive activation of microglia by FGF-2 might predispose to cell death. Other factors leading to neuron loss would promote an ex vacuuo ventriculomegaly.

Absorption of CSF and arachnoid villi. Normally, CSF volume and pressure are critically dependent on the finely regulated balance of fluid movement among three compartments: brain interstitial fluid, large-cavity CSF (ventricular and subarachnoid), and venous blood in the superior sagittal sinus (19, 20). Brain interstitial fluid and ventricular CSF eventually flow into subarachnoid CSF, the egress of which from the CNS, if impeded, can alter CSF volume and/or pressure. Although more than one CSF absorption pathway exists in mammals (20), the arachnoid villi have been widely implicated as a primary route of CSF drainage.

The precise anatomic locus for increased resistance to CSF absorption is unknown, but the functional data
Fig. 8. Ultrastructure of normal (A) and abnormal (B) arachnoid villus after FGF-2 infusion of adult rat for 10 days. Note extensive collagen deposits after FGF-2 treatment (arrows). ASC, arachnoid sinus cell; MEC, myoepithelial cell; Fb, fibroblast; Mp, macrophage; WC, white blood cell; PMN, polymorphonuclear leukocyte. Formed elements of blood are in superior sagittal sinus. Bar = 5 µm.

(Fig. 3) are consistent with the observed fibrosis and collagen deposition in the arachnoid membrane (Fig. 8). Occlusion of drainage channels by collagen deposits in the arachnoid membrane would likely impede bulk flow of CSF across the arachnoid villi. Consequently, because CNS lacks a lymphatic drainage system, it is dependent on having open conductive channels that allow relatively unimpeded convection of extracellular fluid.

Growth factor balance in CNS. FGF-2 and TGF-β participate in processes ranging from the modulation of fetal brain development to the repair of neuropil subjected to ischemia, trauma, and disease (13, 21). Upregulation of FGF-2 may be beneficial in Alzheimer's disease (44), and exogenously-administered FGF-2 can be neuroprotective (21, 26). In addition to bestowing neurotrophic effects, growth factors can also harm the CNS extracellular milieu (16, 47). This raises the issue of the ability of growth factors, when overexpressed or presented therapeutically in excess, to disturb the brain extracellular fluid. Optimal levels of growth factors therefore are essential to maintain the milieu in a state conducive for free-flowing CSF.

Brain extracellular fluid is vulnerable to states of fibrosis. With elevation of FGF-2 and TGF-β, there is often a buildup of collagen and other fibrotic material in the extracellular matrix. With deposition of collagen in the interstitium, there is consequently increased resistance to the free percolation of extracellular fluid with concomitant hydrodynamic consequences.

Growth factors and the onset of hydrocephalus. Hydrocephalus development has now been linked to enhanced CSF concentrations of FGF-2 (9, 42) and TGF-β (28, 45) and to brain upregulation of TGF-β (16, 47). In view of growth factor-induced effects on the CP, ependymal wall, and arachnoid villi, the mechanisms associated with the onset of this normal pressure hydrocephalus are multifactorial. Augmented levels of FGF-2 and TGF-β in various CNS disorders (13, 44) may contribute to ventriculomegaly and altered CSF turnover in these states.

FGF-2 in CSF was calculated to be a few hundred nanograms per milliliter; although this is above reported control values for CSF in the subnanomolar range (32), it is comparable to FGF-2 titers in CSF of glioma patients (32). Attention needs to be directed toward analyzing choroidal (5, 12, 15) as well as extrachoroidal growth factors, both normally and when elevated in hydrocephalus (9, 42, 47), brain trauma (41), CNS tumors (32), and cerebral hypoperfusion (49).

Perspectives

Peptides modulate diverse functions of the blood-brain and blood-CSF barriers. FGF-2 and AVP colocalization in many choroid epithelial cells suggest a neuroendocrine role in regulating CSF formation. These two peptides decrease CSF secretion rate and increase the dark epithelial cells in CP, suggesting integrated roles in controlling CSF dynamics. Studies of CP are needed to ascertain if neuropeptides like AVP interact with growth factors to modify ion transport, as has been demonstrated in the kidney tubule (17).

Augmented concentrations of growth factors in the CNS cause ventriculomegaly as well as changes in CSF turnover. The development of ex vacuo hydrocephalus after FGF-2 infusion intimates that the brain's viscoelasticity is altered by growth factors. The difficult challenge in predicting outcomes in hydrocephalus models...
likely stems from complex compensatory mechanisms involving peptide responses to altered CSF pressure or volume. Therefore, more analyses are needed of CSF growth factors and how they affect the CNS extracellular matrix.

What is the secretory role of the CP in regulating growth factor concentrations in CSF? Because an excess of FGF-2 and TGF-β can induce severe fibrosis and collagen deposition along CSF bulk flow pathways, thereby interfering with brain function, it is essential to elucidate how certain factors, such as hepatocyte growth factor (which is also expressed in CP), may be able to counter adverse effects by activating matrix degradation pathways (35). Clearly, CSF growth factors can impact brain fluid balance at many sites within the CNS.

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