Published in final edited form as:

Mol Pharm. 2013 May 6; 10(5): 1522–1532. doi:10.1021/mp300474m.

Physiology of the intrathecal bolus: the leptomeningeal route for macromolecule and particle delivery to CNS

Mikhail I. Papisov¹, Vasily V. Belov¹, and Kimberley S. Gannon²

Mikhail I. Papisov: papisov@helix.mgh.harvard.edu; Vasily V. Belov: vbelov@partners.org; Kimberley S. Gannon: kgannon@neurophage.com

¹Massachusetts General Hospital, Shriners Hospitals for Children – Boston, and Harvard Medical School, 51 Blossom St, Boston, MA 02114 USA

²NeuroPhage Pharmaceuticals, Inc. 3222 Third Street, Suite 31203 Cambridge, MA 02142 USA

Abstract

Presently, there are no effective treatments for several diseases involving the CNS, which is protected by the blood-brain, blood-CSF and blood-arachnoid barriers. Traversing any of these barriers is difficult, especially for macromolecular drugs and particulates. However, there is significant experimental evidence that large molecules can be delivered to the CNS through the cerebro-spinal fluid (CSF). The flux of the interstitial fluid in the CNS parenchyma, as well as the macro flux of CSF in the leptomeningeal space, are believed to be generally opposite to the desirable direction of CNS-targeted drug delivery. On the other hand, the available data suggest that the layer of pia mater lining the CNS surface is not continuous, and the continuity of the leptomeningeal space (LMS) with the perivascular spaces penetrating into the parenchyma provides an unexplored avenue for drug transport deep into the brain via CSF. The published data generally do not support the view that macromolecule transport from the LMS to CNS is hindered by the interstitial and CSF fluxes. The data strongly suggest that leptomeningeal transport depends on the location and volume of the administered bolus and consists of four processes: (i) pulsation-assisted convectional transport of the solutes with CSF, (ii) active “pumping” of CSF into the periarterial spaces, (iii) solute transport from the latter to and within the parenchyma, and (iv) neuronal uptake and axonal transport. The final outcome will depend on the drug molecule behavior in each of these processes, which have not been studied systematically. The data available to date suggest that many macromolecules and nanoparticles can be delivered to CNS in biologically significant amounts (>1% of the administered dose); mechanistic investigation of macromolecule and particle behavior in CSF may result in a significantly more efficient leptomeningeal drug delivery than previously thought.

Keywords

CNS; Intrathecal; Leptomeningeal; cerebrospinal fluid; CSF; Drug Delivery; biopharmaceuticals; proteins; bacteriophage; drug carriers; Imaging

---

Correspondence to: Mikhail I. Papisov, papisov@helix.mgh.harvard.edu.

AUTHOR CONTRIBUTIONS

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

INTRODUCTION

Diseases involving the CNS are of high social significance due to high prevalence and/or high morbidity and mortality. Presently, there are no effective therapies for many of them, not least because of the poor drug access to the CNS. It has been estimated that for greater than 98% of small molecules and nearly 100% of large molecules, systemic delivery to the CNS is not effective.¹ As a result, several conditions involving CNS remain untreatable. Examples include neurodegeneration (e.g. amyotrophic lateral sclerosis), “diseases of age” (Alzheimer’s and Parkinson’s disease), genetic deficiencies (e.g., lysosomal storage diseases²), and several types of brain cancer (e.g. childhood brainstem glioma³).

If the problem of their delivery to CNS is solved, then biopharmaceuticals (proteins^{4,5,6}, oligonucleotides^{7,8}, gene vectors^{9,10,11}) may potentially provide highly effective therapies for many diseases involving the CNS. The attempts of circumventing or traversing the blood-brain barrier (BBB), including the use of direct transcranial administration and transport across the BBB using endogenous receptors (insulin, transferrin, LDL receptors, LDLR-related proteins)^{12,13,14} and nanocarriers¹⁵ hold significant promise but have not yet resulted in clinically accepted solutions.

The fact that large molecules can penetrate into the brain parenchyma from the cerebrospinal fluid (CSF) has been established four decades ago.¹⁶ However, only recently it has been shown that the fraction of therapeutic macromolecules or particles (gene vectors) delivered to the CNS from CSF may be biologically significant.^{17,18,19,20,21,22,23} Our recent studies using PET as a method of non-invasive tracking of intrathecally administered proteins and particles, such as particles of bacteriophage M13 (below in the text “phage”)^{24,25,26} have shown a high and rapid entrance into the brain parenchyma, which may appear surprising, considering the prevailing views on the physiology of the leptomeningeal space¹ (LMS). This paper is intended to discuss the mechanistic aspects of the macromolecule and particle translocation in the leptomeningeal space and their role in the intrathecal delivery to the CNS, particularly to the brain.

The current clinical paradigm of leptomeningeal (intrathecal) drug administration is rooted in the pioneering work of J. Corning (1885) on spinal anesthesia with cocaine²⁷, and has been greatly influenced by the subsequent studies in anesthesiology, in particular investigating the risk of respiratory distress when the anesthetic is administered at a higher location than the lower thoracic area²⁸. Recent significant developments were based on the recognition that hydrophobic anesthetics introduced in LMS induce rapid segmental anesthesia, while hydrophilic anesthetics induce more gradual onset of anesthesia, with actions extending from the administration site^{29,30}. The published observations generally fit into the mechanistic scheme where the drug administered into the spinal CSF acts locally, being contained by its association with the lipids of the arachnoid and, presumably, by the hypothetical caudad CSF flow. The cephalad (upwards) spread of an anesthetic from injection sites close to the head could be risky because of the proximity of the medullary vasomotor centers³¹. Thus, the development of intrathecal delivery of anesthetics was focused on slow infusion in the lumbar region. Coincidentally, due to the anatomy of the skull and vertebrae, lumbar administration is the most clinically feasible and least invasive way for drug introduction to the LMS. As a result, in anesthesiology the lumbar intrathecal administration has become prevalent. Implantable slow infusion devices have been developed, capable of chronic (years) uninterrupted delivery of pain or spasticity

---

¹Leptomeningeal space (LMS) is understood here as the entire space occupied by CSF, including all spaces continuous with the subarachnoid space, such as perivascular spaces and ventricles.

management therapies with the action focused mostly on the distal segments of the spinal cord^{32,33}.

In other fields of medicine, the use of the leptomeningeal route for access to the CNS has not yet been systematically explored; the number of both preclinical and clinical studies where this route is tested is not very large. This, apparently, is partially due to the perceived (and likely overestimated) dependence of the drug transport in the CSF on the CSF “circulation”³⁴ and other perceived limitations³⁵.

In several animal models of CNS disease the intrathecal drug delivery route has shown promising results for large molecules. This includes intrathecal delivery of enzyme replacement therapeutics^{36,21,37,38,39} antibodies⁴⁰, nerve growth factor,⁴¹ Sonic Hedgehog,⁴² siRNA⁴³ and dynorphins⁴⁴.

One recent paper describing the results of early clinical studies states: [Intrathecally administered] BDNF can be delivered cranially against CSF flow⁴⁵. In fact, the most recent data suggest that the CSF “flow” is perhaps the least important factor of leptomeningeal transport of macromolecules. The solute transport in CSF, as discussed below, is much more influenced by the initial spread of the injected solution in the CSF, subsequent active transport of the solute in the LMS due to pulsatile remixing, drainage of the CSF outside the LMS, and active, pulsation-assisted translocation of the solute into the perivascular space (Virchow-Robin space, VRS), and the subsequent transfer from VRS to the parenchyma and uptake and transport by cells.

THE ANATOMY OF THE LMS AND THE ROLE OF THE INJECTED VOLUME IN THE INITIAL DRUG SPREAD

The brain and spinal cord are suspended in the CSF-filled LMS on avascular membranes and ligaments (trabeculae), which are collectively called “arachnoid” due to their web-like appearance (Figure 1). On the outside, the membranes and trabeculae are attached to the dura -- the outermost of the meninges. Dura is essentially a seamless sac containing the cerebrospinal fluid.

Dura, for the most part, is surrounded by bones (skull, vertebrae) and relatively rigid cartilage (intervertebral disks) and thus has no space to expand (or contract). However, there are two exceptions to the latter: (a) the area (“cisterna magna”) near the atlanto-occipital and atlanto-axial joints, where dura has to accommodate for the movements of the head and neck, and (b) to a lesser degree, intervertebral openings (foramina) through which nerves and blood vessels exit the LMS. The firmly confined boundaries of the LMS define the initial behavior of any additional liquid introduced to the CSF by injection. Thus, regardless of the injection site (e.g., cerebroventral, lumbar), the injected liquid will “push” the CSF towards the expandable area (and towards major veins that can contract and accommodate extra volume under pressure⁷⁴) and spread towards the same direction, i.e., mainly towards the neck area. If the administered volume exceeds the volume of the compartment located between the injection site and the flexible section of the dura, the respective fraction of the dose is delivered directly into the cisterna magna area. The “distances” (expressed in volume units) between various potential injection points and cisterna magna in various animals can be measured experimentally either by CSF sampling or, preferably, non-invasively (by imaging). To the best of our knowledge, this still has not been done systematically. Rieselbach et al. administered large volumes of radiolabeled preparations into the lumbar sac, [¹³¹I] Rose Bengal in *Macaca Mulatta* and [¹⁹⁸Au] colloidal gold in human patients.⁴⁷ Their data suggest that in both humans and monkeys the administration of over 10% of the

total estimated CSF volume results in the immediate appearance of the radioactivity in the intracranial CSF (cisterna magna and basal cisterns).

In our studies in cynomolgus monkeys (*M. Fascicularis*), the estimated “distance” from the lumbar injection point (at L1) to cisterna magna was approximately 0.4 ml/kg of body weight; administration of a 0.5–1 ml bolus followed by a 0.5 ml/kg flush resulted in the immediate delivery of 50% of the administered dose to the cranial CSF pool for all studied proteins and particles.^{24,25,26} Intracerebroventricular administration of the same protein resulted in a very similar initial protein distribution pattern in the CSF. The pattern was significantly different only for the distal thoracic and lumbar regions, where the initial protein content was much lower than after lumbar administration.²⁴ We should note that the position of the needle or catheter opening relative to the spinal cord (dorsally or ventrally) may influence the apparent “distance” to the cisterna magna, because the ventral LMS is relatively open and the dorsal space is crossed by multiple membranes (Figure 1), the continuity and variability of which still have not been studied in detail⁴⁸. Our current studies in rats suggest that catheter tip position can change the initial pattern of bolus translocation very significantly⁴⁹.

Thus, the initial spread of the drug administered by bolus injection is defined by the anatomy of the LMS and depends on the injected volume. The influence of other physical factors (e.g., body position, reinjection) is unlikely to be important. Rieselbach et al. observed that withdrawal and reinjection of [¹⁹⁸Au] did not unequivocally improve distribution in comparison with patients receiving a similar volume with one rapid injection⁴⁷, which is not unexpected since withdrawal and reinjection only moves the solution back and forth along the same segment of LMS. We should note, however, that withdrawal of a CSF volume equal to the subsequently injected dose may result in a safer procedure due to the prevention of the excessive buildup of the CSF pressure (Rieselbach’s data suggest that bolus injection of up to at least 33% and 42% of the total CSF volume is well tolerated by humans and monkeys, respectively).

The influence of the “baricity” (solution density as compared to the CSF) on the initial distribution of the injectate was first suggested by A. Barker⁵⁰ in 1907. He has shown that “hyperbaric” (more dense than CSF) and “hypobaric” solutions (less dense than CSF) can flow under the influence of gravity in the spinal canal. Combined with various degrees of body tilt (Trendelenburg position), injection of air and/or direction of the needle opening this observation has resulted in various techniques for localizing the action of intrathecally administered anesthesia^{51,52,53}. The feasibility of gravity-assisted transport of macromolecules and particles in the LMS has not been investigated.

Thus, the volume of the intrathecal bolus appears to be the most important factor of the initial drug spread in the CSF. After the initial distribution, or after the administration of a small volume, the solute distributes within the LMS relatively slowly. The mechanism and directionality of this distribution are discussed in the following section.

THE “FLOW” OF CSF

The CSF is believed to be produced mostly by the choroid plexus, a highly vascularized tissue located in the brain ventricles. The formation of CSF and the functions of choroid plexus are very well studied and extensively reviewed.^{54,55} Approximately 18–20% of the CSF appear to originate from the interstitial fluid of the CNS in rabbits,⁵⁶ with higher interstitial contribution in non-human primates and other species.⁵⁷

An early view suggested that the circulation of the fluid is a movement from its point of production towards the arachnoid villi to replace fluid which has been absorbed in this

situation; later it was suggested that the cerebrospinal fluid circulation is aided by a vascular pump which drives fluid from the ventricles and through the cranial subarachnoid space.⁵⁸ Although there are alternative points of view⁵⁹, it is generally accepted (Figure 2) that CSF flows from the ventricles into the basal channels and then into other cranial subcompartments of LMS.^{60,61}

Early experiments with direct observation of the movement of dye administered to the CSF of laminectomized dogs resulted in the conclusion that there is no directional CSF flow⁶². In the same publication, the authors conclude that the substances in the CSF spread by diffusion and that there is no evidence that pulse and respiration play a role in the movement of CSF (we must note that the method was not precise enough to adequately support the latter conclusions).

In a later imaging study⁶³, a descent of a radiolabeled solute in the spinal LMS was interpreted as descent of CSF; the interpretation remained uncontested.

For a drug administered in the lumbar region, a downward CSF flow direction in the spine would hinder the intended drug transport. Thus, it may appear that only a very small fraction of the intrathecally injected drug can reach the cerebral CSF pool and the brain surface. This view, however, does not take into account that the “flow” of the CSF is not laminar.

The pulsatile movements of the CSF, mainly in the cervical/ventricular area⁷⁴, create longitudinal oscillations in the CSF along the entire LMS. Most recently, noninvasive flow measurement with phase contrast MRI enabled the in vivo evaluation of the basic parameters of this CSF movement, such as flow velocities and waveforms.^{65,66,67,68,69} Computational Flow Dynamics (CFD) models have been used to complement MRI measurements in explaining the complex fluid flow patterns in the CSF-filled spaces.^{70,71,72} Generally, CSF flow waveform reflects that of the arterial cardiac cycle and has its own systole (spinal column inflow) and diastole (outflow). Peak pulsatile flow rates measured at the cervical level by different authors^{65,66,72,73} range from 120 mL/min to 360 mL/min in humans. The variations in the velocity are significant along the spinal canal and range from 0.5–8 cm/s with the maximum at ca. 20 cm below the skull.⁷² CFD studies show that inertial effects should dominate the flow field causing significant turbulence, especially in the cervical LMS.⁶⁶

In a turbulent liquid compartment, the flux of the solute is not necessarily governed by the bulk flux of the liquid. Turbulence induced in the CSF by the pulsation of the arteries and tissues can propagate the solute along the compartment when there is no solvent flux and even against the flux. For mechanistic reasons, it can be expected that in a small segment of LMS the pulsatile movements should result in the solute flux proportional to the concentration gradient between the ends of the segment, in a simplified form

$$J = k_{pd} \bullet \text{grad}(C), \quad (1)$$

where the “pseudodiffusion” constant k_{pd} is a function of the intensity and patterns of the local pulsatile turbulence. The form of this equation is similar to the Fick’s diffusion equation; however, the underlying process is non-diffusional and does not depend on the hydrodynamic diameters of the solute molecules and particles. In studies performed using quantitative Positron Emission Tomography (PET), the real-time in vivo data obtained using proteins and phage particles, which differed in sizes by about 2 orders of magnitude (ca. 12–14 nm and 900 nm, respectively), showed that in the distal spinal CSF of *M. Fascicularis* the rate of solute transport is much higher than of diffusion, 0.3–2.1 cm/hr for both smaller and larger solutes, as estimated by the front propagation (Figure 3); the range of values reflects

variations within the same animal's LMS as well as between the animals. In rats, a very small volume (3 μ l) injected at L1 region in the rat's spine expanded both cranially (at ca. 2.8 cm/hr) and caudally (at ca. 1.4 cm/hr). As a result, the solute distributed over the entire spinal CSF of the rat within approximately 1 hour.

Thus, the experimentally observed rates of solute transport in the CSF are by orders of magnitude faster than diffusional transport. On the other hand, the imaging data suggest that macromolecules (or particles) spread in the CSF from the administration point in all directions (e.g., to the cerebral LMS from the spine and to the spinal CSF from the ventricle), which excludes a directional CSF flux as the driving force. Therefore, the pulsatile remixing of CSF appears to be the main if not the only driving force in the macromolecule spread in the LMS.

The pulsatile turbulence of the CSF has a much more significant effect on the solute spread in the liquid phase than the CSF "flow" because the directional flux of the CSF is very slow as compared to the pulsatile remixing in all parts of the LMS that is responsible for the solute flux. The local turbulences of CSF, which drive the spread of the solute, are most intense in the area identified by Du Boulay et al⁷⁴, and, respectively, lower (but not absent) where the arteries branch and become thinner, as shown in Figure 4.

The above suggests that a mechanistic pharmacokinetic model can be developed based on the physiology and configuration of the leptomeningeal space, with parameters essentially based on Equation 1. To the best of our knowledge, these parameters were never measured for any animal, although the approaches are presently being developed.⁷⁵ The availability of experimentally validated quantitative models for different species would greatly facilitate both preclinical development and translation to human studies.

The pool of the drug substance dissolved in the CSF may, during and after the initial distribution, further translocate into the CNS or out of the leptomeningeal space following the physiological avenues. Also, depending on the structure of the macromolecule or particle surface, the mobility of the drug substance in the LMS can potentially be attenuated by the molecule or particle interactions with cells lining the leptomeningeal tissue interfaces. Some of these cells may potentially bind or endocytose the drug. The mechanisms and rates of these processes, which may change in pathological states, are of the key importance for the overall results of leptomeningeal transport, as discussed in the following sections.

DRAINAGE OF CSF

CSF drainage is a process that removes the excess of the fluid from the leptomeningeal space and maintains the leptomeningeal volume constant. The rate of fluid replacement varies in different species, from 0.89% of the total volume per minute in the mouse to 0.38% per minute in man.⁷⁶ The drainage routes have been investigated for nearly a century, but there are still conflicting views due to the significant methodological difficulties.⁷⁷

The principal CSF draining route is believed to be in the venous sinuses through physical pores found in specialized structures, arachnoid granulations⁷⁹ (we must note the debate continues even now⁸⁰). In humans and non-human primates, the arachnoid granulations were found in the superior sagittal and lateral sinuses⁸¹, and at some nerve roots⁸², while in rabbits they were found only at the cranial base⁸³. In rats, the drainage site is in the superior sagittal sinus, although arachnoid granulations are not as defined as in other animals.⁸⁴ In the preparations of arachnoid granulations, multiple pores are apparently formed by merged giant vacuoles (Figure 5). Whether these channels are permanent or dynamic remains unknown. R. Tripathi⁸⁵ suggests that filtration proceeds through 0.1–2.3 μ m temporary openings of giant vacuoles, but makes no suggestions on the duration of the existence of

such channels. In rats, the administered solute (peroxidase) was also found in numerous transendothelial vesicles, suggesting a dual filtration mechanism⁸⁶.

In addition to the direct drainage, paraneural CSF drainage into the interstitium with subsequent lymphatic drainage was suggested (as shown, in particular, in Figure 2, arrows 3 and 3a), e.g., in rabbits⁸⁷. The data on the lymphatic drainage, obtained using different (mostly invasive) methods and in different species, diverge very widely (reviewed in [88]). Our PET data suggest that in the absence of invasive procedures there is no or little such drainage in primates, and in rats there is definitely no lymphatic drainage in the spine; the cerebral lymphatic drainage in rats is apparently significant (based on the accumulation of intrathecally administered radiolabeled material in deep submandibular and/or cervical lymph nodes) and needs further investigation.

Our preliminary studies in rats with radiolabeled microspheres with calibrated diameters indicate that the functional size of the pores is at least 1 μm , which is in agreement with the previous electron microscopy data. We have also found size dependence in the macromolecule drainage in the 2–20 nm region⁴⁹, which suggests the presence of another set of either true pores or transcytosis processes functionally indistinguishable from pores (not unlike the “large endothelial pores”⁸⁹).

The PET imaging data also demonstrates the immediate, without a delay, start of accumulation of the phage and protein substance leaving the LMS. The accumulation occurs in the same organs that accumulate the same materials from the systemic circulation after intravenous administration. At the same time, there is no accumulation in any lymph nodes (which avidly accumulate these materials from the interstitium). Thus, the CSF appears to drain predominantly or almost exclusively from the leptomeningeal space directly to the blood and not through consecutive transfer to the extradural interstitium and then to the lymphatics and only then to the blood.

Overall, the available data suggest that the anatomical location and distribution of pores in the LMS varies in different species very significantly. The mechanisms of both pore formation and CSF drainage through them are not known. Thus, the non-human primate model is presently the only animal model for which extrapolation of the pharmacokinetic data to humans is relatively straightforward. The continuity of the physiology of CSF drainage (and, in turn, of the data on drug behavior in CSF) between primates and other species remains to be established.

DRUG ENTRANCE FROM CSF TO CNS PARENCHYMA

The direction of the flow of the interstitial fluid from the deep regions of the brain is believed to be outwards.^{90,91} This may appear to prevent drug access from CSF to the parenchyma, especially in large animals. However, the available data suggest that macromolecules and nanoparticles do penetrate deep into the brain, and the transfer from CSF to the brain is too fast^{24,26} to be explained by diffusion. Hence, an active mechanism appears to participate in the parenchymal translocation of the macromolecular solutes from CSF. At this time, perivascular (perhaps mostly periarterial) transport appears to be responsible for the penetration.

a. Perivascular transport into the brain

Arteries and veins of the brain, unlike the blood vessels in other tissues, run inside liquid-filled “tubes” (Virchow-Robin spaces^{92,93}). In spite of a large amount of anatomical studies with light and electron microscopy, the exact relations of the perivascular and leptomeningeal space remains unclear and may vary between mammalian species.⁹⁴ While

in some reports the continuation of the leptomeningeal sheath into the perivascular space is clearly seen, in others it is not^{95,96,97,98,99}. It has been suggested that the perivascular space is connected with the lymphatic system¹⁰⁰. Some studies suggest that there is a subpial space extending into the perivascular spaces^{98,101}. Functional studies, however, clearly demonstrated the continuity of the leptomeningeal and perivascular spaces^{101,102, 103} (Figure 6).

Protein penetration from the CSF into the perivascular space was detected as early as at 4 minutes after the injection¹⁰², with spread to sub-pial space and with indications of back-and-forth flow¹⁰¹. The data, however, were qualitative, and the fraction of the solute delivered to the perivascular space was not determined. Our PET data shows that in *M. Fascicularis* at 2.5 hours after the injection up to ca. 10–15% of the intrathecally administered dose of proteins and phage particles can be localized in the brain volume (excluding the ventricles), and by 24 hours this value decreases to 1.5–2% of the injected dose, in some animals up to 6%. The subsequent washout is slower (estimated half-life 15–20 hours). This is in agreement with the entrance of the solute from CSF to the perivascular space during the first hours after the injection, when the solute concentration in the CSF is still high, followed by partial exit of the solute back to the CSF when its concentration in the latter is significantly reduced due to the CSF replacement (drainage). The residual fraction is most likely transferred to the parenchyma and taken up by the cells, and the slower process of the label (¹²⁴I) washout relates to the metabolism and deiodination of the administered material. If the above is the case, the fraction of the dose remaining in the parenchyma may possibly be increased via enhancing the transport of the drug from the VRS to the parenchyma.

b. Transport from VRS to the brain parenchyma

While the data of H. Wagner et al¹⁰³ and more recent studies^{104,105,24} unequivocally demonstrate translocation of large molecules from CSF to the brain parenchyma along perivascular spaces, the mechanism(s) of their exit from the perivascular space is unknown. According to the current understanding of the structures surrounding VRS, they have to be transferred through glia limitans (formed by astrocyte endfeet) in smaller vessels and, in addition to that, through pia mater (formed by pial cells) in relatively large vessels. Only large vessels have perivascular space layered with pia¹⁰⁶; the perivascular spaces of smaller vessels have only glial sheaths.

The properties of the pial cells lining VRS have not been studied in detail. They are possibly of the same type as meningeal cells for which the presence of endocytosis- and transcytosis-associated receptors have been demonstrated. Thus it is possible that the transfer of large molecules and particles from the VRS to the CNS parenchyma may be enhanced through chemical modification enabling receptor binding and transcytosis.

The most recent structural studies suggest that glia limitans (a.k.a. glial limiting membrane, GLM) is formed by monolayers of astrocytic processes and/or somata irrespective of the types of blood vessel. However, the thickness of the layer decreased in the order of arterioles, venules and capillaries.¹⁰⁷ The exact functionality of the glial layer surrounding perivascular spaces has not been studied in detail. However, the published data^{24,103,104,105,108} clearly demonstrate that both pia and perivascular glia limitans are penetrable for macromolecules and particles, whether the penetration is a result of passive convectional process or transcytosis. Penetration was observed around both periarterial and perivenous space.¹⁰⁵ In a study utilizing advanced in vivo imaging methods¹⁰⁸ in mice, paravenous accumulation appeared much later (>1 hr after intracisternal administration) than paraarterial accumulation (immediately after the administration).

Ependymal cells lining ventricles are very different from leptomeningeal cells. They are of neuroglial origin¹⁰⁹ and have stem cell characteristics¹¹⁰. Thus, solute penetration to the parenchyma from the ventricles can be expected to have different kinetics and different dependence on structural and physiological factors than solute transport from VRS. They are known to express ICAM-1 and VCAM-1¹⁴⁴, which can possibly be used for enhanced drug transport if these cells have the same CAM-associated mechanisms as the “classical” epithelial cells¹¹¹.

DRUG TRANSPORT IN THE BRAIN PARENCHYMA

While the fact that macromolecules can enter the brain parenchyma through the perivascular space is well supported by experimental data, the direction(s) and rate(s) of their movement within the parenchyma is not yet fully understood. The available data suggests this process is dominated by convective transport (“bulk flow”), and the role of diffusion in the flux of the solute is much less significant. The latter was established in studies where various compounds were injected into the brain through a catheter or a needle in a small volume. Their translocation from the injection site was found to be practically independent of the molecular weight.¹¹² Infusion of therapeutic preparations through this route (“convection-enhanced delivery”¹¹³) is being actively studied clinically, predominantly in neuro-oncology.¹¹⁴

Investigation of the interstitial transport of various compounds injected in the brain provided valuable data on the parenchymal transport routes, including estimates for distribution volumes of the injectate (which can be determined by imaging)¹¹⁵ and information on the directionality and reproducibility of the injectate transport, including translocation along white matter tracts¹¹⁶ and the existence of optimal injection sites for injectate delivery to certain regions.¹¹⁷ It was also shown that drug forms with lower tissue affinity enable wider tissue coverage than ones with higher affinity,¹¹⁸ and that the kinetic parameters of transport may depend on anesthesia and other factors.¹¹⁹ Although the physiology of intrathecal bolus suggests drug entrance to the parenchyma at multiple “injection points” (perivascular sites), the locations of which have not been studied in detail, the information obtained in single injection studies can be highly useful in the development and optimization of drugs intended for intrathecal delivery to CNS.

AXONAL TRANSPORT

Anterograde axonal transport of gene vectors, detected by the expression of intrathecally administered gene vectors in dorsal root ganglia, was demonstrated by Wang et al.¹²⁰ In our studies²⁴ with iduronate-2-sulfatase (I2S), a 6-phosphomannosylated recombinant human protein, the observed association of I2S with neurofilaments was indicative of active axonal transport. The latter likely began with protein interaction with neuronal mannose-6-phosphate receptors¹²¹, which are widely expressed on cells of the spinal cord and brain¹²². Thus, axonal transport is another active process that can potentially be used for enhanced (through molecule/particle modification) macromolecule and particle delivery from the leptomeningeal space to the CNS.

LEPTOMENINGEAL CELLS AND DRUG TRANSPORT

The leptomeningeal space contains several variably membranous and fibrous structures crossing the reservoir filled with CSF (Figure 1). Drug molecules and particles moving along the compartment may interact with these structures, and (if there is such interaction) their transport may be attenuated. Hypothetically, higher affinity to the leptomeningeal structures, lower dose and slower injection/infusion should generally result in a greater “anchoring” or localization. (This is most likely the underlying mechanism of the well

localized spinal action when hydrophobic anesthetics are delivered by slow infusion from implanted pumps¹²³). Our preliminary data showed that hydrophilic proteins, pegylated nanoparticles (up to 1.2 μm tested) and phage particles do not bind the leptomeningeal structures and readily translocate along the compartment in all directions for several hours (see, e.g., Figure 5 in [25] and Figure 7 in [26]). However, hydrophobic nanoparticles remained within 2 cm from the lumbar injection point, presumably due to non-specific binding to the arachnoid. Thus, adhesivity of the macromolecules and particles can be used to regulate their transport in the LMS.

The meningeal structures are lined with cells of neuroectodermal origin¹²⁴ that have not been extensively studied. Cellular phenotypes are variable, though typically consist of elongated, spindle-shaped epithelial cells.¹²⁵ They express epithelial and mesothelial membrane markers, EMA/MUC1 and mesothelin¹²⁶, and also cytokeratin, desmoplakin and vimentin¹²⁵. They are capable of cytochalasin B inhibitable endocytosis¹²⁷ and known to express ICAM-1¹²⁸, and somatostatin¹²⁹, but there is no information on the expression of other receptors, e.g. endocytosis-associated ones, which can play important roles in molecule/particle pharmacokinetics in CSF. Many cell surface proteins may potentially interact with the administered macromolecules and particles, thus further investigation of the meningeal cells to understand their potential role in the leptomeningeal drug delivery is warranted. The same relates to the cells lining the perivascular space (which are likely of the same type). Better understanding of the character and functions of these cells would allow targeted modification of drug molecules and particles enhancing their transport from the LMS and VRS to the parenchyma. The technological approaches for such modification are well developed³⁵.

PATHOLOGY AND LEPTOMENINGEAL TRANSPORT

Pathological processes, such as cancer and inflammation, are known to affect the structure and function of tissues that potentially can influence the leptomeningeal drug delivery.

In many pathologies, the VRS are dilated, which may result in an enhanced drug delivery to CNS parenchyma. Brain trauma results in pathologically dilated VRS^{130,131,132}. Dilated VRS were also associated with migraine¹³³, mucopolysaccharidosis¹³⁴, cryptococcosis¹³⁵, age,¹³⁶ hypertension, dementia, incidental white matter lesions¹³⁷ and other conditions; the mechanism of dilation is believed to be associated with inflammatory changes, but has not been established¹³². The permeability of the dilated VRS walls, which may differ from the normal perivascular permeability, have not been studied.

Meningeal cancer can affect CSF movement, which can significantly alter the leptomeningeal pharmacokinetics.¹³⁸ Although some cranial cancers reportedly do not invade VRS¹³⁹, most meningeal and brain cancers invade and/or occlude the VRS^{140,141,142,143}, which may limit or halt drug transport. Infection-related factors can induce expression of inflammation associated receptors in the meninges^{128,144} and potentially alter the functionality of the leptomeningeal pores, which can also attenuate drug transport. On the other hand, the inflammation induced transcytotic pathways in the VRS could enhance the transport from the VRS to the CNS parenchyma.

Thus, a variety of pathology related factors may affect the leptomeningeal transport of large molecules and particles to CNS, and the influence of these factors depends also on the character of the macromolecule/particle surface, which should be taken into account in drug development.

ADEQUACY OF ANIMAL MODELS

Interspecies variations of several physiological factors defining the intrathecal drug delivery route are very significant. Physical dimensions of the liquid compartments and the brain, intensity of the pulsatile remixing of CSF and perivascular liquid, CSF and interstitial fluid generation and turnover, and protein concentration in CSF¹⁴⁵ are different and must be taken into account in projecting animal data to humans. There is no reason to believe that mechanism-based comparative modeling cannot provide reliable data scaling, provided that quantitative data are available on all stages of transport in humans and the respective model species, of which rodents would be of highest priority in view of the availability of several developed model of disease and relatively low cost. However, until such data become available, non-human primates will likely remain the most reliable model for pharmacokinetics studies.

CONCLUSION

The available data on the physiology of the leptomeningeal space suggests that the leptomeningeal (intrathecal) route may be useful for delivery of drugs of macromolecular and particulate nature to CNS.

The efficacy of the leptomeningeal transport is greatly affected by the volume of the intrathecal bolus. The transport is assisted by pulsation-induced turbulence in the leptomeningeal and perivascular liquid compartments. The leptomeningeal phase is followed by, hypothetically, either active or passive translocation from the VRL to the parenchyma. The latter may be followed by axonal transport.

The outcome of the leptomeningeal transport depends on the pulsatile turbulence of the leptomeningeal space, drug molecule/particle interactions with a variety of leptomeningeal and perivascular cells, and CSF drainage. These factors are not fully studied and understood, which, in view of the potential benefits of the leptomeningeal drug delivery to the CNS, warrants further studies.

Acknowledgments

Authors thank Drs. P. Calias, R. Penn, M. Heartlein, T. McCauley, A.J. Fischman and R. Fischer for valuable discussions, and E. Papisov for assistance with editing the manuscript. Previously unpublished data were from studies supported by NIH grant R21 CA152384 and DoD grant BC100684.

ABBREVIATIONS

CNS	Central Nervous System
CSF	cerebrospinal fluid
LMS	leptomeningeal space
VRS	Virchow-Robin space
CAM	cell adhesion molecule

References

1. Pardridge WM. The blood-brain barrier: Bottleneck in brain drug development. *NeuroRx*. 2005; 2:3–14. [PubMed: 15717053]
2. Bruni S, Loschi L, Incerti C, Gabrielli O, Coppa GV. Update on treatment of lysosomal storage diseases. *Acta Myol*. 2007; 26:87–92. [PubMed: 17915580]

3. Smith MA, Seibel NL, Altekruze SF, Ries LA, Melbert DL, O'Leary M, Smith FO, Reaman GH. Outcomes for children and adolescents with cancer: challenges for the twenty-first century. *J Clin Oncol*. 2010; 28:2625–2634. [PubMed: 20404250]
4. Barinaga M. Neurotrophic factors enter the clinic. *Science*. 1994; 264:772–774. [PubMed: 8171331]
5. Zhang Y, Pardridge WM. Delivery of β -Galactosidase to Mouse Brain via the Blood-Brain Barrier Transferrin Receptor. *JPET*. 2005; 313:1075–1081.
6. Cheng SH, Smith AE. Gene therapy progress and prospects: gene therapy of lysosomal storage disorders. *Gene Ther*. 2003; 10:1275–1281. [PubMed: 12883523]
7. Banks WA, Farr SA, Butt W, Kumar VB, Franko MW, Morley JE. Delivery across the blood-brain barrier of antisense directed against amyloid beta: reversal of learning and memory deficits in mice overexpressing amyloid precursor protein. *J Pharmacol Exp Ther*. 2001; 297:1113–21. [PubMed: 11356936]
8. Vinogradov SV, Batrakova EV, Kabanov AV. Nanogels for oligonucleotide delivery to the brain. *Bioconjug Chem*. 2004; 15:50–60. [PubMed: 14733583]
9. Fink DJ, DeLuca NA, Goins WF, Glorioso JC. Gene transfer to neurons using herpes simplex virus-based vectors. *Ann Rev Neurosci*. 1996; 19:265–287. [PubMed: 8833444]
10. Kaplitt MG, Leone P, Samulski RJ, Xiao X, Pfaff DW, OMalley K, During MJ. Long-term expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain. *Nature Gen*. 1994; 8:148–154.
11. Pardridge WM. Drug and gene delivery to the brain: the vascular route. *Neuron*. 2002; 36:555–558. [PubMed: 12441045]
12. Pardridge WM. Biopharmaceutical drug targeting to the brain. *J Drug Target*. 2010; 18:157–167. [PubMed: 20064077]
13. Dickson PI. Delivering drugs to the central nervous system: an overview. *Drug Deliv Transl Res*. 2012; 2:145–151.
14. Muro S. Strategies for delivery of therapeutics into the central nervous system for treatment of lysosomal storage disorders. *Drug Deliv Transl Res*. 2012; 2:169–186.
15. Vinogradov SV, Bronich TK, Kabanov AV. Nanosized cationic hydrogels for drug delivery: preparation, properties and interactions with cells. *Adv Drug Deliv Rev*. 2002; 54:135–147. [PubMed: 11755709]
16. Wagner HJ, Pilgrim C, Brandl J. Penetration and removal of horseradish peroxidase injected into cerebrospinal fluid. *Acta Neuropath*. 1974; 27:299–315. [PubMed: 4366438]
17. Meuli-Simmen C, Liu Y, Yeo TT, Liggitt D, Tu G, Yang T, Meuli M, Knauer S, Heath TD, Longo FM, Debs RJ. Gene expression along the cerebral-spinal axis after regional gene delivery. *Hum Gene Ther*. 1999; 10:2689–2700. [PubMed: 10566897]
18. Shi L, Tang GP, Gao SJ, Ma YX, Liu BH, Li Y, Zeng JM, Ng YK, Leong KW, Wang S. Repeated intrathecal administration of plasmid DNA complexed with polyethylene glycol-grafted polyethylenimine led to prolonged transgene expression in the spinal cord. *Gene Ther*. 2003; 10:1179–1188. [PubMed: 12833127]
19. Ishigaki A, Aoki M, Nagai M, Warita H, Kato S, Kato M, Nakamura T, Funakoshi H, Itoyama Y. Intrathecal Delivery of Hepatocyte Growth Factor From Amyotrophic Lateral Sclerosis Onset Suppresses Disease Progression in Rat Amyotrophic Lateral Sclerosis Model. *J Neuropath Exp Neurol*. 2007; 66:1037–1044. [PubMed: 17984685]
20. Tsai SY, Markus TM, Andrews EM, Cheatwood JL, Emerick AJ, Mir AK, Schwab ME, Kartje GL. Intrathecal treatment with anti-Nogo-A antibody improves functional recover in adult rats after stroke. *Exp Brain Res*. 2007; 182:261–266. [PubMed: 17717658]
21. Kakkis E, McEntee M, Vogler C, Le S, Levy B, Belichenko P, Mobley W, Dickson P, Hanson S, Passage M. Intrathecal enzyme replacement therapy reduces lysosomal storage in the brain and meninges of the, canine model of MPS I. *Mol Genet Metab*. 2004; 83:163–174. [PubMed: 15464431]
22. Hemsley KM, Hopwood JJ. Delivery of recombinant proteins via the cerebrospinal fluid as a therapy option for neurodegenerative lysosomal storage diseases. *Int J Clin Pharmacol Ther*. 2009; 47(Suppl 1):S118–S123. [PubMed: 20040322]

23. Milligan ED, Sloane EM, Langer SJ, Hughes TS, Jekich BM, Frank MG, Mahoney JH, Levkoff LH, Maier SF, Cruz PE, Flotte TR, Johnson KW, Mahoney MM, Chavez RA, Leinwand LA, Watkins LR. Repeated intrathecal injections of plasmid DNA encoding interleukin-10 produce prolonged reversal of neuropathic pain. *Pain*. 2006; 126:294–308. [PubMed: 16949747]
24. Calias P, Papisov M, Pan J, Savioli N, Belov V, Huang Y, Lotterhand J, Alessandrini M, Liu N, Fischman AJ, Powell JL, Heartlein MW. CNS Penetration of Intrathecal-Lumbar Idursulfase in the Monkey, Dog and Mouse: Implications for Neurological Outcomes of Lysosomal Storage Disorder. *PLoS ONE*. 2011; 7(1):e30341.10.1371/journal.pone.0030341 [PubMed: 22279584]
25. Papisov M, Belov V, Fischman AJ, Belova E, Titus J, Gagne M, Gillooly C. Delivery of proteins to CNS as seen and measured by Positron Emission Tomography. *Drug Deliv Transl Res*. 2012; 2:201–209.
26. Papisov M, Belov V, Belova E, Fischman AJ, Fisher R, Wright JL, Gannon KS, Titus J, Gagne M, Gillooly CA. Investigation of intrathecal transport of NPT002, a prospective therapeutic based on phage M13, in non-human primates. *Drug Deliv Transl Res*. 2012; 2:210–221.
27. Corning JL. Spinal anesthesia and local medication of the cord. *NY Med J*. 1885; 42:483–485.
28. Koster H, Kasman LP. Spinal anaesthesia for the head, neck and thorax; its relations to respiratory paralysis. *Surg Gynecol Obstet*. 1929; 49:617–630.
29. Yaksh TL, Reddy SVR. Studies in the primates on the analgetic effects associated with intrathecal actions of opiates, a-adrenergic agonists and baclofen. *Anesthesiology*. 1981; 54:451–467. [PubMed: 6112935]
30. Cousins MJ, Mather LE. Intrathecal and epidural administration of opioids. *Anesthesiology*. 1984; 61:276–310. [PubMed: 6206753]
31. Mackey, DC. The history of spinal drug delivery: the evolution of lumbar puncture and spinal narcosis. In: Yaksh, TL., editor. *Spinal Drug Delivery*. Elsevier; 1999. p. 1-41.
32. Maythaler, JM. Intrathecally delivered medications for spasticity and dystonia. In: Yaksh, TL., editor. *Spinal Drug Delivery*. Elsevier; 1999. p. 513-529.
33. Penn RD, Kroin JS. Continuous intrathecal baclofen for severe spasticity. *Lancet*. 1985; 326:125–127. [PubMed: 2862320]
34. Di Chiro G. Observations on the circulation of the cerebrospinal fluid. *Acta Radiol Diagn (Stockh)*. 1966; 5:988–1002. [PubMed: 5297333]
35. Soderquist RG, Mahoney MJ. Central nervous system delivery of large molecules: challenges and new frontiers for intrathecally administered therapeutics. *Expert Opin Drug Deliv*. 2010; 7:285–293. [PubMed: 20201735]
36. Dickson P, McEntee M, Vogler C, Le S, Levy B, Peinovich M, Hanson S, Passage M, Kakkis E. Intrathecal enzyme replacement therapy: successful treatment of brain disease via the cerebrospinal fluid. *Mol Genet Metab*. 2007; 91:61–68. [PubMed: 17321776]
37. Xu S, Wang L, El-Banna M, Sohar I, Sleat DE, Lobel P. Large-volume Intrathecal Enzyme Delivery Increases Survival of a Mouse Model of Late Infantile Neuronal Ceroid Lipofuscinosis. *Mol Ther*. 2011; 19:1842–1848. [PubMed: 21730969]
38. Dickson PI, Ellinwood NM, Brown JR, Witt RG, Le SQ, Passage MB, Vera MU, Crawford BE. Specific antibody titer alters the effectiveness of intrathecal enzyme replacement therapy in canine mucopolysaccharidosis I. *Mol Genet Metab*. 2012; 106:68–72. [PubMed: 22402327]
39. Crawley AC, Marshall N, Beard H, Hassiotis S, Walsh V, King B, Hucker N, Fuller M, Jolly RD, Hopwood JJ, Hemsley KM. Enzyme replacement reduces neuropathology in MPS IIIA dogs. *Neurobiol Dis*. 2011; 43:422–434. [PubMed: 21550404]
40. Okuda Y, Sakoda S, Fujimura H, Nagata S, Yanagihara T, Bernard CC. Intrathecal administration of neutralizing antibody against Fas ligand suppresses the progression of experimental autoimmune encephalomyelitis. *Biochem Biophys Res Commun*. 2000; 275:164–168. [PubMed: 10944459]
41. Cahill CM, Dray A, Coderre TJ. Intrathecal nerve growth factor restores opioid effectiveness in an animal model of neuropathic pain. *Neuropharmacology*. 2003; 45:543–552. [PubMed: 12907315]
42. Bambakidis NC, Petrullis M, Kui X, Rothstein B, Karampelas I, Kuang Y, Selman WR, LaManna JC, Miller RH. Improvement of neurological recovery and stimulation of neural progenitor cell

- proliferation by intrathecal administration of Sonic hedgehog. *J Neurosurg.* 2012; 116:1114–1120. [PubMed: 22324418]
43. Im YB, Jee MK, Choi JI, Cho HT, Kwon OH, Kang SK. Molecular targeting of NOX4 for neuropathic pain after traumatic injury of the spinal cord. *Cell Death Dis.* 2012 Nov 15.3:e426.10.1038/cddis.2012.168 [PubMed: 23152062]
  44. Vanderah TW, Laughlin T, Lashbrook JM, Nichols ML, Wilcox GL, Ossipov MH, Malan TP Jr, Porreca F. Single intrathecal injections of dynorphin A or des-Tyr-dynorphins produce long-lasting allodynia in rats: blockade by MK-801 but not naloxone. *Pain.* 1996; 68:275–281. [PubMed: 9121815]
  45. Ochs G, Penn RD, York M, Giess R, Beck M, Tonn J, Haigh J, Malta E, Traub M, Sendtner M, Toyka KV. A phase I/II trial of recombinant methionyl human brain derived neurotrophic factor administered by intrathecal infusion to patients with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord.* 2000; 1:201–206. [PubMed: 11464953]
  46. Nauta HJW, Dolan E, Yasaargil MG. Microsurgical anatomy of spinal subarachnoid space. *Surg Neurol.* 1983; 19:431–437. [PubMed: 6845156]
  47. Rieselbach R, DiChiro G, Freireich EJ, Rall DP. Subarachnoid distribution of drugs after lumbar injection. *New Engl J Med.* 1962; 267:1273–1278. [PubMed: 13973811]
  48. Lu J, Zhu XL. Cranial arachnoid membranes: some aspects of microsurgical anatomy. *Clin Anat.* 2007; 20:502–511. [PubMed: 17149739]
  49. Belova E, Papisov M, et al. 2012 unpublished data.
  50. Barker AE. A report on clinical experiences with spinal analgesia in 100 cases, and some reflections on the procedure. *Br Med J.* 1907; 1:665–674. [PubMed: 20763134]
  51. Kirshner M. Spinal zone anesthesia. Placed at will and dosage individually graded. *Surg Gynecol Obstet.* 1932; 55:317–329.
  52. Brown DT, Wildsmith JA, Covino BG, Scott DB. Effect of baricity on spinal anaesthesia with amethocaine. *Br J Anaesth.* 1980; 52:589–96. [PubMed: 7426230]
  53. Stienstra R, Gielen M, Kroon JW, Van Poorten F. The influence of temperature and speed of injection on the distribution of a solution containing bupivacaine and methylene blue in a spinal canal model. *Reg Anesth.* 1990; 15:6–11. [PubMed: 2275917]
  54. Davson, H.; Segal, MB. *Physiology of the CSF and Blood-brain barriers.* CRC; Boca Raton: 1995.
  55. Zheng, W.; Chodobski, A., editors. *The blood-cerebrospinal fluid barrier.* Chapman & Hall/CRC; Boca Raton: 2005.
  56. Oldendorf WH, Davson H. Brain extracellular space and the sink action of the cerebrospinal fluid. Measurement of rabbit brain extracellular space using sucrose labeled with carbon 14. *Arch Neurol.* 1967; 17:196–205. [PubMed: 6028247]
  57. Kimelberg HK. Water homeostasis in the brain: basic concepts. *Nat Neurosci.* 2004; 129:851–860.
  58. O'Connell JEA. Vascular factor in intracranial pressure and maintenance of cerebrospinal fluid circulation. *Brain.* 1943; 66:204–228.
  59. Oreskovic D, Klarica M. Development of hydrocephalus and classical hypothesis of cerebrospinal fluid hydrodynamics: facts and illusions. *Prog Neurobiol.* 2011; 94:238–258. [PubMed: 21641963]
  60. Dandy WE, Blackfan KD. Internal hydrocephalus. An experimental, clinical and pathological study. *Am J Dis Child.* 1914; 8:406–482.
  61. Ingraham FD, Matson DD, Alexander E, Woods PP. Studies in the treatment of experimental hydrocephalus. *J Neuropathol Exp Neurol.* 1948; 7:123–143. [PubMed: 18915129]
  62. Sachs E, Wilkins H, Sams CF. Studies on cerebrospinal circulation by a new method. *Arch Neurol Psych.* 1930; 23:130–151.
  63. Di Chiro G, Hammock MK, Bleyer WA. Spinal descent of cerebrospinal fluid in man. *Neurology.* 1976; 26:1–8. [PubMed: 942765]
  64. Reid, A.; Marchbanks, RJ.; Ernst, A. *Intracranial and Inner Ear Physiology and Pathophysiology.* Whurr Publishers; London: 1998. p. 9
  65. Enzmann DR, Pelc NJ. Normal flow patterns of intracranial and spinal cerebrospinal fluid defined with phase-contrast cine MR imaging. *Radiology.* 1991; 178:467–474. [PubMed: 1987610]

66. Yoshida K, Takahashi H, Saijo M, Ueguchi T, Tanaka H, Fujita N, Murase K. Phase-contrast MR studies of CSF flow rate in the cerebral aqueduct and cervical subarachnoid space with correlation-based segmentation. *Magn Reson Med Sci*. 2009; 8:91–100. [PubMed: 19783872]
67. Henry-Feugeas MC. Temporal and spatial assessment of normal cerebrospinal fluid dynamics with MR imaging. *Magn Reson Imaging*. 1993; 11:1107–1118. [PubMed: 8271897]
68. Zhu DC, Xenos M, Linninger AA, Penn RD. Dynamics of lateral ventricle and cerebrospinal fluid in normal and hydrocephalic brains. *J Magn Reson Imaging*. 2006; 24:756–770. [PubMed: 16958068]
69. Bhadelia RA, Bogdan AR, Kaplan RF, Wolpert SM. Cerebrospinal fluid pulsation amplitude and its quantitative relationship to cerebral blood flow pulsations: a phase-contrast MR flow imaging study. *Neuroradiology*. 1997; 39:258–264. [PubMed: 9144672]
70. Linge SO, Haughton V, Løvsgren AE, Mardal KA, Langtangen HP. CSF flow dynamics at the craniovertebral junction studied with an idealized model of the subarachnoid space and computational flow analysis. *Am J Neuroradiol*. 2010; 31:185–192. [PubMed: 19729542]
71. Sweetman B, Linninger AA. Cerebrospinal fluid flow dynamics in the central nervous system. *Ann Biomed Eng*. 2011; 39:484–96. [PubMed: 20737291]
72. Loth F, Yardimci AM, Alperin N. Hydrodynamic modeling of cerebrospinal fluid motion within the spinal cavity. *J Biomech Eng*. 2001; 123:71–79. [PubMed: 11277305]
73. Linninger AA, Xenos M, Sweetman B, Ponshe S, Guo X, Penn R. A mathematical model of blood, cerebrospinal fluid and brain dynamics. *J Math Biol*. 2009; 59:729–759. [PubMed: 19219605]
74. Du Boulay GH, O'Connell J, Currie J, Bostick T, Verity P. Further investigation of pulsatile movements in the cerebrospinal fluid pathways. *Acta Radiol Diagn (Stockh)*. 1972; 13:496–523. [PubMed: 4209972]
75. Hettiarachchi HD, Hsu Y, Harris TJ Jr, Penn R, Linninger AA. The effect of pulsatile flow on intrathecal drug delivery in the spinal canal. *Ann Biomed Eng*. 2011; 39:2592–2602. Erratum in: *Ann. Biomed Eng* 2011, 39, 2603. [PubMed: 21751071]
76. Davson, H.; Segal, MB. *Physiology of the CSF and Blood-brain barriers*. CRC; Boca Raton: 1995. p. 201
77. Kapoor KG, Katz SE, Grzybowski DM, Lubow M. Cerebrospinal fluid outflow: an evolving perspective. *Brain Res Bull*. 2008; 77:327–334. [PubMed: 18793703]
78. Levine JE, Povlishock JT, Becker DP. The Morphological Correlates of Primate Cerebrospinal Fluid Absorption. *Brain Res*. 1982; 241:31–41. [PubMed: 7104707]
79. Weed LH. The absorption of cerebrospinal fluid into the venous system. *Am J Anat*. 1923; 31:191–221.
80. Maurizi CP. Arachnoid granules: Dandy was Dandy, Cushing and Weed were not. *Med Hypotheses*. 2010; 75:238–240. [PubMed: 20299159]
81. Welch K, Friedman V. The cerebrospinal fluid valves. *Brain*. 1960; 83:454–469. [PubMed: 13784191]
82. Welch K, Pollay M. The spinal arachnoid villi of the monkeys *Cercopithecus aethiops* and *Macaca irus*. *Anat Record*. 1963; 145:43–48. [PubMed: 13999821]
83. McComb JG, Davson H, Hollingsworth JR. Attempted separation of blood-brain and blood-cerebrospinal fluid barriers in the rabbit. *Exp Eye Res*. 1977; 25(s):333–343. [PubMed: 590395]
84. Butler A, Mann J, Bass N. Identification of the major site for cerebrospinal fluid efflux in the albino rat. *Anat Record*. 1975; 181:323.
85. Tripathi R. Tracing the bulk outflow route of cerebrospinal fluid by transmission and scanning electron microscopy. *Brain Res*. 1974:503–506. [PubMed: 4214009]
86. Butler AB, Mann JD, Bass NH. Vesicular transport of peroxidase across the endothelium of the rat arachnoid villus. *Anat Record*. 1977; 187:543.
87. Yamada S, DePasquale M, Patlak CS, Cserr HF. Albumin outflow into deep cervical lymph from different regions of rabbit brain. *Am J Physiol*. 1991; 261(4 Pt 2):H1197–204. [PubMed: 1928403]
88. Koh L, Zakharov A, Johnston M. Integration of the subarachnoid space and lymphatics: Is it time to embrace a new concept of cerebrospinal fluid absorption? *Cerebrospinal Fluid Res*. 2005; 2:6. [PubMed: 16174293]

89. Michel CC. Transport of macromolecules through microvascular walls. *Cardiovasc Res.* 1996; 32:644–653. [PubMed: 8915183]
90. Cserr HF, Cooper DN, Milhorat TH. Flow of cerebral interstitial fluid as indicated by the removal of extracellular markers from rat caudate nucleus. *Exp Eye Res.* 1977; 25 (Suppl):461–473. [PubMed: 590401]
91. Cserr HF, Ostrach LH. Bulk flow of interstitial fluid after intracranial injection of blue dextran 2000. *Exp Neurol.* 1974; 45:50–60. [PubMed: 4137563]
92. Virchow R. Ueber die Erweiterung kleinerer Gefaesse. *Archiv Pathol Anat Physiol Klin Med.* 1851; 3:427–462.
93. Robin C. Recherches sur quelques particularités de la structure des capillaires de l'encephale. *J Physiol Homme Anim.* 1859; 2:537–548.
94. Davson, H.; Segal, MB. *Physiology of the CSF and Blood-brain barriers.* CRC; Boca Raton: 1995. p. 507
95. Woollam DHM, Millen JW. The perivascular spaces of the mammalian central nervous system. *Biol Rev.* 1954; 29:251–283.
96. Millen JW, Woollam DH. On the nature of the pia mater. *Brain.* 1961; 84:514–520. [PubMed: 14473930]
97. Hutchings M, Weller RO. Anatomical relationships of the pia mater to cerebral blood vessels in man. *J Neurosurg.* 1986; 65:316–325. [PubMed: 3734882]
98. Zhang ET, Inman CB, Weller RO. Interrelationship of the pia mater and the perivascular (Virchow-Robin) spaces in the human cerebrum. *J Anat.* 1990; 170:111–123. [PubMed: 2254158]
99. Pollock H, Hutchings M, Weller RO, Zhang ET. Perivascular spaces in the basal ganglia of the human brain: their relationship to lacunes. *J Anat.* 1997; 191:337–346. [PubMed: 9418990]
100. Schley D, Carare-Nnadi R, Please CP, Perry VH, Weller RO. Mechanisms to explain the reverse perivascular transport of solutes out of the brain. *J Theor Biol.* 2006; 238:962–974. [PubMed: 16112683]
101. Ichimura T, Fraser PA, Cserr HF. Distribution of extracellular tracers in perivascular spaces of the rat brain. *Brain Res.* 1991; 545:103–113. [PubMed: 1713524]
102. Rennels ML, Gregory TF, Blaumanis OR, Fujimoto K, Grady PA. Evidence for a paravascular fluid circulation in the mammalian central nervous system, provided by rapid distribution of tracer protein throughout the brain from subarachnoid spaces. *Brain Res.* 1985; 326:47–63. [PubMed: 3971148]
103. Wagner HJ, Pilgrim C, Brandl J. Penetration and removal of horseradish peroxidase injected into cerebrospinal fluid. *Acta Neuropath.* 1974; 27:299–315. [PubMed: 4366438]
104. Crawley AC, Marshall N, Beard H, Hassiotis S, Walsh V, King B, Hucker N, Fuller M, Jolly RD, Hopwood JJ, Hemsley KM. Enzyme replacement reduces neuropathology in MPS IIIA dogs. *Neurobiol Dis.* 2011; 43:422–434. [PubMed: 21550404]
105. Jolly RD, Marshall NR, Perrott MR, Dittmer KE, Hemsley KM, Beard H. Intracisternal enzyme replacement therapy in lysosomal storage diseases: routes of absorption into brain. *Neuropathol Appl Neurobiol.* 2011; 37:414–422. [PubMed: 21175738]
106. Hager H. Elektronenmikroskopische Untersuchungen über die Feinstruktur der Blutgefäße und perivascular Räume im Säugetiergehirn. *Acta Neuropath.* 1961; 1:9–33. [PubMed: 13903619]
107. Watanabe K, Takeishi H, Hayakawa T, Sasaki H. Three-dimensional organization of the perivascular glial limiting membrane and its relationship with the vasculature: a scanning electron microscope study. *Okajimas Folia Anatomica Japonica.* 2010; 87:109–121. [PubMed: 21174940]
108. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M. A Paravascular Pathway Facilitates CSF Flow Through the Brain Parenchyma and the Clearance of Interstitial Solutes, Including Amyloid β . *Sci Transl Med.* 2012; 4:147ra111.
109. Gotz M, Huttner WB. The cell biology of neurogenesis. *Nat Rev Mol Cell Biol.* 2005; 6:777–788. [PubMed: 16314867]
110. Carlén M, Meletis K, Göritz C, Darsalia V, Evergren E, Tanigaki K, Amendola M, Barnabé-Heider F, Yeung MS, Naldini L, Honjo T, Kokaia Z, Shupliakov O, Cassidy RM, Lindvall O,

- Frisén J. Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. *Nat Neurosci.* 2009; 12:259–267. [PubMed: 19234458]
111. Ghaffarian R, Bhowmick T, Muro S. Transport of nanocarriers across gastrointestinal epithelial cells by a new transcellular route induced by targeting ICAM-1. *J Control Release.* 2012; 163:25–33. [PubMed: 22698938]
  112. Cserr HF, Cooper DN, Suri PK, Patlak CS. Efflux of radiolabeled polyethylene glycols and albumin from rat brain. *Am J Physiol.* 1981; 240:F319–F328. [PubMed: 7223889]
  113. Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH. Convection-enhanced delivery of macromolecules in the brain. *Proc Natl Acad Sci USA.* 1994; 91:2076–2080. [PubMed: 8134351]
  114. Bidros DS, Vogelbaum MA. Novel drug delivery strategies in neuro-oncology. *Neurother.* 2009; 6:539–546.
  115. Iyer RR, Butman JA, Walbridge S, Gai ND, Heiss JD, Lonser RR. Tracking accuracy of T2- and diffusion-weighted magnetic resonance imaging for infusate distribution by convection-enhanced delivery. *J Neurosurg.* 2011; 115:474–480. [PubMed: 21663409]
  116. Geer CP, Grossman SA. Interstitial fluid flow along white matter tracts: A potentially important mechanism for the dissemination of primary brain tumors. *J Neuro-Oncol.* 1997; 32:193–201.
  117. Yin D, Valles FE, Fiandaca MS, Bringas J, Gimenez F, Berger MS, Forsayeth J, Bankiewicz KS. Optimal region of the putamen for image-guided convection-enhanced delivery of therapeutics in human and non-human primates. *Neuroimage.* 2011; 54 (Suppl. 1):S196–S203. [PubMed: 19761848]
  118. Inoue T, Yamashita Y, Nishihara M, Sugiyama S, Sonoda Y, Kumabe T, Yokoyama M, Tominaga T. Therapeutic efficacy of a polymeric micellar doxorubicin infused by convection-enhanced delivery against intracranial 9L brain tumor models. *Neuro Oncol.* 2009; 11:151–157. [PubMed: 18755917]
  119. Groothuis DR, Vavra MW, Schlageter KE, Kang EW, Itskovich AC, Hertzler S, Allen CV, Lipton HL. Efflux of drugs and solutes from brain: the interactive roles of diffusional transcapillary transport, bulk flow and capillary transporters. *J Cereb Blood Flow Metab.* 2007; 27:43–56. [PubMed: 16639426]
  120. Wang X, Wang C, Zeng J, Xu X, Hwang P, Yee W-C, Ng Y-K, Wang Sh. Gene Transfer to Dorsal Root Ganglia by Intrathecal Injection: Effects on Regeneration of Peripheral Nerves. *Mol Ther.* 2005; 12:314–320. [PubMed: 15925545]
  121. Kar S, Poirier J, Guevara J, Dea D, Hawkes C, Robitaille Y, Quirion R. Cellular distribution of insulin-like growth factor-II/mannose-6-phosphate receptor in normal human brain and its alteration in Alzheimer's disease pathology. *Neurobiol Aging.* 2006; 27:199–210. [PubMed: 16399207]
  122. Hawkes C, Kar S. Insulin-like growth factor-II/Mannose-6-phosphate receptor in the spinal cord and dorsal root ganglia of the adult rat. *Eur J Neurosci.* 2002; 15:33–39. [PubMed: 11860504]
  123. Yaksh TL, Horais K, Tozier N, Allen J, Rathbun ML, Rossi S, Sommer C, Meschter C, Richter PJ, Hildebrand KR. Chronically Infused Intrathecal Morphine in Dogs. *Anesthesiology.* 2003; 99:174–187. [PubMed: 12826858]
  124. O'Rahilly R, Muller F. The Meninges in Human Development. *J Neuropathol Exp Neurol.* 1986; 45:588–608. [PubMed: 3746345]
  125. Janson C, Romanova L, Hansen E, Hubel A, Lam C. Immortalization and functional characterization of rat arachnoid cell lines. *Neuroscience.* 2011; 177:23–34. [PubMed: 21195136]
  126. Petricevic J, Forempoher G, Ostojic L, Mardesic-Brakus S, Andjelinovic S, Vukojevic K, Saraga-Babic M. Expression of nestin, mesothelin and epithelial membrane antigen (EMA) in developing and adult human meninges and meningiomas. *Acta Histochem.* 2011; 113:703–711. [PubMed: 20943257]
  127. Feurer DJ, Weller RO. Barrier functions of the leptomeninges: a study of normal meninges and meningiomas in tissue culture. *Neuropathology and Appl Neurobiol.* 1991; 17:391–405.
  128. Endo H, Sasaki K, Tonosaki A, Kayama T. Three-dimensional and ultrastructural ICAM-1 distribution in the choroid plexus, arachnoid membrane and dural sinus of inflammatory rats

- induced by LPS injection in the lateral ventricles. *Brain Res.* 1998; 793:297–301. [PubMed: 9630685]
129. Feindt J, Krisch B, Lucius R, Mentlein R. Meningeal cells are targets and inactivation sites for the neuropeptide somatostatin. *Brain Res Mol Brain Res.* 1997; 44:293–300. [PubMed: 9073171]
  130. Esiri MM, Gay D. Immunological and neuropathological significance of the Virchow-Robin space. *J Neurol Sci.* 1990; 100:3–8. [PubMed: 2089138]
  131. Inglese M, Bomsztyk E, Gonen O, Mannon LJ, Grossman RI, Rusinek H. Dilated Perivascular Spaces: Hallmarks of Mild Traumatic Brain Injury. *Am J Neuroradiol.* 2005; 26:719–724. [PubMed: 15814911]
  132. Inglese M, Grossman RI, Diller L, Babb JS, Gonen O, Silver JM, Rusinek H. Clinical significance of dilated Virchow-Robin spaces in mild traumatic brain injury. *Brain Inj.* 2006; 20:15–21. [PubMed: 16403696]
  133. Schick S, Gahleitner A, Wöber-Bingöl C, Wöber C, Ba-Ssalamah A, Schoder M, Schindler E, Prayer D. Virchow-Robin spaces in childhood migraine. *Neuroradiol.* 1999; 41:283–287.
  134. Murata R, Nakajima S, Tanaka A, Miyagi N, Matsuoka O, Kogame S, Inoue Y. MR imaging of the brain in patients with mucopolysaccharidosis. *Am J Neuroradiol.* 1989; 10:1165–1170. [PubMed: 2556907]
  135. Miszkiel KA, Hall-Craggs MA, Miller RF, Kendall BE, Wilkinson ID, Paley MN, Harrison MJ. The spectrum of MRI findings in CNS cryptococcosis in AIDS. *Clin Radiol.* 1996; 51:842–850. [PubMed: 8972648]
  136. MacLulich AMJ, Wardlaw JM, Ferguson KJ, Starr JM, Seckl JR, Deary IJ. Enlarged perivascular spaces are associated with cognitive function in healthy elderly men. *J Neurol Neurosurg Psychiatry.* 2004; 75:1519–1523. [PubMed: 15489380]
  137. Heier LA, Bauer CJ, Schwartz L, Zimmerman RD, Morgello S, Deck MD. Large Virchow-Robin spaces: MR-clinical correlation. *Am J Neuroradiol.* 1989; 10:929–936. [PubMed: 2505536]
  138. Mason WP, Yeh S, DeAngelis LM. ¹¹¹Indium-diethylenetriamine pentaacetic acid cerebrospinal fluid flow studies predict distribution of intrathecally administered chemotherapy and outcome in patients with leptomeningeal metastases. *Neurology.* 1998; 50:438–444. [PubMed: 9484369]
  139. Hassin GB. Histopathology of carcinoma of the cerebral meninges. *Arch Neurol Psych.* 1919; 1:705–716.
  140. Mammoser AG, Groves MD. Biology and therapy of neoplastic meningitis. *Curr Oncol Rep.* 2010; 12:41–49. [PubMed: 20425607]
  141. Mirfakhraee M, Crofford MJ, Guinto FC Jr, Nauta HJ, Weedn VW. Virchow-Robin space: a path of spread in neurosarcoidosis. *Radiology.* 1986; 158:715–720. [PubMed: 3945745]
  142. Kooistra KL, Rodriguez M, Powis G, Yaksh TL, Harty GJ, Hilton JF, Laws ER Jr. Development of experimental models for meningeal neoplasia using intrathecal injection of 9L gliosarcoma and Walker 256 carcinosarcoma in the rat. *Cancer Res.* 1986; 46:317–23. [PubMed: 3753551]
  143. Herman C, Kupsky WJ, Rogers L, Duman R, Moore P. Leptomeningeal Dissemination of Malignant Glioma Simulating Cerebral Vasculitis. *Stroke.* 1995; 26:2366–2370. [PubMed: 7491666]
  144. Deckert-Schluter M, Schluter D, Hof H, Wiestler OD, Lassmann H. Differential expression of ICAM-1, VCAM-1 and their ligands LFA-1, Mac-1, CD43, VLA-4 and MHC class II antigens in murine toxoplasma encephalitis: A light microscopic and ultrastructural immunohistochemical study. *J Neuropathol Exp Neurol.* 1994; 53:457–468. [PubMed: 7521903]
  145. Maurer MH. Proteomics of brain extracellular fluid (ECF) and cerebrospinal fluid (CSF). *Mass Spectrom Rev.* 2010; 29:17–28. [PubMed: 19116946]

Figure 1. Internal structures of the leptomeningeal space. Dura (1), spinal cord (2), longitudinal membrane (3), dorsal and ventral nerve rootlets (4,5), dorsolateral septum (6). Transverse membranes and the mesh of ligaments (trabeculae) are not shown. From [46], with permission.

Figure 2.

One of the most reprinted schemes depicting the “circulation and drainage” of CSF. Red arrows: examples of CSF “flows” the significance of which for drug delivery can be misinterpreted (see text). From [64] with permission.

Figure 3.
Solute front propagation in the distal lumbar section of the LMS: ca. 0.5–0.7 mm per hour in this animal (derived from data obtained in [26])

Figure 4.

Scheme of the CSF remixing zones. Red arrow: the “CSF pump” [74]: pulsation of major arteries cause pulsatile contraction of the 3rd ventricle transmitted through the entire liquid compartment. Blue, light blue and green areas: zones of very fast, slower and very slow remixing of CSF, according to our PET data. These zones will have different k_{pd} . Graphics based on [74], with permission.

Figure 5.
Leptomeningeal pore (P), from [78] with permission. Scanning electron micrograph.

Figure 6.
Penetration of peroxidase molecules from CSF to VRS (left) and them to parenchyma (right). From [103] with permission.