AD_______________

Award Number: W81XWH-11-1-0387

TITLE: Viral Oncolytic Therapeutics for Neoplastic Meningitis

PRINCIPAL INVESTIGATOR: Mikhail Papisov, PhD

CONTRACTING ORGANIZATION: Massachusetts General Hospital,
Boston, MA 02114

REPORT DATE: July 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
1 July 2013	Annual	1 July 2012 – 30 June 2013

4. TITLE AND SUBTITLE
Viral Oncolytic Therapeutics for Neoplastic Meningitis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0387

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Mikhail Papisov
Betty Diamond

E-Mail: Papisov@helix.mgh.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Massachusetts General Hospital
Boston, Massachusetts 02114

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of this collaborative exploratory project is to develop novel, safe and efficient therapy for neoplastic meningitis (meningeal metastasis of breast cancer). The proposed therapy will be based on direct (intrathecal) administration of oncolytic viruses into the cerebrospinal fluid (CSF). During the first research year, physiology of the intrathecal delivery of particvles was studied in rats. The data was compared with the results obtained in non-human primates in our parallel studies. The key findings are the following. (1) The key factor defining the patterns of the initial particle distribution between the cerebral and spinal CSF is the volume of the bolus. (2) The key physiological factors defining further transport of the particles in the leptomeningeal space (and further into perivascular spaces) are tissue pulsation and particle surface structure. The data of the functional investigation of the diameter of meningeal pores draining CSF to the systemic circulation suggests a new, previously unreported subset of leptomeningeal pores that require further investigation. The new mechanistic model of particle transport in the leptomeningeal space supports the initial idea of the study and suggests further development in accordance with the original plan.

15. SUBJECT TERMS
Neoplastic meningitis, leptomeningeal space, viral oncolysis, cerebrospinal fluid, drug delivery

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	25	

Table of Contents

Page

Introduction……………………………………………………………….……..…..3

Body……………………………………………………………………………………..3

Key Research Accomplishments………………………………………….……..5

Reportable Outcomes…………………………………………………………6

Conclusion……………………………………………………………………………7

References…………………………………………………………………………….8

Appendices……………………………………………………………………………9

INTRODUCTION:

The goal of the proposed study is to develop novel, safe and efficient therapy for neoplastic meningitis. Neoplastic meningitis is a devastating complication of breast cancer caused by the spread of breast cancer cells into layers of tissues surrounding the brain that are normally filled with fluid. the space between two layers of isolating tissues enveloping the brain where neoplastic meningitis develops in isolated from the rest of the body and is not accessible to drug molecules circulating in blood. This study is aimed at the development of a drug of a novel type that would not escape from the space where the cancer cells spread causing neoplastic meningitis. We propose that an engineered virus hrR3, capable of killing cancer cells but harmless for normal tissues, will stay between the isolating tissue layers where the cancer cells spread, and kill them much more effectively than conventional drugs. The objectives of this exploratory study are to determine whether the hrR3 suspensions can stay in the area surrounding the brain for a period of time sufficient to infect the meningeal cancer cells and evaluate the safety and estimate the most effective schedule of hrR3 administration. Finally, we will determine the efficacy of hrR3 in an animal model of neoplastic meningitis.

BODY:

Task: Initiate PET imaging and photoimaging studies of viral expression in meningeal cancer cells. This task includes methodological development and investigating animals with implanted human cancer cells under BSL-2 provisions. Due to the closure of the BSL-2 facility for renovations for nearly the entire period covered by this report, we concentrated on methodological studies not requiring BSL-2. A no-cost extension was requested and granted by DoD to complete the planned BSL-2 work in 2013-14.

(1) Implantation of cancer cells into the subarachnoid (leptomemingeal) space of nude rats requires intrathecal infusion of cell suspensions. Originally, based on our previous experience with outbred rats, direct non-surgical injection into cisterna magna through the atlanto-occipital joint was planned as the method of implantation. We have found in pilot experiments with Rnu/nu rats that the configuration of the atlanto-occipital region in this strain is somewhat different, and the direct injection results in a higher frequency of experimental failure (ca. 40% vs. 20% for CD rats as performed by the same personnel). The main sign of the injection failure was blood in the CSF, which indicated a closer proximity of major veins to the injection point. To develop an alternative cell infusion method, we worked with our animal vendor, Charles River Laboratories (CRL), to adopt their intrathecal catheterization service for our purpose. Cannulated animals from CRL were administered with model radiolabeled particles through the catheters, and the particulate distribution was studied by PET. It was found that the conventional lumbar cannulation (with caudad catheter tip placement) resulted in frequent (~ 50%) blockage of particle passage towards the cerebral CSF volume, which is not acceptable for our purposes. On our request, CRL changed the

direction of the catheter tip placement to the anteriad. With anteriorly oriented catheters, particle passage towards the cranium was observed in about 80% of the animals. This can e considered satisfactory, provided that every injection is monitored for quality by PET. The catheter patency, however, was found to be less than a week, which precludes the use of these (polyolefine) catheters for studies where the catheter needs to be used more than ones over a more than one week period, e.g., for cancer cell infusion and then for oncolythic virus administration.

(2) Using PET for in vivo imaging of traslocation of the injected material in CSF, we attempted to correlate the parameters of lumbar bolus in intrathecally catheterized rats with those in non-human primates. We have found that in cynomolgus monkeys the hydrostatic compliance of the compartment is almost exclusively cerebral, i.e., the added volume translocates from any injection point towards the head. This would be in agreement that the function of absorbing the extra volume is performed by large cerebral veins that respond to the increased pressure. The CSF volume between the lumbar injection point and the cisterna magna was found to be ca. 0.5 ml/kg body weight, i.e., lumbar bolus of more than 0.5 ml/kg results in the immediate translocation of the injected substance to the cerebral CSF. In rats, the cerebral CSF compartment is also largely responsible for the hydrostatic compliance. However, there is apparently some compliance in the distal spine, which accepts at least 20 μl of the injected liquid (150 g animals) if the anteriad passage is blocked. The data on the CSF volume between the lumbar injection point and cisterna magna was less consistent than in monkeys. In some animals, a 10 μl bolus extended to the cisterna magna, whereas in some animals a >50 μl bolus was needed to "push" the injected material to the cerebral CSF. This may be explained by either partial blockage of the spinal CSF compartment in catheterized animals or by variable posterior hydrodynamic compliance (or both) and needs further investigation. Our tentative hypothesis is that in rats with unobstructed spinal subarachnoid space the CSF volume between the lumbar injection point and cisterna magna is ca. 70 μl/kg, i.e., the spinal subarachnoid space is relatively tighter than in primates. This has to be taken into account in extrapolating the pharmacokinetics data from rodents to humans.

Task: Efficacy studies (single and multiple injections). This Task includes pharmacokinetics and pharmacodynamics modeling and the actual efficacy studies. The latter were delayed until 2013-14 research year due to the closure of the BSL-2 compliant facility, as explained above. Thus, during the 2012-13 we concentrated on data analysis, non-BSL-2 model experiments and pharmacokinetic modeling.

(1) Since our data obtained during the previous year strongly suggested that the meningeal pores through which the CSF drains to venous blood have multimodal size distribution and virion-sized particles will stay in the CSF longer than smaller molecular entities (which supports the idea of this study), we expanded the studies towards investigation of small molecule behavior in the CSP. Model small molecules (D- and L- isomers of glucose labeled with fluorine-18) were administered into lumbar CSF of a cynomolgus monkey (n=1), and their clearance from the subarachnoid space and translocation to the systemic circulation was studied by

PET. It was found that 45% of the administered material is transferred to the blood with a half-life of 15 minutes. The rest of L-glucose is transferred slower ($t_{1/2}$=110 min), whereas the rest of D-glucose remains in the meninges. The same monkey was used (outside DoD sponsored studies) in analogous experiments with radiolabeled M13 phage, where phage particles were transferred to the blood with a half-life of ca. 5 hours. Thus, the data suggest that particles are retained in the CSF by at least an order of magnitude longer than small molecules, which further supports the original idea of this project. The initial data warrants further investigation of the size dependence of the cerebrospinal pharmacokinetics.

(2) We have analyzed all our data obtained in rats and monkeys (obtained within this study as well as in earlier and parallel studies) to outline the general physiological mechanisms of solute transport in the CSF. The emerging model very significantly diverges with the prevalent views, mostly due to the earlier unknown cerebral localization of the hydrostatic compliance and the non-directional non-diffusional solute transport in the CSF. The complete analysis of the physiology of intrathecal bolus based on our data (and vs. published data) is given in our paper "Physiology of Intrathecal Bolus" (enclosed in the Appendix).

KEY RESEARCH ACCOMPLISHMENTS:

- New knowledge on the physiology of the solute transport in the CSF have been obtained, in particular on the cerebral hydrodynamic compliance of the subarachnoid compartment and size-dependence of solute clearance from the CSF.
- A new physiological paradigm of intrathecal bolus has been developed.
- Animal models were analyzed and improved for the ongoing studies.

REPORTABLE OUTCOMES:

Full size research paper:

1. Papisov M, Belov VV and Gannon KS. Physiology of the intrathecal bolus: the leptomeningeal route for macromolecule and particle delivery to CNS. Molecular Pharmaceutics 2013, 10:1522-1532, DOI: 10.1021/mp300474m

Abstracts:

2. Belova E, V. Belov V, Gagne M, Gillooly C, Fischman AJ, Papisov MI. Pharmacokinetics of macromolecules in spinal CSF: PET and modeling. 2013 SNM Annual Meeting, Vancouver, BC, Canada.

3. Gillooly C, V. Belov V, Belova E, Gagne M, Fischman AJ, Titus J, Papisov MI. Assessment of CSF drainage to the lymphatic system using positron emission tomography in rats and nonhuman primates. 2013 SNM Annual Meeting, Vancouver, BC, Canada.

4. Bonab A, Fischman AJ, Belov V, Papisov MI. Biokinetics of FDG after intrathecal administration. 2013 SNM Annual Meeting, Vancouver, BC, Canada.

5. Belov V, Gagne M, Titus J, Gillooly C, Belova E, Fischman AJ and Papisov M. Monitoring of drug concentration in the cerebrospinal fluid by PET. 2013 SNM Annual Meeting, Vancouver, BC, Canada.

Invited lectures:

6. "CSF solute dynamics as seen by PET", 2nd International Cerebrospinal fluid Dynamics Dynamics, Manhasset, NY. Sponsor: Chiari and Syringomyelia Foundation, 2013
7. "Macromolecular drug development and the role of PET imaging." Northeastern University School of Pharmacy, Department of Pharmaceutical Sciences Colloquium, 2012

Career development:

8. Two BS-level participants, Matthew Gagne and Caitlin Gillooly, were accepted to a PhD program and Medical School, respectively.
9. The PI, M. Papisov, has been accepted as full member of Dana Farber/Harvard Cancer Center (Breast Cancer, Neuro-Oncology)

Grant applications:

10. DoD Idea Extension Award based on the present study, Viral Oncolytic Therapeutics for Neoplastic Meningitis (PIs: Papisov, Kuruppu)

11. R01 grant application “Fully biodegradable functional polyal-based materials”, NIH, 2013 (PI: Papisov)
12. NIH R21 grant application, entitled “Size dependent mechanisms of drug clearance from the cerebrospinal fluid” (PI: Belov)
13. NIH R21 grant application, entitled “Modeling of leptomeningeal transport of macromolecules and particles” (PI: Papisov)

CONCLUSION:

The research completed to date demonstrates feasibility of the original idea of this exploratory project. The new physiological data further suggests that complete coverage of the leptomeningeal space with intrathecally administered oncolytic viral suspensions is possible, optimally with a high-volume intrathecal bolus.

The obtained data, as well as the progress achieved by the co-PI’s research team (see Annual Report W81XWH-11-1-0388) suggest that the project should continue in accordance with the original plan.

The obtained data warrants further research on the physiological mechanisms of solute transport in the CSF and development of new animal models.

REFERENCES:

(1) Papisov M, Belov VV and Gannon KS. Physiology of the intrathecal bolus: the leptomeningeal route for macromolecule and particle delivery to CNS. Molecular Pharmaceutics 2013, 10:1522-1532, DOI: 10.1021/mp300474m

(2) Belova E, V. Belov V, Gagne M, Gillooly C, Fischman AJ, Papisov MI. Pharmacokinetics of macromolecules in spinal CSF: PET and modeling. 2013 SNM Annual Meeting, Vancouver, BC, Canada.

(3) Gillooly C, V. Belov V, Belova E, Gagne M, Fischman AJ, Titus J, Papisov MI. Assessment of CSF drainage to the lymphatic system using positron emission tomography in rats and nonhuman primates. 2013 SNM Annual Meeting, Vancouver, BC, Canada.

(4) Bonab A, Fischman AJ, Belov V, Papisov MI. Biokinetics of FDG after intrathecal administration. 2013 SNM Annual Meeting, Vancouver, BC, Canada.

(5) Belov V, Gagne M, Titus J, Gillooly C, Belova E, Fischman AJ and Papisov M. Monitoring of drug concentration in the cerebrospinal fluid by PET. 2013 SNM Annual Meeting, Vancouver, BC, Canada.

(6) Video publication of the invited lecture “CSF solute dynamics as seen by PET” (#6 in the list above), http://vimeo.com/channels/570494/72653181

APPENDICES:

Published papers and abstracts:

(1) Papisov M, Belov VV and Gannon KS. Physiology of the intrathecal bolus: the leptomeningeal route for macromolecule and particle delivery to CNS. Molecular Pharmaceutics 2013, 10:1522-1532, DOI: 10.1021/mp300474m

(2) Belova E, V. Belov V, Gagne M, Gillooly C, Fischman AJ, Papisov MI. Pharmacokinetics of macromolecules in spinal CSF: PET and modeling. 2013 SNM Annual Meeting, Vancouver, BC, Canada.

(3) Gillooly C, V. Belov V, Belova E, Gagne M, Fischman AJ, Titus J, Papisov MI. Assessment of CSF drainage to the lymphatic system using positron emission tomography in rats and nonhuman primates. 2013 SNM Annual Meeting, Vancouver, BC, Canada.

(4) Bonab A, Fischman AJ, Belov V, Papisov MI. Biokinetics of FDG after intrathecal administration. 2013 SNM Annual Meeting, Vancouver, BC, Canada.

(5) Belov V, Gagne M, Titus J, Gillooly C, Belova E, Fischman AJ and Papisov M. Monitoring of drug concentration in the cerebrospinal fluid by PET. 2013 SNM Annual Meeting, Vancouver, BC, Canada.

(6) Link to video publication of the invited lecture “CSF solute dynamics as seen by PET” 2nd CSF Dynamics symposium, Manhassett, NY, 2013: http://vimeo.com/channels/570494/72653181

SUPPORTING DATA

N/A

molecular pharmaceutics

Review

pubs.acs.org/molecularpharmaceutics

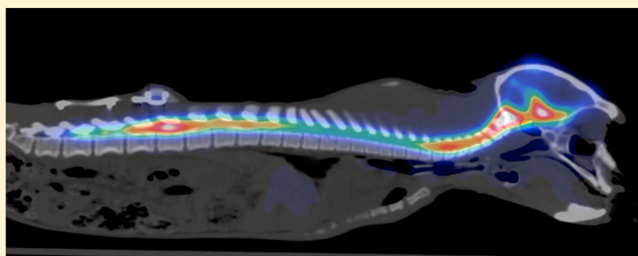
Physiology of the Intrathecal Bolus: The Leptomeningeal Route for Macromolecule and Particle Delivery to CNS

Mikhail I. Papisov,*,† Vasily V. Belov,† and Kimberley S. Gannon‡

†Massachusetts General Hospital, Shriners Hospitals for Children—Boston, and Harvard Medical School, 51 Blossom Street, Boston, Massachusetts 02114, United States

‡NeuroPhage Pharmaceuticals, Inc., 222 Third Street, Suite 3120 Cambridge, Massachusetts 02142, United States

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 cerebrospinal 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*

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 diseases), genetic deficiencies (e.g., lysosomal storage diseases[2]), and several types of brain cancer (e.g., childhood brainstem glioma[3]).

If the problem of their delivery to CNS is solved, then biopharmaceuticals (proteins,[4−6] oligonucleotides,[7,8] gene vectors[9−11]) 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)[12−14] and nanocarriers,[15] 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) was 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.[17−23] Our recent studies using PET as a method of noninvasive tracking of intrathecally administered proteins and particles, such as particles of bacteriophage M13 (below in the text "phage"),[24−26] 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 space. (Leptomeningeal space (LMS) is

Special Issue: Drug Delivery across the Blood-Brain Barrier

Received: August 27, 2012
Revised: January 10, 2013
Accepted: January 14, 2013

 © XXXX American Chemical Society

understood here as the entire space occupied by CSF, including all spaces continuous with the subarachnoid space, such as perivascular spaces and ventricles.) 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 cocaine[27] 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 area.[28] 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 (upward) spread of an anesthetic from injection sites close to the head could be risky because of the proximity of the medullary vasomotor centers.[31] 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 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 limitations.[35]

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,[36,21,37−39] antibodies,[40] nerve growth factor,[41] Sonic Hedgehog,[42] siRNA,[43] and dynorphins.[44]

One recent paper describing the results of early clinical studies states: "[Intrathecally administered] BDNF can be delivered cranially against CSF flow."[45] 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 weblike 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.

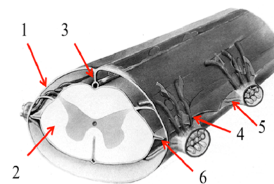


Figure 1. Internal structures of the leptomeningeal space. Dura (1), spinal cord (2), longitudinal membrane (3), dorsal and ventral nerve rootlets (4, 5), dorsolateral septum (6). Transverse membranes and the mesh of ligaments (trabeculae) are not shown. Reprinted with permission from ref 46. Copyright 1983 Elsevier.

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 toward the expandable area (and toward major veins that can contract and accommodate extra volume under pressure[74]) and spread toward the same direction, i.e., mainly toward 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, [^{131}I] Rose Bengal in *Macaca mulatta* and [^{198}Au] 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 (*Macaca 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.[24−26] 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 detail.[48] Our current studies in rats suggest that catheter tip position can change the initial pattern of bolus translocation very significantly.[49]

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 [^{198}Au] did not unequivocally improve distribution in comparison with patients receiving a similar volume with one rapid injection,[47] 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. Barker[50] in 1907. He showed 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.[51−53] 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 appears 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 toward 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 view,[59] 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]

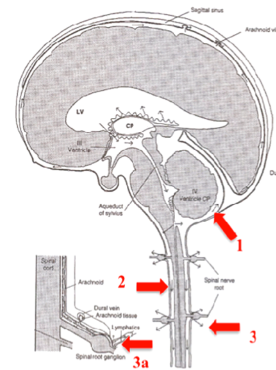


Figure 2. One of the most reprinted schemes depicting the "circulation and drainage" of CSF. Red arrows: examples of CSF "flows" the significance of which for drug delivery can be misinterpreted (see text). Reprinted with permission from ref 62. Copyright 1930 American Medical Association.

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 flow.[63] 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 study,[64] 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 area,[74] 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.[65−69] Computational flow dynamics (CFD) models have been used to complement MRI measurements in explaining the complex fluid flow patterns in the CSF-filled spaces.[70−72] 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 to 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,

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

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 Fick's diffusion equation; however, the underlying process is nondiffusional 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 size by about 2 orders of magnitude (ca. 12−14 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/h 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 μL) injected at L1 region in the rat's spine expanded both cranially (at ca. 2.8 cm/h) and caudally (at ca. 1.4 cm/h). As a result, the solute distributed over the entire spinal CSF of the rat within approximately 1 h.

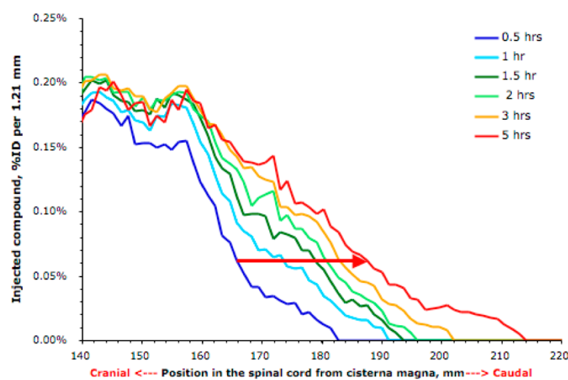


Figure 3. Solute front propagation in the distal lumbar section of the LMS: ca. 0.5−0.7 mm per hour in this animal (derived from data obtained in ref 26).

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 al.[74] 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

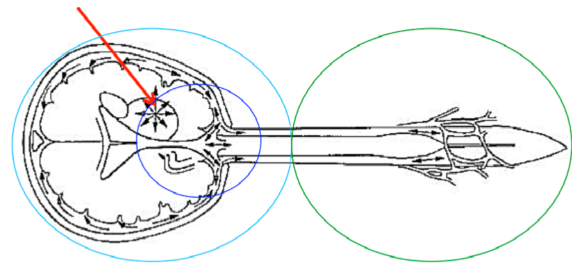


Figure 4. Scheme of the CSF remixing zones. Red arrow: the "CSF pump": [74] pulsation of major arteries causes pulsatile contraction of the 3rd ventricle transmitted through the entire liquid compartment. Blue, light blue, and green areas: zones of very fast, slower, and very slow remixing of CSF, according to our PET data. These zones will have different k_{pd}. Graphics based on ref 75, with permission. Copyright 2011 Biomedical Engineering Society.

configuration of the leptomeningeal space, with parameters essentially based on eq 1. To the best of our knowledge, these parameters were never measured for any animal, although the approaches are presently being developed.[74] 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 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 human.[77] The drainage routes have been investigated for nearly a century, but there are still conflicting views due to the significant methodological difficulties.[78]

The principal CSF draining route is believed to be in the venous sinuses through physical pores found in specialized structures, arachnoid granulations[79] (we must note the debate continues even now[80]). In humans and non-human primates, the arachnoid granulations were found in the superior sagittal and lateral sinuses,[81] and at some nerve roots,[82] while in rabbits they were found only at the cranial base.[83] 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. Tripathi[85] suggests that filtration proceeds through 0.1−2.3 μm 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

dx.doi.org/10.1021/mp300474m | Mol. Pharmaceutics XXXX, XXX, XXX−XXX

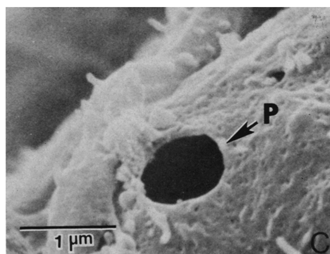


Figure 5. Leptomeningeal pore (P). Reprinted with permission from ref 76. Copyright 1995 CRC Press. Scanning electron micrograph.

also found in numerous transendothelial vesicles, suggesting a dual filtration mechanism.[86]

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 rabbits.[87] The data on the lymphatic drainage, obtained using different (mostly invasive) methods and in different species, diverge very widely (reviewed in ref 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 region,[49] 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 vary 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 outward.[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 number of anatomical studies with light and electron microscopy, the exact relations of the perivascular and leptomeningeal spaces 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.[95−99] It has been suggested that the perivascular space is connected with the lymphatic system.[100] 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[101−103] (Figure 6).

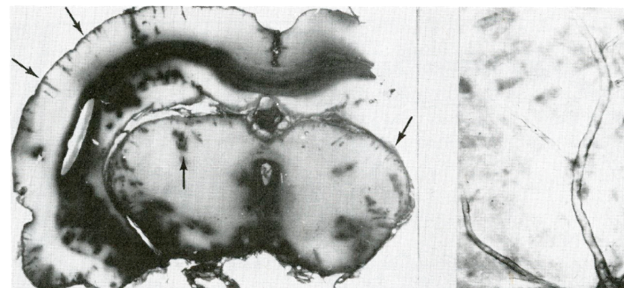


Figure 6. Penetration of peroxidase molecules from CSF to VRS (left) and them to parenchyma (right). Reprinted with permission from ref 103. Copyright 1974 Springer-Verlag.

Protein penetration from the CSF into the perivascular space was detected as early as at 4 min after the injection,[102] with spread to subpial space and with indications of back-and-forth flow.[101] 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 h 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 h 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 h). 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 (^{124}I) washout relates to the metabolization 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.

dx.doi.org/10.1021/mp300474m | *Mol. Pharmaceutics* XXXX, XXX, XXX−XXX

b. Transport from VRS to the Brain Parenchyma. While the data of H. Wagner et al.[103] and more recent studies[24,104,105] 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 are 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 pia;[106] 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 (also known as 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[24,103−105,108] 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 methods[108] in mice, paravenous accumulation appeared much later (>1 h after intracisternal administration) than paraarterial accumulation (immediately after the administration).

Ependymal cells lining ventricles are very different from leptomeningeal cells. They are of neuroglial origin[109] and have stem cell characteristics.[110] 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-1,[144] which can possibly be used for enhanced drug transport if these cells have the same CAM-associated mechanisms as the "classical" epithelial cells.[111]

■ 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 are 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 tracts[116] 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 studies[24] 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 receptors,[121] which are widely expressed on cells of the spinal cord and brain.[122] 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 pumps[123]). 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 ref 25 and Figure 7 in ref 26). However, hydrophobic nanoparticles remained within 2 cm from the lumbar injection point, presumably due to nonspecific 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 origin[124] 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 mesothelin,[126] and also cytokeratin, desmoplakin, and vimentin.[125] They are capable of cytochalasin B inhibitable

 dx.doi.org/10.1021/mp300474m | Mol. Pharmaceutics XXXX, XXX, XXX−XXX

endocytosis[127] and known to express ICAM-1[128] and somatostatin,[129] 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 developed.[35]

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 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.[130−132] Dilated VRS were also associated with migraine,[133] mucopolysaccharidosis,[134] cryptococcosis,[135] age,[136] hypertension, dementia, incidental white matter lesions,[137] and other conditions; the mechanism of dilation is believed to be associated with inflammatory changes, but has not been established.[132] 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 VRS,[139] most meningeal and brain cancers invade and/or occlude the VRS,[140−143] 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 CSF[145] 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 models 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 VRS 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.

AUTHOR INFORMATION

Corresponding Author

*E-mail: papisov@helix.mgh.harvard.edu.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Authors thank Drs. P. Calias, R. Penn, M. Heartlein, T. McCauley, A. J. Fischman, and R. Fisher 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 USED

CNS, central nervous system; CSF, cerebrospinal fluid; LMS, leptomeningeal space; VRS, Virchow−Robin space; CAM, cell adhesion molecule

REFERENCES

(1) Pardridge, W. M. The blood-brain barrier: Bottleneck in brain drug development. *NeuroRx* **2005**, *2*, 3−14.

(2) Bruni, S.; Loschi, L.; Incerti, C.; Gabrielli, O.; Coppa, G. V. Update on treatment of lysosomal storage diseases. *Acta Myol.* **2007**, *26*, 87−92.

(3) Smith, M. A.; Seibel, N. L.; Altekruse, S. F.; Ries, L. A.; Melbert, D. L.; O'Leary, M.; Smith, F. O.; Reaman, G. H. Outcomes for children and adolescents with cancer: challenges for the twenty-first century. *J Clin. Oncol.* **2010**, *28*, 2625−2634.

(4) Barinaga, M. Neurotrophic factors enter the clinic. *Science* **1994**, *264*, 772−774.

(5) Zhang, Y.; Pardridge, W. M. Delivery of β-Galactosidase to Mouse Brain via the Blood-Brain Barrier Transferrin Receptor. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 1075−1081.

(6) Cheng, S. H.; Smith, A. E. Gene therapy progress and prospects: gene therapy of lysosomal storage disorders. *Gene Ther.* **2003**, *10*, 1275−1281.

(7) Banks, W. A.; Farr, S. A.; Butt, W.; Kumar, V. B.; Franko, M. W.; Morley, J. E. 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*, 1113−1121.
(8) Vinogradov, S. V.; Batrakova, E. V.; Kabanov, A. V. Nanogels for oligonucleotide delivery to the brain. *Bioconjugate Chem.* **2004**, *15*, 50−60.
(9) Fink, D. J.; DeLuca, N. A.; Goins, W. F.; Glorioso, J. C. Gene transfer to neurons using herpes simplex virus-based vectors. *Annu. Rev. Neurosci.* **1996**, *19*, 265−287.
(10) Kaplitt, M. G.; Leone, P.; Samulski, R. J.; Xiao, X.; Pfaff, D. W.; OMalley, K.; During, M. J. Long-term expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain. *Nat. Genet.* **1994**, *8*, 148−154.
(11) Pardridge, W. M. Drug and gene delivery to the brain: the vascular route. *Neuron* **2002**, *36*, 555−558.
(12) Pardridge, W. M. Biopharmaceutical drug targeting to the brain. *J. Drug Targeting* **2010**, *18*, 157−167.
(13) Dickson, P. I. Delivering drugs to the central nervous system: an overview. *Drug Delivery 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 Delivery Transl. Res.* **2012**, *2*, 169−186.
(15) Vinogradov, S. V.; Bronich, T. K.; Kabanov, A. V. Nanosized cationic hydrogels for drug delivery: preparation, properties and interactions with cells. *Adv. Drug Delivery Rev.* **2002**, *54*, 135−147.
(16) Wagner, H. J.; Pilgrim, C.; Brandl, J. Penetration and removal of horseradish peroxidase injected into cerebrospinal fluid. *Acta Neuropath.* **1974**, *27*, 299−315.
(17) Meuli-Simmen, C.; Liu, Y.; Yeo, T. T.; Liggitt, D.; Tu, G.; Yang, T.; Meuli, M.; Knauer, S.; Heath, T. D.; Longo, F. M.; Debs, R. J. Gene expression along the cerebral-spinal axis after regional gene delivery. *Hum. Gene Ther.* **1999**, *10*, 2689−2700.
(18) Shi, L.; Tang, G. P.; Gao, S. J.; Ma, Y. X.; Liu, B. H.; Li, Y.; Zeng, J. M.; Ng, Y. K.; Leong, K. W.; 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.
(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. Neuropathol. Exp. Neurol.* **2007**, *66*, 1037−1044.
(20) Tsai, S. Y.; Markus, T. M.; Andrews, E. M.; Cheatwood, J. L.; Emerick, A. J.; Mir, A. K.; Schwab, M. E.; Kartje, G. L. Intrathecal treatment with anti-Nogo-A antibody improves functional recover in adult rats after stroke. *Exp. Brain Res.* **2007**, *182*, 261−266.
(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.
(22) Hemsley, K. M.; Hopwood, J. J. 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.
(23) Milligan, E. D.; Sloane, E. M.; Langer, S. J.; Hughes, T. S.; Jekich, B. M.; Frank, M. G.; Mahoney, J. H.; Levkoff, L. H.; Maier, S. F.; Cruz, P. E.; Flotte, T. R.; Johnson, K. W.; Mahoney, M. M.; Chavez, R. A.; Leinwand, L. A.; Watkins, L. R. Repeated intrathecal injections of plasmid DNA encoding interleukin-10 produce prolonged reversal of neuropathic pain. *Pain* **2006**, *126*, 294−308.
(24) Calias, P.; Papisov, M.; Pan, J.; Savioli, N.; Belov, V.; Huang, Y.; Lotterhand, J.; Alessandrini, M.; Liu, N.; Fischman, A. J.; Powell, J. L.; Heartlein, M. W. 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 DOI: 10.1371/journal.pone.0030341.
(25) Papisov, M.; Belov, V.; Fischman, A. J.; Belova, E.; Titus, J.; Gagne, M.; Gillooly, C. Delivery of proteins to CNS as seen and measured by Positron Emission Tomography. *Drug Delivery Transl. Res.* **2012**, *2*, 201−209.
(26) Papisov, M.; Belov, V.; Belova, E.; Fischman, A. J.; Fisher, R.; Wright, J. L.; Gannon, K. S.; Titus, J.; Gagne, M.; Gillooly, C. A. Investigation of intrathecal transport of NPT002, a prospective therapeutic based on phage M13, in non-human primates. *Drug Delivery Transl. Res.* **2012**, *2*, 210−221.
(27) Corning, J. L. Spinal anesthesia and local medication of the cord. *N.Y. Med. J.* **1885**, *42*, 483−485.
(28) Koster, H.; Kasman, L. P. Spinal anaesthesia for the head, neck and thorax; its relations to respiratory paralysis. *Surg. Gynecol. Obstet.* **1929**, *49*, 617−630.
(29) Yaksh, T. L.; Reddy, S. V. R. Studies in the primates on the analgetic effects associated with intrathecal actions of opiates, a-adrenergetic agonists and baclofen. *Anesthesiology* **1981**, *54*, 451−467.
(30) Cousins, M. J.; Mather, L. E. Intrathecal and epidural administration of opioids. *Anesthesiology* **1984**, *61*, 276−310.
(31) Mackey, D. C., The history of spinal drug delivery: the evolution of lumbar puncture and spinal narcosis. In *Spinal Drug Delivery*; Yaksh, T. L., Ed.; Elsevier: 1999; pp 1−41.
(32) Maythaler, J. M. Intrathecally delivered medications for spasticity and distonia. In *Spinal Drug Delivery*; Yaksh, T. L., Ed.; Elsevier: 1999; pp 513−529.
(33) Penn, R. D.; Kroin, J. S. Continuous intrathecal baclofen for severe spasticity. *Lancet* **1985**, *326*, 125−127.
(34) Di Chiro, G. Observations on the circulation of the cerebrospinal fluid. *Acta Radiol.: Diagn.* **1966**, *5*, 988−1002.
(35) Soderquist, R. G.; Mahoney, M. J. Central nervous system delivery of large molecules: challenges and new frontiers for intrathecally administered therapeutics. *Expert Opin. Drug Delivery* **2010**, *7*, 285−293.
(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.
(37) Xu, S.; Wang, L.; El-Banna, M.; Sohar, I.; Sleat, D. E.; 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.
(38) Dickson, P. I.; Ellinwood, N. M.; Brown, J. R.; Witt, R. G.; Le, S. Q.; Passage, M. B.; Vera, M. U.; Crawford, B. E. Specific antibody titer alters the effectiveness of intrathecal enzyme replacement therapy in canine mucopolysaccharidosis I. *Mol. Genet. Metab.* **2012**, *106*, 68−72.
(39) Crawley, A. C.; Marshall, N.; Beard, H.; Hassiotis, S.; Walsh, V.; King, B.; Hucker, N.; Fuller, M.; Jolly, R. D.; Hopwood, J. J.; Hemsley, K. M. Enzyme replacement reduces neuropathology in MPS IIIA dogs. *Neurobiol. Dis.* **2011**, *43*, 422−434.
(40) Okuda, Y.; Sakoda, S.; Fujimura, H.; Nagata, S.; Yanagihara, T.; Bernard, C. C. Intrathecal administration of neutralizing antibody against Fas ligand suppresses the progression of experimental autoimmune encephalomyelitis. *Biochem. Biophys. Res. Commun.* **2000**, *275*, 164−168.
(41) Cahill, C. M.; Dray, A.; Coderre, T. J. Intrathecal nerve growth factor restores opioid effectiveness in an animal model of neuropathic pain. *Neuropharmacology* **2003**, *45*, 543−552.
(42) Bambakidis, N. C.; Petrullis, M.; Kui, X.; Rothstein, B.; Karampelas, I.; Kuang, Y.; Selman, W. R.; LaManna, J. C.; Miller, R. H. Improvement of neurological recovery and stimulation of neural progenitor cell proliferation by intrathecal administration of Sonic hedgehog. *J. Neurosurg.* **2012**, *116*, 1114−1120.
(43) Im, Y. B.; Jee, M. K.; Choi, J. I.; Cho, H. T.; Kwon, O. H.; Kang, S. K. Molecular targeting of NOX4 for neuropathic pain after traumatic injury of the spinal cord. *Cell Death Dis.* **2012**, *3*, e426. doi: 10.1038/cddis.2012.168.
(44) Vanderah, T. W.; Laughlin, T.; Lashbrook, J. M.; Nichols, M. L.; Wilcox, G. L.; Ossipov, M. H.; Malan, T. P., 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.

(45) Ochs, G.; Penn, R. D.; York, M.; Giess, R.; Beck, M.; Tonn, J.; Haigh, J.; Malta, E.; Traub, M.; Sendtner, M.; Toyka, K. V. A phase I/II trial of recombinant methionyl human brain derived neurotrophic factor administered by intrathecal infusion to patients with amyotrophic lateral sclerosis. *Amyotrophic Lateral Scler. Other Mot. Neuron Disord.* **2000**, *1*, 201−206.
(46) Nauta, H. J. W.; Dolan, E.; Yasaargil, M. G. Microsurgical anatomy of spinal subarachnoid space. *Surg. Neurol.* **1983**, *19*, 431−437.
(47) Rieselbach, R.; DiChiro, G.; Freireich, E. J.; Rall, D. P. Subarachnoid distribution of drugs after lumbar injection. *New Engl. J. Med.* **1962**, *267*, 1273−1278.
(48) Lu, J.; Zhu, X. L. Cranial arachnoid membranes: some aspects of microsurgical anatomy. *Clin. Anat.* **2007**, *20*, 502−511.
(49) Belova, E.; Papisov, M.; et al. 2012, unpublished data.
(50) Barker, A. E. A report on clinical experiences with spinal analgesia in 100 cases, and some reflections on the procedure. *Br. Med. J.* **1907**, *1*, 665−674.
(51) Kirshner, M. Spinal zone anesthesia. Placed at will and dosage individually graded. *Surg. Gynecol. Obstet.* **1932**, *55*, 317−329.
(52) Brown, D. T.; Wildsmith, J. A.; Covino, B. G.; Scott, D. B. Effect of baricity on spinal anaesthesia with amethocaine. *Br. J. Anaesth.* **1980**, *52*, 589−596.
(53) Stienstra, R.; Gielen, M.; Kroon, J. W.; 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.
(54) Davson, H.; Segal, M. B. *Pysiology of the CSF and Blood-brain barriers*; CRC: Boca Raton, 1995.
(55) Zheng, W., Chodobski, A., Eds. *The blood-cerebrospinal fluid barrier*; Chapman & Hall/CRC: Boca Raton, 2005.
(56) Oldendorf, W. H.; 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.
(57) Kimelberg, H. K. Water homeostasis in the brain: basic concepts. *Nat. Neurosci.* **2004**, *129*, 851−860.
(58) O'Connell, J. E. A. 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.
(60) Dandy, W. E.; Blackfan, K. D. Internal hydrocephalus. An experimental, clinical and pathological study. *Am. J. Dis. Child.* **1914**, *8*, 406−482.
(61) Ingraham, F. D.; Matson, D. D.; Alexander, E.; Woods, P. P. Studies in the treatment of experimental hydrocephalus. *J. Neuropathol. Exp. Neurol.* **1948**, *7*, 123−143.
(62) Sachs, E.; Wilkins, H.; Sams, C. F. Studies on cerebrospinal circulation by a new method. *Arch. Neurol. Psychiatry* **1930**, *23*, 130−151.
(63) Di Chiro, G.; Hammock, M. K.; Bleyer, W. A. Spinal descent of cerebrospinal fluid in man. *Neurology* **1976**, *26*, 1−8.
(64) Reid, A., Marchbanks, R. J., Ernst, A. *Intracranial and Inner Ear Physiology and Pathophysiology*; Whurr Publishers: London, 1998; p 9.
(65) Enzmann, D. R.; Pelc, N. J. Normal flow patterns of intracranial and spinal cerebrospinal fluid defined with phase-contrast cine MR imaging. *Radiology* **1991**, *178*, 467−474.
(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.
(67) Henry-Feugeas, M. C. Temporal and spatial assessment of normal cerebrospinal fluid dynamics with MR imaging. *Magn. Reson. Imaging* **1993**, *11*, 1107−1118.
(68) Zhu, D. C.; Xenos, M.; Linninger, A. A.; Penn, R. D. Dynamics of lateral ventricle and cerebrospinal fluid in normal and hydrocephalic brains. *J. Magn. Reson. Imaging* **2006**, *24*, 756−770.
(69) Bhadelia, R. A.; Bogdan, A. R.; Kaplan, R. F.; Wolpert, S. M. 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.
(70) Linge, S. O.; Haughton, V.; Løvgren, A. E.; Mardal, K. A.; Langtangen, H. P. 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.
(71) Sweetman, B.; Linninger, A. A. Cerebrospinal fluid flow dynamics in the central nervous system. *Ann. Biomed. Eng.* **2011**, *39*, 484−496.
(72) Loth, F.; Yardimci, A. M.; Alperin, N. Hydrodynamic modeling of cerebrospinal fluid motion within the spinal cavity. *J. Biomech. Eng.* **2001**, *123*, 71−79.
(73) Lininger, A. A.; Xenos, M.; Sweetman, B.; Ponkshe, S.; Guo, X.; Penn, R. A mathematical model of blood, cerebrospinal fluid and brain dynamics. *J. Math. Biol.* **2009**, *59*, 729−759.
(74) Du Boulay, G. H.; O'Connell, J.; Currie, J.; Bostick, T.; Verity, P. Further investigation of pulsatile movements in the cerebrospinal fluid pathways. *Acta Radiol.: Diagn.* **1972**, *13*, 496−523.
(75) Hettiarachchi, H. D.; Hsu, Y.; Harris, T. J., Jr; Penn, R.; Linninger, A. A. 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.
(76) Davson, H., Segal, M. B. *Physiology of the CSF and Blood-Brain Barriers*; CRC: Boca Raton, 1995; p 201.
(77) Kapoor, K. G.; Katz, S. E.; Grzybowski, D. M.; Lubow, M. Cerebrospinal fluid outflow: an evolving perspective. *Brain Res. Bull.* **2008**, *77*, 327−334.
(78) Levine, J. E.; Povlishock, J. T.; Becker, D. P. The Morphological Correlates of Primate Cerebrospinal Fluid Absorption. *Brain Res.* **1982**, *241*, 31−41.
(79) Weed, L. H. The absorption of cerebrospinal fluid into the venous system. *Am. J. Anat.* **1923**, *31*, 191−221.
(80) Maurizi, C. P. Arachnoid granules: Dandy was Dandy, Cushing and Weed were not. *Med. Hypotheses* **2010**, *75*, 238−240.
(81) Welch, K.; Friedman, V. The cerebrospinal fluid valves. *Brain* **1960**, *83*, 454−469.
(82) Welch, K.; Pollay, M. The spinal arachnoid villi of the monkeys Cercopithicus aethiopis and Macaca irus. *Anat. Rec.* **1963**, *145*, 43−48.
(83) McComb, J. G.; Davson, H.; Hollingsworth, J. R. Attempted separation of blood-brain and blood-cerebrospinal fluid barriers in the rabbit. *Exp. Eye Res.* **1977**, *25*, 333−343.
(84) Butler, A.; Mann, J.; Bass, N. Identification of the major site for cerebrospinal fluid efflux in the albino rat. *Anat. Rec.* **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.
(86) Butler, A. B.; Mann, J. D.; Bass, N. H. Vesicular transport of peroxidase across the endothelium of the rat arachnoid villus. *Anat. Rec.* **1977**, *187*, 543.
(87) Yamada, S.; DePasquale, M.; Patlak, C. S.; Cserr, H. F. Albumin outflow into deep cervical lymph from different regions of rabbit brain. *Am. J. Physiol.* **1991**, *261* (4 Part 2), H1197−H204.
(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.
(89) Michel, C. C. Transport of macromolecules through microvascular walls. *Cardiovasc. Res.* **1996**, *32*, 644−653.
(90) Cserr, H. F.; Cooper, D. N.; Milhorat, T. H. 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.
(91) Cserr, H. F.; Ostrach, L. H. Bulk flow of interstitial fluid after intracranial injection of blue dextran 2000. *Exp. Neurol.* **1974**, *45*, 50−60.

(92) Virchow, R. Ueber die Erweiterung kleinerer Gefaesse. *Arch. 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, M. B. *Physiology of the CSF and Blood-brain barriers*; CRC: Boca Raton, 1995; p 507.
(95) Woollam, D. H. M.; Millen, J. W. The perivascular spaces of the mammalian central nervous system. *Biol. Rev.* **1954**, *29*, 251−283.
(96) Millen, J. W.; Woollam, D. H. On the nature of the pia mater. *Brain* **1961**, *84*, 514−520.
(97) Hutchings, M.; Weller, R. O. Anatomical relationships of the pia mater to cerebral blood vessels in man. *J. Neurosurg.* **1986**, *65*, 316−325.
(98) Zhang, E. T.; Inman, C. B.; Weller, R. O. Interrelationship of the pia mater and the perivascular (Virchow-Robin) spaces in the human cerebrum. *J. Anat.* **1990**, *170*, 111−123.
(99) Pollock, H.; Hutchings, M.; Weller, R. O.; Zhang, E. T. Perivascular spaces in the basal ganglia of the human brain: their relationship to lacunes. *J. Anat.* **1997**, *191*, 337−346.
(100) Schley, D.; Carare-Nnadi, R.; Please, C. P.; Perry, V. H.; Weller, R. O. Mechanisms to explain the reverse perivascular transport of solutes out of the brain. *J. Theor. Biol.* **2006**, *238*, 962−974.
(101) Ichimura, T.; Fraser, P. A.; Cserr, H. F. Distribution of extracellular tracers in perivascular spaces of the rat brain. *Brain Res.* **1991**, *545*, 103−113.
(102) Rennels, M. L.; Gregory, T. F.; Blaumanis, O. R.; Fujimoto, K.; Grady, P. A. 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.
(103) Wagner, H.-J.; Pilgrim, C.; Brandl, J. Penetration and removal of horseradish peroxidase injected into cerebrospinal fluid: Role of cerebral perivascular spaces, endothelium and microglia. *Acta Neuropathol.* **1974**, *27*, 299−315.
(104) Crawley, A. C.; Marshall, N.; Beard, H.; Hassiotis, S.; Walsh, V.; King, B.; Hucker, N.; Fuller, M.; Jolly, R. D.; Hopwood, J. J.; Hemsley, K. M. Enzyme replacement reduces neuropathology in MPS IIIA dogs. *Neurobiol. Dis.* **2011**, *43*, 422−434.
(105) Jolly, R. D.; Marshall, N. R.; Perrott, M. R.; Dittmer, K. E.; Hemsley, K. M.; Beard, H. Intracisternal enzyme replacement therapy in lysosomal storage diseases: routes of absorption into brain. Neuropathol. *Appl. Neurobiol.* **2011**, *37*, 414−422.
(106) Hager, H. Elektronenmikroskopische Untersuchungen über die Feinstruktur der Blutgefässe und perivascüären Räume im Säugetiergehirn. *Acta Neuropathol.* **1961**, *1*, 9−33.
(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 Anat. Jpn.* **2010**, *87*, 109−121.
(108) Iliff, J. J.; Wang, M.; Liao, Y.; Plogg, B. A.; Peng, W.; Gundersen, G. A.; Benveniste, H.; Vates, G. E.; Deane, R.; Goldman, S. A.; Nagelhus, E. A.; 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, W. B. The cell biology of neurogenesis. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 777−788.
(110) Carlén, M.; Meletis, K.; Göritz, C.; Darsalia, V.; Evergren, E.; Tanigaki, K.; Amendola, M.; Barnabé-Heider, F.; Yeung, M. S.; Naldini, L.; Honjo, T.; Kokaia, Z.; Shupliakov, O.; Cassidy, R. M.; Lindvall, O.; Frisén, J. Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. *Nat. Neurosci.* **2009**, *12*, 259−267.
(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. Controlled Release* **2012**, *163*, 25−33.
(112) Cserr, H. F.; Cooper, D. N.; Suri, P. K.; Patlak, C. S. Efflux of radiolabeled polyethylene glycols and albumin from rat brain. *Am. J. Physiol.* **1981**, *240*, F319−F328.
(113) Bobo, R. H.; Laske, D. W.; Akbasak, A.; Morrison, P. F.; Dedrick, R. L.; Oldfield, E. H. Convection-enhanced delivery of macromolecules in the brain. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2076−2080.
(114) Bidros, D. S.; Vogelbaum, M. A. Novel drug delivery strategies in neuro-oncology. *Neurotherapeutics* **2009**, *6*, 539−546.
(115) Iyer, R. R.; Butman, J. A.; Walbridge, S.; Gai, N. D.; Heiss, J. D.; Lonser, R. R. Tracking accuracy of T2- and diffusion-weighted magnetic resonance imaging for infusate distribution by convection-enhanced delivery. *J. Neurosurg.* **2011**, *115*, 474−480.
(116) Geer, C. P.; Grossman, S. A. 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, F. E.; Fiandaca, M. S.; Bringas, J.; Gimenez, F.; Berger, M. S.; Forsayeth, J.; Bankiewicz, K. S. 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.
(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.
(119) Groothuis, D. R.; Vavra, M. W.; Schlageter, K. E.; Kang, E. W.; Itskovich, A. C.; Hertzler, S.; Allen, C. V.; Lipton, H. L. 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.
(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.
(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.
(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.
(123) Yaksh, T. L.; Horais, K.; Tozier, N.; Allen, J.; Rathbun, M. L.; Rossi, S.; Sommer, C.; Meschter, C.; Richter, P. J.; Hildebrand, K. R. Chronically Infused Intrathecal Morphine in Dogs. *Anesthesiology* **2003**, *99*, 174−187.
(124) O'Rahilly, R.; Muller, F. The Meninges in Human Development. *J. Neuropathol. Exp. Neurol.* **1986**, *45*, 588−608.
(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.
(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.
(127) Feurer, D. J.; Weller, R. O. Barrier functions of the leptomeninges: a study of normal meninges and meningiomas in tissue culture. *Neuropathol. 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.
(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.

(130) Esiri, M. M.; Gay, D. Immunological and neuropathological significance of the Virchow-Robin space. *J. Neurol. Sci.* **1990**, *100*, 3−8.
(131) Inglese, M.; Bomsztyk, E.; Gonen, O.; Mannon, L. J.; Grossman, R. I.; Rusinek, H. Dilated Perivascular Spaces: Hallmarks of Mild Traumatic Brain Injury. *Am. J. Neuroradiol.* **2005**, *26*, 719−724.
(132) Inglese, M.; Grossman, R. I.; Diller, L.; Babb, J. S.; Gonen, O.; Silver, J. M.; Rusinek, H. Clinical significance of dilated Virchow-Robin spaces in mild traumatic brain injury. *Brain Inj.* **2006**, *20*, 15−21.
(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. *Neuroradiology* **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.
(135) Miszkiel, K. A.; Hall-Craggs, M. A.; Miller, R. F.; Kendall, B. E.; Wilkinson, I. D.; Paley, M. N.; Harrison, MJ.. The spectrum of MRI findings in CNS cryptococcosis in AIDS. *Clin. Radiol.* **1996**, *51*, 842−850.
(136) Maclullich, A. M. J.; Wardlaw, J. M.; Ferguson, K. J.; Starr, J. M.; Seckl, J. R.; Deary, I. J. Enlarged perivascular spaces are associated with cognitive function in healthy elderly men. *J. Neurol. Neurosurg. Psychiatry* **2004**, *75*, 1519−1523.
(137) Heier, L. A.; Bauer, C. J.; Schwartz, L.; Zimmerman, R. D.; Morgello, S.; Deck, M. D. Large Virchow-Robin spaces: MR-clinical correlation. *Am. J. Neuroradiol.* **1989**, *10*, 929−936.
(138) Mason, W. P.; Yeh, S.; DeAngelis, L. M. 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.
(139) Hassin, G. B. Histopathology of carcinoma of the cerebral meninges. *Arch. Neurol. Psychiatry* **1919**, *1*, 705−716.
(140) Mammoser, A. G.; Groves, M. D. Biology and therapy of neoplastic meningitis. *Curr. Oncol. Rep.* **2010**, *12*, 41−49.
(141) Mirfakhraee, M.; Crofford, M. J.; Guinto, F. C., Jr.; Nauta, H. J.; Weedn, V. W. Virchow-Robin space: a path of spread in neurosarcoidosis. *Radiology* **1986**, *158*, 715−720.
(142) Kooistra, K. L.; Rodriguez, M.; Powis, G.; Yaksh, T. L.; Harty, G. J.; Hilton, J. F.; Laws, E. R., 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*, 317−323.
(143) Herman, C.; Kupsky, W. J.; Rogers, L.; Duman, R.; Moore, P. Leptomeningeal Dissemination of Malignant Glioma Simulating Cerebral Vasculitis. *Stroke* **1995**, *26*, 2366−2370.
(144) Deckert-Schluter, M.; Schluter, D.; Hof, H.; Wiestler, O. D.; 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 ultra-structural immunohistochemical study. *J. Neuropathol. Exp. Neurol.* **1994**, *53*, 457−468.
(145) Maurer, M. H. Proteomics of brain extracellular fluid (ECF) and cerebrospinal fluid (CSF). *Mass Spectrom. Rev.* **2010**, *29*, 17−28.

Pharmacokinetics of macromolecules in spinal CSF: PET and modeling

E. Belova,[1-3] V. Belov,[1-3] M. Gagne,[1] C. Gillooly[1], A.J. Fischman[3] and M. Papisov[1-3]

[1]Massachusetts General Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA and [3]Shriners Hospitals for Children–Boston, MA

Objectives. Delivery of therapeutics through the leptomeningeal space is promising for the treatment of diseases involving CNS and meninges, especially given the availability of implantable ports and pumps designed for drug administration to CSF. However, intrathecal pharmacokinetics of drugs is complex and still poorly studied, which hinders development of the method. The objective of this study was to characterize drug transfer in the liquid phase of the spinal leptomeningeal space.

Methods. Rats (250-275 g) with pre-implanted lumbar catheters were used as model animals. Model protein was labeled with ^{124}I (0.01-0.04 mCi/animal) and administered into CSF in a very small volume (3-9 µl) followed by flush (6-12 µl) of saline. Imaging was carried out using a custom PET/CT system (microPET Focus 220, Siemens, and CereTom, Neurologica). PET imaging was started immediately after injection. The first 30 min of acquired data was reconstructed into a dynamic imaging file, 2 min per frame. CT scans were used for attenuation correction and anatomical reference.

Results. The protein was initially located in a short (1 cm) segment of the spine. The rate of the protein front translocation along the spine from that segment was ca. 2 mm/min and reached the cranial CSF compartment 1.5-2.5 hours after the injection. PET data were segregated into small (vertebra size) compartments and diffusion distribution model was applied to calculate the apparent "pseudo-diffusion" coefficients.

Conclusions. Protein distribution in the spinal leptomeningeal space is faster than diffusion by 1.5-2.5 orders of magnitude and is in agreement with pulsation assisted remixing of the liquid compartment.

Qualitative assessment of CSF drainage to the lymphatic system using Positron Emission Tomography in rats and nonhuman primates

Caitlin Gillooly,[2] Vasily Belov,[1] Elena Belova,[1] Matthew Gagne,[2] James Titus,[2] and Mikhail Papisov[1]

1. Massachusetts General Hospital, Harvard Medical School and Shriners Hospitals for Children, Boston, MA, United States. 2. Massachusetts General Hospital, Boston, MA

Objectives

In order to develop effective treatments for diseases affecting the brain, spinal cord and meninges, it is necessary to understand the biokinetics of drugs after intra-CSF administration. Current literature suggests two pathways by which these drug molecules exit the leptomeningeal space to enter systemic circulation: (1)through micron-range pores in arachnoid granulations that drain directly to the blood and (2)uptake by the lymphatic system. The extent and mechanism of lymphatic drainage is still widely debated and requires further investigation. Therefore, the goal of the present study was to provide a qualitative and quantitative description of this lymphatic uptake using a PET-based approach.

Methods

We administered various radiolabeled proteins and nanoparticles into the CSF of rats and nonhuman primates using surgically installed catheters placed in the lumbar spinal region. Several nonhuman primates also had intracerebral-ventricular ports. Static PET images were acquired over 24 hours, with those within the first four hours post-administration considered most representative. Using these images, we assessed the extent of lymphatic drainage from the CSF using protein and particle uptake in the lymph nodes as a drainage marker.

Results

Our data indicates only minor uptake in the lymphatics, mainly in cervical lymph nodes, though differences appear across species. In rats, the total lymphatic uptake was much higher (4.3±2.0%ID, n=9) than in primates (0.27±0.20%ID, n=9), four hours after administration.

Conclusions

Our findings suggest that CSF drainage directly to the blood is the main pathway by which the vast majority of particles exit the CSF into systemic circulation as the total amount accumulated in the lymph nodes of both species was much less than some previously reported data would suggest. The lymphatic route is therefore insignificant physiologically and pharmacologically, at least in higher mammals, though it may facilitate the immune response to CSF-borne antigens.

Biokinetics of FDG after intrathecal administration

Bonab Ali A,[1,2] Fischman Alan J[2], Belov Vasily V[1,2], and Papisov Mikhail I[1,2]

[1]Massachusetts General Hospital, [2]Shriners Hospitals for Children, Boston, MA

Objectives. Intrathecal administration of therapeutics is promising for the treatment of diseases involving CNS and meninges. The potential of the method has not been fully realized, largely because there is still no clarity with respect to the mechanistic factors governing the biokinetics in the cerebrospinal fluid (CSF). One of the challenges relates to the difficulties in measuring the fraction of the administered drug that can be delivered into the brain from CSF through the periarterial spaces. The goal of the present study was to investigate the entrance and retention of 18FDG, an agent with excellent intracellular retention after the uptake, into the brain from the cerebrospinal fluid.

Methods. Rhesus monkeys were anesthetized with isofluorane/N2O2 and positioned prone on the imaging bed of a custom Siemens focus 220 PET/CereTom CT system. Approximately 2.0 mCi of 18FDG was administered by direct injection into cisterna magna. Whole body images were acquired at 5 min per position at several time points. Images were reconstructed using the OSEM 3D/MAP algorithm and ROIs selected manually. The images and numerical data were compared with those for IV administration of 18FDG.

Results. The data demonstrated 18FDG accumulation in the CNS, concurrently with exit from the CSF and accumulation in all organs and tissues, the latter as after IV administration. The patterns of FDG uptake after the IT and IV administration in CNS were similar, suggesting that a significant part of the CNS uptake was from the systemic circulation rather than from CSF. The kinetics of 18FDG exit to the system was in agreement with the rate of CSF replacement. The data were further processed to determine the fraction entering CNS from the CSF.

Conclusions. 18FDG administration IT provides helpful data for understanding of the entrance of intrathecally administered drugs to CNS, and for the estimation of the maximal fraction of the administered drug that can be delivered to the brain by this route.

Monitoring of drug concentration in the cerebrospinal fluid by PET

V. Belov,[1-3] M. Gagne[1-3], J. Titus[1,2], C. Gillooly[1-3], E. Belova[1-3], A.J. Fischman[3] and M. Papisov[1-3]

[1]Massachusetts General Hospital, [2]Harvard Medical School, Boston, MA and [3]Shriners Hospitals for Children, Boston, MA

Objectives. Administration of therapeutics to the cerebrospinal fluid (CSF) is a promising method for the therapy of diseases involving CNS and meninges. Investigation of drug pharmacokinetics in CSF is difficult and generally requires quantitative real-time imaging, such as PET. However, in the PET image, drug associated with arachnoid structures cannot be resolved from the liquid phase fraction. Sampling of CSF can supplement the PET data, but removal of CSF can distort the kinetics. We report an alternative method based on in-catheter "sampling" of CSF by PET.

Methods. *Macaca Fascicularis* with implanted subcutaneous lumbar ports equipped with catheters leading to CSF were imaged with the MicroPET Focus 220 and CereTom NL 3000. The model drugs were labeled with I-124 were administered through the ports and the latter were flushed. To obtain liquid phase activity values, a small volume of CSF (<0.1 ml) was pooled back into the catheter. Drug concentration in the catheter segment filled with CSF was determined from the total radioactivity as measured by PET and the known volume of the segment. CSF was then flushed back into the leptomeningeal space (LMS). Accuracy validation was carried out in vitro, using analogous injection port.

Results. In vitro, the accuracy of the obtained values was shown to deviate from the true value by not more than ±10%. In contrast, values obtained by CSF sampling were not accurate unless an unacceptably large volume of CSF was taken (>1 ml). Animal data demonstrated the utility of the proposed approach for analysis of drug distribution between the liquid and solid phases in the LMS at multiple time points.

Conclusions. PET imaging enables non-invasive real-time selective quantification of the radiolabeled drug concentration in the liquid phase of the LMS.