Progress or Final Report (delete one)

Author: Dr Shinuo Liu
Qualification: MBBS, BSci(Med) Hons
Institution: Macquarie University
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Title of Project:
Fluid physiology in the spinal cord and subarachnoid space with relevance to syringomyelia pathogenesis

Summary: (approximately 1,000 words)

Project One: Fluid outflow in the rat spinal cord: the role of perivascular and paravascular pathways

Background

Cerebrospinal fluid (CSF) is thought to flow into the brain via perivascular spaces around arteries, where it mixes with interstitial fluid. The precise details concerning fluid outflow remain controversial. Although fluid dynamics have been studied in the brain, little is known about spinal cord fluid inflow and outflow. Understanding the normal fluid physiology of the spinal cord may give insight into the pathogenesis of spinal cord oedema and CSF disorders such as syringomyelia. We therefore aimed to determine the fluid outflow pathways in the rat spinal cord.

Methods

A fluorescent tracer, Alexa-Fluor®-647 Ovalbumin, was injected into the extracellular space of either the cervicothoracic lateral white matter or the grey matter in twenty-two Sprague Dawley rats over 250 s. The rats were sacrificed at 20 or 60 min post injection. Spinal cord segments were sectioned and labelled with vascular antibodies for immunohistochemistry.

Results

Fluorescent tracer was distributed over two to three spinal levels adjacent to the injection site. In grey matter injections, tracer spread radially into the white matter. In white matter injections, tracer was confined to and redistributed along the longitudinal axonal fibres. Tracer was conducted towards the pial and ependymal surfaces along vascular structures. There was accumulation of tracer around the adventitia of the intramedullary arteries, veins and capillaries, as well as the extramedullary vessels. A distinct layer of tracer was deposited in the internal basement membrane of the tunica media of arteries. In half the grey matter injections, tracer was detected in the central canal.

Conclusions

These results suggest that in the spinal cord interstitial fluid movement is modulated by tissue diffusivity of grey and white matter. The central canal, and the compartments around or within blood vessels appear to be dominant pathways for fluid drainage in these experiments. There may be regional variations in fluid outflow capacity due to vascular and other anatomical differences between the grey and white matter.

Project One has been published in a peer-reviewed journal.

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Project Two: Intrathoracic pressure and arterial pulsations exert different driving forces on spinal cerebrospinal and interstitial fluid flow

Background

There is mounting evidence that disruption of CSF circulation and CSF/interstitial fluid exchange is likely to contribute to a number of CNS diseases including syringomyelia. However, the physiological factors that govern CSF flow in the SAS and fluid transport in the spinal cord are poorly understood. The aims of this study were to determine the effects of heart rate, blood pressure and respiration on the flow of fluid in the SAS, as well as into and out of the spinal cord interstitium.

Methods

In Sprague Dawley rats, physiological parameters were manipulated such that the effects of free breathing (generating alternating positive and negative intrathoracic pressures), mechanical ventilation (positive intrathoracic pressure only), tachy/bradycardia, as well as hyper/hypotension were separately studied. To investigate spinal CSF hydrodynamics, *in vivo* near infrared imaging of intracisternally infused indocyanine green was performed. Spinal fluid inflow at a microscopic level was quantitatively characterised by *ex vivo* epifluorescence imaging of fluorescent ovalbumin, AFO-647, injected into the SAS. Fluid and solute transport at the level of the perivascular space was further characterised with *in vivo* two-photon intravital imaging of intracisternally delivered fluorescent ovalbumin and microspheres. To assess fluid outflow, AFO-647 was injected into the cervicothoracic spinal grey or white matter for epifluorescence analysis.

Results

Compared to controls, free breathing animals had significantly higher flow of CSF in the SAS. There was also greater inflow of AFO-647 into the spinal cord interstitium. This correlated with higher microsphere tracer velocity and displacement. Hypertension and tachycardia had no significant effect on SAS CSF flow. In hypertensive animals, there was reduced AFO-647 influx compared to some of the control cohorts, although higher microsphere velocities and displacement were noted. Tachycardia did not result in greater AFO-647 inflow compared to some of the control animals. There was no difference in microsphere velocity or displacement compared to control groups. Tachycardia and hypertension stimulated AFO-647 tracer efflux, while respiration did not affect spinal interstitial clearance.

Conclusions

Intrathoracic pressure has a significant effect on spinal CSF flow and cord parenchymal fluid influx. Arterial pulsations play a smaller role in SAS hydrodynamics but have profound effects on spinal cord interstitial fluid homeostasis, particularly in outflow.

Project One has been prepared for publication in a peer-reviewed journal.

Shinuo Liu, Neftali Flores Rodriguez, Courtney Wright, Marcus A. Stoodley, Lynne E. Bilston, Rob Lloyd, Simon McMullan, Sarah J. Hemley
Intrathoracic pressure and arterial pulsations exert different driving forces on spinal cerebrospinal and interstitial fluid flow

Note that Projects One and Two have been submitted as part of a thesis to fulfil the requirements of Doctor of Philosophy at the Faculty of Medicine and Health Sciences, Macquarie University.

Hypothesis vs Findings

There is an urgent need to elucidate the pathophysiology of syringomyelia and other fluid disorders in the CNS. Efforts to achieve this end have been hindered by an incomplete understanding of the fundamental details surrounding fluid physiology in and around the spinal cord. In an attempt to fill these gaps, the experimental work described here tested the following hypotheses:

1. Fluid outflow to the SAS is predominantly via a perivenular route in the grey and white matter of the spinal cord.

2. Increases in arterial pulse pressure and heart rate will increase perivascular flow into and out of the spinal cord.

3. Negative intrathoracic pressure will increase perivascular flow into and out of the spinal cord.

The aims are to:

1. Determine the fluid outflow pathways in the grey and white matter of the normal rat spinal cord.

2. Determine the effects of heart rate, blood pressure and respiration on fluid flow in and around the rat spinal cord.

Findings (summarized):
Free breathing animals (in which cycles of negative and positive intrathoracic pressure are generated) had significantly greater flow of CSF in the SAS as well as inflow of tracer into the spinal cord compared with mechanically ventilated control rats (positive intrathoracic pressure only). Hypertension and tachycardia had no significant effect on CSF flow in the SAS. Hypertension produced conflicting results but likely had a modest effect on inflow. Increased tracer influx was not observed with tachycardia. Both tachycardia and hypertension stimulated tracer efflux, but respiration was not found to affect spinal interstitial clearance. Spinal intramedullary movement of tracer was slow, and its redistribution was limited by isotropic and anisotropic properties of white and grey matter. Perivascular spaces of all vessel types provided preferential pathways for both tracer influx and efflux to pial and ependymal surfaces. Tracer deposited within the internal basement membrane of the tunica media of arteries and arterioles.

Unanswered Questions

One of the most important unanswered questions has been raised by our findings of tracer within the “vascular basement membrane” of blood vessels, and the fact that arterial pulsations appear to

drive ISF/CSF exchange. The synthesis of these observations imply that fluid and solute transport occurs in a fluid filled, compressible perivascular space. However, the precise anatomical configuration of the perivascular space is still a point of intense contention, and its resolution will likely lie in future in vivo investigations of the ultrastructure of the perivascular space, ideally free of the biases of tracer injections. No such study currently exists. Furthermore, a consensus should be established on the precise definition and nomenclature surrounding the perivascular space. Whether the perivascular space extends beyond the pia into the SAS, for example, is controversial.

In this thesis, the cervicothoracic spinal cord down to T4 was the focus of the studies. It is conceivable that SAS fluid dynamics are markedly different in the rat thoracolumbar spine. Modelling studies in human have demonstrated that CSF flow velocities differ greatly between the upper and lower cervical levels. There is also some evidence from MRI studies suggesting SAS flow differences exist between upper thoracic and lower thoracolumbar spine. Thus, future experiments, especially in silico ones, need to incorporate a more holistic view of the vertebral column. Outflow has been investigated exclusively near the cervicothoracic junction, but future investigations also need to consider other regions of the spinal cord.

Recently, brain dural lymphatics and their role in fluid outflow have garnered significant attention. There is likely a role for spinal dural lymphatics in CSF/ISF clearance in at least some mammalian species. It is possible that respiration and cardiovascular pulsations have profound effects on lymphatic CNS fluid drainage. Immunofluorescent labelling of lymphatic vessels and in vivo characterisation of lymphatic outflow have already been well described in the literature. Future studies could appropriate these techniques to the vertebral column, the superior sagittal sinus and the peripheral circulation. The role of the spinal nerve roots in facilitating fluid outflow has also not been investigated. These structures are richly vascularised and traverse the interface between dura, lymphatic vessels and CSF. They may even provide insight into the connection between extradural lymphatics and ISF (if ones exist).