Measurement of the Brain Extracellular Space Using Tracer-Based MRI

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Neurons have been considered the most important functional unit of the brain and have attracted the most attention in neurobiology. Neural cells occupy 70%-80% of the whole brain volume, but until now, our current knowledge of neural circuit cannot explain all the neurological conditions of the brain. Thus, it is also important to consider the influence of the extracellular space (ECS) around neural cells, which contains the brain interstitial fluid (ISF). This paper includes the anatomy of the brain ECS under physiological condition, the in vivo measurement techniques of brain ECS, and the divisions of brain ECS. Moreover, the implications of ECS knowledge of basic neuroscience and clinical applications are introduced, including the brain tissue engineering and the local drug delivery via ECS.

Key words: brain microenvironment, brain interstitial space, in vivo measurement

Developing new techniques and recording activities of individual neurons and neural circuits at high spatial and temporal resolutions are the focus of brain science research for the past century. In a long period of time, neural cells, as the most important function unit of the brain, occupy 70%-80% of the whole brain volume. While vascular system and the interstitial system (ISS) occupy the rest. The brain ISS exists between neural cells and blood vessels as a regular and tortuous narrow gap, composed of interstitial fluid (ISF) and extracellular matrix (ECM). In the brain ISS, space between adjacent neural cells is called extracellular space (ECS). Recent studies have shown that the brain ECS plays more active roles in brain functions, including, interactive information between neural cells, the processing and integrating of information, and coordinating the respond of the brain to the external and internal environment changes.

In brain ISS, the ECM is produced and secreted by cells, and it surrounds cell membrane and attached to it. Brain ISF is a water solvent that soaks and surrounds the neural cells, containing ions, gaseous and organic molecules. There is extensive interaction between the cerebrospinal fluid (CSF) and ISF. CSF has a role as the “reservoir” of ISF and clears the most garbage produced by ISF. There are three drainage ways at present. Two of them involve a direct exchange between ISF and CSF. One exists in the wall of ventricle through ependymal cells, the other exists at the surface of the brain and spinal cord through pia mater–glial membranes. The last way is the blood vessel wall. ISF can flow within the basement membrane in contrast to the flow directions, and...
finally reach extracranial lymph nodes\textsuperscript{18, 20}. Over the past few decades, a large number of science and technology have been developed in brain research, and among these techniques, three special techniques are developed to measure or image brain ECS, including the ion-selected micro-electrode (ISM)\textsuperscript{21}, integrative optical imaging (IOI)\textsuperscript{22}, and tracer-based MRI techniques\textsuperscript{23, 26}. The ISM uses ion selective microelectrode technology to detect brain potential change of the ECS. IOI technology measures light signals generated by a fluorescent probe in ECS. Tracer-based MRI method uses magnetic sensitive contrast agents as a probe to tag and track water molecules in ISF\textsuperscript{24}. Microelectrode (ISM) technology, commonly known as voltammetry, is a branch of electrochemistry technology. ISM is responsible for recording the geometry of brain ECS which is quantitatively measured with exogenous electrical ion. The measured potential can be converted to probe concentration for further calculation of ECS parameter. According to the ECS probe-driven approach, ISM can be divided into real time iontophoresis (RTI) and real-time pressure ejector method (RTP). The selective charged ISM probes include tetramethylammonium (TMA), tetraethylammonium (TEA), Hexafluoroarsenate (AsF$_6$), and α-naphthalenesulfonate\textsuperscript{27, 28}. RTI-TMA $+$ is commonly used to measure brain ECS, which takes TMA $+$ as a probe. Application of iontophoresis enables TMA $+$ imported into the brain tissue through a special microcatheter\textsuperscript{29}. Micro catheter is a monocular capillary glass tube containing the TMA $+$, with a silver chloride wire attached to a constant current generator. It produces a positive charge and drives the TMA $+$ into the ECS\textsuperscript{30}. ISM is placed in 20–200 microns far, converts the local electrical signals to the TMA $+$ ions concentration\textsuperscript{31}. Taking the known distance and measured TMA $+$ ion concentration – time relationship into the classical diffusion equation, diffusion parameters could be obtained\textsuperscript{30}.

The fluorescent molecules of Optical imaging are injected into the brain ECS, with a series of optical imaging device to detect them. Unlike the micro-electrode probe, with biological activity, fluorescent probe can bind to the receptor and cause the deterioration of some measured ECS parameter accuracy. Because of its high water solubility and biocompatibility, Dextran is the most commonly used probes. With the external pulse pressure, the fluorescent probe is injected into the ECS in the cortex though a micro-catheter, and inspire the light and “bright field” which outline the brain tissue with probe in ECS. The fluorescent probe molecules are excited fluorescence light, the fluorescence through the objective lens and the dichroic mirror, and finally received by a charge coupled device (CCD) camera and forms an image\textsuperscript{29, 31, 32}. The Fluorescence signal is conveted into the fluorescent probe concentration, so dynamic record for distribution process of probe comes ture, and then calculate the ECS diffusion coefficient D and tortuosity $\lambda$. Using RTI-TMA $+$ technology to integrate an optical image and quantitatively measure ECS parameter\textsuperscript{33}. Because of the limitation of the optical microscope detection, integrated optical imaging (IOI) technology used to detect ISF flow or material transport in cerebral cortex, range from the surface of the brain is 200 microns\textsuperscript{34, 35}.

Currently, MRI is the only one technique that can detect and image the ECS within the scope of whole brain among all ECS measurement techniques, especially for the middle of the deep brain ECS\textsuperscript{24, 36}. Gd-DTPA is the most commonly used clinical positive contrast enhancer. Compared with other ion $+$ or fluorescent probes, Gd-DTPA has many advantages, such as biological inertness, thermal stability, small molecular weight, and extracellular distribution\textsuperscript{24, 37}. Gd-DTPA probe also could shorten the proton spin–lattice relaxation time of water molecules within the effective range of 2.5 angstroms to track endogenous water molecules, showing high signal on MR images\textsuperscript{23}. MRI tracer techniques would achieve a “one-stop” access to many ECS transport parameters, such as $V_d$\textsubscript{max}, $t_1/2$, D, $\lambda$\textsuperscript{24, 36, 38}. After injection in ECS, the tracer is slowly diluted. MRI signal intensity is gradually decreased, and finally present a series of MR signal intensity continuously attenuated image\textsuperscript{23}. Then, with self-developed software, signal strength will get converted into real-time tracer concentration values. The appropriate method to convert the equation with MRI data is applied to calculate the diffusion or flow parameters\textsuperscript{38, 39}.

Tracer–based MRI technique is used to study the rat deep brain tracer flow solution, and brain ECS was found to be a physical partition structure, with
the unique ISF distribution and removal rates for characteristics. This location-dependent property of the ISF drainage system not only increases the understanding of ECS, but also optimizes the local drug delivery \[40\] \[41\]. Until now, tracer-based MRI is the only technology which can provide 3D visualization of the dynamic drainage flow of brain ISF in the whole brain. In addition, this technology also enables measurement of multi-point diffusion parameters along any direction around the injection point. Moreover, tracer-based MRI techniques has a unique advantage in the analysis and prediction of dynamic distribution of water-soluble drug through potential independence and labeled endogenous water molecules \[38\].

Recently, a new division system has been confirmed by the study of tracking the brain ISF flow in the deep center of rat brain. The brain ISF flows from the caudate nucleus to ipsilateral cortex and then into subarachnoid space \[23\] \[42\]. The other studies found that ISF tracer in the caudate nucleus does not flow to the thalamus, although the thalamus located nearby the caudate nucleus, it flows in the opposite direction to the cerebral cortex. A boundary exists between the thalamus and the caudate nucleus. The tracer from the thalamus cannot cross the boundary into caudate nucleus \[24\] \[36\]; this is also validated by optical techniques \[43\]. This finding suggests that the ISF in brain ISS is not randomly distributed within the brain in the whole range and mobility, but limited in certain areas, exist divisions.

Several studies demonstrated the alteration of ECS in neuropathology in glioma, ischemic stroke and Alzheimer’s Disease. These alternations can be classified based on changes in (1) ECS geometry, including the changes of volume fraction or tortuosity; (2) ECM and its components \[41\] \[43\]; (3) the components and biochemical properties of the ISF; (4) substance transport and ISF flow in ISS \[40\]-\[50\]. Besides mentioned common brain disorders, the alternations of ECS have also been reported in some other diseases, such as Parkinson’s disease \[51\], multiple sclerosis \[52\] \[53\], and epilepsy \[54\]. With progress of ECS measurement techniques, the links between ECS properties and brain disorders will be further clarified at cellular or sub-cellular level.

The success rate of the clinical applications of new CNS drugs is only 7%, compared with 15% for other kind of drugs \[35\] \[36\]. One example of the dilemma in developing CNS drugs is Neuro-protective drugs, which took several decades and cost billions of dollars, and finally failed in clinical application \[37\] \[38\]. Although the vascular system takes only 3% to 5% of the brain volume, nearly all of the previous CNS drug investigation or clinical trials considered it as the exclusive route to the target \[29\] \[40\]. With the routine oral or intravenous administration, the drug is difficult to reach the ischemic or penumbra regions where no blood supply or low perfusion has lasted for a period of time. Additionally, blood–brain barrier (BBB) prevents most of penetrations. Finally, even if the drug can pass BBB, it needs to move from the capillary bed to the target area. Since ECS provides direct space for substance transport and exchange among neural cells, local drug delivery seems attractive for the treatment of brain disorders. Recently, the administration of low dose CDPC through brain ISS has been demonstrated to be more efficient than the systemic drug delivery \[40\].

Although the ISF flow and transport at whole brain scale were far from clear in the early 1990s, scientists had begun to investigate the possibility of administration of drugs via ECS to circumvent the BBB. ECS –based drug delivery, also known as convection enhanced delivery (CED) in the literature, is conducted with a continuous injection of the therapeutic agent under positive pressure via a catheter implanted into the brain \[61\] \[62\]. A retrospective clinical study reported that infusion parameters may improve the efficiency of drugs delivered by CED \[63\].

CED shows several advantages over conventional drug delivery methods, including bypass of BBB, lower systemic toxicity and better efficacy. Despite promising effect in the treatment of high-grade glioma, PD and AD, substantial refinement works are necessary before its successful clinical application, which mainly include the following points: (1) The back flow of injection in CED may degrade its performance; (2) The concentration and distribution of injected drug cannot be easily predicted and controlled; (3) Mechanical damage to brain tissue may cause significant side effects in practical applications of CED \[41\].

Simple diffusion delivery (SDD) has been a recently developed local brain drug delivery
method, in which pharmaceutical agents are driven to the target zone via brain ECS due to a concentration gradient. SDD is based on the above findings of the brain ISS divisions and the properties of substance transport in each division. Before or during SDD, the tracer-based MRI technique is used to trace the dynamic distribution of the water soluble probes Gd-DTPA, along with the transportation and flow of water molecules in the brain ISF. Due to the inertness, the biophysical parameters of Gd-DTPA provides net background or reference for the other water soluble agents, like CDPC. Therefore, the unique advantage of SDD over CED is that the dynamic distribution of drugs in any specific division can be simulated and the full immersion and contact with target cells makes the treatment more effective with only a very small dose of drug. Meanwhile, the delivery process without continuous pressures also avoids the weaknesses of CED method, e.g., unwanted flow back, uncontrollable distribution, and tissue damage.

CED and SDD are promising method for locally delivering the drugs to deep brain. However, a couple of challenging works have to be solved before their clinical applications. One work is to clarify the influence factors (e.g., injection pressure) on the distribution and spread rate of drugs in various brain divisions. The other is the manual method for regulating the distribution and transport of drugs in each brain division. We have successfully applied prophylactic treatment for ischemic stroke in rats by injecting CDPC based on SDD method. Very attractively, the effective dose via brain ECS is only around 0.1% comparing to that via the vascular system.

ECS is a complex system with a lot of unknown mechanisms and properties. We need to develop new techniques to study brain ECS, including biophysical modeling, novel brain imaging technique, and techniques for large-scale recording and signal modulation in the CNS which were not discussed in this review. ECS research results can be used to improve treatment of brain disorders through the development of novel drugs and systems for drug and gene delivery.

References

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