NVFU (NeuroVascular cerebrospinal Fluid Unit)

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Abstract

We have come to the time to reconsider the bulk theory that have been hitherto upheld in explaining the cerebrospinal fluid as the third circulatory system next to the blood and lymph. There are possibilities that the cerebral capillaries serve as the site of generation and absorption of tissue and cerebrospinal fluids and as the drainage pathway for metabolic and waste products. If we assume that these functions of cerebrospinal fluid are disturbed when individuals enter pathological states or become aged, there are possibilities that the behaviors of the cerebrospinal fluid involve the neurovascular unit (NVU). Thus it is necessary to examine the correlation of these possibilities with the results of past experiments and clinical findings. If we are to believe that astrocytes, a component constituting NVU, are related to the water channel such as aquaporine, it becomes necessary to re-examine the cerebral blood circulation, which has hitherto been defined by the accepted theories and knowledge, in line with the added concept of cerebrovascular circulation. In the past, the studies on metabolism related to cerebrovascular circulation tended to lean heavily on blood circulation. For the future studies of neuroprotection, complex and comprehensive approaches, such as the concept of the NVFU (NeuroVascular cerebrospinal Fluid Unit) with spacial and functional expansion encompassing not only cerebrovascular but also cerebrospinal fluid circulations may be necessary.

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Key words: NVU, cerebral ischemia, CSF, aquaporine, NVFU

1. Introduction

History of the study on cerebrospinal fluid (CSF) goes back to the early days of medicine and many once believed that it had already been exhausted. Therefore there was a tendency to disregard it in recent years. Believed as the third circulatory system following the blood and lymph, however, we have now come to re-evaluate the bulk flow theory. Although the brain lacks a lymphatic system, interstitial fluid is actively produced: for the drainage mechanism of this interstitial fluid (i.e.,

lymph), it is believed that the perivascular space around the cerebral arteries functionally corresponds to the lymphatic vessels and serves as a drainage pathway for waste products from the neurons. On the other hand, studies on the protection of the brain against situations such as apoplexy are incomplete if limited to neurons; rather, a neurovascular unit (NVU) has been proposed as a conceptual structural unit including the surrounding cells (together with those of the cerebral capillaries)¹⁾. As for the production, absorption and circulation of the cerebrospinal fluid, new concepts have lately been introduced: not only the blood circulation but also the cerebrospinal fluid circulation with the aid of the interstitial fluid and lymph as the solvents are considered necessary for the drainage pathway for metabolic wastes in various disease conditions such as cerebral ischemia.

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2. NVU (Neurovascular Unit)

When I was conducting experiments on cerebral protection by applying methods mild hypothermia in association with energy metabolic disturbances and slow neuronal death in animals such as gerbils with transient fore-brain ischemia²⁾, the main focus of the study was neurons and physiopathological mechanisms such as excitatory toxicity and oxidative stress and apoptotic pathways in the post-ischemic neurons were being elaborated. However, clinically effective therapeutic measures were yet to be introduced. Since then, the concept of the NVU—composed of neurons, astrocytes, vascular endothelial cells, microglia, pericytes, and oligodendrocytes—was proposed. Thus it was recognized that for normal functioning of the brain in cerebral diseases, protection of the neurons alone was not sufficient; rather, cross-talking among different cells is necessary. During the past 10 years, the focus has been on the elaboration of physiopathology of cerebral disorders and studies on their treatment. To clarify the status of the NVU, the focal point has been on the supply of nutrients to the cerebral capillaries, especially the supply of glucose as the substance responsible for energy metabolism. The topic still constitutes a focal point as the astrocyte-neuron lactate shuttle³⁾. Furthermore, in the pathological changes of the substantia alba also, dynamic interaction was noted between glia cells and neurons, indicating that the glutamate-glutamine shuttle manifests different functions in different disease conditions. The findings suggest structural integrity of the glial matrix⁴⁾.

Thus, many research results were introduced through interpretation of physiopathology applying a comprehensive approach represented by the NVU. However, very few therapeutic agents that could be used in clinical scenes resulted from these studies.

In search of therapeutic methodologies that are effective in stroke, it is necessary to keep in mind that the environment within the NVU may be diverse dependent on the situation. When injured, the astrocytes that are intended to support the function of the surrounding cells are transformed to reactive astrocytes, suppressing the vascular regeneration that are associated with the recovery stage⁵⁾, while they promote the recovery of the cerebral function that has been lost⁶⁾. On the other hand, it has been reported that precursor cells of oligodendro-

cytes become oligodendrocytes themselves in order to supplement those that have been lost through the disease process, thus contributing to the recovery of the cerebral function⁷⁾; on the other hand, the precursor cells of oligodendrocytes often exacerbate the surrounding area by releasing malignant factors to exacerbate the surrounding area in the acute stage following the disturbances⁸⁾. Furthermore, interactions among the cells themselves are often under the influence of nutritional factors or humoral factors (such as cytokines). However, the factors that trigger cerebral dysfunction following an acute stage may contribute to the restoration of the cerebral tissue-cerebral functions during the recovery stage⁹⁻¹¹⁾.

Thus proposals have been made to explain the physiopathology of stroke based on an all-inclusive concept of NVU, a single unit that functionally unites cellular entities such as neurons, astrocytes, vascular endothelial cells, microglia, pericytes and oligodendrocytes. At present, the concept has been applied to studies of various brain diseases beyond the framework of cerebral apoplexy.

3. Recent reports on CSF

On the other hand, new opinions have recently been introduced on the production, absorption, and circulation of cerebrospinal fluid. The bulk flow theory, which was proposed by Cushing¹²⁾ and Weed¹³⁾, has been an accepted concept that is still described in textbooks. However, the arachnoid granules along the superior sagittal sinus, the site believed to be the major place of cerebrospinal fluid absorption by these predecessors, are now considered to be the pathway for the high pressure system, but not the main pathway¹⁴⁾. Specifically, Weed et al., in their experiment, injected ink at pressures as high as 150 mmHg. This was considered to be physiologically unlikely. It has traditionally been believed that the cerebrospinal fluid is also absorbed into the lymphatic system of the spinal arachnoid granules, constituting a minor pathway for drainage. Currently, the release of the cerebrospinal fluid into the nasal lymphatic pathway is considered to constitute the main pathway for the low pressure system (the lymphatic drainage)¹⁵⁾. Furthermore, intracranial pressure did not rise and ventricular enlargement failed to develop in a model for mesencephalic aqueduct obstruction. Because of these findings, some hold that the cerebral capillaries also constitute a main

pathway for cerebrovascular absorption (perivascular drainage). In recent clinical studies, the intracranial pressure was continuously monitored during normal daily activities and it was found that the normal pressure was 0 mmHg. The findings suggested a possible mechanism in which the low pressure system operates in a normal condition and the arachnoid granules around the superior sagittal sinus are activated at a high pressure system at emergencies.

For the dynamics of the interstitial fluid (ISF) in the brain parenchyma, a passage has been envisioned in which the ISF is expelled from the brain via a perivascular drainage pathway with the aid of arterial pulsatile force as the driving force and it ultimately reaches cervical lymph nodes¹⁶⁾. According to a recent study of the autopsy brains of patients with Alzheimer's disease, amyloid β ($A\beta$) from capillaries accumulates selectively in the arterial perivascular area of the cerebral subarachnoid space. Thus a mechanism was proposed in which a reduction in the driving force and the resultant increase in the flow resistance interfere with the excretion of $A\beta$, culminating in neuronal dysfunction^{17–19)}. On the other hand, another finding has been introduced in a study in which a fluorescent tracer was injected into the subarachnoid space. In this study, the CSF enters the cerebral parenchyma via the para-arterial pathway around arteries and having refluxed into the cerebral parenchyma, it travels through the para-venous pathway and is released from the brain²⁰⁾. Furthermore, the glia cells play an important role in the transport of ISF and the route is called the glymphatic pathway. However, these observations are inconsistent with the earlier findings for several points, i.e., there is no confirmation of the presence of structures that would constitute a discharging route around the veins and amyloid β ($A\beta$) protein, the substance responsible for the development of Alzheimer's disease, is deposited at the arterial wall but not on the venous wall. Furthermore, it was recently reported that the cerebral interstitial space is enlarged during sleep, the glymphatic pathway is enhanced, and cerebral excretion of the waste products increases²¹⁾. Thus perivascular drainage may be interpreted as a mechanism to remove substances such as $A\beta$ and tau proteins. Or it may be one of the mechanisms in which the accumulation of $A\beta$ obstructs the drainage and compromises the drive to expel fluid due to the arteriosclerotic process. Such processes may culminate in functional disturbances and lead to the development of

comorbidity of 3 disease entities—small vessel diseases of the brain, Alzheimer's and iNPH.

As for the cerebrospinal fluid circulation, there are critical opinions on the bulk flow theory that upholds the uni-directional flow. It has been said that there is no reflux in the cerebral ventricles in normal individuals. At the level of the foramen of Monro, however, intraventricular reflux is noted, synchronized with heart beat. In patients with ventricular enlargement, however, no intraventricular reflux of the cerebrospinal fluid is observed. It is believed that the intraventricular reflux observed in cisternography is due to diffusion of the tracer that has been kept at a high concentration in the cerebral ventricle while the cerebrovascular fluid absorbing capacity has been compromised.

It has been believed that the cerebrovascular fluid is produced in the choroid plexus. However, it has been found that cerebrovascular fluid production is maintained at a certain level even after the excision of choroid plexus. In fact, it has recently been proposed that the cerebrovascular fluid is produced not only in the choroid plexus but also in the stromal cells of the NVU²²⁾. Furthermore, it is believed that aquaporin (AQP), a water channel protein, exists abundantly in the choroid plexus of the brain, the foot process of glia cells and immediately below the surface of the pia mater, suggesting its important role in the production of cerebrospinal fluid. However, its mechanism of water control has not been completely elucidated. According to recent findings, the major mechanism of cerebrospinal fluid production is not through the choroid plexus—via AQP-1—as it has been believed. Rather, it is produced in the perivascular space by AQP-4, presumably in a form more advanced than that of AQP-1 that is localized in the foot process of glia cells, in a manner better controlled in the perivascular space^{22, 23)}.

4. Verification and new development

We have come to the time to reconsider the bulk theory that have been hitherto upheld in explaining the cerebrospinal fluid as the third circulatory system next to the blood and lymph. There are possibilities that the cerebral capillaries serve as the site of generation and absorption of tissue and cerebrospinal fluids and as the drainage pathway for metabolic and waste products. If we assume that these functions of cerebrospinal fluid are disturbed

when individuals enter pathological states or become aged, there are possibilities that the behaviors of the cerebrospinal fluid involve the NVU. Thus it is necessary to examine the correlation of these possibilities with the results of past experiments and clinical findings. The study of the cerebrospinal fluid goes back in the history and we tend to consider that all possibilities have been exhausted, thus abandoning further studies. However recent findings indicate that the area constitutes an old and yet a new area of studies pointing to a vast and unexplored possibilities.

If we are to believe that astrocytes, a component constituting NVU, are related to the water channel such as aquaporine²³⁾, it becomes necessary to re-examine the cerebral blood circulation, which has hitherto been defined by the accepted theories and knowledge, in line with the added concept of cerebrovascular circulation. In the past, the studies on metabolism related to cerebrovascular circulation tended to lean heavily on blood circulation. For the future studies of neuroprotection, complex and comprehensive approaches, such as the concept of the NVFU (NeuroVascular cerebrospinal Fluid Unit) with spacial and functional expansion encompassing not only cerebrovascular but also cerebrospinal fluid circulations may be necessary.

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