

An Investigation into the CSF Absorption in Cats

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Abstract

Objective

Cerebrospinal fluid (CSF), its secretion, circulation and absorption have been the subject of recent researches. There is a general agreement that the main site of CSF secretion is in the choroids plexus. However, the main absorption site is controversial. Some believe that arachnoid granulations are the principle sites of CSF absorption. In this study, different locations of CSF absorption in cats are investigated.

Materials and Methods

2 ml (100 mg), of ferrous dextran was injected into the subarachnoid compartment of seven cats. After 15 days the animals were euthanized, and the central nervous system (CNS) and its relative tissues were analyzed for any iron amlumulation. Several tissue samples were taken and stained with Prussian blue and Hematoxylin-eosin.

Results

The light microscopic study of different tissues showed amlumulation of the tracer in olfactory and optic nerves, dorsal root ganglia as well as spinal nerve roots.

Conclusion

The main locations of CSF absorption were the spinal nerve roots and their surrounding lymphatic tissue. There is reason to believe that some endothelial fenestrate within these roots were involved in this process.

Keywords: Absorption, Cat, Cerebrospinal fluid

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Introduction

First identified by Pamlhioni in the 18th century, the microscopic arachnoid villi and macroscopic arachnoid granulations have been recognized by most scientists as the main sites of Cerebrospinal Fluid (CSF) absorption for about three centuries. Retzius (1985), Weed (1914), Spatz (1933), Schaltenbrand (1959), and others reported excretion of foreign bodies by phagocytes within these granulations as well (1).

Although the central nervous system parenchyma does not contain lymphatic vessels, numerous studies suggest a physiological link between cerebral interstitial fluid, CSF, and extracranial lymph (2). Protein tracers injected into the brain interstitium or CSF exit the cranium and enter lymphatic vessels. The injected molecules exit out of the cranium and move alongside the prolongations of the subarachnoid space associated with several cranial nerves. The observation that tracers injected into the ventricles enter the spinal lymph nodes suggests that similar CSF-to-lymph pathways exist in the spinal cord as well.

Today it is known that arachnoid villi, or granulations, are not the only site of absorption, as other places such as the cribriform plate, nasal submucosa (3, 4), and to a lesser extent, the optic nerve (5, 6), and the endolymph of the inner ear (7), also contribute in this process. There are also some reports about CSF absorption via the meningium of spinal nerve roots in rats (8, 9), and the quantitative contribution of spinal cord to total CSF transport (10). Because of the different anatomy and the fine structure of spinal roots in rats in comparison to humans, as well as greater anatomical similarities between human and cat spinal roots (11), in the present study the same research is continued in cats.

Materials and Methods

The animals were adopted randomly at a healthy group from a veterinary institute and thoroughly examined by a veterinarian beforehand. The animals were fed water,

ground meat, yoghurt and fish, but fasted for 24 hours before the operation. All ethical and behavioral aspects of this study were observed and respected according to ethics committee of MUMS. All animal experiments were carried out in accordance with MUMS ethical committee acts.

The instrument used was an optic microscope (Zeiss, with standard: 20), the same one routinely used in the pathology ward. The main materials were specific stain for iron containing pigments (Prussian blue), formalin as fixative (Merck), pure ethanol (Merck), and xylol, pure paraffin, with a melting point of 57-60° C (Merck), ketamine, rampone, ferrous dextran and ethanol 96° (Meykadeh co.).

Since we didn't find similar studies with the same tracer as we used, seemingly it was for the first time that ferrous dextran was used as a tracer; the dosage and appropriate time were assessed during the procedure. The tracer contained Fe³⁺, and was injected into the cerebello-medullary cistern via a subomlital puncture. The procedure was performed in 3 steps:

To begin with, the cats were divided into two groups of seven. The volume of injection was equal in both groups, and it was investigated at various times. We injected 0.5 ml of diluted ferrous dextran (0.5 ml ferrous dextran + 0.5 ml distilled water), (Fe= 12.5 mg). Group one was killed after one week, while the second group wasn't killed until 15 days later. The relevant tissues were then excised for analysis. The results were negative in all of the samples; therefore, the amount of injected substance and the investigation times had to be revised.

In the second step we increased the amount of substance to 0.5 ml ferrous dextran (Fe= 25 mg) with the same investigation times as before, but with four cats in each group. Again, the results were not desirable; however there were more amleptable responses in 15 days samples rather than one week samples.

In the third step the injected volume was 2 ml and the samples were seven cats for a 15 days period. After perfusion (15 days later), the histological investigations showed changes and amleptable responses in samples.

The cats were anesthetized with ketamine (110 mg/kg) and rampone (5.5 mg/kg). The perfusion system was used in order to keep the nervous tissue intact. After anesthetizing, the chest was incised, and a catheter entered the aorta through the heart. The catheter was connected to a reservoir which pumped 10% formalin into aorta. The thoracic aorta was obstructed to prevent perfusion of other organs. Therefore, formalin entered the brain via the aorta and then after circulating capillaries exited via sinuses, brain veins and finally the superior vena cava (SVC) to right ventricle (RV), and thus all of the blood was replaced by formalin. Because of the low volume of the subarachnoid space of the spinal cord in cats, a smaller amount of formalin via the anterior spinal artery was sufficient. Following perfusions, the animal was decapitated, and the scalp with the whole vertebrae was separated from the animal's body and immersed in 10% formalin for 20 days. Then the brain with the spinal cord and the spinal roots was removed carefully.

A. Subarachnoid absorption of CSF in the brain

The injected substance and the iron containing

Sampling and staining

After the removal of the brain and spinal cord they were kept in 10% formalin for 5 days. We sectioned the tissue in transverse and longitudinal planes and different slices with a diameter of 7 - 8 microns were taken. The samples were stained with Hematoxylin-eosin (H&E) and Prussian blue. As Prussian blue shows a ferrous containing pigment better but makes the tissue appears vague, H&E was also used to show the tissue structures more clear.

Results

The Fe³⁺ in ferrous dextran turned into hemosiderin in the CSF and was taken up by macrophages and then exited through different pathways which were marked by the blue color in relevant locations. Thus, we were able to find out the different locations of CSF absorption and the rate of its contribution in absorption. More iron accumulation meant more absorption of CSF (Figure 1).

Figure 1. Different sites of CSF absorption by the presence of tracer.

A. Subdural and subarachnoid veins B. Olfactory nerve with macrophages containing iron in perineurium and mucosal glands spaces C. Nasal sinus with accumulation of tracer D. Connective tissue around the optic nerve with lower tracer and, macrophages carrying iron to venules E. Iron containing phagocytes in connective tissue around the spinal root ganglion F. Lymph nodes around the spinal nerve roots.

macrophages were seen along the arachnoid membrane on the surface of the brain cortex.

B. Absorption of CSF via the olfactory nerve

Absorption was relatively higher in this place and was mostly around the olfactory sheath and its connective tissue.

C. Absorption of CSF via the optic nerve

Although rare, we saw the iron containing macrophages around the optic nerve.

D. Absorption of CSF via the spinal nerve roots

The major portion of the injected tracer was seen here. The density around the spinal nerve roots was so high that we could see it in the perineural connective tissue, especially its lymph nodes. We observed the tracer even in the connective tissue of muscles around the spinal nerve roots.

Discussion

The arachnoid projections into the cranial venous system have long been considered the primary location where CSF absorption occurs; however, it would seem that the

experimental and clinical evidence favoring this view is far from persuasive. One of the difficulties in reconciling physiological and pathophysiological realities with an arachnoid projection-centric view of CSF absorption is the limited data and anatomically based evidence supporting a role for arachnoid projections in CSF transport and its mechanism, which remains speculative even after many years of investigation. Besides, some arachnoid projections are not associated with veins and whether or not these elements function to absorb CSF is unknown. In rats, a CSF-venous sinus pressure gradient favoring absorption does not seem to exist until 20 days after birth. Without this pressure gradient, CSF must be absorbed in other locations or via mechanisms unrelated to arachnoid projections. Another important point is the little evidence linking developmental deficiencies of arachnoid projections with hydrocephalus. Perhaps the most important problem with the conventional view is that arachnoid villi, or granulations, do not appear to exist prenatally (12). In two microscopic studies of autopsy specimens from individuals up to 56 days old (13), and from 18 weeks of gestation to 80 years (14), no arachnoid villi or granulations were observed before birth. The choroid plexus develops relatively early in gestation, and by the third gestational month it nearly fills both of the lateral ventricles. This suggests significant CSF production in the fetus and implies that the neonate needs effective mechanisms to absorb CSF. It seems unlikely that arachnoid projections play a significant role at this level of development. However, at or around the time of birth, arachnoid projections start to become visible in the dura (15). As the infant ages, the villi and granulations increase in number, and in the adult they exist in abundance. Therefore, arachnoid projections may have some role in CSF transport in the older individual.

One of the important locations of CSF absorption is the nasal route. By injection of horse radish peroxidase (HRP), into CSF of rabbits, the substance is shown in lymph vessels, ducts and mucosal glands, intercellular space of the nasal epithelium, and

vessels (4). Investigators have known for some time that CSF converts along the extensions of the subarachnoid compartment associated with the olfactory nerves, transports through the cribriform plate, and is ultimately absorbed by lymphatics in the nasal submucosa. Morphological evidence also supports the cribriform-lymphatic CSF transport pathway in human and nonhuman primates. In some species, the perineural extensions of the subarachnoid space appear to open directly into the tissue spaces from which CSF is absorbed into prenodal lymphatics. In rats, CSF passes directly from arachnoid channels into nasal lymphatics (16). Investigation shows that the perineural spaces provide an efficient drainage route from the subarachnoid space to the nasal mucosa in cases with haemorrhagic cerebral lesions (17). There are many potential locations where CSF may gain access to extracranial lymphatic vessels; however most attention has focused on the cribriform plate and the nasal submucosa. In our study, absorption of the tracer was relatively high in this location.

Another important pathway is the orbital route. By injection of ferritin (400000 IU) into the ventricles, the tracer is found in optic nerve connective tissue (5). Manzo et al (7) injected HRP into CSF of 14 rabbits and showed that HRP spreads in perineural and epineural spaces and also in the surface of lymphatics. In our study the amount of absorption was relatively low in this location.

As mentioned before, the central nervous system parenchyma does not contain lymphatic vessels, and it is assumed that a special CSF-to-lymph pathway is present in both the brain and the spinal cord. What encouraged us to do this research were the few reports about the role of spinal cord in CSF transport. The spinal cord is known to contribute significantly to the total cranial/spinal system compliance in response to volume infusions (18, 19), but the routes by which CSF exits the spinal subarachnoid compartment have not been defined clearly. It has been assumed generally that relatively little cerebrospinal fluid is

drained from the spinal CSF space, although anatomic evidence suggests that several possibilities exist for CSF clearance out of the spinal subarachnoid compartment. Arachnoid proliferations similar to those described in the cranium have been observed at the sites of emerging spinal nerve roots in dogs and sheep (20), monkeys (21), and humans (22). Additionally, lymphatic vessels may also play a role in draining spinal CSF. Boulton et al. (23) injected radiolabeled human serum albumin into lumbar CSF and observed elevated radioactivity in the lumbar and intercostals lymph nodes and also collected lymph from the thoracic duct. There are also a few reports in rats by Zencker (8, 9). In any event, arachnoid proliferations resembling the villi and granulations of the cranial system have been described in spinal tissues; however in many cases, these structures were not associated with veins and their significance may be questionable (21).

Our study confirms that at least in cats the main portion of CSF is absorbed via the spinal nerve roots, probably by endothelial fenestrae and the phagocytes of these areas. Therefore, one of the main locations of CSF absorption lies in the spinal nerve roots. However, the results of one quantitative measurement suggest that under normal circumstances, approximately 25% of the total CSF transport omits from the spinal CSF space; although the spinal contribution to CSF clearance may become more significant in pathophysiological conditions (10). Our opinion is that, probably the spinal nerve roots are the main site of CSF absorption in humans.

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