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Original scientific paper

Transventricular and Transpial Absorption of Cerebrospinal Fluid into Cerebral Microvessels

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A B S T R A C T

It is generally accepted that volume of cerebrospinal fluid (CSF) is secreted in brain ventricles and flows to subarachnoid space to be absorbed into dural venous sinuses or/and into lymphatics via perineural sheats of cranial nerves. Since 99% of CSF volume is water, in experiments on cats ^{3}H-water was slowly infused into lateral ventricle and found that it does not flow to subarachnoid space but that it is rapidly absorbed transventricularly into periventricular capillaries. When ^{3}H-water was infused in cortical subarachnoid space, it was absorbed locally into cerebral capillaries via pia mater. On the contrary, when macromolecule ^{3}H-inulin is applied in CSF, it is very slowly eliminated in bloodstream, and, with time, is carried by systolic-diastolic pulsations and mixing of CSF bidirectionally along CSF system. Thus, CSF volume (water) is absorbed rapidly into adjacent cerebral capillaries while inulin is distributed bidirectionally due to its long residence time in CSF. Previously, the macromolecules have been used to study CSF volume hydrodynamics and with this misconception of CSF physiology arose.

Key words: *cerebrospinal fluid, absorption, cerebral microvessels, water absorption*

Introduction

Absorption of the cerebrospinal fluid (CSF) is still puzzling despite of the intensive investigation of this phenomenon. The classical concept of the CSF absorption across arachnoid villi into dural nervous sinuses is dubious[1]. On the other hand, experiments of CSF absorption from subarachnoid space into lymphatics via perineural sheats of cranial nerves, most particularly the olfactory nerve, are generally performed under high CSF pressure which can damage meningeal layers and destroy the barrier function of arachnoid membrane[2] so that such results should be critically evaluated.

In the study of CSF absorption two relevant questions arise: a) which marker of CSF absorption should be used, and b) under which experimental conditions the absorption of CSF should be investigated. So far, very often the macromolecules (proteins, dextrans, inulin) have been used as markers of CSF volume absorption. It is questionable, however, whether the macromolecules can show the absorption of CSF volume since 99% of this volume constitutes water. Since fate of water in CSF may be different from that of macromolecules we assume that labelled water (^{3}H-water) is to be used, hopefully to trace absorption of CSF volume. Furthermore, any marker should be applied into CSF in such a way that CSF pressure is not significantly increased to avoid damage of integrity of CSF system.

Brain interstial fluid (ISF) and CSF are in continuity and constitute cerebral extracellular fluid. Our working hypothesis assumes that volume of extracellular fluid (water) is controlled across the walls of brain microvessels (arterial and venous capillaries and postcapillary venules): water filtration through arterial capillaries into ISF-CSF and its absorption from ISF-CSF into venous capillaries and postcapillary venules. If this is so then ^{3}H-water infused into lateral brain ventricle should be absorbed into adjacent periventricular capillaries which drain via great vein of Galen in confluence of sinuses (torcular Herophili) but it will not be carried to cisterna

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magna and other subarachnoid spaces to be absorbed via perineural sheats of cranial nerves or arachnoid villi. To test our hypothesis, ^{3}H-water was slowly infused into lateral ventricle or cortical subarachnoid space and its absorption into cerebral microvessels was investigated in cats. For comparative purpose ^{3}H-inulin, a macromolecule (m.w. 5500) which passes poorly across cerebral microvascular walls, was applied in the same way as ^{3}H-water. Our results indicate that fate of ^{3}H-inulin is different from that of ^{3}H-water in the CSF.

Material and Methods

Acute and subchronic experiments were performed on adult cats (2.5–4.2 kg body weight) according to the Croatian Animal Welfare Act and the approval of the institutional Ethical Committee.

Acute experiments

The cats were anaesthetized with thiopenton sodium (50 mg/kg i.p.) and femoral artery and femoral vein were cannulated. The head of the animal was fixed in a stereotaxic frame (D. Kopf, USA). For infusion of substances into lateral ventricle, a drill hole (1.5 mm d.) in parietal bone, 2.5 mm lateral of midsagittal line and 8 mm caudal from the coronal suture, was performed and dura exposed. A stainless-steel cannula (25 gauge) was introduced by micromanipulator into lateral ventricle (10–12 mm below the level of dura)[3] and connected to infusion pump (Harvard 975, USA). The hole was closed by acrylate.

For sampling of blood (0.5 mL) from confluence of sinuses, a drill hole (1.5 mm) through the bone overlaying the confluence was performed and the confluence punctured by 25 gauge needle. After blood sampling, the puncture site was covered with Gelfoam to prevent bleeding. Arterial blood samples (0.5 mL) were obtained from cannulated femoral artery. The samples of cisternal CSF (70 μL) were obtained after skin incision by direct puncture of cisterna magna with 25 gauge cannula which was fixed in position by a holder.

^{3}H-water (spec. act. 25 mCi/g, New England Nuclear, USA) or ^{3}H-inulin (metoxy – ^{3}H-inulin, spec. act. 100–500 mCi/g, New England Nuclear, USA) were appropriately diluted in physiological saline (see Results) for infusion by pump either in lateral ventricle or in cortical subarachnoid space over 3 hours. At the start of experiment the rate of infusion was 10 μL/min during first 2 minutes for priming and thereafter rate of infusion was 1.77 μL/min during 3 hours. It was observed in another series of experiments that infusion of physiological saline up to the rate of 5 μL/min does not influence the CSF pressure recorded in cisterna magna via pressure transducer (P 23, Gold Electronics, USA) and polygraph (R 511A, Beckman, USA).

At the end of experiment, a few drops of trypan blue were given through infusion cannula, and after killing the animal with overdose of anaesthetic, the position of cannula in situ was verified by dissection of brain. The radioactivity of samples (50 μL) of CSF, arterial and confluence plasma was measured in 5 mL of Bray's solution by a liquid scintillation counter (Beckman LS 1701, USA).

Subchronic experiments

To explore how physical activity of animal could affect fate of ^{3}H-water and ^{3}H-inulin, these substances were slowly infused by osmotic minipump over 5 days into lateal ventricle of freely moving cats. The cat was anaesthetized by thiopenton sodium (50 mg/kg i.p.) and its head was fixed in stereotaxic frame. The infusion canula was introduced stereotaxically into lateral ventricle with the same coordinates as in acute experiments (see above). The external end of cannula was connected by plastic tubing to osmotic minipump (model 1701, Alza Corp., USA). The minipump and connection tubing were filled previously either with ^{3}H-water (0.5 mCi/mL) or ^{3}H-inulin (0.25 mCi/mL) in physiological saline. To fix the cannula in position, two stainless steel screws were implanted into the bone and thereafter dental acrylate was poured over the bone hole, screws, external end of canula and connection of cannula to plastic tubing, while the osmotic minipump was positioned subcutaneously. At the end, the wound was sprayed with local anaesthetic (lidocaine), and the cat received 50 mL of 5% dextrose intraperitoneally. The next day the cat recovered, moved freely and began to eat spontaneously.

The osmotic minipump (model 1701) has a volume of 170 μL and ejects the solution at the rate of 1 μL per hour. Five days after the operation, the cat was anaeszhetized by thiopenton sodium and samples of arterial blood, venous blood from confluence of sinuses, urine and CSF from different parts of the CSF system were taken for analysis. CSF samples were obtained from cortical, cisternal, thoracic and lumbar regions. To obtain cortical CSF, a trephine hole in parietal bone (0.5 cm d.) 1.5 cm lateral to midsagittal line and 0.5 cm posterior to coronal suture was performed, dura punctured by 25 gauge needle which was positioned over cortical surface, and under light negative pressure the cortical CSF was collected in tuberculine syringe[3]. Cisternal CSF was obtained as in acute experiments (see above). After laminectomy of thoracic (Th8-Th9) and lumbar (L4-L5) vertebrae, thoracic and lumbar CSF samples were obtained from subarachnoid space after the puncture of dura and arachnoid. Urine was obtained by direct puncture of urinary bladder. The radioactivity of plasma, urine and CSF samples was measured as in acute experiments. The animals were killed by overdose of anaesthetic, and position of cannula in lateral ventricle verified by application of Evans blue and brain dissection, as in acute experiments.

The results are shown either as single experiments or as mean and standard error of mean (SEM) with number of experiments (n). For statistical evaluation Student's t-test was used and $p<0.05$ was taken as statistically significant.

Results

Fate of ^{3}H-water in the CSF

During infusion of ^{3}H-water into lateral ventricle, its concentrations in confluence plasma water increased several times over those in cisternal CSF and arterial plasma water when either lower (Figure 1a and b) or five times higher (Figure 1c and d) concentrations of ^{3}H-water were infused. Thus, it appears that ^{3}H-water is absorbed from brain ventricles into periventricular capillaries which drain in confluence.

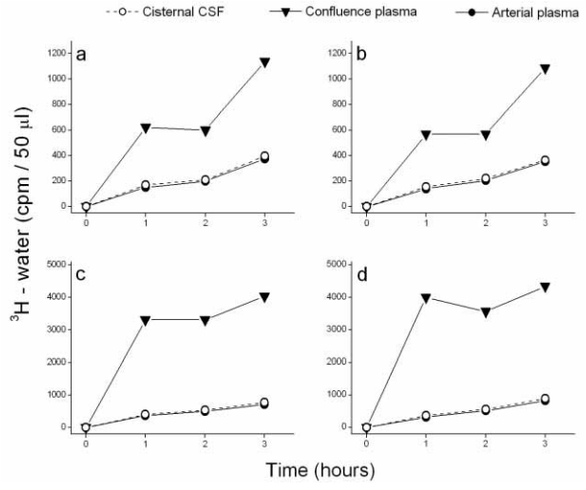

Fig. 1. Concentration of ^{3}H-water in the cisternal CSF, confluence and arterial plasma (cpm/50 µL) during infusion (1.77 µL/min) of ^{3}H-water in saline into lateral ventricle at lower (0.025 mCi/mL, a and b) and five times higher (0.125 mCi/mL, c and d) concentration of ^{3}H-water.

Furthermore, concentrations of ^{3}H-water in arterial plasma water and cisternal CSF were practically equal during experiment (Figure 1). The fact that the concentrations of ^{3}H-water are much higher in confluence than in arterial plasma and cisternal CSF (Figure 1) indicates that ^{3}H-water is carried from confluence to systemic bloodstream where it is diluted by large volume of the blood, and than it passes across cerebral capillaries walls into ISF and CSF so that the concentration of ^{3}H-water in arterial plasma and CSF reaches a rapid equilibrium. To explore such a possibility, ^{3}H-water was applied intravenously as a bolus and samples of arterial plasma and cisternal CSF were taken over short time intervals (Figure 2). Figure 2 shows that the concentration of ^{3}H-water in arterial plasma falls and increases in cisternal CSF during time and that these concentrations reach equilibrium in about 5 min. This supports our assumption that during intraventricular infusion ^{3}H-water in cisternal CSF is derived from bloodstream. When ^{3}H-water was infused into cortical CSF, its concentration in confluence plasma was several times higher than in arterial plasma and cisternal CSF, the last two concentrations being practically equal ($p>0.05$, Figure 3). Thus, a similar phe-

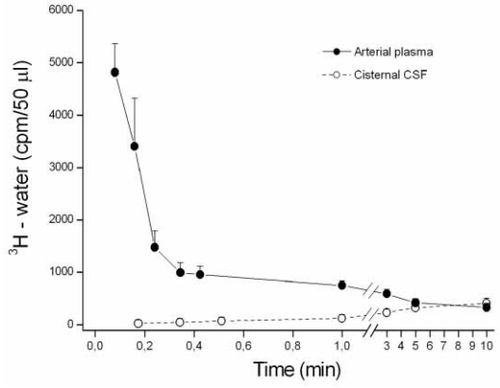

Fig. 2. Concentration of ^{3}H-water in arterial plasma and cisternal CSF (cpm/50 µL) after intravenous injection of ^{3}H-water (0.034 mCi in 2 mL of saline) as a bolus during 4 seconds. Therafter, samples of arterial blood from femoral artery and cisteral CSF were taken at indicated time intervals. Results are shown as mean and SEM (n=4).

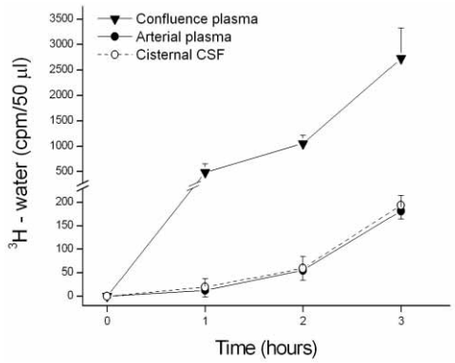

Fig. 3. Concentration of ^{3}H-water in the cisternal CSF, confluence and arterial plasma (cpm/50 µL) during infusion (1.77 µL/min) of ^{3}H-water in saline (0.125 mCi/mL) into cortical subarachnoid space. Results are shown as mean and SEM (n=4).

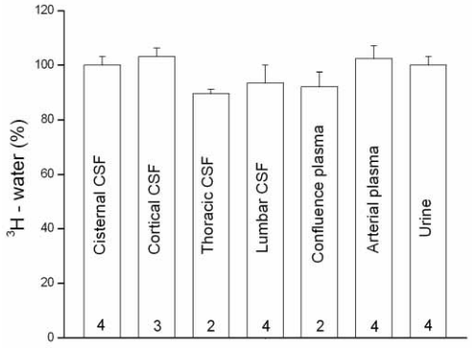

Fig. 4. Concentration of ^{3}H-water in different compartments of CSF, confluence and arterial plasma, and urine after 5 days of ^{3}H-water in saline (0.50 mCi/mL) infusion into lateral ventricle by osmotic minipumps at rate 1 µL/hour. The concentrations of ^{3}H-water are expressed in percentage of its concentration in cisternal CSF which was taken as 100%. Results are shown as mean and SEM. The numbers in bottom of columns show the number of successful experiments.

nomenon was observed as during intraventricular infusion of ^{3}H-water (see Figure 1).

In subchronic experiments ^{3}H-water was slowly infused (1 μL/hour) by osmotic minipump over 5 days in freely moving cats. Concentrations of ^{3}H-water in different fluid compartments are shown in percentages of its concentration in cisternal CSF which was taken as 100% (Figure 4). No statistical difference in ^{3}H-water concentration was detected between various CSF compartments (cisternal, cortical, thoracic and lumbar CSF), confluence and arterial plasma and urine ($p>0.05$). It seems that ^{3}H-water was absorbed into periventricular capillaries and distributed by bloodstream over 5 days to all fluid compartments so that an equilibrium of ^{3}H-water concentration was reached between these compartments.

Fate of ^{3}H-inulin in the CSF

^{3}H-inulin was infused into lateral ventricle at different concenrations (Figure 5) and its concentrations measured in cisternal CSF, confluence and arterial plasma. Concentrations of ^{3}H-inulin in cisternal CSF increase over time depending on infused concentrations. At the same time its concentrations in confluence and arterial plasma were near background activity so that they could not be precisely measured. In one animal urinary bladder was punctured and considerable concentration of ^{3}H-inulin was detected in urine. These results indicate that ^{3}H-inulin is carried from lateral ventricle to cisternal CSF and very slowly passes in bloodstream and from blood eliminated into urine (see below).

It was shown previously that ^{3}H-inulin was distributed from cisternal to cortical CSF[4]. To explore the potential distribution in the opposite direction ^{3}H-inulin was infused in cortical CSF and its concentration measured in cisternal CSF. It can be seen that during cortical infusion ^{3}H-inulin increases in cisternal CSF over 3 hours of experiment (Figure 6) indicating its bidirectional distribution between cortical and cisternal CSF.

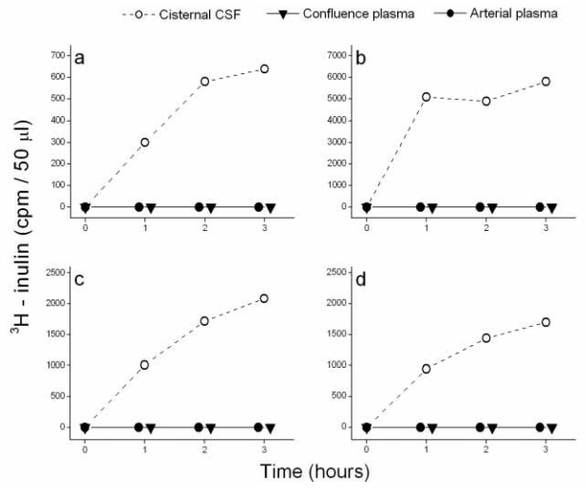

Fig. 5. Concentration of ^{3}H-inulin in the cisternal CSF, confluence and arterial plasma (cpm/50 μL) during infusion (1.77 μL/min) of ^{3}H-inulin in saline into lateral ventricle. Radioactivities infused were 0.0013 mCi/mL (a), 0.018 mCi/mL (b), and 0.004 mCi/mL (c and d), respectively.

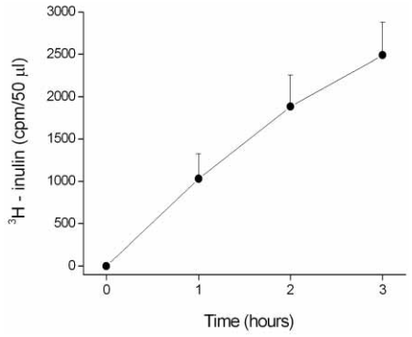

Fig. 6. Concentration of ^{3}H-inulin in the cisternal CSF (cpm/50 μL) during infusion (1.77 μL/min) of ^{3}H-inulin in saline (0.004 mCi/mL) into cortical subarachnoid space. Results are shown as mean and SEM (n=4).

In subchronic experiments ^{3}H-inulin was slowly infused (1 μL/hour) by osmotic minipump in lateral ventricle over 5 days in freely moving cats. Concentrations of ^{3}H-inulin in different fluid compartments are shown in percentages of its concentrations in cisternal CSF which was taken as 100% (Figure 7). No statistical difference in ^{3}H-inulin concentrations was detected between cisternal, cortical, thoracic and lumbar CSF ($p>0.05$). Concentrations of ^{3}H-inulin in confluence and arterial plasma were much lower than in CSF (Figure 7), while concentration of ^{3}H-inulin in urine (not shown in Figure 7) was 340± 96% (n=4). These data indicate that ^{3}H-inulin is slowly removed from CSF into bloodstream, distributed from the blood to various peripheral tissues and efficiently eliminated in urine.

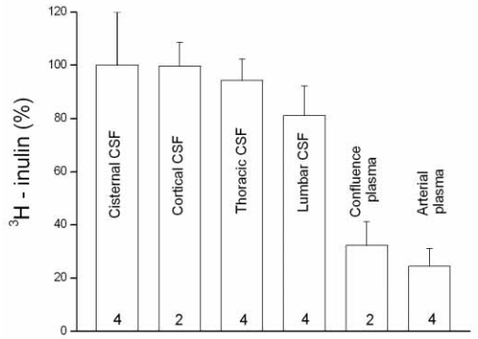

Fig. 7. Concentrations of ^{3}H-inulin in different compartments of CSF, confluence and arterial plasma, and urine after 5 days of ^{3}H-inulin in saline (0.25 mCi/mL) infusion into lateral ventricle by osmotic minipumps at rate 1 μL/hour. The concentrations of ^{3}H-inulin are expressed in percentage of its concentration in cisternal CSF which was taken as 100%. Results are shown as mean and SEM. The numbers in bottom of columns show number of successful experiments.

Discussion

The communication between the CSF compartments and cerebral interstial fluid (ISF) shows some anatomical and physiological specificities. The ependymal single layer of cells in the ventricles offers no serious impediment to the exchange of substances between CSF and its adjacent ISF where blood microvessels are located. These microvessels (arterial and venous capillaries and postcapillary venules) are characterized by endothelial cells with tight intercellular junctions which encircle completely each endothelial cell, forming blood-brain barrier, which controls the passage of substances across microvascular walls[5]. Most microvessels in choroid plexus are fenestrated, but these fenestrae are covered with thin cellular diaphragm. It seems that the exchange of substances between these microvessels and choroidal ISF is made easier due to fenestrae, but the exchange of substances between choroidal ISF and adjacent CSF is strictly controlled by choroidal epithelial cells, which are connected by tight intercellular junctions[5]. The subarachnoid CSF and nervous parenchyma are separated by a layer of thin pial cells without specialized intercellular junctions[5], so that the exchange of substances across pial layer is not significantly restricted[6,7].

Water absorption from CSF

To elucidate the absorption of water into cerebral capillaries the passage of water and solutes across blood-brain barrier should be considered. Water passage between cerebral capillaries and ISF is relatively free[8,9], while the passage of proteins and inorganic electrolytes is greatly restricted[10]. Since proteins and electrolytes contribute 0.4% and 94%, respectively, to total plasma osmolarity, it has been recently proposed that electrolytes, mostly Na and Cl which contribute 83% plasma osmolarity, are main regulators of water filtration and reabsorption[11]. During water filtration in arterial capillaries under high hydrostatic pressure, the electrolytes are sieved (retained) so that plasma osmotic counterpressure is generated due to water loss. This osmotic counterpressure rises along the lenght of capillary and when it reaches the level of capillary hydrostatic pressure, water filtration is brought to a halt[11]. However, when such hyperosmolar plasma reaches venous capillaries and postcapillary venules in which hydrostatic pressure is low, osmotic counterpressure is instrumental in osmotic water reapsorption from the ISF so that osmotic counterpressure is dissipated[11]. Thus, a continuous and rapid turnover of water volume across microvascular walls occurs: filtration in arterial capillaries and reabsorption in venous capillaries and postcapillary venules. Since ISF and CSF are in continuity, we assume that the absorption of ISF and CSF is a directly connected process. This idea is supported by the observation that after the intravascular application of hyperosmolar mannitol, a fall of hydrostatic pressure in both ISF and CSF[12] and an increase of electrolytes in CSF[13] are observed, indicating that the microvascular absorption of water volume from ISF-CSF takes place.

Furthermore, when hyperosmolar arteficial CSF is applied in brain ventricles, an influx of water volume[14] and an augmentation of CSF pressure[15] are observed. Thus, it appears that the direction of the osmotic pressure gradient across microvascular walls controls the influx and the efflux of water volume in ISF-CSF. Water volume asorption from ISF-CSF into microvessels is determined inter alia by capillary surface area. Taking that capillary surface area is 240 cm^2/g [16] and brain weight of cat 26 g[17], total cerebral capillary surface area in cats would be 6,240 cm^2. On the other hand, the surface area of arachnoid villi and perineural sheats of cranial nerves in cats is probably not higher than a few cm^2 at best. Thus, capillary surface area is incomparably higher than surface area of classically postulated sites of CSF absorption, i.e. arachnoid villi and perineural sheats of cranial nerves.

Our results show that during slow infusion into lateral ventricle ^{3}H-water reached several times higher concentration in confluence plasma than that in cisternal CSF and arterial plasma which had practically equal concentrations (Figure 1). This phenomenon is observed when lower (Figure 1a and b) and five times higher (Figure 1c and d) concentrations of ^{3}H-water were infused into lateral ventricle indicating that ^{3}H-water is absorbed into periventricular capillaries which drain via great vein of Galen into the confluence of sinuses. When confluence blood is delivered to systemic circulation, the concentration of ^{3}H-water is diluted by large blood volume so that the concentration of ^{3}H-water is lowered in arterial plasma from which ^{3}H-water enters into cerebral ISF and equlibrates with cisteranl CSF. To explore such possibility, ^{3}H-water was given as a bolus intravenously and its concentration in arterial plasma and cisternal CSF measured at short time intervals. It appears that in 5 min the equilibrium of ^{3}H-water concentration between arterial plasma and cisternal CSF is reached (Figure 2). It was shown previously in dogs that deuterium water (D_2O) reaches half-time of equilibrium between plasma and various parts of brain in about 20 sec and between plasma and cisternal CSF in 3 min[8]. Delay in a equilibrium of D_2O between brain parenchyma and CSF is explained by larger distance of CSF than ISF from cerebral capillaries.

Total volume of ^{3}H-water in physiological saline infused over 3 hours into lateral ventricle was 339 μL what is somewhat higher than half of the volume of a lateral ventricle in cats[18]. If any part of infused volume reached cisternal CSF via brain ventricles, the ^{3}H-water concentration in cisternal CSF should be higher than in arterial plasma, which was not found (Figure 1). Furthermore, previously we have observed that when ^{3}H-water was slowly infused into lateral ventricle over several hours, its concentration in arterial plasma and cisternal CSF were equal even when cisternal CSF was drained under negative pressure for one hour[19]. This indicates that the absorption of water volume in brain ventricles is much higher than its postulated unidirectional flow (»circulation«) between lateral ventricle and cisterna magna. In

other words, higher concentration of ^{3}H-water in confluence plasma than in arterial plasma and cisternal CSF (Figure 1) is not due only to the exchange of ^{3}H-water molecules across walls of periventricular capillaries but to the absorption of CSF-ISF volume (water), as elaborated above.

When infused into cortical CSF, ^{3}H-water concentration in confluence plasma was several times higher than in arterial plasma and cisternal CSF which had equal concentrations (Figure 3). Thus, a similar phenomenon is observed as during the infusion of ^{3}H-water into lateral ventricle (Figure 1), indicating that ^{3}H-water passes from subarachnoid space across pia mater and is absorbed into cerebral microvessels, which drain in adjacent superior sagittal sinus, and by blood flow it reaches the confluence of sinuses.

After 5 days of slow infusion of ^{3}H-water in physiological saline (1 μL/min) by osmotic minipump in lateral ventricle of freely moving cats, its concentrations in various CSF compartments, plasma and urine were equal (Figure 4). In this case, ^{3}H-water absorption from brain ventricles in periventricular capillaries and its delivery to bloodstream (see above) leads to increase of ^{3}H-water concentration over 5 days in the whole organism and its equilibrium between all body fluids. Thus, all our experiments with ^{3}H-water support the idea that CSF volume (water) does not flow unidirectionaly along CSF spaces but is locally absorbed in adjacent microvessels located in brain parenchyma.

Bidirectional distribution of ^{3}H-inulin in the CSF

In contrast to water, inulin is a macromolecule which passes poorly across cerebral microvascular walls[16] what should affect their different distribution in brain and CSF. How the restricted passage of substances across microvascular walls determines their distribution along CSF spaces is best demonstrated by the fate of organic acids such as 5-hydroxyindoleacetic acid (main metabolite of serotonin), penicillin and phenolsulfonphthalein, which are eliminated by active transport into capillaries, and this transport can be competitively inhibited by probenecid. This offers an unique opprtunity to study the fate of substances in CSF and ISF when their transport across microvascular walls is normal and inhibited. When organic acids are applied into cisternal CSF in dogs and cats under control conditions, they enter adjacet brain parenchyma, from where they are rapidly eliminated into microvessels[7], so that their concentration in cisternal CSF rapidly falls and they are distributed only in traces to ventricular, cortical and lumbar CSF[20]. However, in probenecid pretreted animals the organic acids after application in cisternal CSF[20] maintain high concentration in cisternal CSF[20] and adjacent brain parenchyma[7] so that half-life and residence time in cisternal CSF are greatly increased in comparison to control animals[20]. Under such condition the distribution of organic acids to lumbar, cortical and ventricular CSF greatly increases so that their concentration overshoots (lumbar CSF) or equals (cortical CSF) concentration in cisternal CSF after 5 hours[20]. Thus, there is a correlation between the rate of removal of substances across capillary walls and their distribution along CSF spaces, i.e., a slower rate of removal leads to better distribution in CSF system. Since inulin poorly passes across microvascular walls[16], its distribution along CSF spaces should be efficient and similar to the distribution of organic acids when their transport into microvessels is blocked by probenecid. Our subchronic experiments show that ^{3}H-inulin is efficaciously distributed from lateral ventricle to all CSF compartments over 5 days (Figure 7). At the same time ^{3}H-inulin is slowly eliminated into bloodstream, distributed to peripheral tissues and removed in urine (see Results).

It was previously shown that, when applied in cisternal CSF, ^{3}H-inulin is efficiently distributed to lumbar and cortical CSF as well as to the CSF of lateral ventricle[4]. Present experiments show that after the application of ^{3}H-inulin in lateral ventricle (Figure 5) or cortical CSF (Figure 6), it is distributed to cisternal CSF. Thus, bidirectional ^{3}H-inulin distribution is observed: between cisterna magna and lateral ventricle[4] and in the opposite direction (Figure 5), and between cisterna magna and cortical CSF[4] and in the opposite direction (Figure 6). It should be mentioned that in humans the distribution of contrast media in CSF is bidirectional: from lateral ventricle to subarachnoid space but also in the opposite direction, i.e. from subarachnoid space to lateral ventricles[21].

The bidirectional distribution of substances in CSF system is a consequence of CSF mixing[20] due to systolic-diastolic to-and-fro displacement of CSF volume [22,23]. Since diffusion is a very slow process[24], it does not contribute significantly to substance distribution between CSF compartments because diffusion distances between compartments are long[25]. It was shown that blood and CSF pulsations in cranium are instrumental for rapid distribution of proteins such as horseradish peroxidase (m.w. 40,000) from CSF along perivascular spaces of all blood vessels in parenchyma[26] and that this protein is eliminated slowly across capillary walls by the process of micropinocytosis[5]. Thus, it appears that substances, which are slowly eliminated into microvessels and have a long residence time, are efficiently distributed not only along CSF spaces but also between CSF and brain parenchyma. Since microvascular surface area in parenchyma is large (see above), such substances are eliminated during time across capillary walls into bloodstream. The elimination of substances from CSF across arachnoid villi into dural venous sinuses and via perineural sheats of cranial nerves into lymphatics is probably very small under normal CSF pressure due to small surface area of these »closed« structure. However, under high increase of CSF pressure, these structures may become damaged and »open«[2] so that the elimination of substances from CSF may increase.

CSF physiology and hydrocephalus

Our results indicate that neither the net formation of CSF volume (water) in brain ventricles nor its unidirectional flow in subarachnoid space are present. Thus, it appears that the formation and absorption of CSF in ventricles are in balance. This is supported by observation that, when the aqueduct of Sylvius was cannulated in cats, no outflow of ventricular CSF from cannula was observed at physiological CSF pressure[27].

Generally accepted hypothesis of net CSF formation in brain ventricles is mostly due to the development of widely used ventriculocisternal perfusion method for the measurement of ventricular formation of CSF[28]. In this method one of lateral ventricles and cisterna magna are cannulated and ventricles perfused with perfusate containing a macromolecular marker (inulin, albumin or dextran) assuming that the marker is not lost from perfusate so that its dilution in outflowing fluid from cisternal cannula is taken as the measure of net CSF formation in ventricles. However, it is known that markers can pass in brain parenchyma[5,26] and in nonperfused contralateral lateral ventricle[29], and it can also be distributed from cisternal region to cervical and cranial CSF. All these losses of the marker from perfusate should give a false calculation of net CSF formation in ventricles. Furthermore, at lower rates of ventriculocisternal perfusion the higher rates of CSF formation are calculated than at higher perfusion rates[30], indicating that an inherent defect in the method is present. It was also observed during ventriculocisternal perfusion with perfusate containing ^{3}H-water and blue dextran (m.w. 2×10^6), that the increase of hydrostatic pressure in perfusate from negative (–10 cm H_2O) to positive (20 cm H_2O) values has opposite effects on these two substances: the augmentation of blue dextran and the decrease of ^{3}H-water concentrations in perfusate[31]. This indicates that under rised hydrostatic pressure much higher absorption of ^{3}H-water, in comparison to blue dextran, occurs across microvascular walls. Anyhow, we beleive that ventriculocisternal perfusion method should be used with caution and that it cannot be accepted as a valid method for the determination of net CSF formation.

How stenosis or obstruction of CSF pathways leads to the development of hydrocephalus, is a question without an adequate answer at present. Generally accepted idea that transmantle pressure gradient between ventricles and cortical subarachnoid space is the cause of hydrocephalus was questioned recently, since no such gradient was present in patients with communicating and noncommunicating hydrocephalus[32]. Furthermore, after obstructed shunt was removed in some children with communicating and noncommunicating hydrocephalus, patients proved to be compensated and shunt-independent[33]. It was also shown in cats that the obstruction of cervical subarachnoid space or aqueductal stenosis lead to development of hydrocephalus after three weeks without changes in ventricular CSF pressure[34].

It is not clear which factor triggers the development of acute hydrocephalus during the initial expansion of obstructive process in the CSF system. It was proposed that such trigger may be a decrease of systolic displacement of CSF volume out of ventricles, what could impair periventricular perfusion with ischemic damage of periventricular tissue and consequently dilatation of ventricles[34]. Furthermore, the decrease of arterial pulsations and cerebral blood flow are postulated factors responsible for the development of hydrocephalus, what is supported by the observation that after shunting cerebral blood flow is improved[35]. Further investigation of the potential role of cerebral ischemia in the development of hydrocephalus may shed new light on the pathogenesis of this process.

Conclusion

Our results support the idea that CSF system functions like a closed box which operates via ISF and cerebral microvessels: the volume of ISF and CSF is continously formed by water filtration from arterial capillaries and reabsorbed in venous capillaries and postcapillary venules so that the volume of ISF and CSF is constantly renewed. Water, which constitutes 99% of ISF and CSF volume, does not flow unidirectionally along CSF spaces but is locally formed and reabsorbed. In contrast to water, substances which poorly pass across microvascular walls have a long residence time in ISF and CSF and are slowly carried bidirectionally along all CSF spaces by the systolic-diastolic to-and-fro displacement of CSF volume and its mixing.

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APSORPCIJA CEREBROSPINALNOG LIKVORA U MOŽDANE KAPILARE

S A Ž E T A K

Općenito se smatra da se volumen cerebrospinalnog likvora secernira u moždanim komorama i teče u subarahnoidni prostor, te se apsorbira u duralne venske sinuse ili/i u limfu putem perivaskularnih ovojnica kranijskih živaca. Budući da voda sačinjava 99% volmena likvora, u pokusima na mačkama sporo je infundirana ^{3}H-voda u lateralne moždane komore, te je pokazano da ona ne teče u subarahnoidni prostor nego biva brzo apsorbirana u priležeće periventrikularne krvne kapilare. Kada je ^{3}H-voda bila infundirana u kortikalni subarahnoidni prostor ona je kroz piju mater bila apsorbirana lokalno u priležeće cerebralne kapilare. Nasuprot tome, kada je ^{3}H-inulin bio infundiran u likvorske prostore, ta je makromolekula bila veoma sporo odstranjivana u krv, te je tijekom vremena bila raznesena dvosmjerno uzduž likvorskog sustava uslijed sistoličko-dijastoličkih, naprijed-natrag pulzacija likvora i njegova miješanja. Dakle, volumen likvora (voda) se brzo apsorbira lokalno u priležeće moždane kapilare, dok makromolekule (inulin) bivaju dvosmjerno raznesene zbog dugotrajnog boravka u likvoru. Do sada su makromolekule bile korištene za izučavanje hidrodinamike likvora, što je dovelo do krivog shvaćanja fiziologije likvora