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Potential Error in Ventriculocisternal Perfusion Method for Determination of Cerebrospinal Fluid Formation Rate in Cats

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A B S T R A C T

The cerebrospinal fluid (CSF) formation rate (Vf) has been extensively studied by the ventriculocisternal perfusion, a method still regarded as the most precise one. This method as well as the equation for the calculation of the CSF formation rate (Vf) was established by Heisey et al[4] on indicator dilution in perfusate. They assumed that the dilution of the indicator substance in perfusion is a consequence of newly formed CSF i.e. a higher CSF formation rate would result in a higher degree of dilution of the indicator substance. Therefore, such method is indirect and any mistake in the interpretation of the degree of indicator dilution would lead to questionable and often contradictory results regarding CSF formation rates. According to Heisey's equation, Vf shoud not depend on the rate of ventriculocisternal perfusion. However it has been shown that Vf is perfusion dependt value[10], and also that during perfusion the indicator substance is partially absorbed into surrounding tissue. It is possible that obtained Vf dependence on perfusion rate was caused by observed absorption of indicator substances. For that reason, in anaesthetised cats ventriculocisternal perfusion was performed at higher (252.0 μL/min) and at lower perfusion rate (65.5 μL/min) and Vf was calculated at both experimental and corrected (just for absorbed amount) values of indicator substance. Since (inspite of the correction) the difference of 12.4 μL/min between lower (15.0 μL/min) and higher perfusion rate (27.4 μL/min) was obtained, it is obvious that ventriculocisternal perfusion method cannot be considered reliable for measuring CSF formation rate.

Key words: *cerebrospinal fluid, cerebrospinal fluid formation, ventriculocisternal perfusion, indicator substance*

Introduction

According to the generally accepted hypothesis of cerebrospinal fluid (CSF) dynamics, CSF is produced within the cerebral ventricular system, and circulates slowly from the brain ventricles towards the cortical subarachnoid space, to be absorbed into the venous sinuses across the arachnoid villi. Today it is generally believed[4,5,12,13] that CSF is formed mainly by the secretory activity of the choroid plexuses in the brain ventricles and that the passage through the choroidal epithelium is an active energy consuming metabolic process which transforms the ultrafiltrate into a secretion, i.e. CSF. The CSF formation rate (Vf) has been extensively studied by the ventriculocisternal perfusion technique, which is still regarded as the most precise method[1–4]. This method is widely accepted and represents basic tool by which CSF physiology has been studied. Mostly thanks to the experimental results obtained by such method general hypothesis of the CSF hydrodynamics has been confirmed. The method and the equation for the calculation of the CSF formation rate have been established by Heisey et al.[4] and Pappenheimer et al.[5], who assumed that the dilution of the indicator substance is a consequence of the newly formed CSF, i.e. that a higher CSF formation rate would result in a higher degree of dilution of the indicator substance. Therefore, any mistake in the interpretation of the degree of dilution of the indicator

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substance in the perfusate caused by other reasons (escape of indicator substances or water into the brain tissue, or irregular mixing) would result in questionable and often contradictory conclusions regarding CSF formation rates[6–11].

However, it is known that an indicator substance can pass from the CSF into brain parenchyma[7,8,12–14] and that inulin enters rapidly perivascular CNS spaces, reaches a very large surface area of capillaries and, by slow diffusion across microvascular walls, reaches the bloodstream to be rapidly eliminated in the urine[15]. Since it has been shown that calculation of Vf depends on ventriculocisternal perfusion rate[10] we wanted to investigate if these obtained differences were caused by absorption of indicator substance in ventricular tissue. Accordingly, it was investigated how the change of outflow concentration (Co) caused by such surrounding absorption should reflect on calculation of CSF formation rate (Vf) at lower (65.5 μL/min) and higher perfusion rate (252.0 μL/min) with 3H-inulin as indicator substance. The inulin was used because the way and amount of inulin absorption into surrounding tissue are known[7,15]. If results represent CSF formation, after correction of absorbed indicator, obtained values should be the same regardless of the perfusion rate.

Material and methods

Animals

Experiments were performed on domestic cats of both sexes weighing between 1.9 and 4.0 kg (n=10) in compliance with the Law on Animal Rights and Protection of the Republic of Croatia. Animal quarters were kept at a temperature of 23°C, with natural light–dark cycles, and entered between noon and two p.m. for cleaning and supplying fresh water and food. The animals were housed in individual cages and fed commercial cat food (SP215 Feline, Hill's, Topeka, Kansas, USA). Before any experimental procedure was undertaken, the cats were quarantined for 30 days.

The animals were anesthetized with chloralose (Chloralose, Fluka Chemika, Buchs, Switzerland, 100 mg/kg, i.p.) and the anesthesia was maintained by the administration of the anaesthetic via a polyethylene cannula in the femoral vein. The cats were positioned in a stereotaxic frame (Cat model, D. Kopf, Tujunga, California, USA) with their heads elevated, the external auditory meatus being at 15 cm above the stereotaxic table (sphinx position). Ventriculocisternal perfusion was performed with ^{3}H-inulin (spec.act. 1–5 Ci/mmol; Amersham, England) as an indicator substance dissolved in artificial CSF using the method of Heisey et al.[4], modified for use in cats[6]. A 22-gauge needle was placed by a micromanipulator into the left lateral ventricle at coordinates 4.5 mm anterior and 9 mm lateral to the zero point of the stereotaxic atlas, and 8–10 mm vertically from the dural surface, until free communication with the CSF was established. The needle was connected via a polyethylene tubing to a perfusion pump (Palmer, London, England) and the perfusion solution infused at a desired rate. From a second needle, which punctured the cisterna magna, 20-min samples of the perfusate were collected in glass tubes (Figure 1). The perfusion was performed for 60 min in order to stabilize the rate of perfusion prior to collecting the first sample, as well as after changing the perfusion rate. Intracranial pressure (ICP) was measured at the inflow needle, using a Statham strain gauge leading into a polygraph (7D, Grass, Quincy, Massachusetts, USA). ICP was adjusted at +20 cm H_2O by positioning the outflow tubing 20 cm above the external auditory meatus. The level of the external auditory meatus was taken as pressure zero.Ventriculocisternal perfusion were done at lower (65.5 μL/min) and higher perfusion rate (252.0 μL/min).

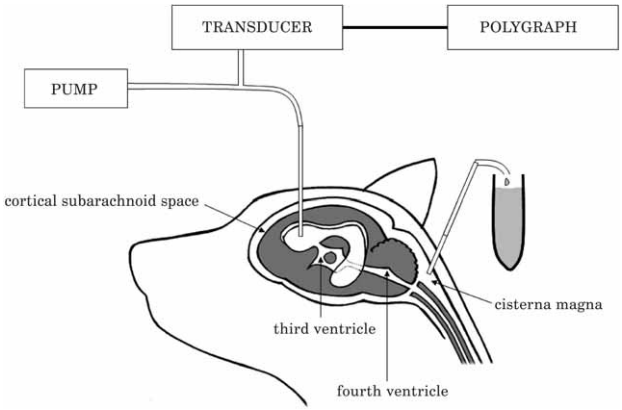

Fig. 1. Sheme of the ventriculocisternal perfusion in cats.

Body temperature was maintained at 37°C using an infrared lamp connected to an electronic thermometer placed in the rectum. The femoral artery was cannulated for blood pressure recording and blood sampling for acid-base balance determination. During the experiments, no significant changes in the monitored physiological parameters were observed while the cats were breathing spontaneously. At the end of experiment the animals were sacrificed by an anesthetic overdose.

After the collection, the samples were centrifuged at 3000 rpm for 5 min to remove particular matter, and radioactivity of sample (50 μL) was measured in 5 mL of Bray's solution by liquid scintilation counter (Beckman LS 1701, USA)

Calculation of CSF formation rate

The CSF formation rate (Vf) was calculated according to the equation derived from Heisey et al.[4]: Vf=Vi(Ci–Co)/Co, where Vi is the inflow perfusate rate, Ci is the concentration of the indicator substance in the inflow perfusate, and Co is the concentration of the indicator substance in the outflow perfusate. The calculation of Vf is based on the dilution of the indicator in the outflow perfusate. Therefore it is of utmost importance that the dilution of the indicator should be caused only by newly

TABLE 1
THE CONCENTRATION OF THE INDICATOR SUBSTANCE (^{3}H-INULIN) AND THE CEREBROSPINAL FLUID FORMATION (V_F) OBTAINED BY DIFFERENT VENTRICULOCISTERNAL PERFUSION RATES AT EXPERIMENTAL AND CORRECTED VALUES IN FIVE CATS

	EXPERIMENTAL VALUES Rate of perfusion		CORRECTED VALUES Rate of perfusion	
	65.5 μL/min	252.0 μL/min	65.5 μL/min	252.0 μL/min
Inflow concentration (C_i)	100%	100%	100%	100%
Outflow concentration (C_o)	74.0%	82.0%	81.4%	90.2%
Rate of CSF formation (V_f)*	23.0[a]	55.3[b]	15.0[c]	27.4[d]

*V_f difference (μL/min): b-d=27.9
a-c=8.0
d-c=12.4

formed CSF. Only in that case would the obtained result of CSF formation rate (Vf) by using equation of Heisey et al[4] be correct. The CSF formation rate (Vf) was expressed as μL of CSF per min. The correction of outflow concentration (C_{ocor}) was performed in the following manner (see Table 1); the experimental values of outflow concentration (C_o) have been increased for 10% i.e. 74.0 (C_o)+ 7.4(10%)=81.4 (C_{ocor}) at low or 82.0(C_o)+8.2(10%) =90.2 (C_{ocor}) at high rate of perfusion. For statisctical evaluation Student's t-test was used and $p<0.05$ was taken as statistically significant.

Results

An experiment in a single cat showed the effect of the perfusion rates on the calculated cerebrospinal fluid formation (Vf) within the entire cerebrospinal fluid system studied by ventriculocisternal perfusion at different time intervals (20 min) and the same intracranial pressure of +20 cm H_2O (Figure 2). The change in the perfusion rates from 65.5 μL/min to 252.0 μL/min resulted in an increase in the outflow concentration of ^{3}H-inulin and an increase in the calculated cerebrospinal fluid formation (Vf).

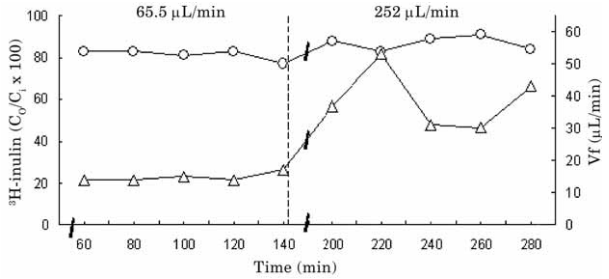

Fig. 2. Concentration of the indicator substance and the cerebrospinal fluid (CSF) formation rate at different perfusion rates presented in time. Effect of different ventriculocisternal perfusion rates (65.5 and 252.0 μL/min) on the CSF formation rate (V_f) at the same intracranial pressure (+20 cm H_2O) with ^{3}H-inulin in the artificial CSF in a single cat. Open circles show the outflow concentration of ^{3}H-inulin as a percentage of the inflow concentration (C_o/C_i x 100); the closed triangles show the calculated V_f at 20 min intervals. Vertical broken line represents the time when the rates of perfusion were changed.

We measured the outflow concentration of ^{3}H-inulin (C_o/C_ix100) and calculated cerebrospinal fluid formation (V_f) at different ventriculocisternal perfusion rates (65.5 and 252.0 μL/min) in five cats at the same intracranial pressure of +20 cm H_2O (Figure 3). An increase in the perfusion rate from 65.5 μL/min to 252.0 μL/min resulted in significant increase in the outflow concentration of ^{3}H-inulin and significant increase in the calculated cerebrospinal fluid formation. An analogous result was observed with perfusion initiated at 252.0 μL/min and subsequently reduced to 65.5 μL/min, i.e. decrease in outfow concentration of ^{3}H-inulin and the calculated cerebrospinal fluid formation.

Table 1 shows outflow concentration (C_o) and rate of CSF formation (V_f) obtained by ventriculocisternal perfusion with ^{3}H-inulin as indicator substance at low (65.5 μL/min) and high (252.0 μL/min) perfusion rates in five

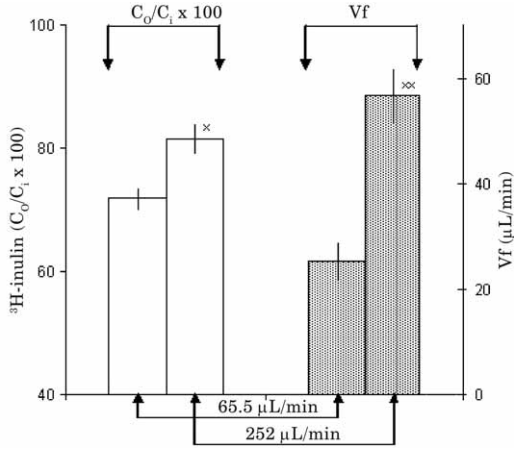

Fig. 3. Concentration of the indicator substance and the cerebrospinal fluid (CSF) formation rate at different perfusion rates.Outflow concentration of ^{3}H-inulin (open bars) as a percentage of the inflow concentration (C_o/C_i x 100) and the calculated CSF formation rate (V_f; closed bars) during different rates of ventriculocisternal perfusion (65.5 and 252.0 μL/min) with ^{3}H-inulin at the same intracranial pressure (+20 cm H_2O) in five cats. Each values represent mean ± S.E.M. for five cats. $^x p<0,05$; $^{xx} p<0.01$ versus low rate of perfusion.

cats. The results have shown, by experimental and corrected outflow concentration (C_o) of ^{3}H-inulin, that the increase in the perfusion rate (from 65.5 to 252.0 μL/min) resulted in increase in the outflow concentration of ^{3}H-inulin and increase in the calculated cerebrospinal fluid formation. The corrected values were attained in a way that experimental Co was increased for 10% of apsorbed indicator substance[7] i.e. from 74% to 81.4% at low perfusion rate, respectively from 82% to 90.2% at high perfusion rate, respectively. It can be seen that differences between experimental and corrected formation rates (V_f) are higher at high (55.3–27.4=27.9 μL/min) than at low perfusion rate (23.0–15.0=8.0 μL/ min). The corrected difference between high and low perfusion rate was 12.4 μL/min (27.2–15.0 μL/min).

Discussion

Generally accepted hypothesis of CSF physiology is based on formation, circulation and absorption of CSF as its crucial assumptions. The results of CSF formation and absorption obtained by Heisey method have been most frequently used as the key evidence to confirm such hypothesis. On the other hand, the authors of this method (Heisey et al.[4]) have determined the conditions to be met for the successful application of the method, although these conditions have actually not been the subject of serious scientific analysis. In the meantime some warranted doubts have been put forward regarding both the hypothesis itself[12–16] and the method of perfusion[6,8–10], therefore it is essential that both of them be a subject to a serious scientific evaluation. Among mentioned doubts is also the rate of ventriculocisternal perfusion. Namely, in accordance with proclamated conditions the calculation of cerebrospinal fluid formation rate (V_f) shoud be independent of perfusion rate used. Since it was shown by recent experiments[10] that Vf depends on ventriculocisternal perfusion rates, in this study it was analised if the reason for this unexpected results was the consequence of indicator substance behavior during perfusion. Because an indicator substance can pass from the CSF into brain parenchyma[7,8,12–14] we decided to investigate whether the indicator absorption is the cause of the observed change (dependance of Vf on the perfusion rate). Therefore the experiments were standarized for each cat; 60 minutes of steady-state period (see Figure 2), the same molecule was used as the indicator substance (^{3}H-inulin), the different perfusion rates were used always in the same cat, the experiments were conducted under a constant pressure of +20 cm H_2O, and ventriculocisternal perfusion was used in the same animal position (sphing position). The ^{3}H-inulin was chosen as indicator substance since the nature and amount of inulin absorption (10%) into surrounding tissue during ventriculocisternal perfusion is known[7,15].

Our study has shown that the calculated cerebrospinal fluid formation rate and outflow concentration of indicator substance (^{3}H-inulin) were affected by the changes in the perfusion rate (Figures 2 and 3). The same tendency in the behavior of the indicator concentration in the outflow perfusate (C_o) and the calculated cerebrospinal fluid formation rates (V_f) was observed (increased C_o and increased V_f) at lower (65.5 μL/min) as well as higher (252.0 μL/min) perfusion rates. An analogous phenomenon was observed when pefusion was initiated at higher (252.0 μL/min) and subsequently reduced to lower (65.5 μL/min) rate of perfusion, i.e. decrease in C_o and decrease in V_f.

When outflow concentration (C_o) was corrected for absorbed indicator (10%; Table 1), the V_f difference of 12.4 μL/min (d–c) still persisted, and calculated V_f at high perfusion rate was nearly two times greater (d/c= 1.8). By correction, the difference of V_f was diminished in correlation to experimental values (b/a=2.4) but was not annuled yet. Only if absorption of indicator into surrounding tissue was extrapolated to 17%, the calculation of Vf would be the same (10 μL/min) during the high and low perfusion rate. Thus, the absorption of inulin in the CSF system of a cat should be nearly two times higher (17%) than it really is (10%) in order to obtain equal calculated values of CSF formation at different perfusion rates (65.5 and 252.0 μL/min). If we take into consideration that in ventricular system besides indicator substance the water is also significantly absorbed[17], and that water absorption changes indicator concentration, we should ask ourselves what the outflow concentration (C_o) in ventriculocisternal perfusion really represents? It is obvious that this is not a new formation of CSF.

In conclusion, the obtained results have clearly demonstrated that the perfusion method by Heisey et al.[4] cannot be used with precision to calculate CSF formation (Vf) because an increase in the perfusion rate causes an increase in the calculated formation rate (V_f) and correction of experimental results for absorbed indicator substance does not abolish this difference in V_f at high perfusion rate.

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REFERENCES

1. ARTRU AA, Anesthesiology, 69 (1988) 541. — 2. HARNISH PP, SAMUEL K, Invest Radiol 23 (1988) 534. — 3. LORENZO AV, PAGE LK, WATERS V, Brain, 93 (1970) 679. — 4. HEISEY SR, HELD D, PAPPENHEIMER JR, Am J Physiol, 203 (1962) 775. — 5. PAPPENHEIMER JR, HEISEY SR, JORDAN EF, DOWNER JC, Am J Physiol, 203 (1962) 763. — 6. OREŠKOVIĆ D, KLARICA M, LUPRET V, VUKIĆ M, Neurosci Res Commun, 26 (2000) 69. — 7. CURRAN RE, MOSHER MB, OWENS ES, FENSTERMACHER JD, Exp Neurol, 29 (1970) 546. — 8. OREŠKOVIĆ D, BULAT M, Hydrostatic force in regulation of CSF volume. In: AVEZAT CJJ, VAN EIJNDHOVEN JHM, MAAS AIR, TANS JTJ (Eds), Intracranial pressure VIII (Springer Verlag, Berlin, 1993). — 9. MARTINS AN, NEWBY N, DOYLE TF, J Neurol Neurosurg, 40 (1977) 645. — 10. OREŠKOVIĆ D, KLARICA M, VUKIĆ M, MARAKOVIĆ J, Croat Med J, 44 (2003) 161. — 11. OREŠKOVIĆ D, VUKIĆ M, KLARICA M, BULAT M, Acta Neurochir, 95 (2005) 433. — 12. DAVSON H, WELCH K, SEGAL MB, Physiology and pathophysiology of the cerebrospinal fluid (Churchill-Livingstone, Edinburgh, 1987). — 13. FENSTERMACHER JD, PATLAK CS, BLASBERG RG, Fed Proc, 33 (1974) 2070. — 14. BERGSNEIDER M, Neurosurg Clin Am, 12 (2001) 631. — 15. VLADIĆ A, KLARICA M, BULAT M, Brain Res, 1248 (2009) 127. — 16. KLARICA M, OREŠKOVIĆ D, BOŽIĆ B, VUKIĆ M, BUTKOVIĆ V, BULAT M, Neurosci, 158 (2009) 1397. — 17. BULAT M, LUPRET V, OREŠKOVIĆ D, KLARICA M, Coll Antropol, Suppl 3 (2008) 45.

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MOGUĆA GREŠKA KOD ODREĐIVANJA STVARANJA CEREBROSPINALNE TEKUĆINE POMOĆU METODE VENTRIKULOCISTERNALNE PERFUZIJE KOD MAČKE

SAŽETAK

Količina stvorenog cerebrospinalnog likvora (CSF) se najčešće određuje pomoću ventrikulocisternalne perfuzije, metode koja se smatra jednom od najpreciznijih metoda. Tu metodu i jednadnadžbu za izračunavanje količine stvorenog likvora (V_f) su razvili Heisey i sur[4]. na temelju razređenja indikatora u perfuzatu. Oni su predpostavili da je razređenje indikatora u perfuzatu posljedica novostvorenog CSF, tj.veća količina stvorenog CSF će dovesti do većeg razređenja indikatora. Zbog toga je ta metoda indirektna i bilo koja greška koja dovodi do krive interpretacij stupnja razređenja indikatora će dovesti do upitnih i često kontradiktornih rezultata vezanih uz količinu stvorenog CSF. U skladu s Heiseyevom jadnadžbom, V_f nebi trebala ovisiti o brzini ventrikulocisternalne perfuzije. Međutim, pokazano je da V_f ovisi o brzini perfuzije[10] i da se za vrijeme perfuzije indikator također djelomično apsorbira u priležeće tkivo. Moguće je da je opažena ovisnost V_f o brzini perfuzije uzrokovana upravo opaženom apsorpcijom indikatora. Zbog toga je na anesteziranim mačkama vršena ventrikulocisternalna perfuzija kod visokih (252,0 μL/min) i niskih brzina perfuzije (65,5 μL/min) i izračunavana je V_f pomoću eksperimentalnih, kao i korigiranih vrijednosti indikatora, korigiranih upravo za količinu apsorbiranog indikatora. Budući da je (usprkos korekciji) dobivena razlika od 12,4 μL/min između niskih (15,0 μL/min) i visokih (27,4 μL/min) brzina perfuzije, očito je da se metoda ventrikulocisternalne perfuzije ne može uzeti u obzir kao pouzdana metoda za mjerenje količine stvorenog CSF.