CROATIAN MEDICAL JOURNAL
CMJ

44(2):161-164,2003

BASIC SCIENCES

Evaluation of Ventriculo-cisternal Perfusion Model as a Method to Study Cerebrospinal Fluid Formation

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Aim. To evaluate ventriculo-cisternal perfusion as a method for measuring cerebrospinal fluid formation rate, calculated by means of the Heisey et al equation.

Method. All experiments were carried out on anesthetized domestic cats fixed in the sphinx position in a stereotaxic frame. Ventriculo-cisternal perfusion was used at an intracranial pressure of -10 cm H_2O at different perfusion rates (32.0, 65.5, 125.0, and 252.0 μL/min). Dextran blue was applied as an indicator substance and the concentration of the indicator was measured with a spectrophotometer at a wavelength of 635 nm. Cerebrospinal fluid formation rate was calculated with the equation of Heisey et al.

Results. The indicator substance was less diluted at a higher perfusion rate, and the calculated cerebrospinal fluid formation rate was lower. The increase in perfusion rate from 65.5 to 125.0 to 252.0 μL/min increased the concentration of indicator substance from 0.75 to 0.89 to 0.97 mg/mL and decreased calculated cerebrospinal fluid formation rate from 21.8 to 15.4 to 7.8 μL/min. This reduction was linear and an increase in the perfusion rate by 1.0 μL/min decreased the cerebrospinal formation rate by 0.05 μL/min.

Conclusion. The calculated cerebrospinal fluid formation rate depends on different perfusion rates. The increase in the perfusion rate diminishes the calculated formation rate. Ventriculo-cisternal perfusion may not be a suitable method to calculate the cerebrospinal fluid formation rate according to the equation of Heisey et al.

Key words: *cerebrospinal fluid; intracranial pressure; spectrophotometry; stereotaxic techniques*

According to the generally accepted hypothesis of cerebrospinal fluid dynamics, cerebrospinal fluid circulates slowly from the brain ventricles towards the subarachnoid space cortex, where the venous sinuses absorb it through the arachnoid villi. Cerebrospinal fluid is produced within the cerebral ventricular system, mainly by the secretory activity of the choroid plexuses in the brain ventricles. Its passage through the choroidal epithelium is an active metabolic process – secretion, which makes cerebrospinal fluid secretion rather than ultrafiltrate. The cerebrospinal fluid formation rate in animals has been extensively studied by the ventriculo-cisternal perfusion technique, which is still regarded as the most precise method (1-4). This method and the equation for the calculation of the cerebrospinal fluid formation rate (Vf) 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 newly formed cerebrospinal fluid, ie, that a higher cerebrospinal fluid formation rate will result in a higher degree of dilution of the indicator substance. Therefore, any error in the interpretation of the degree of dilution of the indicator substance in the perfusate caused by other reasons (escape of indicator substances or water into the brain tissue, or irregular mixing) will result in questionable and often contradictory conclusions regarding cerebrospinal fluid formation rates (6-9).

In experimental studies of cerebrospinal fluid formation by the ventriculo-cisternal perfusion method, a wide range of perfusion rates have been used (4,10). However, questions arise as to whether different perfusion rates result in variable or stable calculated formation rates.

For this reason, we measured the perfusate outflow concentration of dextran blue, the indicator substance, using different perfusion rates in cats (32.0, 65.5, 125.0, and 252.0 μL/min), and compared the calculated formation rates. If the method were correct and precise, no significant differences should be found between the formation rates.

Material and Methods

Experimental Procedure

Experiments were performed in 6 domestic cats of both sexes, weighing between 1.9 and 4.3 kg. Animal quarters were kept at a temperature of 23°C, with natural light-dark cycles. Every day between noon and two p.m., the animal quarters were entered for cleaning and supplying fresh water and food. The animals were housed in individual cages sized 0.80x0.45x0.40 m and fed commercial cat food (SP215 Feline; Hill's, Topeka, KS, USA). Before any experimental procedure, the cats were quarantined for 30 days.

The animals were anesthetized with sodium thiopental (Nesdonal; Specia, Paris, France; 60 mg/kg, intraperitoneally) and the anesthesia was maintained by the administration of the anesthetic via a polyethylene cannula in the femoral vein. The cats were positioned in a stereotaxic frame (Cat model; D. Kopf, Tujunga, CA, USA) with their heads elevated, the external auditory meatus 15 cm above the stereotaxic table (sphinx position). Ventriculo-cisternal perfusion was performed with an indicator substance, dextran blue (Pharmacia, Uppsala, Sweden), dissolved in artificial cerebrospinal fluid (1 mg/mL) according to 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 from the zero point of the stereotaxic atlas, and 8-10 mm vertically from the dural surface, until free communication with the cerebrospinal fluid was obtained. The needle was connected via a polyethylene tubing to a perfusion pump (N° 6135; 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 (Fig. 1). The perfusion was allowed to proceed for a 60-min period to stabilize before the first sample was collected, as well as after the perfusion rate was changed. Intracranial pressure was measured at the inflow needle, with a Statham strain gauge feeding into a polygraph (7D; Grass, Quincy, MA, USA). Intracranial pressure was adjusted at -10 cm H_2O by positioning the outflow tubing 10 cm below the external auditory meatus. The pressure level of the external auditory meatus was taken as pressure zero. No significant changes of intracranial pressure were observed at different ventriculo-cisternal perfusion rates (32.0, 65.5, 125.0, and 252.0 μL/min).

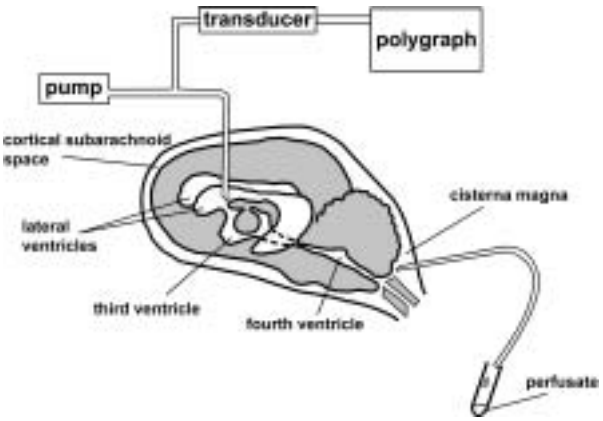

Figure 1. Scheme of the ventriculo-cisternal perfusion in cats.

Body temperature was maintained at 37°C by means of 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.

After collection, the samples were centrifuged at 3,000 rpm for 5 min to remove particular matter, and the optical density of the perfusate was measured with a spectrophotometer (55B; Perkin-Elmer, Norwalk, CT, USA) at a wavelength of 635 nm.

Calculation of Cerebrospinal Fluid Formation Rate

The cerebrospinal fluid formation rate (Vf) was calculated by 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 (sample; mg/mL). The calculation of cerebrospinal fluid formation rate is based on the dilution of the indicator in the outflow perfusate. For this reason, it is of utmost importance that the indicator does not diffuse from the perfusate into the surrounding nervous tissue. Therefore, dextran blue, as a large molecule ($2x10^6$ molecular weight), was used as the indicator substance in our experiments.

The calculated cerebrospinal fluid formation rate was expressed in μL of cerebrospinal fluid per min and the concentration of the indicator substance in the outflow perfusate as a percentage of the inflow concentration (Co/Ci x 100; Fig. 2) or mg of Dextran Blue per μL of perfusate (Fig. 3). For statistical comparison of the values, Kruskal-Wallis test was used. The correlation coefficient (Pearson's r) and regression analysis were used to compare changes in the calculated cerebrospinal fluid formation against different perfusion rates (65.5, 125.0, and 252.0 μL/min).

Results

An experiment in a single cat showed the effect of the four perfusion rates on the calculated cerebrospinal fluid formation (Vf) within the entire cerebrospinal fluid system studied by ventriculo-cisternal perfusion at different time intervals (20 min) and at the same intracranial pressure of -10 cm H_2O (Fig. 2). The change in the perfusion rates from 32.0 to 65.5, from 65.5 to 125.0, and from 125.0 to 252.0 μL/min resulted in an increase in the outflow concentration of dextran blue and a decrease in the calculated cerebrospinal fluid formation.

We measured the outflow concentration of dextran blue (mg/mL) and calculated cerebrospinal fluid formation during different ventriculo-cisternal perfusion rates (65.5, 125.0, and 252.0 μL/min) in five cats at the same intracranial pressure of -10 cm H_2O (Fig. 3). An increase

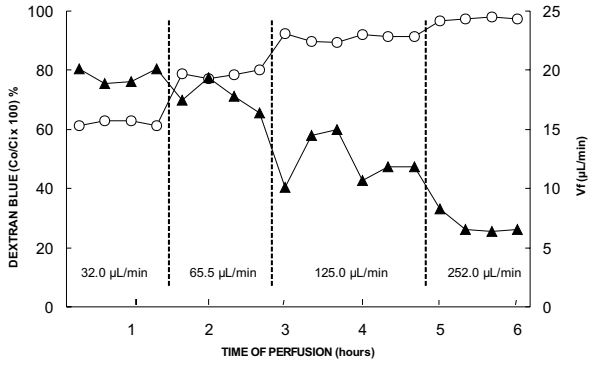

Figure 2. Concentration of the indicator substance and the cerebrospinal fluid (CSF) formation rate at different perfusion rates presented in time. Effect of different ventriculo-cisternal perfusion rates (32.0, 65.5, 125.0, and 252.0 μL/min) on the CSF formation rate (Vf) at the same intracranial pressure (-10 cm H_2O) with dextran blue in the artificial CSF in a single cat. Open circles show the outflow concentration of dextran blue as a percentage of the inflow concentration (Co/Ci x 100); the closed triangles show the calculated Vf at 20-min intervals. Vertical broken lines represent the time when the rates of perfusion were changed.

in the perfusion rate from 65.5 to 125.0 and to 252.0 μL/min resulted in a highly significant increase in the outflow concentration of dextran blue and a decrease in the calculated cerebrospinal fluid formation. An analogous result was observed with the perfusion initiated at 252.0 μL/min and subsequently reduced to 125.0 and 65.5 μL/min, ie, a decrease in the outflow concentration of dextran blue and an increase in the calculated cerebrospinal fluid formation.

We observed the effects of different perfusion rates (65.5, 125.0, and 252.0 μL/min) on the cerebrospinal fluid formation on fourteen samples in five cats at the same intracranial pressure of -10 cm H_2O (Fig. 4). When the calculated cerebrospinal fluid formation rate was plotted against different perfusion rates, a linear relationship was obtained ($r = -0.65$, $p < 0.01$), ie,

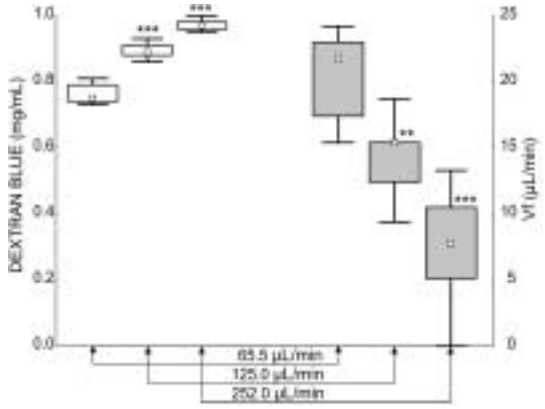

Figure 3. Concentration of the indicator substance and the cerebrospinal fluid (CSF) formation rate at different perfusion rates. Outflow concentration of dextran blue (mg/mL; open square) and the calculated CSF formation rate (Vf; closed square) during different rates of ventriculo-cisternal perfusion (65.5, 125.0, and 252.0 μL/min) with dextran blue at the same intracranial pressures (-10 cm H_2O) in five cats. Each box shows the median value of 25 samples (**$p < 0.01$; ***$p < 0.001$). The analysis was carried out with the Kruskal-Wallis test. Small square – median; large square (open or gray) – interquartile range; error bars – non-outlier minimum and maximum.

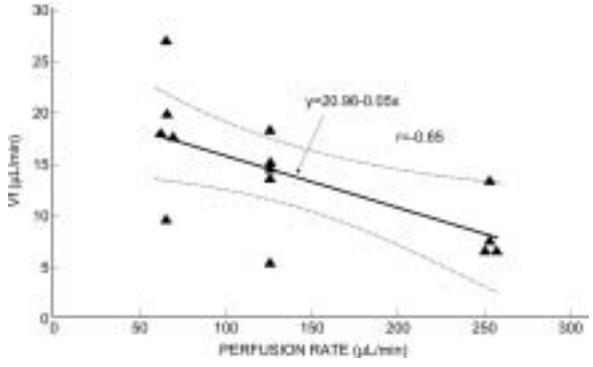

Figure 4. Effect of different perfusion rates on cerebrospinal fluid (CSF) formation rate. CSF calculated formation rate (Vf; μL/min) plotted against the 65.5, 125.0, and 252.0 perfusion rate (μL/min) with the correlation coefficient (r) and the equation of the regression line at the same intracranial pressure (-10 cm H_2O). Each triangle represents the CSF formation rate (Vf) for a single cat. The broken lines show 95% confidence intervals.

an elevation in the perfusion rate from 65.5 to 125.0, and further to 252.0 μL/min resulted in decreased calculated cerebrospinal fluid formation.

Discussion

Our study showed that the calculated cerebrospinal fluid formation rate and the outflow concentration of indicator substance (dextran blue) were affected by the changes in the perfusion rate. An increase in the perfusion rate significantly diminished the calculated cerebrospinal fluid formation rate and increased the outflow concentration of indicator substance studied by ventriculo-cisternal perfusion in the whole cerebrospinal fluid system. The decrease of calculated cerebrospinal fluid formation rate was linear and an increase in the ventriculo-cisternal perfusion rate by 1.00 μL/min resulted in a decrease in formation rate by 0.05 μL/min.

The experiments were standardized for each cat: 60 min of steady-state period, the same large molecule was used as the indicator substance, ventriculo-cisternal perfusion was used under the same animal position, and the different perfusion rates were used always in the same cat. The change of the perfusion rate was the only exemption; all other experimental parameters were the same. For this reason, the experiments were done under a constant pressure of -10 cm H_2O of intracranial pressure, because a free, equilibrated, constant, and long-lasting escape (6 h) of the newly formed cerebrospinal fluid is established (16 μL/min; ref. 16) at this pressure in cats (17). In addition, the ventriculo-cisternal perfusion method with perfusion rates of 65.5, 125.0, and 252.0 μL/min was chosen to evaluate the results, because at the lowest rate more time (about 2 h) was spent to reach the steady-state values. The same tendency in the behavior of the indicator concentration in the outflow perfusate (Co) and the calculated cerebrospinal fluid formation rates (increased concentration of dextran blue and decreased calculated cerebrospinal fluid formation rate) was observed at lower (32.0 and 65.5 μL/min) as well as higher (125.0, and 252.0 μL/min) perfusion rates.

Assuming that the cerebrospinal fluid formation rates are constant (16 μL/min; ref. 16), we expected that, at a constant intracranial pressure (-10 cm H_2O), the same volume of cerebrospinal fluid per time unit should be available to dilute the indicator substance during perfusion. If higher perfusion rates were used (e.g., 125.0 instead of 65.5 μL/min), then more indicator per time units would flow through the cerebrospinal fluid system, and in the same period of time, there would be more indicator substance for dilution by the same volume of cerebrospinal fluid. However, according to the equation of Heisey et al (4), at the highest perfusion rate (ie, highest Vi), the outflow concentration of the indicator (Co) should increase, so that the formation rate (Vf) remains unchanged. That can be simply shown by including the experimental values (Vf = 16 μL/min; Vi = 125.0, and 252.0 μL/min; Ci = 1 mg/mL) in the equation for the outflow concentration (Co; derived from equation of Heisey et al; ref. 4) where:

$Co = ViCi/(Vf + Vi)$

The Co values obtained by the calculation were 0.80 mg/mL at a perfusion rate of 65.5 μL/min, 0.88 mg/mL at a perfusion rate of 125.0 μL/min, and 0.94 mg/mL at a perfusion rate of 252.0 μL/min. But at the same time, the measured Co values under the same perfusion rates as calculated were 0.75, 0.89, and 0.97 mg/mL. The observed discrepancy between the calculated and the experimental Co might be caused by different mechanisms, which are difficult to detect and control. Three possible mechanisms that could have caused it are variable mixing of the cerebrospinal fluid across the whole cerebrospinal fluid system and the indicator in the perfusate, the different spread of perfusate through the cerebrospinal fluid system, and possible different absorption of the indicator or water from the perfusate into the surrounding tissue. Although there is no final answer, the fact remains that an increase in the perfusion rate decreases the calculated cerebrospinal fluid formation rate.

The results obtained by the perfusion method in the studies of the same physiological phenomenon have often been contradictory, e.g., when the influence of intracranial pressure on calculated cerebrospinal fluid formation rate were studied (1,4,10-13). However, ventriculo-cisternal perfusion persists despite its limitations as the most reliable method to determine the cerebrospinal fluid formation rate, and the major body of knowledge available to explain the classical hypothesis of cerebrospinal fluid formation rate rests on this method. These contradictory results have been explained by the loss of fluid distally to the fourth ventricle (14), an incomplete mixing of the newly secreted cerebrospinal fluid and the perfusion fluid (9), effect of pressure (upon +30 cm H_2O) exerted at the first stage of the cerebrospinal fluid formation – ultrafiltration across the choroidal capillaries and partly variation in Vf among animals (15,16). Our experiments indicate that these contradictory results are at least partly a consequence of the different perfusion rates used in different studies, which has not been previously evaluated.

In conclusion, our results showed that the ventriculo-cisternal perfusion model as a method to study the cerebrospinal fluid formation and calculate cerebrospinal fluid formation rate could be used as a precise method, because an increase in the perfusion rate causes a decrease in the calculated formation rate (Vf).

Acknowledgment

We thank Mrs Katarina Karlo for her skilled technical assistance. This work has been supported by the Ministry of Science and Technology, Republic of Croatia (Project No. 0098069).

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Received: July 24, 2002

Accepted: January 31, 2003

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