Cerebral Blood Flow and Effects of Cerebrospinal Fluid on Calcium Transport in Patients With Cerebral Infarction

S.E. Akopov, MD; G.S. Grigorian, MD; E.S. Gabrielian, MD

**Background and Purpose** In this study we investigated whether cerebrospinal fluid in patients with brain infarction possesses an activity that contributes to the evolution of brain ischemia. As a test, the effect of cerebrospinal fluid on Ca$^{2+}$ influx into the intracellular space was chosen because this process is a mechanism for vasospasm, platelet aggregation as thrombi, and neuron damage.

**Methods** Effects of cerebrospinal fluid taken from 48 patients with cerebral hemispheric infarction on the concentration of cytosolic free Ca$^{2+}$ in platelets were studied using the fluorescent probe quin-2. Hemispheric cerebral blood flow was measured using $^{133}$Xe intravenous injection.

**Results** Cerebrospinal fluid in 19 of 48 patients with cerebral hemispheric infarction increased the level of cytosolic free Ca$^{2+}$ in platelets. The course of the disease in the patients who showed a positive effect of cerebrospinal fluid on Ca$^{2+}$, when compared with that of patients who showed a negative effect, was characterized by a more severe clinical manifestation and mortality. The decrease in hemispheric cerebral blood flow was more marked in both ischemic and contralateral hemispheres in patients with positive effects of cerebrospinal fluid on the level of Ca$^{2+}$.

**Conclusions** These data suggest that the ability of cerebrospinal fluid to evoke Ca$^{2+}$ influx into the intracellular space in patients with brain infarction is a factor that aggravates ischemic brain damage. (Stroke. 1994;25:608-610.)

**Key Words** • cerebral blood flow • cerebrospinal fluid • calcium

**Subjects and Methods**
A total of 48 patients (27 men and 21 women, aged 45 to 60 years) with hemispheric infarction were observed for 3 days after the onset of symptoms. The diagnosis of cerebral infarction was based on history, full neurological examination, and lumbar puncture. Computed tomographic scanning was not routinely undertaken except when there was doubt about the clinical diagnosis. The inclusion criterion was acute onset of neurological deficit due to a presumed vascular occlusion. The deficit was required to be moderate to severe, lasting more than 24 hours. The clinical exclusion criteria were past medical history of focal neurological disease with residual deficit, presence of any disease that might interfere with the assessment of disability (eg, dementia, parkinsonism); presence of a major life-threatening illness that might interfere with survival (diabetes, myocardial infarction, renal failure, malignant hypertension, etc); evidence of cerebral hemorrhage or brain tumor; and rapid regression of symptoms. CSF samples were withdrawn during lumbar puncture performed at 48 to 72 hours after the onset of stroke. In all cases, CSF samples were without red blood cell staining and xanthochromia. The routine analyses of the CSF samples were normal: pH, 7.31 ± 0.02; osmolarity, 296 ± 5 osm/L; proteins, 48.5 ± 7.9 mg/dL; and cell count, 4 to 9 cells per cubic millimeter. As a control, 23 samples of CSF from patients without neurological disease (mean age, 49±4 years) were used. Samples of CSF were taken during operation performed with patients under spinal anesthesia; samples were withdrawn before anesthetic injection.

Hemispheric CBF (CBFh) was measured using $^{133}$Xe intravenous injection and the multidetector device BI-1400 (Valmet, Finland) according to Austin et al. CBFh was calculated as a mean of measurements of CBF in 12 regions of the ischemic hemisphere and in 12 symmetrically placed regions in the contralateral hemisphere. There was no difference between the level of PaO$_2$ and PaCO$_2$ in the patients during the CBF investigation. As a control, CBF was studied in 29 healthy volunteers (mean age, 48 ± 5.2 years) without a history of diseases associated with increased risk for cerebrovascular
The concentration of cytosolic free Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) was studied by Rink et al\(^8\) with the fluorescent probe quin-2. The platelets were isolated from freshly drawn human blood and resuspended in the standard phosphate buffer at a final concentration of 200,000 cells per microliter. The measurements of [Ca\(^{2+}\)]\(_i\) level were performed in the buffer (normal [Ca\(^{2+}\)]\(_i\) level) and in the buffer with tested CSF (20% vol/vol). Intracellular quin-2 fluorescence was monitored using an RF-540 Shimadzu spectrofluorometer. Effects of CSF on [Ca\(^{2+}\)]\(_i\) were determined in duplicate. CSF activity was measured by a specialist who did not know which CSF samples were withdrawn from control patients or patients with stroke. Differences between means were analyzed by Kruskal-Wallis test (nonparametric ANOVA) using SPSS PC+ statistical software.\(^9\)

Results

Control CSF samples did not change [Ca\(^{2+}\)]\(_i\) in platelets; however, in 19 of 48 (39.6%) patients with cerebral infarction, the ability of CSF to increase [Ca\(^{2+}\)]\(_i\) in platelets was observed (Figure). The Table presents the comparative clinical and CBF characteristics of patients with cerebral infarction depending on CSF ability to increase [Ca\(^{2+}\)]\(_i\) level. The severity of disturbances of consciousness and mortality were more evident in patients with a positive effect of CSF on [Ca\(^{2+}\)]\(_i\). These patients tended to have a poorer clinical course.

Analysis of the values of CBF in the patients showed that in patients with CSF-positive effects, CBF\(_h\) was lower than with CSF-negative effects (Table). It should be noted that in the patients with CSF-positive effects on [Ca\(^{2+}\)]\(_i\), the total depression of CBF was revealed in both ischemic and contralateral hemispheres, whereas in the patients with CSF-negative effects the decrease in CBF was observed only in some regions, mainly in the ischemic hemisphere.

Discussion

Ca\(^{2+}\) influxes from the extracellular fluid into the intracellular space is an important phenomenon in different mechanisms of cerebral ischemia. In vascular smooth muscle, Ca\(^{2+}\) influx leads to vessel constriction and increased vascular resistance,\(^3\) delaying reperfusion that typically follows ischemia.\(^10\) A shift of Ca\(^{2+}\) in platelets results in their activation and aggregation as microthrombi.\(^4\) These processes in the microcirculatory bed in the ischemic region seem of importance in aggravating ischemic brain damage, resulting in an increase in infarct size.\(^11\) In addition, Ca\(^{2+}\) influx into neurons and synapses (calcium overload) caused by disturbances of cellular ion pumps that maintain intracellular calcium homeostasis is considered a mechanism of the ischemic damage of brain tissue.\(^12,13\) Calcium overload exerts many detrimental effects, particularly an increase in neural arachidonic acid content that leads to the production of eicosanoids,\(^14\) which are capable of causing circulatory disturbances and cell damage.\(^15\)

Based on these data, it may be suggested that a factor that is able to enhance Ca\(^{2+}\) influxes might aggravate brain damage during cerebral ischemia. In this study, we discovered such a factor (factors) in the CSF of some patients with cerebral infarction. The importance of this factor in the evolution of brain infarction is confirmed.
by our investigation of CBFh. Patients with CSF ability to provoke calcium influx into the intracellular space were characterized by the more marked decrease in CBF. CBF in these patients was significantly reduced in both ischemic and contralateral hemispheres, whereas in patients without such CSF activity the decrease in CBF was localized in a part of the ischemic hemisphere. It is probable that the ability of CSF to provoke calcium influxes might lead to the spread of circulatory disturbances in the brain. Accordingly, these patients had severe clinical manifestation and mortality (Table).

Although we demonstrated CSF effects on \( \text{Ca}^{2+} \) fluxes only in platelets, it is not unlikely that in vascular smooth muscle and neurons CSF would evoke similar \( \text{Ca}^{2+} \) transport in platelets are similar to those in the vascular smooth muscle\(^6\) and the monoaminergic neuron.\(^3\) Hence, platelets might be considered as a convenient laboratory assay system indicating the accumulation in CSF of factors that can provoke calcium overload in the brain. However, more experimental studies are needed to clarify a relation between calcium fluxes in platelets and in different brain structures.

The CSF activity appears to be related to the entrance of some substances from the damaged brain tissue into CSF. The nature of these substances remains unclear. Recently, the role of platelet-activating factor as a key mediator of calcium-dependent neuroinjury in ischemia-related disorders has been established.\(^16\) Endogenous production of platelet-activating factor in neural tissue was found to be elevated more than 20-fold after ischemia and reperfusion, resulting in disorders of cerebral circulation and metabolism.\(^17\) Platelet-activating factor also provokes strong platelet activation with an increase in [\( \text{Ca}^{2+} \)]\(_{\text{cyt}}\);\(^12\) hence, it might be assumed that platelet-activating factor can be one of the factors that are released into CSF after stroke and that determine CSF-induced calcium influx in platelets. It is likely that accumulation of such factors in CSF is a mechanism for generalization of circulatory disorders in the brain after stroke, resulting in total depression of CBF in both hemispheres and the aggravation of neurological deficit as it was observed in this study. Certainly, these suggestions are not entirely confirmed and need further investigation.

References
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