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Progressive ventricular enlargement in the absence of high ventricular pressure in an experimental neonatal rat model

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Abstract *Object:* In the present study, we compared ventricular pressures (VP) and the progression of ventricular enlargement in a new experimental neonatal hydrocephalus model, to gain an understanding of how communicating hydrocephalus progresses. *Methods:* Kaolin was injected into the subarachnoid space at the cranial convexity of neonatal rats. Gross examination was performed on the 3rd, 5th and 7th days, and ultrasonographic examination on the 15th day, and at the end of the 1st and 2nd months following the kaolin application. Ventricular size indexes (VSI) were calculated in the case of a large ventricular dilatation. VPs were assessed on the 15th day, and at the end of the 1st and 2nd months, with a computerized data acquisition system. *Conclusions:* In the 1st and 2nd months VSIs were significantly higher than in control rats on the 15th day after kaolin administration. VP on the 15th day was significantly increased compared with that in control rats. VP in the 1st month was still high, but had subsided. In the 2nd month VP was not increased over control. In the late stages, the progression of infantile communicating hydrocephalus is not related to VP levels.

Keywords Experimental rat model · Progressive communicating hydrocephalus · Ventricular enlargement · Ventricular pressure

Introduction

Parenchymal ischemia has been confirmed to cause progression of ventricular enlargement [2, 8, 10, 13, 22, 28]. There are also reports proving that intracellular and extracellular metabolic changes effect the progression of ventricular enlargement [3, 5, 9, 17, 19]. In our previous study in a neonatally acquired hydrocephalus model [7], we were unable to demonstrate conspicuous reduced blood support, but there was progression in the ependymal layer destruction. This has led to doubt as to whether clinicopathological progression is a consequence of ischemia. An initial acute phase with hypertensive hydrocephalus and a late phase with chronic normotensive hydrocephalus in adult dogs have been reported [14]. In order to understand how neonatally acquired communicating hydrocephalus progresses, we needed to investigate the correlation between ventricular pressure (VP) and progression of the ventricular enlargement in same experimental neonatal rat model.

Materials and methods

Animal preparation

The Medical and Surgical Research Center of Osmangazi University and the Committee on Animal Experiments of the Medical Faculty of Osmangazi University approved the present experimental study. All experimental procedures were performed in accordance with the National Institute of Health Principles of Laboratory Animal Care.

Neonatal Sprague-Dawley rats 6–7.5 g in weight were selected from a group of 2- to 3-day-olds. They were fixed on the table and

Table 1 Summary of injection and examination data (*d* days, *K* kaolin, *m* months, *VP* ventricular pressure, *V* ventricle dimension) Number of the rats

Group	Timing of procedure	VP	Status of V[a]
K+3d	3 days after kaolin injection	0	7
K+5d	5 days after kaolin injection	0	7
K+7d	7 days after kaolin injection	0	7
K+15d	15 days after kaolin injection	14	14
K+1m	1 month after kaolin injection	8	8
K+2m	2 months after kaolin injection	7	7
N+3d	3 days after normal saline injection	0	3
N+5d	5 days after normal saline injection	0	3
N+7d	7 days after normal saline injection	0	3
N+15d	15 days after normal saline injection	5	5
N+1m	1 month after normal saline injection	5	5
N+2m	2 months after normal saline injection	5	5

[a] Gross observation or ultrasonographic examination of the ventricles

their scalps were prepped with Betadine solution, after which a 26-G needle was inserted percutaneously through the fontanel into the left subarachnoid space at the cranial convexity. Kaolin, 0.03 ml (200 mg/ml), was then injected over a period of 5 s. The control rats each had 0.03 ml of normal saline injected into the subarachnoid space in the same fashion. All of the rats were weaned at the age of 30 days and then allowed free access to food and water. Rats were anesthetized with intramuscular xylazine hydrochloride (5 mg/kg) and ketamine (30 mg/kg) for the subsequent processes.

Gross and ultrasonographic examination

On the 3rd, 5th, and 7th days (7 rats in each group), after the injections of kaolin and normal saline, the rats were decapitated and their scalps stripped. The craniums were then placed in the 10% neutral buffered formalin for 5 days. Coronal sections through the perpendicular line from the posterior edge of fontanel or the coronal suture were used to observe lateral ventricles.

To calculate the VSI, rats were each subjected to a real-time ultrasonographic examination with a 5-MHz transducer (SSA-340A, Toshiba) on the 15th day (n=14) and at the end of the 1st (n=8) and 2nd (n=7) months. A coronal ultrasonographic view of the lateral ventricles was obtained through the fontanel with the help of a water bag. The ventricular and cerebral sizes were measured, and VSI was calculated as the ratio of the largest ventricular diameter to maximal biparietal brain diameter.

Assessment of the intraventricular pressure

VP was examined in the rats in which the physiological range (including blood pressure and P_aCO_2) was maintained. For the assessment of the VP on the 15th day (n=14) and at the end of the 1st (n=8) and 2nd months (n=7), a computerized data acquisition system (MP 100, Biopac) and pressure transducer (RX 104A, Biopac) were used. After the rats had been anesthetized, they were placed in the stereotactic head holder with their heads in a sphinx position, and their body temperature was maintained at 37±0.5 C with a heat lamp connected to a rectal probe. Blood pressure in the femoral artery was continuously monitored. To ensure P_aCO_2 at between 36 and 40 mmHg, spontaneous or mechanical ventilation was maintained. A saline-filled 26-G needle of the pressure transducer was inserted through a small drill hole at the coronal suture into the lateral ventricle and stabilized pressure values were recorded as centimeters of H_2O. On the 3rd, 5th, and 7th days after injection of kaolin and normal saline, VPs were not assessed owing to technical problems for the maintenance of physiological range, because their weight and body volume were very small.

Groups and statistical analysis

The rats were divided into groups based on the type of procedure (Table 1). If there was a measurable hydrocephalic ventricular enlargement, VSI was calculated by ultrasonographic examination. For statistical analysis, the results were expressed as means ± standard deviation; mean differences between groups were calculated using one-way analysis of variance (ANOVA) and the two-tailed Student's *t*-test.

Results

Gross and ultrasonographic examination

On the 3rd day after the kaolin injection there was no ventricular enlargement, but kaolin bulk and fibrosis were visible in the subarachnoid space at the cranial convexity (Fig. 1a). On the 5th day, only 2 rats showed minimal ventricular changes. On the 7th day, 2 rats showed conspicuous ventricular enlargement, with an average VSI of 0.54; 2 rats had minimal ventricular changes; in 3 rats there was no ventricular change.

VSI values calculated with ultrasonographic examination in kaolin injection groups are shown in Fig. 2 and Table 2. All rats in kaolin-injected groups (after 15th days and in 1st month and 2nd months after injection) showed conspicuous ventricular enlargement. Changes in ventricles of the kaolin-injected groups are shown in Fig. 1b. VSIs in the 1st and 2nd months showed a significant increase from 15 days after the injection (P<0.001). In control groups there were no changes in ventricles or cerebrum.

Ventricular pressure values

VP values in kaolin and saline injection groups are shown in Fig. 3 and Table 2. On the 15th day there was a statistically significant increase in the kaolin group compared with the 1st and 2nd month kaolin-injected rats and with all of the normal saline groups (P<0.01 and P<0.05). On the 15th day, VP values were also above 4 cmH_2O in 6 of the kaolin-injected rats (K+15d). The high VP values in the 2nd week subsided at the end of 1st month and then returned to normal pressure values at the end of 2nd month. VP in the 1st month was still high compared with VP in the control group of the same period (P<0.05). At the end of the 2nd month there was no difference between the kaolin-injected rats and the control group (P>0.05).

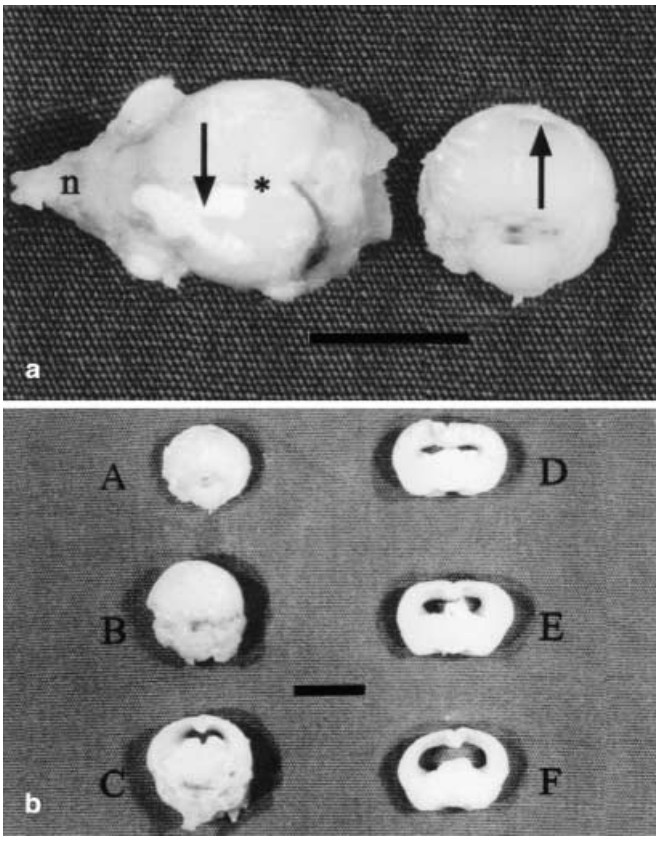

Fig. 1 **a** Photographs showing gross observation from external view (*left*) and from a coronal section (*right*) in rats 3 days after kaolin injection. Kaolin bulk and fibrosis at the injection site are clearly visible (*arrows*). (*n* nasion, *midline, sagittal sinus, *bar* 1 cm). **b** Photograph showing changes in the ventricles of kaolin injected rats. Coronal sections in *A, B,* and *C*. Sections in *D, E* and *F* were obtained from brains embedded in paraffin after transcardial perfusion with 10% neutral buffered formalin. (*K* kaolin, *d* days, *m* months). *A* K+3d, *B* K+5d, *C* K+7d, *D* K+15d, *E* K+1m, *F* K+2m, *bar* 1 cm

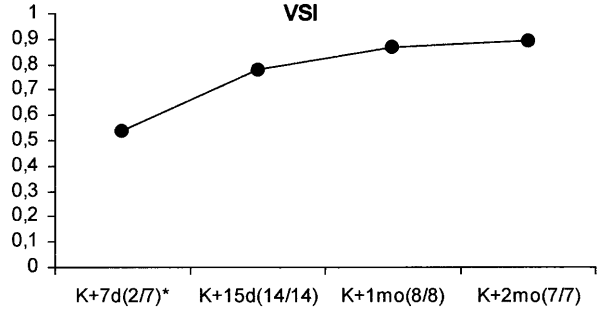

Fig. 2 Graph showing the ventricular size index (*VSI*) in rats with conspicuous ventricular enlargement. *Ventricular dilatation had a measurable diameter in only two rats, and two of the reminder had minimal ventricular dilatation

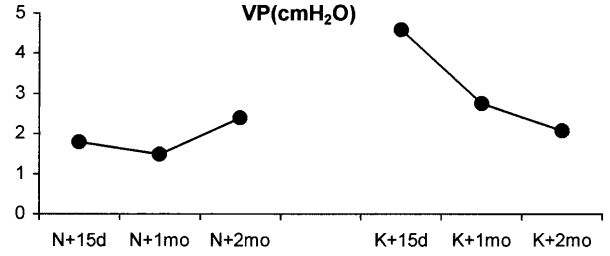

Fig. 3 Graph showing the ventricular pressure (*VP*) values in rats 2 weeks, 1 and 2 months after injection

Table 2 VP and VSI values in rats after injections

Groups	VP (cm H_2O)	VSI
K+15d	4.58±1.79	0.78±0.05
K+1m	2.74±0.88	0.87±0.06
K+2m	2.07±0.36	0.89±0.04
N+15d	1.78±0.93	Normal[a]
N+1m	1.48±0.43	Normal[a]
N+2m	2.38±1.20	Normal[a]

[a] Ventricular dimensions were too small for measurement

Discussion

Although not all the rats had a conspicuous ventricular enlargement at the 1st week after kaolin application, our results showed that changes in ventricular size and pressure had begun in the early stages. We observed an increase in VP at the early stage (K+15d) and a decrease to a steady state at the later stages. High VP subsided in the 1st month, and then returned to normal pressure levels in the 2nd month. Ventricular enlargement, however, did not subside but continued despite lowering of the VP. Previous results in this model have shown that cerebral blood support is undiminished despite high vascular resistance, and that there is advanced ependymal layer destruction in the chronic stage [7].

Pathophysiology of ventricular enlargement

Pathophysiological changes in the hydrocephalic animals occur as early as 12 h after the restriction of the normal flow of CSF [11]. Some authors have emphasized a progressive damage in cerebral white matter and ependymal layer with ventricular enlargement [8, 10, 14, 24]. Cellular destruction may be a possible consequence of the further ventricular enlargement [2, 8, 10]. Hakim's hypothesis stresses that hydrocephalus can develop and progress only if the brain decreases in size and that such a decrease is due to water being squeezed from the extracellular space [15, 26]. Compromising diffusion and perfusion of the extracellular molecules has been demonstrated to

cause an accumulation of waste products [9, 23]. Neurochemical abnormalities in the extracellular space and the consequent breakdown of cellular metabolism may be the main cause of the progression [4, 15, 17, 19, 27].

Value of ventricular pressure

High VP in the early stage and no supporting sign of ischemia in any stage in our previous study may explain why ventricles continue to enlarge in infantile communicating hydrocephalus. Ventricles initially enlarge as a result of high CSF accumulation associated with high VP. It has been reported that brain tissue destruction is inevitable when ventricles are enlarged [17]. Even if the VP begins to return to normal levels, enlarged ventricles may not return to the previous normal diameters. A fundamental question is why a VP should return to normal values. The alteration of the viscoelastic modulus of the brain, a consequence of the squeezing out of the extracellular water from the brain parenchyma and the structural changes in brain tissues due to prolonged overstretching, in its turn may have caused low VP [25].

Timing of surgery

There are a variety of options open to neurosurgeons for the management of hydrocephalus [18]. We may encounter some cases of hydrocephalus with progressive cerebral destruction and increasing ventricular size despite normal VP or shunt insertion [6, 16, 20, 21]. VP need not, therefore, be the only criterion in the choice of treatment modalities at the chronic stage. Our results showed that the size of the ventricles increases as the VP levels off toward normal values. If the VP returns to a normal level in the late stage, it will not be appropriate to base the selection of treatment modalities on the VP level. It has also been reported that a child may present confusing and unpredictable progressive symptoms related to conditions that are nothing to do with shunt malfunctions [12]. Although cerebral tissue reconstitution after shunt procedure has been presented [1, 29], in infantile communicating hydrocephalus progression resulting from irreversible ventricular enlargement may be the main cause of unsuccessful shunt procedures. Before VP subsides in infantile communicating hydrocephalus, shunt insertion has to be performed within a limited time, even urgently [21, 24].

Conclusion

Progression of the ventricular enlargement in neonatal and infantile acquired hydrocephalus is not dependent on VP levels in the late stages. We believe that further investigations on cellular tissue damage and neurochemical changes, and knowledge of the results of further time-related shunt insertions in this neonatal rat model would make a valuable contribution to our understanding of the pathogenesis of infantile communicating hydrocephalus.

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