Extracellular Space Volume Changes and Diffusion Barriers in Rats with Kaolin-Induced and Inherited Hydrocephalus

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Introduction

Hydrocephalus is associated with progressive ventricular dilation leading to brain compression and cerebral ischemia. Both compression and ischemia are associated with brain damage-related ionic changes and amino acid release resulting in fast and pulsatile or long-term cellular (particularly glial) swelling. Cellular swelling is compensated for by extracellular space (ECS) volume shrinkage and by a decrease in the apparent diffusion coefficients (ADC) of

neuroactive substances diffusing in the ECS (see scheme in Fig. **2B**). Pathological changes in cortical gray matter during hydrocephalus vary with age at onset. The increase in cerebrospinal fluid (CSF) volume and pressure causes damage to the surrounding brain tissue and results in an ECS volume decrease, presumably due to both mechanical compression and cell swelling. However, an increase in ESC volume due to secondary brain edema can also occur. To date, the diffusion parameters of hydrocephalic brains of animals and humans were only examined by diffusion-weighted NMR, which can determine the apparent diffusion coefficient of water (ADC_W) in the tissue. This method, however, is unable to measure changes in ECS volume, nor does it distinguish sufficiently between intra- and extracellular diffusion.

Methods

In our study, the degree of compression or expansion of the extracellular space and the extent of diffusion barriers in the extracellular fluid were determined *in vivo* in anesthetized animals using the real-time iontophoretic tetramethylammonium (TMA^+) method. This method measures the apparent diffusion coefficient of TMA (ADC_{TMA}) – a tracer for which the cell membranes are impermeable – and allows the calculation of the extracellular space volume fraction (α = ECS volume/total tissue volume) (2). The apparent diffusion coefficient of water (ADC_W) in the tissue was determined by diffusion-weighted H-1 MR imaging (Bruker 4.7 Tesla spectrometer). The water content was estimated from T_2 relaxation times. The animals were perfused after all measurements, and the extent of hydrocephalus was also established histologically.

Two different animal models of hydrocephalus were used: kaolin-induced hydrocephalus as a model of adult obstructive hydrocephalus and H-Tx rats with inherited hydrocephalus as a model of infantile obstructive hydrocephalus (1). The H-Tx strain (obtained from H. Jones, Univ. of Florida, Gainesville, USA) has a high incidence of pups with inherited hydrocephalus, due to obstruction of the cerebral aqueduct in late gestation. Adult obstructive hydrocephalus was induced by the injection of a kaolin suspension (KI) into the cisterna magna. Two groups of rats were established, depending on the volume of kaolin suspension (KS) administered (0.03 ml or 0.08 ml). TMA and MR measurements were made in the cortex and in the subcortical white matter 24 hours and 7 days after KI. MR measurements of ADC_W and T_2 values were performed in 1 mm thick coronal slices. The H-Tx rats with inherited hydrocephalus were studied 21 days after birth. Hydrocephalus in H-Tx hydrocephalic rats starts to develop in late gestation, and an extreme ventricular dilatation and cortical compression follows.

Results

The results obtained in adult rats with kaolin-induced hydrocephalus are shown in Table **1** and Fig. **1**. Cortical thickness decreased at 24 hours and further at 7 days after kaolin injection. Twenty-four hours after KI, we found a significant decrease in α in the cortex, an increase in ADC_{TMA}, but no significant change in ADC_W or T_2 relaxation times. The mean α in control animals was about 0.20 and ADC_{TMA} about 0.52 (10^{-5} cm^2 s^{-1}). In hydrocephalic animals, the mean α decreased to 0.14 and ADC_{TMA} increased to 0.60 (10^{-5} cm^2 s^{-1}). Seven days after injection of 0.03 ml, and in 60% of animals injected with 0.08 ml KS, α decreased further (α = 0.11), ADC_{TMA} remained at 0.61 while ADC_W also significantly increased above control values (Table **1**). T_2 relaxation times were not changed (T_2 = 63.8 ± 2.5 ms). Seven days after injection of 0.08 ml KS, white matter edema was found in 40% of the animals. In these animals α, ADC_W and T_2 relaxation times increased in both cortex and in white matter (Table **1**).

In 21-day-old H-Tx rats that developed hydrocephalus, the ventricles were grossly enlarged and the cortical thickness decreased from about 1.9 mm to 0.9 mm (Fig. **2**). TMA measurements performed in hydrocephalic H-Tx rats revealed a significant decrease in the ECS volume fraction with respect to control animals of the same age: α being about 0.23 and 0.16, respectively (Fig. **2**). There was no significant change in the ADC_{TMA}, with means of 0.53 and 0.55, respectively.

Discussion

We conclude that there is a significant decrease in ECS volume fraction during kaolin-induced as well as inherited hydrocephalus. While ADC_{TMA} is increased in adult KI hydrocephalus, it remains unchanged if the hydrocephalus is inherited. ADC_W is not yet changed 24 hours after KI, but it is increased after 7 days. There is, therefore, no simple correlation between ECS volume and ADC_W.

ADC_{TMA} decreases from about 0.58 to above 0.30 ($10^{-5}cm^2s^{-1}$) during ischemia (Fig. **2B**). These changes significantly impair extrasynaptic (volume) transmission, which is mediated by the diffusion of neuroactive substances through the ECS and which plays an important role in short- and long-distance communication between neurons, axons and glia (2,4,7). Demyelination and gray matter pathologies result in ECS volume changes and, importantly, in a loss of diffusion anisotropy (5). In damaged tissue, pathologic states and during aging, structural changes, particularly astrogliosis, result in persistent changes in ECS diffusion parameters. A significant increase in diffusion barriers (a decrease in ADC_{TMA}, and/or a decrease of ECS volume fraction) during astrogliosis evoked by excessive stimulation, traumatic injury and aging, was found in rat cortex, corpus callosum, hippocampus, spinal cord and in cortical grafts. The time course of changes in α and λ may, or may not, correlate with the

Table 1 Cortical and white matter thickness, volume fraction, ADC_{TMA}, ADC_W and T_2 relaxation times in control animals and those with kaolin-induced hydrocephalus

	Thickness (mm)	Volume fraction (α)	ADC_{TMA} ($10^{-5}cm^2s^{-1}$)	ADC_W ($10^{-5}cm^2s^{-1}$)	T_2 (ms)	No. of animals
Control cortex	1.9 ± 0.1	0.20 ± 0.01	0.52 ± 0.01	0.65 ± 0.02	65.7 ± 1.6	8
24 hours – cortex	1.6 ± 0.1*	0.14 ± 0.01*	0.60 ± 0.03*	0.63 ± 0.01	64.2 ± 1.6	8
7 days – cortex	1.2 ± 0.1*	0.11 ± 0.01*	0.61 ± 0.04*	0.70 ± 0.01*	63.8 ± 2.5	9
7 days – cortex edema	1.1 ± 0.1*	0.24 ± 0.01*	0.51 ± 0.02	0.78 ± 0.03*	81.7 ± 4.4*	4
Control WM	0.2 ± 0.1	0.23 ± 0.02	0.45 ± 0.02	0.55 ± 0.03	64.5 ± 1.5	6
7 days – WM edema	0.7 ± 0.1*	0.48 ± 0.03*	0.59 ± 0.05*	1.54 ± 0.12*	198.2 ± 8.5*	4

* Significantly different from control values, $p < 0.05$

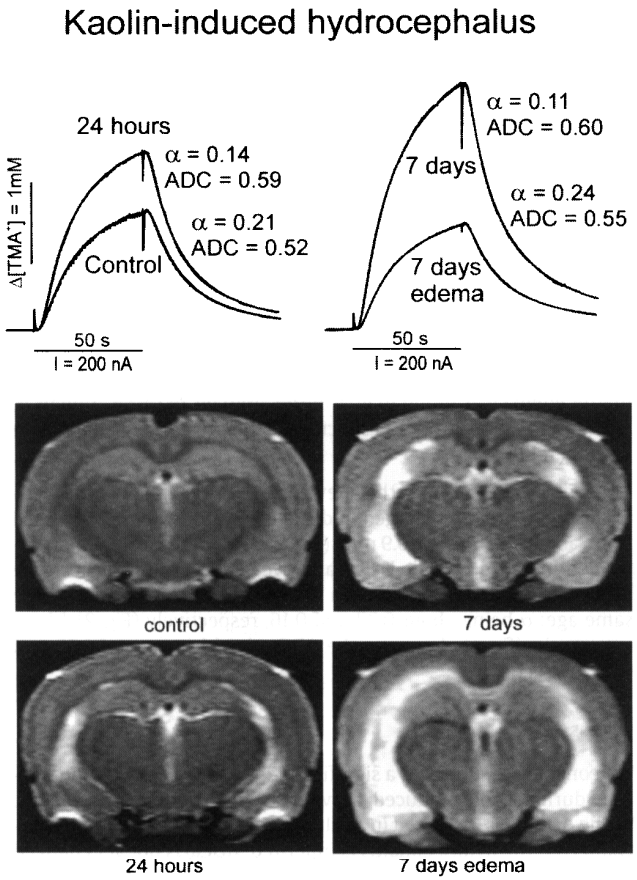

Fig. 1 Typical diffusion curves and T_2-weighted MR images of coronal brain sections in the cortex of an adult rat (control) and in rats with hydrocephalus induced by the injection of either 0.03 ml (24 hours and 7 days) or 0.08 ml (7 days edema) of a kaolin suspension. The higher diffusion curves in the cortex of the 24 hour and 7 day rats indicate a decreased ECS volume fraction (α) as compared with control animals, while their slower rise and fall indicates an increased ADC_{TMA}. The lower diffusion curve in the cortex of the 7 day edema animal indicates an increased volume fraction. In the MR images, note the white matter edema in the animal injected with the larger kaolin dose (7 days edema). The images show enlarged ventricles and cortical compression in the hydrocephalic rats.

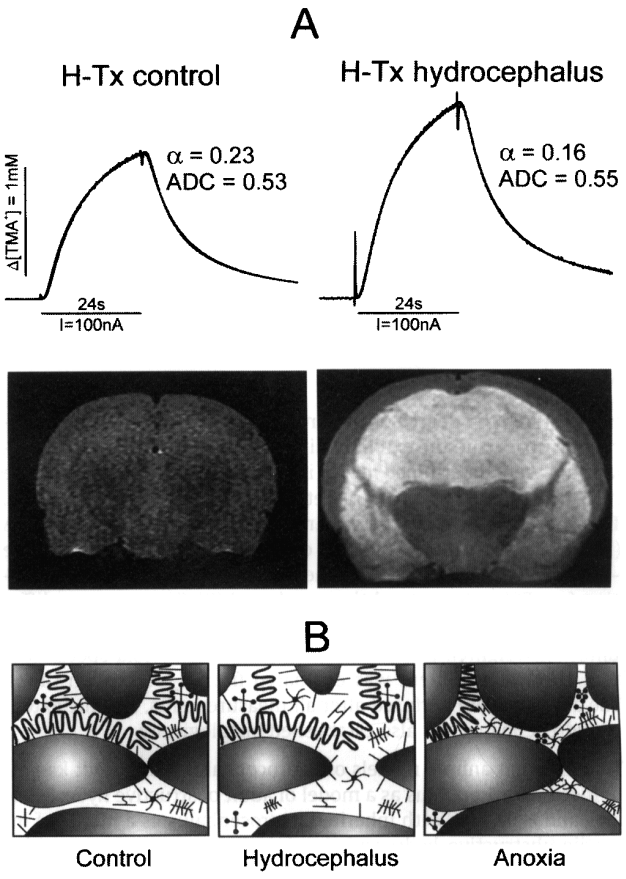

Fig. 2A and **B** **A** Typical diffusion curves and T_2-weighted MR images obtained in 21-day-old H-Tx rats that either did (hydrocephalus) or did not (control) develop inherited hydrocephalus. The higher diffusion curve recorded in the hydrocephalic rat reflects the decrease observed in the ECS volume fraction (α), while the T_2-weighted images illustrate the ventricular enlargement and cortical compression seen in this animal.
B Schematic representation of extracellular space diffusion in a control brain (Control), in a hydrocephalic brain with edema (Hydrocephalus) and in a hydrocephalic brain without edema, the diffusion parameters of which correspond to anoxia (Anoxia).

time course of the decrease in the ADC_W as determined by diffusion-weighted MRI. We found a good correlation during terminal anoxia (8); however, after cortical stab wound injury there was no simple correlation (9). Our previous morphological studies revealed that the movement of substances is hindered not only by the cell (particularly glia) swelling-induced narrowing of intercellular clefts, but also by diffusion barriers formed by the production and/or crowding of molecules of the extracellular matrix, by the swelling of fine glial processes and by astrogliosis (3,6).

The observed changes in ECS volume and in diffusion barriers in the hydrocephalic brain (Fig. **2B**) can therefore affect the efficacy of synaptic as well as extrasynaptic (volume) transmission, as well as be an important factor to consider for therapeutic drug application.

Acknowledgement

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