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Development of Acute Edema Following Cerebral Hypoxia-Ischemia in Neonatal Compared with Juvenile Rats Using Magnetic Resonance Imaging

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ABSTRACT

We hypothesized that the evolution of cerebral edema accompanying cerebral hypoxia-ischemia is dependent on age and that such differences would be detectable using magnetic resonance imaging methods. Thus we examined in immature and juvenile rats the relationship between hypoxic-ischemic changes in T_1 and T_2 and the alterations in brain water content, as assessed by differences in tissue wet-dry weights. One- and 4-wk-old rats were anesthetized and subjected to unilateral carotid artery occlusion and subsequent exposure to hypoxia (8% oxygen). T_1 and T_2 maps were acquired at 9.4 T, and then brain water content was measured in sham controls or in hypoxic-ischemic animals before, during, and 1 or 24 h after hypoxia-ischemia. In sham controls, T_1, T_2, and proton density decreased with increasing age, corresponding to an ontogenic decrease in water content. In 1-wk-old rats, increases in T_1 and T_2 were observed during and at 1 and 24 h after hypoxia-ischemia, corresponding to elevations in water content. In 4-wk-old rats, T_1 and water content increased during and at 1 and 24 h after hypoxia-ischemia whereas T_2 was not increased until 24 h after hypoxia-ischemia. Regression analysis showed that T_1 correlated better with total water content than T_2. In both immature and older brain, an increase in total brain water develops acutely and persists after an episode of cerebral hypoxia-ischemia, and T_1 imaging detects this change better than T_2. Hypoxic-ischemic changes in T_2 are age dependent, reflecting other physicochemical changes of water in the tissue than water content alone. **(*Pediatr Res* 55: 101–106, 2004)**

Abbreviations

HI, hypoxia-ischemia
MR, magnetic resonance
BBB, blood–brain barrier

Cerebral ischemia produces a tissue edema consisting of a combination of intracellular and extracellular water accumulation within the tissue. The edema associated with ischemia has been assessed frequently using one or more noninvasive MR imaging techniques (1) in which the contrast has been based primarily on regional differences in proton density, spin-lattice (T_1), or spin-spin (T_2) proton relaxation times. Considering the fact that the majority of protons within the brain are found in water molecules, changes in intensity in T_1- and T_2-weighted images have often been interpreted to represent edema and have been used to monitor its progression under various pathologic states including cerebral ischemia and brain trauma (2). However, the actual changes in water content or the biophysical properties of the tissue that underlie many of the MR imaging changes remain poorly understood. Indeed, those studies measuring ontogenic or pathologic changes in tissue water content have reported a range of poor, mild, and good correlations between water content and MR variables such as T_2 and T_1 (3–12). The majority of such studies have not examined the correlation of the changes in MR relaxation variables with the evolution of the pathologic edema as a function of time, and even fewer have investigated directly the relationship between changes in MR variables and the edema resulting from a cerebral HI insult. Recently, we reported that there are age-dependent changes in T_2 after an episode of transient hypoxia in immature rats with unilateral carotid artery occlusion. In this model of hypoxia plus incomplete cerebral ischemia, changes in T_2 correlate better to a combined alteration in water content and protein extravasation than to either altered water content or a disruption in the BBB alone (13). Although in this study we did not investigate changes in T_1, such changes have the potential for providing an alternative and perhaps preferred measure of edema. We hypothesized that the T_1 changes detected in edematous tissue would be affected by the maturity of brain and that T_1 would correlate better with water content than T_2. To test this, we investigated the relationship between

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water content and the temporal changes in T_1, T_2, and proton density with MR techniques during and after a cerebral HI insult in 1- and 4-wk-old brain in which the maturity of the rat brain at these ages corresponds roughly to newborn and juvenile (prepuberty) stages of human development, respectively (14).

METHODS

Model of cerebral HI. Pregnant Wistar rats were obtained from Charles River Laboratories (Montreal, Canada) and gave birth approximately 1 wk after their arrival, after which the litter was culled to 9 to 10 pups. All animals were treated in accordance with the guidelines provided by the Canadian Council on Animal Care, and experiments were approved by the local animal care committee. Animals were assigned to experiments to be performed at one of two ages: 1 or 4 wk. Cerebral HI was produced as described previously and involved permanent ligation of the right carotid artery with subsequent exposure to an episode of hypoxia (8% oxygen in nitrogen) (15). In this model of cerebral HI, ligation of the carotid artery alone does not result in ischemia because of collateral blood flow via the circle of Willis. During HI, there is a transient episode of incomplete ischemia produced within the hemisphere ipsilateral to the occlusion (16).

In each animal, the right common carotid artery was ligated and severed under isoflurane (2.5%) anesthesia. The incision site was closed, and saline solution (0.1 mL/10 g, i.p.) was injected to compensate for any fluid losses during surgery. In the sham-control group, the carotid artery was isolated but not ligated. After surgery, the rats were returned to the cage with the mother for 1–2 h of recovery from the anesthesia. Rats were then exposed to hypoxia by spontaneously breathing humidified 8% oxygen in 92% nitrogen for a duration of 1.5 h for 1-wk-old rats or 30 min for 4-wk-old rats, thereby producing a cerebral infarct of similar size (17). Body temperature was maintained at 37.0°–37.5°C during hypoxia with a heating lamp and circulating water blanket.

MR imaging. T_1 and T_2 maps were obtained with a 9.4-T, 21-cm horizontal bore magnet (Magnex, Yarton, U.K.) equipped with an Avance Bruker console (Bruker, Ettlingen, Germany). The animals were anesthetized with isoflurane (0.5–1.25%) and placed in a chamber designed to fit the bore of the magnet. In 1-wk-old rats, the head was restrained with a foam-lined head holder, and in 4-wk-old rats the head was restrained with ear pins and an incisor bar within a quadrature rf coil. Respiration rate was monitored continuously while animals were in the magnet. Within each age group ($n = 20$), images were acquired in sham controls ($n = 5$) or HI rats immediately before HI ($n = 15$, all the HI rats were imaged before HI), during HI ($n = 5$), at 1 h ($n = 5$), or 24 h ($n = 5$) after HI. At the end of imaging at each time point the rats were killed for the assessment of water content. T_2 maps were determined using a spin-echo multiecho imaging sequence consisting of slice-selective 90° and 180° sinc pulses with a length of 4 ms and 3 ms, respectively. The T_2 map was obtained from an eight-echo train with 20 ms of echo spacing and repetition time (TR) of 1200 ms within a slice at the level of striatum having a thickness of 1 mm for 1-wk-old and 1.5 mm for 4-wk-old rats. The field of view was 3 cm × 3 cm and the data matrix was 256 × 128. The signal intensity within each voxel and at each echo was used to determine the T_2 relaxation times using a nonlinear least squares fitting algorithm. T_2-weighted images were acquired at the third echo (TE = 60 ms). T_1 maps were acquired with an inversion-recovery Snapshot-FLASH imaging technique (inversion Sinc pulse and excitation Gauss pulse with a length of 5 ms and 1 ms, respectively): TR = 3.55 ms, TE = 2.1 ms, increasing time of inversion (TI) delays of 234, 503, 831, 1233, 1751, 2480, 3728, 9226 ms between the inversion pulse and the imaging sequence, a field of view of 3 cm × 3 cm, slice thickness of 1.5 mm, and a data matrix of 128 × 128. T_1-weighted images were obtained at a TI delay of 1233 ms. Proton density was estimated from the T_1 fit of the data (18) and normalized to the T_1 of water within tubing adjacent to the head of the rat. T_1, T_2, and proton density were measured from ipsilateral and contralateral parietal cortex using image analysis software (Marevisi, Institute for Biodiagnostics, National Research Council of Canada) (19).

Assessment of changes in brain water. Brain water was determined in 20 1-wk-old and 20 4-wk-old rats by measuring the percentage of the difference in wet and dry weights of the brain samples (13). After the last set of images were obtained within each age group, subgroups of rats were euthanized consisting of sham-control animals ($n = 5$) or HI animals euthanized at one of three times: during HI ($n = 5$), at 1 h ($n = 5$), or at 24 h ($n = 5$) after HI. The animals were injected with pentobarbital (120 mg/kg), the brain was removed, and samples of forebrain containing the striatum from the ipsilateral and contralateral hemispheres were weighed and dried in an oven at 100°C for 4–5 d.

Statistical analysis. Grouped data (*e.g.* ipsilateral and contralateral T_1, T_2, and water content) are presented as their mean ± SD. A paired t test was used to compare ipsilateral-contralateral differences. The mean ratios of values obtained from the ipsilateral *versus* contralateral hemisphere at variable times from different animals during and after HI were compared against the control group with an ANOVA followed by a Newman-Keuls test (Statistica; StatSoft, Tulsa, OK, U.S.A.). Differences were considered significant at $p < 0.05$. A least squares regression analysis was used to analyze the correlation of alterations in brain water compared with changes in T_1 or T_2 relaxation times.

RESULTS

HI changes in MR images. In sham controls, T_1 in brain was similar in both hemispheres and was inversely dependent on age. T_1 in the cerebral cortex was 21% shorter in 4-wk-old than in 1-wk-old animals ($p < 0.01$; Table 1). Despite differences in initial T_1, the effect of cerebral HI on changes in T_1 was similar in both age groups (Figs. 1 and 2, Table 1). During HI, the ipsilateral hemisphere appeared bright and there were increases in the T_1 acquired from the ipsilateral compared with contralateral cortex ($p < 0.01$; Figs. 1 and 2, Table 1). At 1 or

Table 1. *Changes in MR tissue relaxation* during and after an episode of cerebral HI in 1- and 4-wk-old rats*

		1-wk-old			4-wk-old		
		T_1 (ms)	T_2 (ms)	water (%)	T_1 (ms)	T_2 (ms)	water (%)
Sham	Contralateral	2627 ± 83	64 ± 3	88.3 ± 0.4	2080 ± 66	46 ± 3	81.1 ± 0.4
	Ipsilateral	2623 ± 47	66 ± 3	88.4 ± 0.4	2041 ± 49	49 ± 3	81.2 ± 0.3
During HI	Contralateral	2729 ± 167	61 ± 5	88.3 ± 0.2	2064 ± 77	46 ± 3	81.4 ± 0.5
	Ipsilateral	2923 ± 169‡	80 ± 4‡	88.9 ± 0.2‡	2358 ± 103‡	47 ± 3	82.3 ± 0.4‡
1 h after HI	Contralateral	2757 ± 190	64 ± 5	88.5 ± 0.2	2143 ± 87	49 ± 2	81.4 ± 0.3
	Ipsilateral	2993 ± 149†	78 ± 7‡	89.2 ± 0.3‡	2237 ± 97†	50 ± 2	82.1 ± 0.3‡
24 h after HI	Contralateral	2736 ± 228	65 ± 7	88.4 ± 0.2	2022 ± 60	46 ± 3	81.0 ± 0.4
	Ipsilateral	3007 ± 200†	100 ± 20‡	89.4 ± 0.5†	2195 ± 149†	55 ± 6†	82.2 ± 0.6†

* T_1 and T_2 in the cortex were measured from MR imaging maps of T_1 and T_2. Water content in the cerebrum was measured using dry-wet weight of tissue presented as a % of the wet weight of tissue.

† $p < 0.05$, ‡ $p < 0.01$ *vs* the contralateral cortex, paired *t* test.

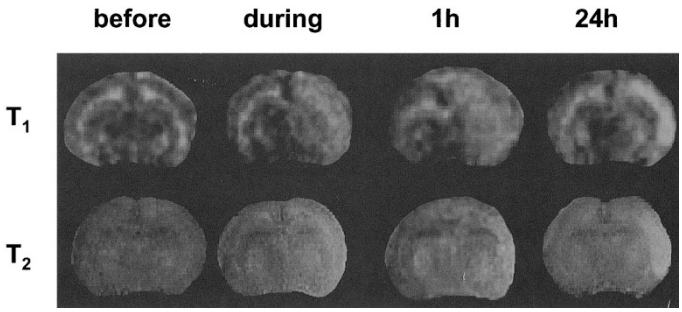

Figure 1. T_1- and T_2-weighted images in 1-wk-old rats obtained before HI, during HI, and at 1 or 24 h after HI. Areas of HI injury appear bright within the hemisphere ipsilateral to the right carotid artery occlusion.

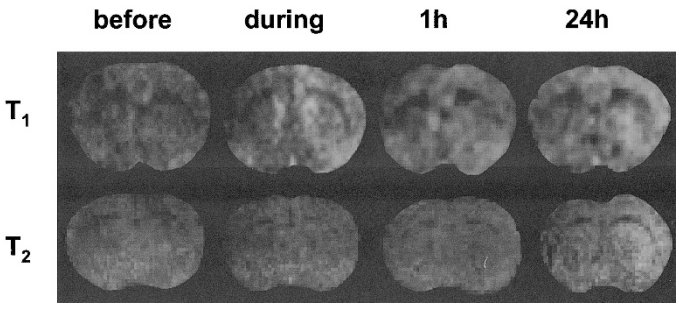

Figure 2. T_1- and T_2-weighted images in 4-wk-old rats obtained before HI, during HI, and at 1 or 24 h after HI. Areas of HI injury appear bright within the hemisphere ipsilateral to the right carotid artery occlusion.

24 h after the termination of HI, T_1 from the ipsilateral cortex remained elevated in both age groups ($p < 0.05$).

In sham controls, T_2 in the rat brain was similar in both hemispheres and also inversely dependent on age. T_2 in the cerebral cortex was 28% shorter in 4-wk-old than in 1-wk-old animals ($p < 0.01$; Table 1). In addition, the time course of HI changes in T_2 differed in young and older rats (Figs. 1 and 2, Table 1). During HI, the ipsilateral hemisphere appeared bright, and there were increases in T_2 in the ipsilateral cerebral cortex in 1-wk-old ($p < 0.01$) but not in 4-wk-old animals. In the 1-wk-olds, the elevations in T_2 remained at 1 h, increasing further at 24 h after the end of HI ($p < 0.01$). In the 4-wk-olds, increases in T_2 ipsilaterally were detected only at 24 h after the end of HI ($p < 0.05$).

In sham controls, cerebral proton density was similar in both hemispheres but decreased with age. Proton density in the cerebral cortex was 7% less in 4-wk-old than in 1-wk-old animals ($p < 0.05$). No ipsilateral-contralateral differences in proton-density were observed during or after HI.

HI changes in water content. In sham controls, brain water content assessed with wet-dry weight differences was similar in both hemispheres and inversely dependent on age. Water content in the forebrain was 8% lower in 4-wk-old than in 1-wk-old animals ($p < 0.01$; Table 1). The time course of HI changes in water content was similar in both age groups (Table 1). During HI, the water content acquired from the ipsilateral compared with the contralateral hemisphere became elevated ($p < 0.01$) and remained elevated in both age groups at 1 or 24 h after the termination of HI ($p < 0.05$).

Correlation of MR relaxation times with water content. Using a regression analysis, a linear relationship was demonstrated to be present between the T_1 and brain water measurements obtained under control and HI conditions ($r^2 = 0.74$, $p = 0.00008$ for the 1-wk-old rats; $r^2 = 0.70$, $p = 0.0003$ for the 4-wk-old rats; Fig. 3). In contrast, the regression analysis for the T_2 data demonstrated a difference between age groups with the correlation between T_2 and water content being higher in 1-wk-old than 4-wk-old animals ($r^2 = 0.68$, $p = 0.001$ for the 1-wk-old rats; $r^2 = 0.45$, $p = 0.04$ for the 4-wk-old rats; Fig. 3).

Whether changes in water content are reflected better by changes in T_1 or T_2 was examined by comparing the changes in the ratios of ipsilateral to contralateral water content and MR relaxation times during the time course of cerebral HI (Figs. 4 and 5). There was a good correspondence between changes in water content and either T_1 or T_2, except for in 4-wk-old rats, in which there was a discrepancy during and 1 h after HI at which times water content increased despite an absence of changes in T_2.

DISCUSSION

The present study demonstrates that T_1 and T_2 maps detect differentially the brain water changes associated with a cerebral HI insult and indicate that there are differences in the cerebral edema evolving after HI in immature compared with more mature brain. There are early changes in water content associated with cerebral HI that are similar in immature and older brain, and T_1 maps or inversion recovery–weighted images are able to detect such changes. In contrast, as has been reported in adult brain (20), in young juvenile rats, T_2 changes occur in

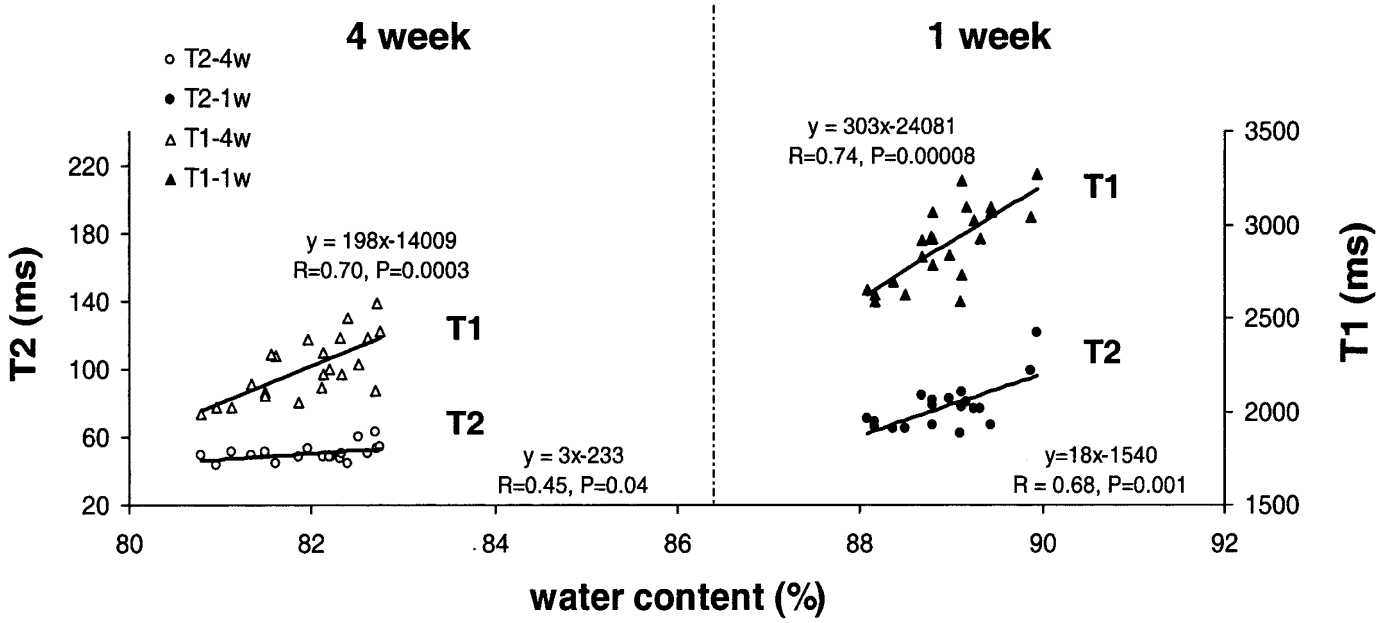

Figure 3. Correlation of T_1 and T_2 relaxation times with water content (wet-dry weight) in the hemisphere ipsilateral to the occlusion in control and HI rats (from the start of HI to 24 h after HI) in 1- and 4-wk-old rats.

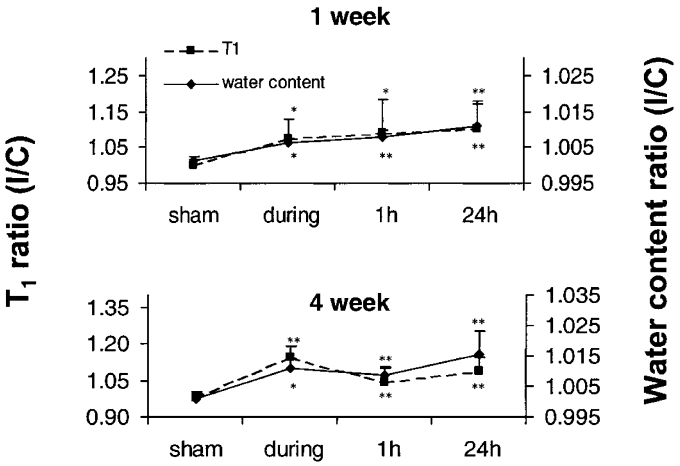

Figure 4. Changes in T_1 relaxation times and water content during the time course of an HI insult in 1- and 4-wk-old rat brain. Shown are mean ratios for T_1 and water content acquired from the hemisphere ipsilateral (*I*) *vs* contralateral (*C*) to the right carotid artery occlusion in sham controls or in HI rats during and at 1 or 24 h after HI. Data are displayed as mean ± SD. $^{**}p < 0.01$, $^{*}p < 0.05$ *vs* sham controls.

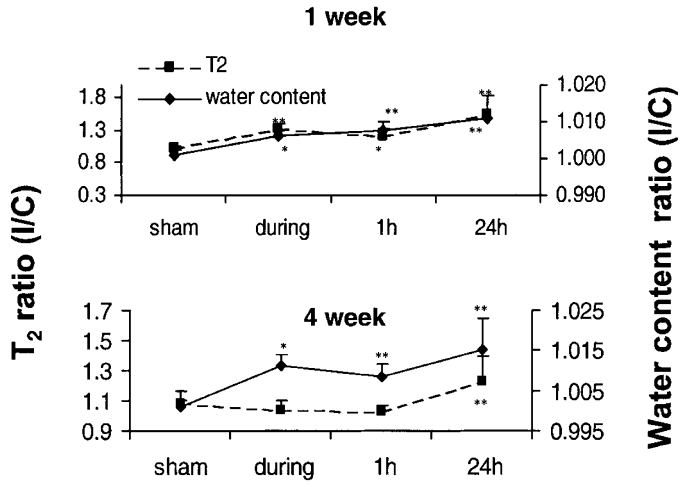

Figure 5. Changes in T_2 relaxation times and water content during the time course of an HI insult in 1- and 4-wk-old rat brain. Shown are mean ratios for T_2 and water content acquired from the hemisphere ipsilateral (*I*) *vs* contralateral (*C*) to the right carotid artery occlusion in sham controls or in HI rats during and at 1 or 24 h after HI. Data are displayed as mean ± SD. $^{**}p < 0.01$, $^{*}p < 0.05$ *vs* sham controls.

areas of edema or infarction only hours to days after an ischemic insult, whereas such changes appear much earlier, already during HI, in neonatal brain. Indeed T_2 appears to detect the cerebral edema that is accompanied by BBB disruption, presumably reflecting changes in the physicochemical properties of water and its environment rather than total water content alone (13).

The ability of T_1 imaging methods to readily detect the edema associated with cerebral ischemia appears to be dependent on a number of factors. The use of standard T_1 spin-echo–weighted imaging in stroke patients has been rather insensitive for detecting abnormalities within the brain at an acute stage of cerebral ischemia (21–24). In contrast, in experimental animals, quantitative increases in T_1 comparable to those in the present study have been observed within minutes to a few hours after focal cerebral ischemia (2, 25, 26). Although some of the initial increase in T_1 (up to 2%) could be caused by reductions in cerebral blood flow during HI (25, 26), the majority of the acute increases in T_1 during and after HI, which ranged from 7 to 14%, likely are related to the increased water content measured in the tissue. One obvious difference between experimental and clinical studies is in the MR sequences used to acquire T_1 images. Inversion-recovery T_1-weighted images or T_1 maps rather than spin-echo T_1-weighted images are often used in animals, in which the former can be more sensitive for detecting edema. Animal MR imaging is also usually performed at higher field, in which the changes in T_1 associated

with focal ischemia are greater than at low field (*e.g.* 4.7 or 9.4 T *versus* 1.5 T, respectively) (25, 27). As high-field clinical magnets and faster T_1 acquisition sequences become more widely available, quantitative T_1 imaging could prove beneficial for diagnosing the acute edema associated with HI or stroke clinically (28).

The present study found a good correlation between HI changes in brain water and T_1. Previous studies correlating T_1 or T_2 with water content have observed a rather narrow range of water content changes, some being maximal many hours after cerebral ischemia (3–7, 29, 30). In these studies a range of insignificant to highly significant correlations between T_2 and water content have been reported. Presently, water content varied widely (81 to 90%) between control animals and after HI. The comparison of edema and MR changes resulted in a good temporal agreement between changes in T_2 and water content during the time course of HI in the 1-wk-old brain but only a partial agreement in the 4-wk-olds. The rather poor overall correlation between T_2 and brain water content in 4-wk-olds contrasts with the highly significant correlation between T_1 and brain water content at either age. This is similar to the better correlation of T_1 than T_2 with changes in water content induced by triethyltin intoxication or cold-injury (8, 9). This suggests that changes in T_1 are an indicator of alterations in the total amount of water, irrespective of ontogenic differences in the tissue, including cell density, cell composition, BBB permeability, the distribution of bound *versus* free water, or whether the edematous fluid is enriched with proteins or electrolytes (13, 31, 32).

Proton-density imaging was able to detect the rather large (8%) ontogenic decreases in water content but not the smaller HI increases in water content. A large variability is inherent in the proton-density measurements made using T_1 (33), resulting in SDs of 3 to 7%. However, even smaller SDs of $\pm 1.5\%$ from proton-density measurements made using spectroscopic methods might be too large for routine determination of cerebral edema (33).

The greatest difference in the HI changes between T_1 and T_2 ratios occurred in 4-wk-old animals in which there was an increase in T_1 and brain water but no change in T_2 during and immediately after HI. The reason for this is uncertain, but it is possible that protein extravasation influences the T_2 obtained (13). Also to be considered are an interaction between endogenous macromolecules and water molecules or changes in the bound and free fractions of water (34). Multicomponent analysis of the fitting curve of T_2 relaxation decay has demonstrated that T_2 can be separated into at least two components in which the fast T_2 likely represents the fraction of water bound to macromolecules, which is predominant in the intracellular compartment, whereas the slow T_2 represents free water, which exists primarily in the extracellular compartment (8, 9, 32). Indeed, the fraction of bound water increases as the brain matures, being associated with ontogenic decreases in extracellular space (31, 32, 35). Furthermore, the patterns of changes in fast T_2 and slow T_2 have been observed to differ greatly in brain edema induced by ischemia or trauma (9, 36). Unfortunately, in our study the echo time of 20 ms was much longer than the minimum that has been suggested for the analysis of the short component of T_2 in the brain, and eight echoes is considered insufficient for an accurate multiexponential fitting of the T_2 data (37). Thus, additional studies with an appropriate selection of MR acquisition variables are needed to analyze the dynamic distribution of slow and fast components of T_2 during and after HI.

CONCLUSIONS

In summary, there are ontogenic changes in brain water content and age-dependent differences in the edematous changes observed in response to an HI insult. By investigating the relationship between changes in cerebral T_1 and T_2 with alterations in brain water content in rats at different ages, we have demonstrated that changes in T_1 but not T_2 best serve as an indicator of edema associated with an elevation in water content. Changes in T_2 appear to best reflect the vasogenic edema associated with cerebral HI.

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