New Insights Into Brain Damage in Stroke-Prone Rats
A Nuclear Magnetic Imaging Study

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Background and Purpose—The spontaneously hypertensive stroke-prone rat (SHRSP) is an animal model for a complex form of cerebrovascular pathology. MRI provides an efficient and noninvasive tool for studying the time course of brain damage. The aim of this study was to gain new insights into the pathological phenomena responsible for the occurrence of brain injury in SHRSP with the use of the apparent diffusion coefficient of water (ADC), one of the most efficient MRI parameters for detecting brain abnormalities. To this end, the pattern of ADC variation observed in SHRSP was compared with that of focal ischemia induced in both SHRSP and Sprague-Dawley rats.

Methods—Four groups of animals were studied: SHRSP developing spontaneous brain lesions fed with a salt-loaded (n=15, group 1) or standard diet (n=3, group 2) and Sprague-Dawley rats (n=8, group 3) and SHRSP (n=8, group 4) with permanent middle cerebral artery occlusion. ADC maps and T2-weighted images of brains were performed by MRI. After the rats were killed, the brains were removed and histologically processed.

Results—There was no decrease in ADC during spontaneous stroke in the SHRSP fed with a normal or salt-enriched diet, while both the SHRSP and Sprague-Dawley rats with middle cerebral artery occlusion showed a marked decrease that lasted for 24 to 48 hours.

Conclusions—Cerebral ischemia cannot be considered a major factor in the onset of spontaneous brain lesions in SHRSP, which show only vasogenic edema after the beginning of the damage with no evidence of metabolic impairment. (Stroke. 2002;33:825-830.)

Key Words: animal models ■ brain injuries ■ diffusion ■ magnetic resonance imaging ■ middle cerebral artery occlusion ■ rats

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he spontaneous hypertensive stroke-prone rat (SHRSP) is generally considered a good experimental model for studying the hypertensive damage leading to stroke, but the similarity between SHRSP and human stroke is still a matter of debate. The model has been used to study vascular and brain tissue alterations both structurally and functionally, and the relationship between histopathological findings and behavioral or metabolic abnormalities (eg, increased aggressiveness, hypokinesia or hyperkinesia, blood-brain barrier damage, proteinuria, a sudden loss of body weight) has also been extensively investigated. However, less information is available concerning the onset of the spontaneous brain damage and the mechanisms responsible for the development of brain abnormalities.

The morphological and histopathological investigations directed at the characterization of the brain lesion in SHRSP did not univocally clarify the pathogenesis of the damage. In fact, the term stroke is not univocally adopted in literature that concerns SHRSP studies, and there is ambiguity regarding whether this term is used to mean hemorrhage, infarct, or hemorrhagic infarct. This is in part due to the difficulty of precisely predicting the timing and the location of the spontaneous brain lesions, in turn making their systematic study in this animal model rather complex.

In the case of ischemic stroke, diffusion-weighted (DW) imaging offers a number of advantages in detecting ischemic diseases, including a prompt variation (of a few minutes) in the apparent diffusion coefficient of water (ADC) at the onset of ischemia-induced cellular metabolic impairment and sensitivity (an initial decrease in ADC of 50% to 60% from the normal value). It also allows the time course of cerebral damage to be assessed. In both experimental animal and human acute stroke, the prompt and significant decrease in ADC lasts for a few days, after which the value generally increases to more than that detected in normal tissue.

Ischemic lesions can also be detected by means of T2-weighted (T2W) MRI, but only in the advanced stage of the disease. Generally, ADC values decrease in the first hours
after the ischemic insult without any changes in T2W images, which show the bright zones revealing the presence of vasogenic edema only after 12 to 24 hours.

Spontaneous cerebral lesions in SHRSP have been localized and measured in vivo by means of T2W MRI,1,2 but no analysis has been made of the evolution of MRI parameters in the follow-up period. To the best of our knowledge, ADC images have only been used in 1 study evaluating the chronic stage of the disease in SHRSP,16 but no information is available concerning this parameter at the time of the onset of brain damage and during the early stages.

In this study we used T2W and DW MRI to investigate the onset and evolution of the damage occurring spontaneously in SHRSP fed with standard or salt-loaded diet in comparison with that induced in SHRSP or Sprague-Dawley rats by middle cerebral artery occlusion (MCAO).

Materials and Methods

Animals and Treatments

The study involved 34 male SHRSP and Sprague-Dawley rats obtained from Charles River (Calco, Italy). The procedures involving the animals and their care were performed at the University of Milan’s Department of Pharmacological Sciences in conformity with the institution’s guidelines, which comply with national and international rules and policies.

The rats were divided into 4 groups: (1) SHRSP fed on standard rat chow and tap water until 6 weeks of age, subsequently switched to a Japanese diet (Laboratorio Dr Piccioni, Gessate, I: 18.7% protein, 0.63% potassium, 0.37% sodium) with 1% NaCl being added to their drinking water (n = 15); (2) SHRSP fed with standard diet for their entire life span (n = 15); (3) Sprague-Dawley rats fed with a standard diet and subjected to MCAO (n = 8); and (4) SHRSP fed with a standard diet and subjected to MCAO (n = 8).

Every week, the rats in groups 1 and 2 were weighed and had their arterial blood pressure measured; they were then housed individually in metabolic cages for 24 hours to measure their food and liquid intake and to collect urine. Twenty-four-hour urinary protein concentrations were measured according to Bradford (Bio-Rad Laboratories, Milan, Italy), with bovine albumin being used as a standard. Proteinuria (protein levels ≥ 40 mg/d) predicts the appearance of brain abnormalities in SHRSP11,12 and was used to schedule the frequency of the MRI investigations. Systolic arterial blood pressure was measured in conscious rats by means of tail-cuff plethysmography (PB Recorder 8006, Ugo Basile), after warming to 37 °C.

The SHRSP fed the Japanese diet and NaCl (group 1) underwent DW and T2W MRI every 3 days until 24-hour proteinuria exceeded 40 mg/d, when MRI was repeated daily. The SHRSP in group 2 underwent DW and T2W MRI every month until 24-hour proteinuria exceeded 40 mg/d, when MRI was repeated daily. MRI measurements in groups 1 and 2 ended 3 to 5 days after the occurrence of brain damage.

The Sprague-Dawley rats (group 3) and SHRSP rats in group 4 were fed a standard diet and drank tap water for the same period of time necessary for the salt-loaded diet to lead to brain abnormalities. MCAO was then performed according to a previously described procedure.13 Briefly, the rats were anesthetized with chloral hydrate (400 mg/kg IP), and then the right MCA was exposed through a subtemporal craniectomy and permanently occluded by means of microbipolar coagulation. The animals were sutured and placed in warmed cages for the next 2 hours and then underwent MRI. The MRI measurements were repeated 24 and 48 hours after MCAO. Physiological variables were continuously monitored during and after the occlusion.

MRI Measurements

For the MRI evaluations, the rats were anesthetized with 2% isoflurane in 70% N2/30% O2, fixed on the animal holder by means of a rod held beneath the teeth, and placed into the 4.7-T, vertical 15-cm bore magnet of a Bruker spectrometer (AMX3 with micro-imaging accessory). A 6.4-cm-diameter birdcage coil was used for the imaging.

A 3-orthogonal-plane, gradient-echo scout acted as a geometric reference for locating the olfactory bulb; then T2W, reference, and DW images were acquired caudally.

The turbo spin-echo T2W device (Bruker RARE), with 16 echoes per excitation, 10-ms interecho time, 85-ms equivalent echo time, and 4-second repetition time, allowed the acquisition of 16 contiguous 1-mm-thick slices. The spin-echo reference and DW images (echo time = 40 ms; repetition time = 1 s) were acquired in 8 contiguous 2-mm-thick slices. The field of view was 4 × 4 cm2 in both the DW and T2W images to ensure that the investigated volume was the same. The in-plane resolution was 128 × 128 points in all of the images.

Diffusion weighting was obtained by adding to a spin-echo multislice sequence two 10-ms-long, 24.7-ms-spaced, 8-G/cm rectangular gradients, giving a b-value of approximately 1000 s/mm2. Four averages were acquired in 8 minutes and 30 seconds per gradient direction. ADC maps were computed from reference and DW images. In many cases, even if not strictly necessary for our purpose, the maps of the trace of apparent diffusion tensor18,19 were computed by adding the maps obtained in 3 orthogonal directions. The trace map, which is rotationally invariant, offers the advantage of being free of anisotropy effects, thus giving a more precise definition of the lesions. Images were analyzed locally with homemade software by thresholding diffusion values and interactively drawing outlines of the region of interest.

Histology

For the histological analyses following the last MRI sessions, the anesthetized rats were killed by cervical dislocation, and their brains were removed and frozen in isopentane or fixed in Carnoy reagent and embedded in Paraplast. Coronal sections with a thickness of 5 μm were stained with hematoxylin-eosin and examined by light microscopy.

Statistical Analysis

Data are expressed as mean±SD. Statistically significant differences were computed with ANOVA followed by post hoc test with Bonferroni adjustment. P<0.05 was taken as statistically significant.

Results

As previously described, the SHRSP fed with the Japanese diet and exposed to 1% NaCl developed proteinuria (40 mg/d) and cerebral lesions after 30±3 and 42±3 days, respectively, from the start of salt loading. The cerebral lesions were first detected in T2W images and, shortly afterward, in ADC maps: both parameters surprisingly increased. Figure 1 shows the MR images (T2 and trace of apparent diffusion tensor) of a representative rat developing spontaneous cerebral damage (group 1) from day −2 to day 5. On day 0, when brain damage was first observed, the T2W image already showed slight hyperintensity in the right striatum; no changes in ADC values were evident at the time but were observed 24 hours later. During the following days, there was a time-dependent increase in both T2 and ADC involving the entire caudate putamen and corpus callosum. The same qualitative MRI changes were observed in all of the rats regardless of the localization of the lesions.

To assess whether the sodium-enriched diet was responsible for the trend in the MRI parameters, we studied SHRSP fed a normal diet and developing spontaneous cerebral damage (group 2). These animals developed proteinuria after approximately 10 months and brain lesions when they were
aged approximately 1 year; however, the behavior of the MRI parameters was the same as that observed in the SHRSP on the salt-loaded diet (Figure 2).

To compare the MRI changes in the rats with spontaneous lesions (groups 1 and 2) with those with lesions due to the lack of blood supply (groups 3 and 4), we performed permanent MCAO in both SHRSP and Sprague-Dawley rats. In both strains of animals, the ischemic damage was evaluated by MRI at 2, 24, and 48 hours after MCAO. Figure 3a shows the qualitative changes in the T2W images and ADC parameters of a Sprague-Dawley rat. The effect of the injury was negligible on the T2W image taken 2 hours after the occlusion, whereas a strong signal was observed at 24 and 48 hours. In contrast, a decrease in ADC was detected after only 2 hours and was still marked after 48 hours. Comparable results were obtained when the same MRI analyses were made on the SHRSP (Figure 3b). The quantitative ADC values in a representative animal from each group are shown in Figure 4; the mean values of all of the animals in each group are given in the Table.

Histological evaluations of the brain areas of the SHRSP developing spontaneous damage (with or without being fed a sodium-enriched diet) indicated that the MR images identified areas of brain damage. The gray matter in these areas was markedly spongy, with loss of neurons, accumulation of astrocytes, and deposition of fibrinoid-eosinophilic material. Perivascular infiltrates, monocytes-macrophages, and occasionally erythrocytes were also detectable, and the white matter was also characterized by a loss of texture. The arterioles in the affected brains showed vessel wall alterations: in particular, the endothelial cell layer seemed to be well maintained but was surrounded by disorganized tissue (Figure 5a, arrow and insert).

The tissue damage induced by MCAO in both rat strains did not show the histological complexity of the spontaneous brain lesions occurring in the SHRSP. In particular, the brain
lesions, limited to the ipsilateral cerebral cortex, presented slight tissue rarefaction, the loss of neuronal cells, and gliosis. No fibrinoid-eosinophilic deposits or vessel wall alterations were detected, and the edematous state was limited to the infarction area (Figure 5b). The histological analysis indicated that the brain lesions induced by MCAO were similar in both SHRSP and Sprague-Dawley rats.

Discussion

The main finding of this study is that the pattern of ADC variation in SHRSP developing spontaneous brain lesions has different features from those reported in the case of ischemic damage induced by MCAO. In particular, ADC values increase in SHRSP during spontaneous brain damage, whereas those of the brain lesions induced by MCAO have the characteristic biphasic pattern previously described for ischemic stroke in every investigated animal species and humans. This biphasic pattern is thought to reflect the evolution of edema in cerebral ischemia. The decrease in ADC is interpreted as the consequence of cytotoxic edema (eg, the transfer of water from the extracellular space into the cells due to cellular energy failure): since ADC is a weighted average between the intracellular (assumed to be lower) and extracellular diffusion coefficients (assumed to be higher), its decrease reflects the changes in the ratio of intracellular and extracellular volume. The later increase in T2 shows that vasogenic edema occurs as a result of the increase in absolute extracellular water content due to the increase in vascular permeability. Meanwhile, a process of irreversible cell death begins and, as the cell membranes became disrupted, the trend of ADC is generally reversed.

Whether SHRSP are fed a normal or sodium-enriched diet, the phase of cytotoxic edema is missing. At the onset of spontaneous cerebral damage, T2 and ADC values increase at about the same time, thus reflecting the occurrence of vasogenic edema.

A number of studies have suggested that vasogenic edema plays a crucial role in the development of spontaneous brain lesions in SHRSP; the spread of plasma constituents into the brain due to the blood-brain barrier has been revealed with the...
use of tracers (e.g., Evans blue) or immunohistochemistry.\textsuperscript{5–7} Other studies have established the presence of functional and structural abnormalities in SHRSP arteries, including vessel wall alterations such as an irregular geometric disorganization and focal degeneration of the medial smooth muscle cell.\textsuperscript{30,31} In particular, electron microscopy studies have revealed widespread medial necrosis and the complete disappearance of medial vascular muscle cells in the damaged areas of brain lesions in SHRSP.\textsuperscript{32} This is followed by the penetration of monocytes through the vascular endothelium (which accumulate in the subendothelial space), thus altering the blood-brain barrier and favoring the penetration of plasma components, which in turn leads to marked edema around the lesions.\textsuperscript{32}

However, the work performed thus far concerning the pathogenesis of the brain damage in SHRSP has not precisely detected the onset of the process because the neurological symptoms appear later than the brain abnormalities. We have previously shown that widespread alterations in vascular permeability occur in this animal model before the appearance of MRI-detected brain abnormalities.\textsuperscript{12} In the present study we used T2W images and ADC maps to record the onset of spontaneous brain damage in SHRSP and its evolution during the following few days. Our data, in particular the unexpected absence of a decrease in the water diffusion coefficient, suggest that the spontaneous brain abnormalities of SHRSP have a vasogenic origin rather than being indicative of ischemic processes as in the case of surgery-induced (MCAO) cerebral damage.

In conclusion, our findings suggest that SHRSP may be a suitable model for studying human pathologies characterized by brain damage due to vasogenic edema, such as hypertensive encephalopathy and leukoencephalopathy,\textsuperscript{33} rather than to ischemic stroke. Nevertheless, other aspect of the process (e.g., the trend of the spectrum of phosphorous metabolite and the role of osmolarity) should be considered to provide greater insights into the pathogenesis of the spontaneous brain damage occurring in SHRSP.

\textbf{Acknowledgments}

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\renewcommand\arraystretch{1.2}
\begin{table}[h]
\centering
\caption{Time Evolution of Diffusion Tensor Trace of Damaged Regions in All Studied Animals}\\
\begin{tabular}{cccccccc}
\hline
Time & TR(D)* & SD & n & TR(D)* & SD & n & TR(D)* & SD & n \\
\hline
\text{-1 d} & 1.38 & 0.12 & 15\textsuperscript{†} & 1.45 & 0.15 & 3 & 1.52 & 0.12 & 8 \\
0 & 1.55 & 0.15 & 15\textsuperscript{†} & 1.65 & 0.2 & 3 & 0.71 & 0.18 & 8 \\
2 h & 1.95 & 0.15 & 15\textsuperscript{†} & 2.05 & 0.2 & 3 & 0.65 & 0.2 & 8 \\
1 d & 2.08 & 0.2 & 13\textsuperscript{‡} & 2.21 & 1 & 7 & 2.05 & 1.2 & 0.25 \\
3 d & & & & 2.18 & 1 & & & & \\
4 d & & & & 1.82 & 0.2 & 5 & & & \\
5 d & 1.79 & 1 & & & & & & & \\
7 d & & & & 2.3 & 0.25 & 4 & & & \\
\hline
\end{tabular}
\footnotesize{Bonferroni’s post hoc test was used to assess whether individual groups were different from one another considering the whole time course of each group. Statistically significant differences: group 1 vs 3, \(P<0.001\); group 1 vs 4, \(P<0.001\); group 2 vs 3, \(P<0.001\); group 2 vs 4, \(P<0.001\).}
\footnotesize{*10\textsuperscript{-3} mm\textsuperscript{2}/s.}
\footnotesize{†In 8 rats only ADC map was acquired, with trace of apparent diffusion tensor (TR(D)) estimated by multiplying by 3.}
\footnotesize{‡In 7 rats only ADC map was acquired, with TR(D) estimated by multiplying by 3.}
\end{table}

\textbf{Figure 5.} Representative histological sections (magnification \(\times 40\)) of T2W hyperintense brain area in a salt-loaded SHRSP 3 days after the first MRI appearance of a spontaneous brain lesion (a) and an SHRSP 3 days after MCAO (b). Histological comparison of the different types of damages show edema-related structural loosening in both cases but the deposition of fibrinoid-eosinophilic material (\(\ast\)) and 2 examples of vessel wall thinning (arrow and insert) in the spontaneously damaged area.
References


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