Diffusion Tensor Imaging Study of Subcortical Gray Matter in CADASIL

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Background and Purpose—In cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), water diffusion changes suggestive of microstructural tissue alterations have been recently reported in abnormal- and normal-appearing white matter as seen on T2-weighted images. In the subcortical gray matter, typical lacunar infarcts are repeatedly observed. Whether microstructural tissue changes are also present outside these lesions within the putamen or thalamus remains unknown.

Methods—We used diffusion tensor imaging, an MRI method highly sensitive to cerebral microstructure, in 20 CADASIL patients and 12 controls. Both the trace of the diffusion tensor \([\text{Tr}(D)]\) and an anisotropic diffusion index (volume ratio) of diffusion were measured within the putamen and thalamus outside typical lacunar infarcts as detected on both T1- and T2-weighted images.

Results—A significant increase in \([\text{Tr}(D)]\) and a decrease in anisotropy were observed in the putamen and thalamus in patients. The right/left indices of \([\text{Tr}(D)]\) in the thalamus, but not in the putamen, were strongly correlated with the corresponding indices calculated in the white matter of the centrum semiovale. In addition, the diffusion increase in the thalamus was positively correlated with \([\text{Tr}(D)]\) and with the load of small deep infarcts within the white matter and negatively correlated with the Mini-Mental State Examination score.

Conclusions—Our results suggest that microstructural tissue alterations are present in the putamen and thalamus, outside the typical lacunar infarcts in CADASIL. In the thalamus, these microstructural changes appear constant and are even observed in asymptomatic subjects. Some of these thalamic changes appear to result from degeneration of thalamocortical pathways secondary to ischemic white matter damage. The importance of this degenerative phenomenon in the pathophysiology of CADASIL requires further investigation. (Stroke. 2001;32:2049-2054.)

Key Words: magnetic resonance imaging ▪ small-vessel disease

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a systemic arteriopathy caused by mutations of the Notch3 gene on chromosome 19. The main clinical manifestations of the disease include attacks of migraine with aura, mood disturbances, recurrent subcortical ischemic strokes, and stepwise or progressive dementia. One of the hallmarks of CADASIL is the presence of white matter (WM) hyperintensities on MR T2-weighted images (T2-WI). These WM lesions are inconsistently associated with small infarcts in basal ganglia and/or thalamus.

Water molecular diffusion, which corresponds to the random motion of water molecules, is determined by both physicochemical properties (viscosity, temperature) and structural features (membranes, macromolecules) of the tissue. In a liquid such as the cerebrospinal fluid, water diffusion is isotropic, ie, identical in all directions in space. This is not the case in WM, where diffusion is faster parallel to the WM fibers than in the perpendicular direction (anisotropic diffusion). In gray matter, anisotropy is much lower since the cellular membranes are not orientated along a preferential direction. MR diffusion tensor imaging (DTI) evaluates diffusion in at least 6 noncolinear directions of space. This MRI method can provide an orientationally averaged measure of water diffusion \([\text{Tr}(D)]\) and the degree of diffusion anisotropy (volume ratio \([\text{VR}]\)). Several studies have shown that this in vivo technique is highly sensitive to variable microstructural changes of the cerebral tissue.

Using DTI, we recently observed an increase in diffusion with a loss of anisotropy in areas of abnormal WM as seen on T2-WI in CADASIL. These diffusion changes may reflect the extent of axonal and/or myelin loss and appear to be related to the clinical severity of the disease. We also detected significant diffusion changes within the normal-appearing WM as defined on both T1- and T2-WI. Whether these tissue alterations are due to ischemia or to secondary wallerian degeneration remains disputed.
Subjects and Methods

Subjects

All subjects had a detailed neurological examination during the 2 hours preceding the MRI examination, including an evaluation of the cognitive deficit with the Mini-Mental State Examination (MMSE) and the degree of handicap with the Rankin Scale score.

Twenty CADASIL patients with a deleterious mutation in the Notch3 gene were studied (mean age, 56.8 ± 8.9 years). All, except 2 asymptomatic subjects, had the typical clinical manifestations of the disease. Eight had a history of recurrent attacks of migraine with aura. Fourteen had previous transient ischemic attacks and/or completed strokes. Eight presented with a focal neurological deficit at onset and/or completed strokes. Eight had a Rankin Scale score > 2.

The control group consisted of 12 healthy subjects (mean age, 51.4 ± 10 years) selected from a larger database with (1) no familial vascular disorder, (2) no history of neurological disorder, (3) normal neurological and general examination, (4) MMSE score > 28, and (5) normal MR T1- and T2-WI.

An independent institutional ethics committee approved the present study (CCPPRB Bicêtre 9724), and informed consent was obtained from each participant.

Magnetic Resonance Imaging

T1-WI and diffusion-weighted images were acquired with the use of a 1.5-T MRI system (Signa General Electric Medical Systems) equipped with gradient hardware allowing up to 22 mT/m. A standard quadrature head coil was used for radio frequency transmission and reception of the MR signal. Reduction of head motion was achieved with pillows placed on either side of the participant’s head and a fixed strap positioned around the forehead.

T1-WI (inversion-recovery) were acquired first in the axial plane with a spoiled gradient echo sequence (124 slices 1.2 mm thick, repetition time = 10.3 ms, echo time = 2.1 ms, inversion time = 600 ms) and 24×24 cm field of view (resolution of 0.937×0.937×1.2 mm). Acquisition time was 7 minutes, 38 seconds.

Diffusion-weighted images (5 mm thick) were acquired with an echo-planar imaging sequence (single shot) sensitized to diffusion by application of gradient pulses on either side of the refocusing radio frequency pulse, in the axial plane, at 26 slice locations. For each slice location, T2-WI with no diffusion sensitization, followed by 11 b values (incremented linearly to a maximum value of 1000 s/mm²), were obtained in 6 noncolinear directions (x, y, z, x-y, x-z, y-z). The image resolution was 128×128, field of view 24×24 cm (in-plane resolution of 1.875×1.875), echo time = 96.4 ms, and repetition time = 3300 ms. Total acquisition time for DTI was 8 minutes, 12 seconds.

The diffusion tensor parameters were calculated on a pixel-by-pixel basis. Tr(D) and VR were calculated as described by Basser et al and Pierpaoli et al, respectively.

Regions of Interest

In all subjects, regions of interest were defined on 3 T2-WI slices in the right and left thalamus and putamen. By visual inspection, we delineated rectangular regions of interest at a distance from lacunar lesions defined as any circumscribed signal abnormality of diameter > 2 mm and isointense to the cerebrospinal fluid signal on T1- and T2-WI. These criteria were based on the results of Bokura et al, who recently showed that the mean size of lacunar infarctions, in 73 postmortem and MRI-identified lesions, was ≥3×2 mm. Because of the variable size of these lesions in our patients, the volume of interest delineated on each side varied from 316 to 844 mm³ in the thalamus and from 160 to 633 mm³ in the putamen. In controls, volumes of fixed size were used, on each side, in the thalamus (900 mm³) and putamen (650 mm³).

To investigate the possible relationships between diffusion changes in the subcortical gray matter and microstructural WM changes, we also calculated Tr(D) and VR within the centrum semiovale, as previously reported. The WM region of interest included areas of increased signal delineated on T2-WI using the 4 consecutive axial planes located just above the lateral ventricles. At the same level, all typical small infarcts, as defined above, were delineated. Total infarct volume was then divided by the corresponding hemispheric volume calculated on the same slices to obtain the load of small infarcts within the centrum semiovale as a fraction of the corresponding hemispheric volume in each patient.

Statistical Analysis

The patients were separated in 2 groups according to the presence or absence of at least 1 associated typical small infarct within the corresponding structure. ANOVA was then performed to compare the diffusion parameters in the putamen between patients and controls. This analysis was repeated for comparison of the data obtained in the thalamus. Tr(D) and VR were calculated in the thalamus and putamen as the mean value of both sides in each subject. The analysis was performed after adjustment for age between groups (covariate = age).

Fisher’s test of protected least significant difference was used for post hoc analysis of multiple comparisons only in the presence of a significant difference between the 3 groups (patients with associated infarcts within the same structure, patients without these lesions, and controls).

When the difference between the groups was not related to the presence of associated small infarcts (lack of a significant difference between the 2 groups of patients), correlation between the significant parameters and Tr(D) or VR within the WM was investigated by multiple linear regression analysis, including age as covariate. Correlation between the same parameters and the load of small infarcts in WM was similarly tested. In addition, relationships with the MMSE score and the Rankin score were investigated with the Spearman rank correlation test.

Values of P < 0.05 were considered statistically significant. Data are presented as mean ± SD. The statistical analysis was performed with Statview software (Abacus Concepts, Inc).

Results

T1- and T2-WI Findings

All patients presented with typical widespread (n = 18) or multiple punctiform (n = 2) T2 hyperintensities located in the centrum semiovale. Eleven patients had thalamic signal abnormalities suggestive of lacunar infarcts (bilateral in 8, unilateral in 3). Five patients presented with a heterogeneous signal of the thalamus on T2-WI but had no focal changes on T1- or T2-WI. Four patients had normal signal on both T1- and T2-WI in the thalamus. Thirteen patients had focal signal
abnormalities suggestive of lacunar infarcts in the putamen (bilateral in 9, unilateral in 4). All 7 other patients had only a few punctiform hyperintensities on T2-WI. The mean increase in water diffusion in both the putamen and thalamus was detected in areas devoid of significant signal changes on T1-WI and with inconsistent or minor signal changes on T2-WI.

**Diffusion Parameters**

ANOVA showed that the mean Tr(D) and VR significantly differed among the 3 groups in both the putamen and thalamus. The Tr(D) in patients was 3.01±0.75 10⁻³ mm²/s in the presence of associated infarcts and 2.48±0.42 10⁻³ mm²/s in their absence. Tr(D) was 2.17±0.15 10⁻³ mm²/s in controls (Figures 1 and 2). The post hoc analysis showed that Tr(D) calculated in both the presence and absence of associated small infarcts in the putamen significantly differed from the mean value calculated in controls (P<0.01 and P<0.05, respectively). The difference between the 2 groups of patients was also statistically significant (P<0.01). The VR index measured in the putamen in the presence of associated infarcts (0.95±0.02; P=0.01), but not that measured in the absence of typical small infarcts (0.92±0.02), differed from the mean value obtained in controls (0.92±0.16).

In the thalamus, Tr(D) was significantly increased in both the presence (3.00±0.42 10⁻³ mm²/s; P<0.0001) and absence of associated small thalamic infarcts (2.82±0.31 10⁻³ mm²/s; P<0.0001) in comparison to controls (2.22±0.085 10⁻³ mm²/s) (Figures 1 and 2). The difference between the 2 groups of patients was not statistically significant. In addition, the mean value of VR was significantly increased in both the presence (0.92±0.02; P<0.0001) and absence of small infarcts (0.93±0.02; P=0.003) compared with controls (0.88±0.018). Individually, 12 of 20 patients had a Tr(D) value in the putamen above the 95% confidence limit of the control mean value. All patients had a Tr(D) value in the thalamus above the 95% confidence limits of the control mean value (Figure 1).

Visual examination of the Tr(D) map in 5 of our patients shows an obvious asymmetry of diffusion, with a larger increase in the thalamus ipsilateral to a unilateral capsular lacunar infarct (Figure 3). The right/left indices of Tr(D) values calculated in the WM were significantly correlated with the corresponding asymmetry indices in the thalamus (r=0.56; P=0.01) but not with those calculated in the putamen (P=0.3). A correlation between the right/left indices of Tr(D) in the thalamus and the right/left indices of VR in the WM was also observed (r=0.55; P=0.01). In addition, Tr(D) in the thalamus was positively correlated with both Tr(D) (r=0.49; P=0.002) and the load of small infarcts (r=0.59; P=0.02) in the centrum semiovale. Multiple regression analysis showed that these results were independent of age effect in the CADASIL group.

Finally, Tr(D) in the thalamus but not in the putamen correlated negatively with the MMSE score (p=0.045; P=0.05). Our analysis revealed only a trend for a correlation between Tr(D) in the thalamus and the Rankin disability score (p=0.4; P=0.075).

**Discussion**

This DTI study was undertaken to investigate the tissue microstructure within subcortical gray matter outside typical small infarcts in CADASIL. Our results show a significant increase in water diffusion in both the putamen and thalamus in our patients. This increase was detected in areas devoid of significant signal changes on T1-W1 and with inconsistent or minor signal changes on T2-W1. The mean increase in water mobility was 35% in both structures but greatly varied in each one, from +13% to +69% in the thalamus and from 0% to +113% in the putamen. In contrast to acute ischemia causing a reduction in diffusion mainly related to cytotoxic edema,26,27 the present diffusion changes are presumably secondary to the loss of structural barriers to water motion and to expansion of the extracellular space.15,16 Furthermore, diffusion anisotropy, which is usually low in these structures, was decreased in both nuclei, which further suggests a loss of the normal microstructural organization within the putamen and thalamus. Postmortem studies have demonstrated varying
degrees of loss of structural components such as astrocytes, oligodendrocytes, neurons, or myelin within the cerebral tissue of CADASIL patients. Therefore, we hypothesize that the variable diffusion increase observed within the noninfarcted thalamus and putamen may also reflect various degrees of ultrastructural tissue changes.

In the putamen, the increase in diffusion was maximal in the presence of associated lacunar infarcts. This suggests that the microstructural alterations underlying the diffusion changes and the lacunar lesions in the putamen might share some common pathophysiological features. One possibility is that the diffusion changes in the putamen correspond to different degrees of tissue ischemic injury up to cavitation. Lammie et al previously observed a selective but variable neuronal loss and demyelination (termed “incomplete lacunar infarcts”) in the basal ganglia, which were associated with multiple small deep infarcts and severe arteriolosclerosis. Identical lesions have been observed in the cerebral tissue of 1 patient with Notch3 gene mutation (M.M. Ruchoux, MD, PhD, personal data). The vessel alterations in CADASIL are widespread and not restricted to the infarcted areas. These vascular lesions are presumably responsible for “chronic ischemia” in the subcortical areas, as supported by previous reports of widespread reduction in cerebral blood flow and increase in oxygen extraction at this anatomic level. In animal models of chronic hypoperfusion, various degrees of tissue damage have been reported, depending on both the duration and amplitude of the blood flow reduction. Underlying histological changes, such as the disappearance of apical dendrites, neuronal loss, demyelination, or gliosis would likely modify water mobility within the cerebral tissue. Alternatively, the microstructural changes underlying the diffusion increase in the putamen may be related to an increased number of very small, dilated perivascular Virchow-Robin spaces (type III lacunes). An unusually high number of these lesions (diameter <200 μm) have been observed by Ruchoux et al within the putamen of 1 CADASIL patient. Because of the limited spatial resolution of our MRI technique, we were unable to detect such lesions. Furthermore, punctiform T2 signal abnormalities of diameter <2 mm, which mainly correspond to small dilated Virchow-Robin spaces, were not excluded from our regions of interest.

Our data from the thalamus suggest that additional processes might underlie the diffusion increase. It is noteworthy that the thalamic diffusion changes appear mostly diffuse, arguing against the putative role of “microscopic” small infarcts. In 5 of our patients, the Tr(D) map shows a greater increase in diffusion in the thalamus ipsilateral to a unilateral infarct within the anterior limb of the internal capsule. In 3 of them, a linearly shaped diffusion increase, with parallel loss of anisotropy, was even clearly visible between the infarction

Figure 2. T1-WI, T2-WI, and map of Tr(D) in 1 control subject (first row) and 3 CADASIL patients. In an asymptomatic patient (patient A, second row), a significant increase in Tr(D) is observed within the thalamus (arrow) but not in the putamen (arrow). In a symptomatic patient (patient B, third row), a significant increase in diffusion was also observed in the putamen at a distance from a small infarct (arrows). In another symptomatic patient (patient C, fourth row), a diffuse increase in diffusion was observed in the thalamus and putamen in the absence of typical infarcts in both structures. Note, in this patient, the moderate but diffuse signal changes observed on T2-WI in these structures.
and the thalamus. Such findings suggest that retrograde and/or anterograde degeneration of axonal bundles from the WM lesion into the thalamus may underlie the thalamic diffusion changes. Microstructural thalamic tissue alterations secondary to remote cerebral ischemic lesions have been extensively reported.\textsuperscript{39–41} In experimental models of middle cerebral artery occlusion and in human studies, a decrease in metabolic activity\textsuperscript{42} followed by a progressive neuronal loss and gliosis with secondary shrinkage and atrophy of the thalamus has been mainly attributed to retrograde degeneration of thalamocortical pathways.\textsuperscript{39,42–44} One main result of this study is the significant correlation found in CADASIL patients between the asymmetry indices of diffusion in the thalamus and those measured in the centrum semiovale. Furthermore, we found a significant correlation between the diffusion increase in the thalamus and that measured in the remote regions of WM, which suggests that the degree of the underlying tissue alterations are actually related. Taken together, these data strongly support the hypothesis of secondary thalamic degeneration in CADASIL. Interestingly, all subjects, including 2 asymptomatic carriers of the mutated gene, presented with an increased diffusion in the thalamus. In addition, significant bilateral diffusion changes were found in the thalamus of 2 asymptomatic subjects who presented with only punctate T2 hyperintensities in the WM, suggesting that even isolated axonal rarefaction within WM tracts may alter the tissue microstructure of the thalamus. Thus, our data support the hypothesis that WM degeneration in CADASIL has early and important consequences for the microstructure of the connected neuronal network and that the thalamus, a major relay for numerous myelinated fibers, might be particularly sensitive to the WM lesions in CADASIL.

The diffusion increase within the noninfarcted thalamus, but not that observed in the putamen, was correlated with the MMSE score in our patients. This finding, together with the previously reported correlation between diffusion changes in the WM and the MMSE score,\textsuperscript{16} suggests that damage of thalamocortical pathways passing through the hemispheric WM has a crucial role in the clinical status of CADASIL. In this regard, secondary degeneration processes, such as those described above, might contribute to the progressive decline of functional and cognitive capabilities in CADASIL.\textsuperscript{19} Finally, further elucidation of these degenerative mechanisms might aid in understanding the pathophysiology of vascular dementia and provide potential targets for future therapeutic interventions,\textsuperscript{45,46} thus highlighting the importance of DTI studies in CADASIL and in other types of vascular dementia.

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