Acta Radiologica 42 (2001) 459–466
Printed in Denmark · All rights reserved

ACTA RADIOLOGICA
ISSN 0284-1851

MR IN PHENYLKETONURIA-RELATED BRAIN LESIONS

M. DEZORTOVÁ[1], M. HÁJEK[1], J. TINTĚRA[1], L. HEJCMANOVÁ[2] and E. SYKOVÁ[3,4]

[1]MR Unit, Department of Diagnostic and Interventional Radiology, Institute for Clinical and Experimental Medicine, [2]Department of Pediatrics, 3rd Medical Faculty Charles University, and [3]Department of Neuroscience, 2nd Medical Faculty Charles University and [4]Institute of Experimental Medicine, AS CR, Prague, Czech Republic.

Abstract

Purpose: Phenylketonuria (PKU) patients were examined by different MR techniques to explain the pathological changes observed in periventricular white brain matter using conventional MR imaging.

Material and Methods: Fifteen patients with treated classical PKU were examined by ^{1}H spectroscopy, relaxometry and diffusion imaging on a whole-body 1.5-T MR imager.

Results: Known PKU lesions characterized by T2 enhancement in periventricular white matter were observed in all patients. The MR spectra from the lesioned areas showed a significant decrease in choline concentration. The mean *ADC* of water decreased and tortuosity increased in PKU lesions compared to control data.

Conclusion: The results support the following hypothesis: The T2 increase in the PKU lesion reflects a raised concentration of free water molecules (about 15%) that have an increased trajectory between collisions compared to the same region in controls. The increase in water mobility might be explained by changes in extracellular space volume and myelin sheaths, which, presumably, have a different geometry with more hydrophobic sites in PKU patients. The changes result in increased tortuosity and may be confirmed by the loss of anisotropy in PKU lesions.

Key words: Brain, phenylketonuria; MR imaging, diffusion; spectroscopy.

Correspondence: Milan Hájek, MR Unit, Department of Diagnostic and Interventional Radiology, Institute for Clinical and Experimental Medicine, Vídeňská 1958/9, CZ-140 21 Prague 4, Czech Republic. FAX +420 2 472 8224.

Accepted for publication 10 May 2001.

Classical phenylketonuria is caused by the absence or insufficient function of phenylalanine hydroxylase, which transforms phenylalanine into tyrosine. As a result of this dysfunction, phenylalanine is accumulated in the body, and toxic levels influence brain development.

MR imaging and MR spectroscopy (MRS) make it possible to observe abnormalities in patients with phenylketonuria (PKU). The observed areas of signal enhancement on T2-weighted images (4, 24, 29, 32) in periventricular white matter correspond to increasing T2 relaxation time (2). Similar findings can be seen in a number of diseases in which demyelination is present. However, our findings and those in the MRS literature do not confirm a demyelination process in the case of PKU because the N-acetylaspartate (NAA) signal as a neuronal marker in the spectra is not decreased (2, 6, 10, 13, 17, 21, 23, 25). Decreased choline (Cho) (6, 10) and inositol (13) signals in PKU patients have been observed, findings that remain largely unexplained at present. The visible signal of phenylalanine (17, 21, 23, 25) in the aromatic part of ^{1}H MR spectra measured for a large volume of interest could reflect the increase in serum phenylalanine from the normal level of about 60 μmol/l in controls to more than 1000 μmol/l in patients. Nevertheless, no significant correlation between serum and brain phenylalanine levels has been found (21, 23).

Several studies have shown that there is no correlation between either IQ or the clinical stage of patients and MR findings (19, 31). Some authors observed a partial reversal of white matter changes in patients (1, 4, 34) and it was hypothesized that the early changes in MR images represent white

matter edema with intramyelinic vacuole formation and the late changes reflect permanent myelin damage and loss (32).

In this study we therefore used different ^{1}H MR techniques, such as MR relaxometry, diffusion-weighted MR imaging, and localized ^{1}H MRS in PKU patients who were treated by strict or moderate diet in an attempt to explain the origin of possible MR findings.

Material and Methods

A group of 15 patients (mean age 23.4 years) with classical PKU and a control group of 14 age-matched healthy volunteers (mean age 21.5 years) were examined. Another group of 32 volunteers (mean age 24.6 years) was used for ^{1}H MRS comparison. Spectra of 1 patient were excluded from the evaluation. The clinical stage of all patients was stable, without any other disease, all patients were on moderate or strict diet for more than 10 years. The description of groups of patients and controls is given in Table 1. The examination protocol was approved by the local Grant Ethics Committee.

MR imaging: MR measurements were performed on a whole-body 1.5-T imager (Siemens Vision) with a standard head coil tuned and matched by the manufacturer. MR protocol: a turbo spin-echo T2-weighted MR sequence (TR/TE 5400/99 ms, flip angle 180°, 5 mm slice thickness, 260 mm field-of-view (FOV), 192×256 matrix) was used to determine the exact position of the volume of interest for MRS examination and T2 measurements using a modified Siemens CPMG (Carr-Purcell-Meiboom-Gill) sequence with 16 echoes (TE 22.5–360 ms, echo-spacing 22.5 ms, TR 3000 ms, 5 mm) was performed. Mono- and biexponential calculations of T2 (area of interest about 1.3 cm^2) were performed from intensities using CurveExpert 1.3 PC software. We used the mean value of T2 calculated from monoexponential fits for the comparison of both groups (controls vs. patients).

Diffusion-weighted images and the apparent diffusion coefficients of water (ADC, 10^{-4} mm^2) in the x, y and z directions ($ADC_{x,y,z}$) were calculated from images obtained using SE EPI sequences (TR/TE 4000/121 ms) with a single bipolar gradient in the x, y and z directions with b=1100 s/mm^2 and compared with a "trace" gradient method (11) with b=65 and 650 s/mm^2. The evaluation of ADC maps was repeated four times by an experienced operator, from the left and right sides of the transversal image, and the means were used for the comparison. Area of interest was chosen from the lesion (A). Frontoparietal white matter and gray matter (B) (Fig. 1c), where no significant changes due to PKU were observed in patients, was chosen as the control area. In the case of controls, the same positions were evaluated.

Two parameters characterizing diffusion in the tissue were calculated. The root mean square displacement of spins during the diffusion measurement (22), which can also be described as the distance that water molecules diffuse between collisions (R) in the x, y and z directions, can be calculated (assuming the fast exchange of water molecules in a soft tissue) by the equation

$$R = \sqrt{2ADC^{*}T_2} \qquad \text{(Eqn. 1)}$$

The tortuosity λ in the lesion can be calculated as

$$\lambda = \sqrt{D/ADC} \qquad \text{(Eqn. 2)}$$

where D is the free water diffusion coefficient D=$22^{*}10^{-4}$ mm^2/s.

MRS: Proton spectra were recorded using a short TE localized STEAM sequence (TR/TE/TM 5000/10/15 ms, TM – mixing time) with one or three CHESS pulses (chemical shift-selective pulse) for water suppression in automatic setup mode from a 3.4 ml volume of interest represented by a cube 15×15×15 mm in periventricular white matter (Fig. 1a). The measured spectra were evaluated automatically using the LCModel program (27). The concentrations of 13 compounds were determined; details are described separately (9).

Table 1

Clinical parameters describing patient groups and controls. Metabolite concentrations in serum (SD)

	n	Age, years (SD)	Phenylalanine, μmol/l (SD)	Tyrosine, μmol/l (SD)	Tryptophane, μmol/l (SD)	IQ (SD)	Genotype	Tremor
Patients	15	23.4 (4.6)	1503.8* (309.7)	47.84 (17.96)	49.93 (23.11)	90.0* (16.4)	Homozygous R408W: 3 Heterozygous R408W: 6 Other: 6	Absent: 12 Minimal: 2 Moderate: 1
Controls	14	21.5 (5.9)	117.9 (57.9)	58.39 (19.84)	59.59 (15.07)	128.43 (14.41)	–	–

* Significant difference from controls, $p<0.05$.

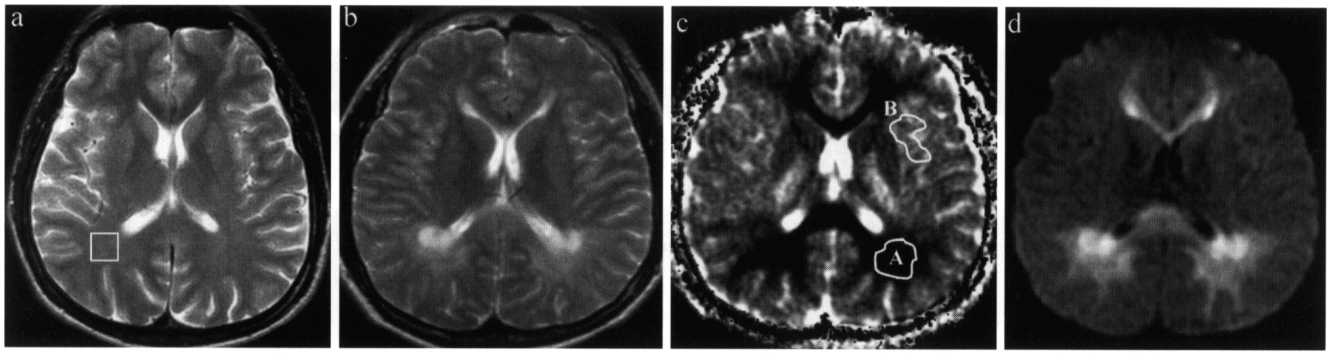

Fig. 1. T2-weighted transversal MR images of a healthy volunteer (a) and a PKU patient (b). *ADC* map (c) and diffusion-weighted (d) MR image of the same PKU patient. Typical pathological lesions are visible in the periventricular white matter. Area A: The mean *ADC* of water measured in PKU lesions in periventricular white matter. Area B: Visibly uninvolved white matter and gray matter in the frontoparietal region. Areas of interest A and B were approximately 1 cm^2. Projection of the volume of interest for the MRS examination is marked in (a).

Results

Relaxation and diffusion: We confirmed previous observations of pathological lesions characterized by symmetrical areas of T2 enhancement, especially in white matter near the posterior parts of the lateral ventricles, in all our patients (Fig. 1b). In the most affected areas, we found the T2 relaxation time in the range from 137 to 315 ms. The mean value of the T2 relaxation time was calculated to be 219.7±46.5 ms in the patient group, compared to 95±6 ms in healthy volunteers (Table 2).

Pathological changes in the periventricular region were observed with even better contrast in the diffusion-weighted images of PKU patients (Fig. 1d). The signal enhancement between lesion and uninvolved white matter was about 100%. *ADCs* of water in the *x*, *y* and *z* directions were calculated in both patients and controls (Fig. 2). Data are summarized in Table 2 from two different types of brain matter (e.g. periventricular white matter and frontoparietal white matter and gray matter tissue in both patients and controls). The absolute values of the *ADC* are in agreement with values previously published for white matter (18), i.e. about 6×10^{-4} mm^2/s. The mean *ADC* of water measured in PKU lesions in periventricular white matter (area A in Fig. 1c) decreased by 29–41% compared to the *ADC* calculated for the same volume of interest in the controls. Measurements of the diffusion coefficient in the frontoparietal region without any lesions revealed that the diffusion parameters in both groups (patients and controls) were not significantly different. The anisotropy observed in the group of controls was not clearly ob-

Table 2

Mean values of ADC (10^{-4} mm^2/s), R and λ in x, y and z directions (and SD) measured by the single bipolar gradient method from volume of interest in periventricular white matter (lesion) and control frontoparietal white and gray matter (FPariet) of patients and healthy volunteers

	Lesion x (SD)	Lesion y (SD)	Lesion z (SD)	Lesion mean (SD)	FPariet x (SD)	FPariet y (SD)	FPariet z (SD)	FPariet mean (SD)
PKU patients								
T2, ms		220				95		
ADC mean	4.59* (0.41)	4.87* (0.82)	4.29* (0.63)	4.59* (0.52)	7.17 (0.54)	6.69 (0.31)	7.00 (0.34)	6.95 (0.28)
R, μm	14.20 (0.62)	14.59 (1.25)	13.70 (1.02)	14.18 (0.81)	11.66 (0.45)	11.27 (0.26)	11.53 (0.28)	11.49 (0.23)
λ	2.19* (0.09)	2.15* (0.19)	2.28* (0.17)	2.20* (0.13)	1.76 (0.07)	1.82 (0.04)	1.77 (0.04)	1.78 (0.04)
Healthy volunteers								
T2, ms		95				95		
ADC mean	6.52# (0.74)	7.77# (0.21)	7.16# (0.19)	7.15 (0.30)	7.42 (0.54)	6.81 (0.75)	6.70 (0.89)	6.97 (0.40)
R, μm	11.11 (0.62)	12.15 (0.16)	11.66 (0.15)	11.65 (0.24)	11.86 (0.43)	11.36 (0.64)	11.25 (0.76)	11.51 (0.33)
λ	1.85 (0.10)	1.68 (0.02)	1.75 (0.02)	1.76 (0.04)	1.73 (0.06)	1.81 (0.11)	1.83 (0.13)	1.78 (0.05)

Statistical analysis by U-test. * Significant difference from control data, from frontoparietal volume of interest of patients and controls, $p<0.01$. # Significant difference between *ADC* values, $p<0.05$. *R*=root mean square displacement (Eqn. 1). λ=tortuosity (Eqn. 2).

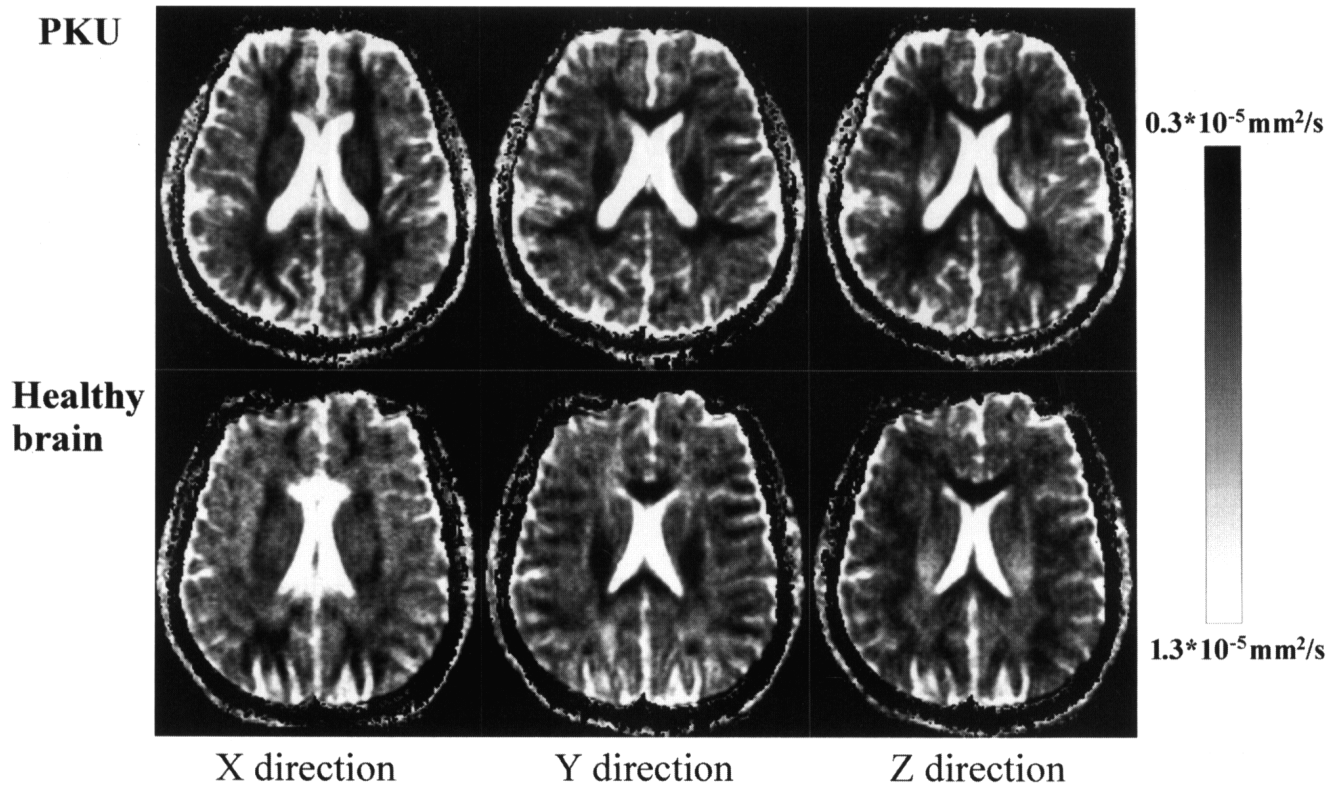

Fig. 2. The *ADCs* of water in the *x*, *y* and *z* directions of a healthy brain and the brain of a PKU patient.

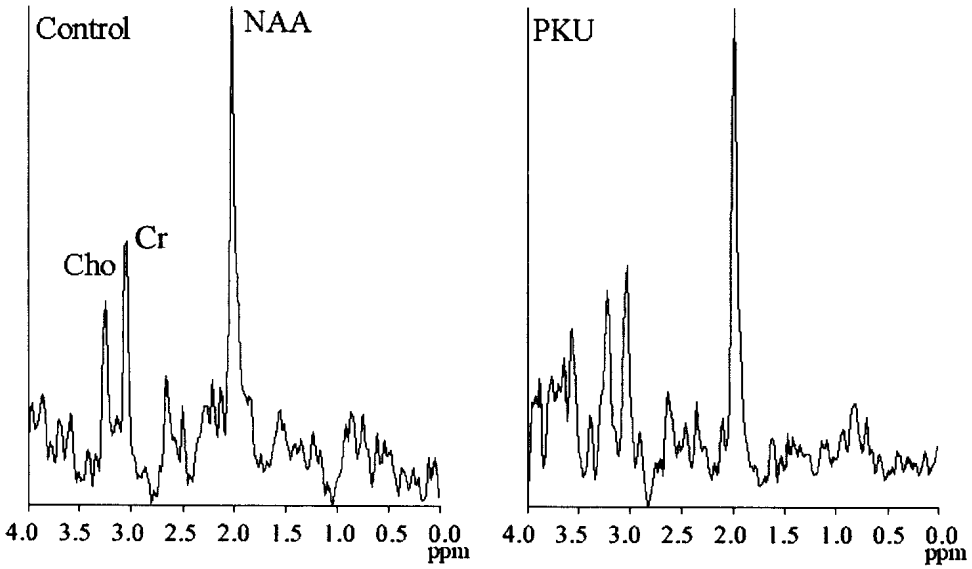

Fig. 3. ^{1}H MR spectra (TR/TE/TM 5000/10/15 ms) measured in the periventricular white matter of a healthy volunteer and a PKU patient in the range of 0–4 ppm (NAA – N-acetylaspartate, Cr – creatine/phosphocreatine, Cho – choline-containing compounds).

served in periventricular areas in the group of PKU patients, i.e. there was no significant difference between the mean ADC_x, ADC_y and ADC_z in PKU patients (Table 2).

Tortuosity calculated in the *x*, *y* and *z* directions in periventricular white matter lesions was significantly increased in PKU patients compared to controls. The results in Table 2 show for the mean distance *R* that water molecules diffuse between collisions (calculated from Eqn. 1) in the *x*, *y* and *z* directions. The trajectory between two collisions due to Brownian motion has in patients an in-

Table 3

Absolute concentrations (and SD) of selected metabolites (mmol/l) from lesion volume of interest in periventricular white matter in patients and controls

	n	Age	Cho	Cr	GABA	Glc	Myo-ins	Lac	NAA	NAAG	Scyllo-ins	Ala	Gln	Glu	Tau
Patients	14	22.8	1.43*	6.20	0.32	0.46	2.56	0.36	9.68	1.63**	0.23	0.39	3.29	4.37	0.19
		(4.9)	(0.27)	(1.02)	(0.44)	(0.85)	(1.50)	(0.44)	(1.48)	(0.71)	(0.34)	(0.64)	(3.07)	(4.40)	(0.34)
Controls	32	24.6	1.62	6.62	0.81	0.38	3.33	1.00	9.89	0.83	0.19	0.76	2.26	6.46	0.10
		(5.9)	(0.23)	(0.85)	(1.21)	(0.65)	(1.07)	(1.10)	(1.40)	(0.71)	(0.18)	(0.86)	(2.93)	(3.22)	(0.26)

Cho=choline-containing compounds. Cr=creatine/phosphocreatine. GABA=g-aminobutyric acid. Glc=glucose. Myo-ins=Myo-inositol. Lac=lactate. NAA=N-acetylaspartate. NAAG=N-acetylaspartylglutamate. Scyllo-ins=Scyllo-inositol. Ala=alanine. Gln=glutamine. Glu=glutamate, Tau=taurine. Statistical analysis by U-test. * significant difference from control data, $p<0.05$. ** significant difference from control data, $p<0.01$.

crease of about 15% (14 μm in PKU lesions compared to 12 μm in controls). No significant differences were observed between R in the x, y or z directions or between R in the areas of uninvolved frontoparietal brain tissue of patients and controls.

MRS: Fig. 3 shows typical ^1H MR spectra of a healthy volunteer and a PKU patient in the range of chemical shifts between 0 and 4 ppm obtained in white matter. The MR spectra of patients are not qualitatively different from the spectra of controls. However, from a quantitative point of view, the absolute molar concentrations of metabolites summarized in Table 3 show a significant decrease in the Cho concentration in PKU patients. This is in agreement with our previous findings in PKU patients (6, 10). The concentration of NAA in PKU lesions was not significantly different from control patients' data, although the CH_3 signal of NAA has to be considered as the signal of NAA with N-acetylaspartylglutamate (NAAG) contribution. In the present study an increased NAAG in PKU patients was observed, but not the sum of both NAA and NAAG compounds.

Discussion

Previously, lesions observed on T2-weighted MR images of PKU patients have been described as demyelination (20, 29). Nevertheless, histological and other studies show that these findings do not represent the typical demyelination that can be found in other lesions such as those seen in multiple sclerosis, tumors etc. A typical example of demyelination was described in the case of a 55-year-old untreated female PKU patient (7). Quite the opposite finding was observed in a 27-year-old patient with typical untreated PKU, where the myelination and neuronal cell population were remarkably similar to those of the controls, except for changes resulting from the associated neural dysfunction (14, 15).

Several animal studies that have been performed can be divided into two groups: experiments with genetically modified mice and experiments with induced hyperphenylalaninemia. Genetically modified mice (deficient in phenylalanine hydroxylase) revealed no changes in myelin (14). Studies with induced PKU in rats (injecting or feeding phenylalanine) showed different findings. Retarded myelination, probably due to slow axonal maturation followed by hypomyelination, and reversible changes in myelin-basic and neurofilament proteins in the rats' brains were found by immunohistochemical analysis (3, 28). In humans, partially reversible changes on T2-weighted MR images in the periventricular white matter have been observed in a group of patients who underwent a strict low-phenylalanine diet (1, 4, 34).

The results of our present study using diffusion-weighted MR imaging and MRS support the hypothesis that the lesions observed in PKU patients are not produced by any demyelination process. Compared to age-matched control data, we measured a significant decrease of about 30% in the mean ADC of water in PKU lesions in periventricular white matter. Such a result is usually consistent with a decreased NAA concentration that is considered as the marker of neuronal loss. This was not the case in PKU patients, in that no significant changes in the NAA signal were observed in the MR spectra, and confirmed that there is no decrease in the concentration of NAA that could be ascribed to neuronal (axonal) damage (2, 6, 10, 13, 25). The unchanged concentrations of NAA and creatine/phosphocreatine (Cr) show that there is probably no change in the total number of axons in lesions; in addition, the same concentrations in PKU patients and control groups explain the good clinical state of patients under a suitable diet. The change that we observed in the group of patients was a decrease in the concentration of Cho-containing compounds. This finding is consistent with

our previous observation of an increased ratio of NAA/Cho in measurements using longer TE sequences (6, 10). The decrease of Cho could also be consistent with the decrease in neurotransmitter levels observed in the brain of PKU patients (26, 30).

In the present study, we also found a significantly increased NAAG in PKU patients. NAAG is associated with a group of neurotransmitters; however, the balance in the neurotransmitter pool is very subtle and only small changes should be expected in ^{1}H MR spectra.

The changes in the extracellular space correspond to T2 changes of water molecules. It is known that the total water concentration in a lesion is unchanged compared to the healthy brain (12).

If we accept the three-fraction hydration model of water distribution in the tissue (8), the measured mean relaxation time contains contributions from the relaxation rates of bulk, hydration and bound water. Bulk water is represented by free water molecules which are in the moment of the measurement not bound to any other water molecule or to the hydrophilic site with a long relaxation time T2 (approximately 500 ms). Hydration and bound water are mostly water molecules interacting with macromolecules with a short T2 (approximately 30 ms and shorter). These values of T2 relaxation

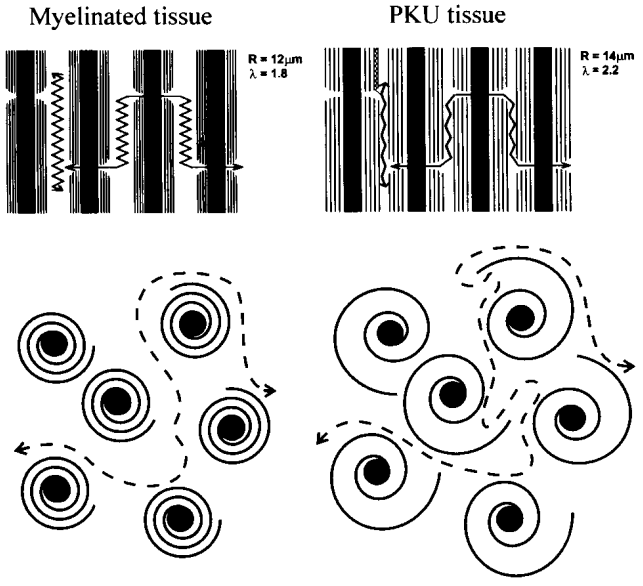

Fig. 4. Projection of the motion of water molecules along and perpendicularly to myelin fibers. The trajectory R of water molecules between two collisions is about 15% longer in PKU tissue compared to controls due to the increasing tortuosity of the extracellular space (tortuosity λ increased about 26%). Water molecules could also diffuse in greater extent into the inner space of myelin sheats.

times can be calculated from the relaxation curves summarizing all relaxation contributions in the form of a multi-exponential curve. The multi-exponentional dependence of signal intensity vs. echo time in CPMG experiments in white matter has been described by several authors (2, 5, 16).

We measured T2 relaxation times in PKU lesions that were more than 100% longer than the T2 times measured in controls. Staying on a semi-quantitative approach, an increase of T2 in lesions (mean T2 value of 220 ms) can be explained by the increasing contribution of free water molecules to the relaxation. The ratio of free to bounded water concentration increased from 2.7 for normal tissue (mean T2 95 ms) to 11.3 in lesions.

Another parameter – the distance between two collisions calculated using the root mean square displacement equation – shows that the displacement (R) of water molecules in PKU lesions is about 15% greater than in controls. The explanation may lie in the hindrances to motion that water molecules encounter in their tortuous path around obstacles such as macromolecules, intracellular organelles and a changed myelin structure. Then, the pathway of free water molecules in cellular tissue can be much longer than the straight distance among cells.

In the white matter of controls, diffusion images show strong anisotropy, which is also characterized by significant differences in the *ADCs* in the area of ascribed PKU lesions (Table 2). In the group of patients, no significant anisotropy in the visible lesions in the periventricular area was demonstrated (Fig. 2) because no statistically significant differences in *ADCs* were observed (Table 2).

The significant decrease of *ADC* seen in PKU lesions corresponds to an increased tortuosity, which is significantly different from the tortuosity calculated in controls. This means that the movement of water molecules is more severely restricted in the extracellular space of PKU lesions. On the other hand, the distance between two collisions calculated using the root mean square displacement equation is about 15% greater in PKU lesions, suggesting an increased extracellular space volume or a greater number of hydrophobic sites, mainly due to changes in the myelin sheaths.

In conclusion, in PKU patients, where T2 enhancement is preferentially observed in periventricular white matter, the results of T2 relaxometry, diffusion measurements and ^{1}H spectroscopy support the hypothesis that the increase of T2 in a patient reflects a greater concentration of free unbound water molecules in the lesion. In addition, the water molecules in the lesion also have increased mobility, as described by R, which is higher

in the lesion than in the same volume of interest in the controls (Fig. 4). The increase in water mobility might be explained by changes in the myelin sheaths, which, presumably, have a different geometry in PKU patients than in healthy controls, and thus water molecules can diffuse between them more easily due to changes in extracellular space geometry (33). The changes in geometry are confirmed by the loss of anisotropy in the lesion as compared to controls and by the increased tortuosity in PKU lesions.

ACKNOWLEDGEMENTS

This study was supported by grants GACR No. 309/97/K048 and No. 309/99/0657, IGA MZ CR No. 1064-7 and VS 96 130, The Czech Republic.

REFERENCES

1. Battistini S., De Stefano N., Parlanti S. & Federico A.: Unexpected white matter changes in an early treated PKU case and improvement after dietary treatment. Funct. Neurol. 6 (1991), 177.
2. Bick U., Ullrich K., Stöber U. et al.: White matter abnormalities in patients with treated hyperphenylalaninaemia. Magnetic resonance relaxometry and proton spectroscopy findings. Eur. J. Pediatr. 152 (1993), 1012.
3. Burri R., Matthieu J. M., Vandevelde M., Lazeyras F., Posse S. & Herschkowitz N.: Brain damage and recovery in hyperphenylalaninemic rats. Dev. Neurosci. 12 (1990), 116.
4. Cleary M. A., Walter J. H., Wraith J. E., White F., Tyler K. & Jenkins J. P.: Magnetic resonance imaging in phenylketonuria. Reversal of cerebral white matter change. J. Pediatr. 127 (1995), 251.
5. Danielsen E. R. & Henriksen O.: Absolute quantitative proton NMR spectroscopy based on the amplitude of the local water suppression pulse. Quantitation of brain water and metabolites. NMR Biomed. 7 (1994), 311.
6. Dezortová M., Hájek M. & Hejcmanová L.: Decreasing choline signal – A marker of phenylketonuria? Magma 4 (1996), 181.
7. Forssman H., Kristensson K., Sourander P. & Svennerholm L.: Histological and chemical studies of a case of phenylketonuria with long survival. J. Ment. Defic. Res. 11 (1967), 194.
8. Fullerton G. D. & Cameron I. L.: Relaxation of biological tissues. *In:* Biological magnetic resonance imaging, p. 115. Edited by F. W. Wehrli et al. VCH Publishers, New York 1988.
9. Hájek M., Burian M. & Dezortová M.: Application of LCModel for quality control and quantitative *in vivo* 1H MR spectroscopy by short echo time STEAM sequence. Magma 10 (2000), 6.
10. Hájek M., Hejcmanová L. & Pradny J.: Proton *in vivo* spectroscopy of patients with hyperphenylalaninaemia. Neuropediatrics 24 (1993), 111.
11. Heid O. & Weber J.: Diffusion tensor trace pulse sequences. *In:* Proceedings 5th ISMRM Meeting (1997), p. 225.
12. Huether G., Neuhoff V. & Kaus R.: Brain development in experimental hyperphenylalaninemia. Disturbed proliferation and reduced cell numbers in the cerebellum. Neuropediatrics 14 (1983), 12.
13. Johannik K., Van Hecke P., Francois B. et al.: Localized brain proton NMR spectroscopy in young adult phenylketonuria patients. Magn. Reson. Med. 31 (1994), 53.
14. Kornguth S., Anderson M., Markley J. L. & Shedlovsky A.: Near-microscopic magnetic resonance imaging of the brains of phenylalanine hydroxylase-deficient mice, normal littermates, and of normal BALB/c mice at 9.4 T. Neuroimage 1 (1994), 220.
15. Kornguth S., Gilbert-Barness E., Langer E. & Hegstrand L.: Golgi-Kopsch silver study of the brain of a patient with untreated phenylketonuria, seizures and cortical blindness. Am. J. Med. Genet. 44 (1992), 443.
16. Kreis R., Fusch C. & Boesch C.: *In vivo* characterization of three water compartments in human white matter using a single voxel technique with short TE. *In:* Proceedings 11th SMRM Meeting (1992), p. 1963.
17. Kreis R., Pietz J., Penzien J., Herschkowitz N. & Boesch C.: Identification and quantitation of phenylalanine in the brain of patients with phenylketonuria by means of localized *in vivo* 1H magnetic-resonance spectroscopy. J. Magn. Reson. Series B 107 (1995), 242.
18. Le Bihan D., Turner R. & Patronas A.: Diffusion MR imaging in normal brain and in brain tumors. *In:* Diffusion and perfusion magnetic resonance imaging. Application to functional MRI, p. 134. Edited by D. Le Bihan. Raven Press, New York 1995.
19. Leuzzi V., Gualdi G. F., Fabbrizi F., Trasimeni G., Di Biasi C. & Antonozzi I.: Neuroradiological (MRI) abnormalities in phenylketonuric subjects. Clinical and biochemical correlations. Neuropediatrics 24 (1993), 302.
20. Malamud N.: Neuropathology of phenylketonuria. J. Neuropathol. Exp. Neurol. 25 (1966), 254.
21. Möller H. E., Vermathen P., Ullrich K., Weglage J., Koch H.-G. & Peters P. E.: *In-vivo* NMR spectroscopy in patients with phenylketonuria. Changes of cerebral phenylalanine levels under dietary treatment. Neuropediatrics 26 (1995), 199.
22. Neil J. J.: Measurement of water motion (apparent diffusion) in biological systems. Concepts Magn. Reson. 9 (1997), 385.
23. Novotny E. J. Jr, Avison M. J., Herschkowitz N. et al.: *In vivo* measurement of phenylalanine in human brain by proton nuclear magnetic resonance spectroscopy. Pediatric Res. 37 (1995), 244.
24. Pearsen K. D., Gean-Marton A. D., Levy H. L. & Davis K. R.: Phenylketonuria. MR imaging of the brain with clinical correlation. Radiology 177 (1990), 437.
25. Pietz J., Kreis R., Schmidt H., Meyding-Lamadé U. K., Rupp A. & Boesch C.: Phenylketonuria. Findings at MR imaging and localized *in vivo* H-1 MR spectroscopy of the brain in patients with early treatment. Radiology 201 (1996), 413.
26. Potempska A., Loo Y. H. & Wisniewski H. M.: On the possible mechanism of phenylacetate neurotoxicity. Inhibition of choline acetyltransferase by phenylacetyl-CoA. J. Neurochem. 42 (1984), 1499.
27. Provencher S. W.: Estimation of metabolite concentrations from localized *in vivo* proton NMR spectra. Magn. Reson. Med. 30 (1993), 672.
28. Reynolds R., Burri R. & Herschkowitz N.: Retarded development of neurons and oligodendroglia in rat forebrain produced by hyperphenylalaninemia results in permanent deficits in myelin despite long recovery periods. Exp. Neurol. 124 (1993), 357.
29. Shaw D. W. W., Maravilla K. R., Weinberger E., Garretson J., Trahms C. M. & Scott C. R.: MR imaging of phenylketonuria. AJNR 12 (1991), 403.
30. Swaiman K. F. & Wu S. R.: Phenylalanine and phenylace-

tate adversely affect developing mammalian brain neurons. Neurology 34 (1984), 1246.
31. THOMPSON A. J., TILLOTSON S., SMITH I., KENDALL B. E., MOORE S. G. & BRENTON D.: Brain MRI changes in phenylketonuria. Associations with dietary status. Brain 116 (1993), 811.
32. VAN DER KNAAP M. S. & VALK J.: Magnetic resonance of myelin, myelination, and myelin disorders, p. 192. Springer, Berlin 1995.
33. VORISEK I. & SYKOVA E.: Evolution of anisotropic diffusion in the developing rat corpus callosum. J. Neurophysiol. 78 (1997), 912.
34. WEGLAGE J., SCHUIERER G., KURLEMANN G., BICK R. & ULLRICH K.: Different degrees of white matter abnormalities in untreated phenylketonurics. Findings in magnetic resonance imaging. J. Inherit. Metab. Dis. 16 (1993), 1047.