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Characterization of CNS Lesions by Using High-Resolution ^{1}H MR Spectroscopy of CSF:
Preliminary Results

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Sixty-six samples of CSF from 66 patients with a variety of diseases, including tumors, arteriovenous malformations, aneurysms, brain infarctions, and lumbar back pain, were studied with ^{1}H MR spectroscopy at 360 MHz. ^{1}H MR spectroscopy offers a simple means to obtain fast information about different metabolites simultaneously. As compared with the control group, which consisted of 19 CSF samples from the same group of 66 patients, but from individuals who had no abnormal findings on neurologic examination, common clinicochemical tests, or neuroradiologic studies, our preliminary results suggest that tumors and hemorrhages may be differentiated by ^{1}H MR spectroscopy. MR peak intensities relative to lactate peak intensity were used as variables in a statistical analysis to determine the significance of individual resonance intensities in predicting CNS abnormalities. The most important factors for diagnosis were analyzed by means of a multivariate general linear hypothesis and a principal component method. The most important factors for predicting CNS abnormalities in the studied diseases were creatinine, glucose, creatine, citrate, protein content, glutamine, amount of cells, and valine. By using this model for discriminant analysis, we could predict hemorrhages correctly in 88% of cases and tumors in 75% of cases. All samples of controls were determined correctly. In cases of brain infarctions, different signals were observed, which may lead to further characterization of such lesions.

^{1}H MR spectroscopy may offer a simple means for further characterizing CNS lesions. However, this needs confirmation by a prospective study, which would include a larger patient population with different diseases.

CSF is a common information source in the diagnosis of diseases of the CNS. To date, common clinicochemical tests—such as determination of cells, protein content, and electrophoresis—are usually employed to study CSF for the purpose of supporting diagnoses of neurologic or neurosurgical diseases. Because CSF reflects the metabolic situation of the brain, it seems reasonable to apply other tests of analytical chemistry, such as MR spectroscopy, to the study of CSF to gain fast information about brain metabolism, and, thus, more data for further characterizing CNS lesions. This article describes our preliminary results with high-resolution ^{1}H MR spectroscopy for human CSF.

Materials and Methods

Sixty-six samples of CSF from 66 patients who were thought to have neurologic/neurosurgical disease were studied by common clinicochemical tests and ^{1}H MR spectroscopy at 360 MHz (Bruker WH 360 and Bruker AM 360 WB, respectively). In 19 patients, who had as a clinical sign only low back pain but no neurologic deficit, clinicochemical tests showed normal protein contents and normal quantities of cells. Since no neurologic disease could be confirmed either by common clinical tests or by neuroradiologic studies, which included myelography and CT, these cases were considered as a representative control group with normal metabolite levels in CSF. No weighting procedure was performed to indicate outliers and unknown pathologic samples. If the concentration of a metabolite deviated more than 2 SD from the mean, the complete sample was considered suspect and was excluded from

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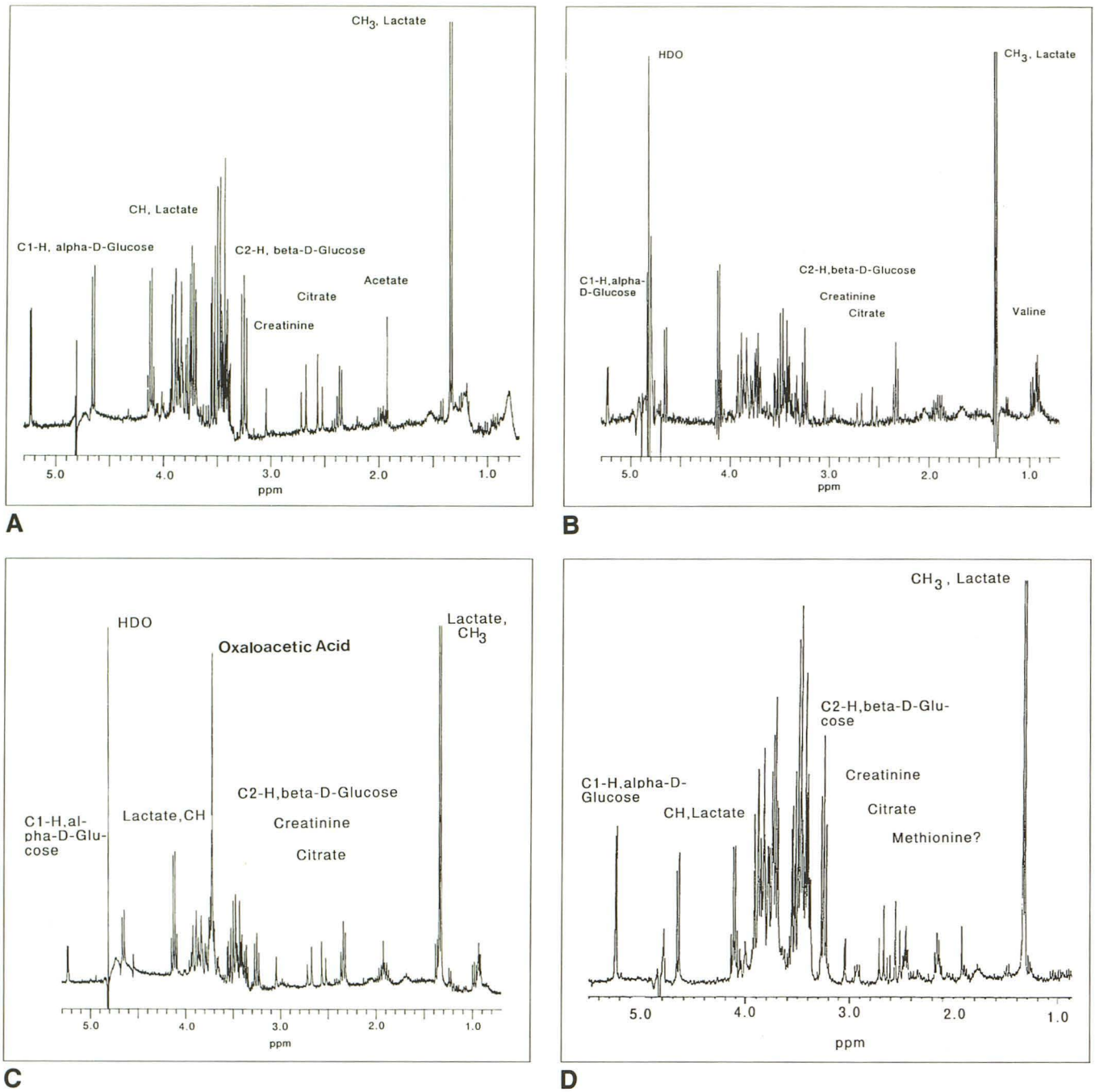

Fig. 1.—^{1}H MR spectra of different samples of human CSF, recorded at 360 MHz. Each spectrum was acquired in approximately 10 min. The vertical scale was selected in such a way that the methyl signal of each lactate had identical intensities in all spectra. This signal is truncated at 50% of its total intensity.

***A*, Normal finding. Lactate served as internal reference for chemical shift scale and for the determination of relative metabolite concentrations. The broad resonance around 4.7 ppm indicates incomplete suppression of the signal of residual H_2O or HDO. The most important metabolites are shown by name. For further assignment, see Table 1. The numerous peaks between 3.3 ppm and 4.0 ppm also belong to glucose, but cannot be quantitated because other metabolites (e.g., fructose) resonate in the same spectral region.**

***B*, Intraspinal, extramedullary tumor. In comparison with normal CSF (Fig. 1A), it is immediately obvious that the glucose level and the citrate level are lower and that the levels of the amino acids α-alanine and valine are higher in tumorous CSF. The spikes near 4.8 ppm arise from the saturation of the large HDO signal.**

***C*, Subarachnoid hemorrhage. Compared with Fig. 1B, at 3.7 ppm oxaloacetic acid is visible as a new signal.**

***D*, Brain infarction. Compared with Figs. 1B and 1C, at 2.45 and 2.6 ppm a new signal is visible, which has been preliminarily assigned to methionine (2.6 ppm). For further signal assignment see Table 1.**

the study. Since in two cases the concentration of acetate deviated roughly more than 600%, these two samples had to be excluded; therefore, only 17 samples were used to form a control group. The pathologic samples consisted of 26 tumors; 18 hemorrhages caused by arteriovenous malformations, aneurysms, or trauma; and three brain infarctions. The diagnosis was confirmed by CT and/or angiography in all cases. MR at 0.5 or 1.5 T was performed in 16 cases of brain tumor. Twenty-four of 26 tumors were treated surgically, and thus could be confirmed histologically. The two cases that were not surgically confirmed included one presumed glioblastoma multiforme, which invaded the midline structures of the brain, and one brainstem tumor, which was considered to be a glioma.

After centrifugation, 1 ml of CSF was lyophilized and resuspended in 0.4 ml of deuterium (D_2O). ^1H MR spectra were recorded at 360 MHz with flip angles of 30° using a superconducting magnet operating at 8.47 T. One hundred to 500 transients were accumulated with a recycle time of 2 sec. The field was locked to D_2O. For each sample experimental conditions were identical (temperature, 25°C; same set of parameters for spectrometer setting). The chemical shifts were referenced to the signal of the lactate methyl group at 1.33 ppm. The abundant signal of residual water protons was selectively saturated before the sampling pulse.

Signals were assigned to molecular substructures by reference to empirical shift tables [1–3]. Conclusive evidence was gained by reconstructing the molecular structure or substructure from two-dimensional spectra shift-correlated spectroscopy (COSY) [4], which were obtained from CSF of the control group and/or from the increase of signal intensity after the addition of authentic material. In some pathologic findings COSY was also performed. Since the baseline had too many broad underlying peaks in several cases, signal intensities were used to measure concentrations. Thus, signal intensities were measured as signal heights and corrected for the number of contributing equivalent protons and hyperfine splitting caused by J-coupling. For each spectrum, these intensities were divided by that of the methyl signal of lactate (signal H8, see Fig. 1) and multiplied by 100. The resulting figures are metabolite/lactate ratios in arbitrary units, which can be compared with each other. In all but two metabolites (signal H2 and H6) the individual linewidth was identical, and thus the ratios were comparable. As the saturation of the water signal was not ideally selective, the glucose signal H20 experienced a partial saturation. The amount of partial saturation was estimated by comparing the lactate signal H19, which also was partially saturated, with signal H8, which was not affected. The intensity of signal H20 was corrected accordingly, allowing for the different frequency offset [5]. No other signal was corrected. In all spectra, the resulting ratio of glucose anomers H20:H18 was near the expected value of 36:64, which can be calculated by changing specific rotations toward equilibrium of the α-glucose and β-glucose anomers (mutarotation). Thus, the correction for partial saturation was right.

Sixty-one CSF samples from as many patients were evaluated statistically. The Kruskal-Wallis test was performed for all available peaks to identify those metabolites that differed at a level of 99%. These variables were used as a model for a multivariate general linear hypothesis to give a discriminant analysis. The principal components were analyzed and the strength of relationship between two variables was read directly from the calculated corrected contingency coefficient (CC_{corr}) according to Pawlik [6], whose values always range from 0 to 1. The value of 1 would mean a 100% relationship, or a complete dependence, while 0 would denote no relationship at all. Thus, the most important factors for diagnosis could be estimated.

Results

Figure 1 gives the proton MR spectrum of a healthy subject. The spectrum is a complete picture of all metabolites, which contain covalently bound protons and occur in a concentration roughly above 10 μM. Urea, which is abundant in CSF [7], remains invisible, because its protons are exchangeable with water and are replaced by deuterons from the solvent. Thus, ^1H MR spectroscopy is a fast and simple means to record many components of CSF. The relative metabolite levels or metabolite/lactate ratios in the group of controls are presented in Table 1.

In cases of tumors, a comparison of the spectra in figs. 1A and 1B immediately indicates distinct differences between normal CSF (Fig. 1A) and tumorous CSF (Fig. 1B), the latter

TABLE 1: Relative Concentrations of Metabolites in CSF of the Control Group (n = 17)

Signal	Chemical Shift (ppm)	Signal Assignment	Relative Concentrations	No. of Subjects
H1	0.17	CH3, not assigned	1.78 ± 2.07	8
H2	0.85	CH3, lipids	2.99 ± 4.38	14
H3			2.06 ± 1.85	12
H4	0.98; 1.03	CH3, valine	0.19 ± 0.43	4
H5	1.18	CH3, β-hydroxybutyric acid	3.42 ± 6.07	12
H6	1.26	CH2, lipids	8.25 ± 21.80	6
H7			0.45 ± 0.87	5
H8	1.33	CH3, lactate	100.00	17
H9	1.45	CH3, α-alanine	3.35 ± 6.72	11
H10	1.92	CH3, acetate	45.49 ± 150.22	17
H11	1.97	Putrescine?	11.15 ± 6.73	14
H12	2.02	CH2?	5.34 ± 3.76	14
H13	2.24	CH3, acetone	6.02 ± 14.62	12
H14	2.39	Glutamine?	15.91 ± 5.20	14
H15	2.62	CH2, citrate	11.88 ± 3.47	15
H16	3.04	CH3, creatinine	3.65 ± 0.80	17
H17	3.05	CH3, creatinine	3.70 ± 1.04	17
H18	3.25	C2-H, β-D-glucose	241.87 ± 54.46	17
+				
H20	5.24	C1-H, α-D-glucose		
H19	4.12	CH, lactate		

Note.—Concentration of lactate set at 100.00.

TABLE 2: Relative Concentrations of Metabolites in Tumorous CSF (n = 24)

Signal	Mean	Standard Deviation	Standard Error	Variance
H1	0.375	1.136	0.232	1.292
H2	3.577	4.647	0.949	21.599
H3	1.502	4.514	0.921	20.376
H4	2.185	2.356	0.481	5.550
H5	1.058	1.304	0.266	1.700
H6	13.513	32.854	6.706	1079.370
H7	0.881	1.376	0.281	1.894
H8	100.00			
H9	6.818	16.730	3.415	279.887
H10	3.825	3.261	0.666	10.637
H11	9.549	5.899	1.204	34.803
H12	13.532	40.772	8.323	1662.348
H13	2.291	6.840	1.396	46.791
H14	13.046	7.348	1.500	53.989
H15	7.015	3.488	0.712	12.166
H16	2.277	1.169	0.239	1.366
H17	1.677	1.025	0.209	1.050
H18	71.663	46.498	9.491	2162.018
H20	42.321	26.759	5.462	716.057
H18 + H20	113.317	71.331	14.560	5088.168

TABLE 3: Relative Concentrations of Metabolites in CSF with Subarachnoid Hemorrhage (n = 17)

Signal	Mean	Standard Deviation	Standard Error	Variance
H1	0.053	0.174	0.042	0.030
H2	4.271	5.928	1.438	35.143
H3	1.400	2.390	0.580	5.714
H4	1.676	1.757	0.426	3.086
H5	2.265	3.951	0.958	15.607
H6	8.918	18.116	4.394	328.177
H7	1.265	2.252	0.546	5.074
H8	100.00			
H9	2.494	2.625	0.637	6.889
H10	3.012	2.517	0.610	6.335
H11	6.359	5.191	1.259	26.943
H12	3.394	2.806	0.680	7.872
H13	1.976	4.847	1.176	23.496
H14	9.376	4.143	1.005	17.163
H15	6.853	2.425	0.588	5.879
H16	2.159	1.046	0.254	1.094
H17	1.688	0.967	0.235	0.935
H18	60.218	29.004	7.034	841.220
H20	37.606	22.342	5.419	499.183
H18 + H20	97.824	50.934	12.353	2594.223

of which was taken from a patient with an intraspinal extramedullary tumor. In the tumorous CSF the signals of glucose (3.2–4.0 ppm) are reduced, while new signals appear between 0.8 and 1.0 ppm and at 1.28 and 1.42 ppm, as compared with the spectra of the control group. These signals were identified as α-alanine and valine, respectively. In other tumorous samples the position of these signals varied slightly because of the individual pH and/or ionic strength. The addition of these α-amino acids was repeated in these samples, which led to the same assignments. By reference to shift tables the broad signals at 0.87 and 1.28 ppm were attributed to various fatty acids. Some of the tumorous samples showed poorly resolved signals between 5 and 0.5 ppm, which are typical for proteins [8, 9]. These signals had a low amplitude and were not quantitated.

In cases of hemorrhage (Fig. 1C), oxaloacetic acid was observed at 3.7 ppm as new signal in seven of 18 cases; in the three cases of brain infarctions (Fig. 1D), two new signals appeared at 2.45 and 2.6 ppm, which were preliminarily assigned to methionine. Tables 2 and 3 give the metabolite/lactate ratios in CSF of tumors and hemorrhage. Since there are significant differences in metabolite/lactate ratios as compared with the control group, it seemed to be reasonable to run discriminant analysis using those metabolites as a model. They differed significantly at a level of 99%.

The results of discriminant analysis are presented in Table 4. The group of controls is perfectly distinguished, whereas hemorrhages are distinguished in 88% of cases and tumors in 75%. The analysis of principal components by calculation of the corrected contingency coefficient CC_{corr} according to Pawlik [6] shows that in this small series of 12 variables eight had a more or less strong relation to the tested diseases (Fig. 2), starting with creatinine, glucose, creatine, and citrate and followed by basic clinicochemical test results, including protein content, glutamine, amount of cells, and valine.

TABLE 4: Results of Discriminant Analysis (n = 58)

	Control Group	Hemorrhage Group	Tumor Group	Total
Control group	17	0	0	17
Hemorrhage group	1	15	1	17
Tumor group	1	5	18	24
Total	19	20	19	58

Note.—The rows show the actual groups; the columns show the prospective registration into each group.

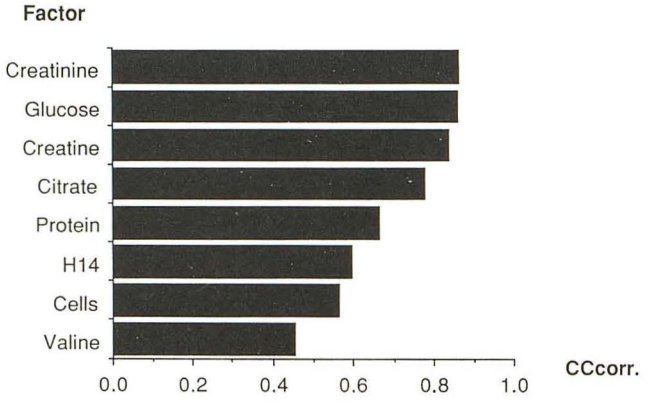

Fig. 2.—Results of analysis of factors for diagnosis and calculation of corrected contingency coefficients according to Pawlik [6].

Discussion

The first aim of this study was to determine whether proton MR spectroscopy is feasible for CSF, and the second aim was to see whether different diseases of the CNS reflect themselves in ^{1}H MR spectra. Therefore, the simplest and fastest experimental procedure was used: we avoided a

complete characterization of the samples, such as measurements of pH, an addition of an internal chemical-shift and/or concentration standard, and a complete identification of all metabolites. Consequently, these data have to be regarded as preliminary results, and care must be taken if one compares these findings with those obtained by common clinical analyses, including enzymatic and chromatographic studies [10–12]. Nevertheless, it seems to be obvious that the levels of some metabolites—such as the level of creatinine, creatine [13], glucose, and citrate [14]—as well as the levels of the α-amino acid valine may serve as important markers in the diagnosis, because these levels differ remarkably among tumorous samples in CSF. This finding is in line with a report on medulloblastomas, gliomas, and neuroectodermal cell lines. In accordance with this report, creatine may be useful as a marker for prognosis, diagnosis, and monitoring of response to therapy [15].

Since the concentrations are relative to the concentration of lactate or else metabolite/lactate ratios, the low level of glucose in tumorous CSF may also be due to an increased amount of lactate in these samples. However, both an increased level of lactate and a decreased level of glucose would indicate an enhanced energy metabolism via anaerobic glycolysis. Nevertheless, we tentatively conclude that either the absolute glucose concentration or the glucose/lactate ratio seems to be an important factor in discriminating healthy from pathologic CSF. To our knowledge, a lowered glucose level in CSF has not yet been related to brain neoplastic disease [7]. There is one article on insulinoma-engrafted rats, which reports depressed levels of brain glucose [16]. However, our report is on intraaxial and extraaxial brain tumors without a case of metastasis of an insulinoma. High levels of α-amino acids may serve as warning signs. The diagnostic value of α-amino acid levels has been discussed elsewhere [7, 17, 18] in relation to several diseases, but not to tumors. In this small series of hemorrhages, oxaloacetic acid was observed only in cases of subarachnoid hemorrhage. Thus, it may be a marker for a hemorrhage. However, one has to look for all metabolite/lactate ratios to get a diagnosis, since the present series is small. Moreover, in the future, the model used for discriminant analysis may have to be changed to serve better as a diagnostic test. Since CSF was centrifugated in preparation for MR spectroscopy, no correlation was calculated between the amount of erythrocytes or other cells and the metabolite/lactate ratios.

The results of the discriminant analysis on this small series indicate that hemorrhages may be clearly delineated by ^{1}H MR spectroscopy. This finding may be important in cases of arteriovenous malformations, since a previous hemorrhage is an indication for surgical treatment. The slight misregistration of tumors into the group of hemorrhages may be due to an undetected hemorrhage within the tumor or to the inhomogeneity within the group of tumors, since the group consisted of both intraaxial and extraaxial lesions.

Conclusions

These first applications indicate that ^{1}H MR spectroscopy is feasible for CSF studies. We believe that ^{1}H MR spectroscopy may offer a simple means for further characterizing CNS lesions, since it measures many metabolites simultaneously, and thus may offer fast information on brain metabolism in different diseases and in different stages of any one disease. A future prospective study will test the most important factors for diagnosis and will include different diseases, because we believe that ^{1}H MR spectroscopy of CSF may become a useful diagnostic tool for treatment monitoring and prognosis.

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