Cerebral Metabolism in Severe Neonatal Hyperbilirubinemia

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ABSTRACT. The metabolism of the basal ganglia was examined by using proton magnetic resonance spectroscopy in 5 neonates with severe hyperbilirubinemia. A decreased *N*-acetylaspartate/choline ratio, indicating neuronal injury, and an abnormally high lactate/*N*-acetylaspartate ratio were found only in the neonate with neonatal magnetic resonance imaging abnormalities and subsequent cerebral palsy. *Pediatrics* 2004;114:291–294; *bilirubin, neonate, kernicterus, magnetic resonance spectroscopy, metabolism.*

ABBREVIATIONS. MRI, magnetic resonance imaging; ^{1}H-MRS, proton magnetic resonance spectroscopy; NAA, *N*-acetylaspartate; Cho, choline; ABR, auditory brainstem response; AABR, automated auditory brainstem response; ADC, apparent diffusion coefficient; G6PD, glucose-6-phosphate dehydrogenase.

Elevated levels of unconjugated bilirubin in neonates can induce changes in the mitochondria of the basal ganglia, resulting in a disturbed mitochondrial respiration, increased apoptosis, and so-called bilirubin encephalopathy.[1,2] Animal experiments have indicated that disruption of the blood-brain barrier, hypoxia, and acidosis contribute to bilirubin toxicity.[3,4] Changes in the *N*-methyl-D-aspartate receptor of neurons may increase bilirubin toxicity.[5] Tissue changes in the basal ganglia can be visualized by using magnetic resonance imaging (MRI) techniques.[6,7]

For a decade cerebral metabolism of neonates has been studied by using proton magnetic resonance spectroscopy (^{1}H-MRS).[8] Reduced *N*-acetylaspartate (NAA)/choline (Cho) ratios and elevated lactate/NAA ratios in asphyxiated term neonates predict an adverse neurodevelopmental outcome.[8,9]

In the present study we tested the hypothesis that alterations in cerebral metabolism could be demonstrated in vivo in neonates with high levels of unconjugated bilirubin.

SUBJECTS

Subjects were all 5 neonates admitted during the last 7 years to our level III neonatal intensive care unit with bilirubin levels of ≥25 mg/dL (≥425 μmol/L). All neonates showed hypotonia on admission. Patient E had a definite abnormal clinical picture, with a high-pitched cry and opisthotonos. Additional clinical details are presented in Table 1. A sixth neonate was excluded from the study because of propionic acidemia. MRI was performed for clinical reasons during the first week after birth. Informed consent was obtained to add ^{1}H-MRS to the MRI examination. The study was approved by the Medical Ethical Committee of Wilhelmina Children's Hospital/University Medical Center Utrecht.

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METHODS

Bilirubin was measured in capillary or arterial blood samples by using Vitros BuBc slides (Ortho-Clinical Diagnostics, Rochester, NY). The highest levels during the first week after birth are reported in Table 1. Albumin was within the normal range for all 5 neonates.

Auditory brainstem responses (ABRs) were obtained during the first week after birth until 1999; since 2000, the automated ABR (AABR) method followed by ABR was used.[10] The aim of the ABR measurements was to find a threshold.

Follow-up of the patients was performed as described[11] by using items from the examinations of Amiel-Tison, Touwen, and the Alberta Infant Motor Scale during the first year after birth, and Griffiths' tests were performed during the second year after birth. Cerebral palsy was graded according to the classification of Hagberg et al.[12]

MRI/^{1}H-MRS

Details of the methods have been reported elsewhere.[11] Briefly, the examinations were performed in sedated neonates. Vacuum pillows (Med-Tec, Orange City, IA) were used to avoid movements of the patient's head while providing ear protection. Heart rates and transcutaneous oxygen saturation were monitored by using pulse oximetry (Nonin, Minneapolis, MN), and respiratory rates were monitored by using an abdominal transducer (Philips Gyroscan ACS-NT, Best, Netherlands).

MRI

Standard MRI was performed for localization of the volume of interest for ^{1}H-MRS by using a 1.5-T Philips ACS-NT system. MRI included sagittal T1, axial turbo spin-echo T2, inversion recovery, and diffusion-weighted MRI. Apparent diffusion coefficient (ADC) of water images were created after diffusion-weighted MRI.[13]

^{1}H-MRS

For the ^{1}H-MRS examination, a volume of interest of ~3.5 × 2.5 × 2.0 cm^3 was placed in the left basal ganglia, including the caudate nucleus, putamen, globus pallidus, and thalamus. A PRESS sequence with a repetition time of 2000 milliseconds was used. The echo time was 144 milliseconds, and 64 measurements were averaged. After zero-filling of the time-domain data points to 4096 data points, Gaussian multiplication of 5 Hz, exponential multiplication of −4 Hz, Fourier transformation, and baseline correction were performed, NAA, (phospho-)creatine, and Cho peaks were identified at 2.02, 3.02, and 3.24 ppm, respectively. Lactate was measured as an inverted doublet at 1.33 ppm. Peak area ratios of NAA/Cho were calculated. Furthermore, the presence of a lactate peak was noted. Results were compared with normal values obtained in our institute.[11]

RESULTS

Results of MRI, ^{1}H-MRS, ABR, and follow-up are shown in Table 2.

TABLE 1. Patient Clinical Data

Patient	Diagnosis	Gestational Age at Birth, wk	Age on Admission, d	Birth Weight/ Weight on Admission, g	Apgar 1 min/ 5 min Score	Age at Peak Bilirubin, h	Bilirubin Total/ Conjugated, mg/dL	Albumin, g/L
A	Rhesus incompatibility	38	0.5	3030/3030	8/9	24	29.2/1.5	30.8
B	Cephalohematoma	41.3	1	2350/2340	8/9	36	25.8/0.6	20.3
C	ABO incompatibility	39.3	4	3520/3240	9/10	77	47.5/0	31.0
D	G6PD deficiency	37.9	3	2915/2815	9/10	54	34.6/0	30.5
E	G6PD deficiency	38.7	4	3430/3185	7/9	81	41.8/0	22.7

MRI and ^{1}H-MRS

Abnormal neonatal MRI findings were seen only in the child who developed athetoid cerebral palsy (patient E; Fig 1 A and B). The bright signal in the globus pallidus (Fig 1A) is abnormal and cannot be seen in normal, term neonates. In addition, the posterior limb of the internal capsule does not show a high signal suggestive of myelination.

Diffusion-weighted MRI in this patient, however, did not show abnormalities. ^{1}H-MRS showed the presence of lactate in 4 of the 5 neonates. Lactate/NAA ratios were low (<0.10) in patients A, B, C, and D, which is within the normal range, but too high in patient E (0.20; Fig 1C). A low NAA/Cho ratio was demonstrated only in patient E, who developed athetoid cerebral palsy. At present, none of the patients had a second MRI.

ABR

All 5 neonates showed signs of bilirubin toxicity, because the ABR was abnormal, ie, an increased threshold, in all 5. The ABR normalized in 3 neonates (A, B, and C) during the first 3 months after birth, improved in patient D at 4 months after birth, and remained definitely abnormal in patient E. In patients D and E, other causes of hearing loss were excluded.

Neurodevelopment

Three neonates (A, B, and C) were completely normal at follow-up at 24 months of age, and 1 (patient E) had severe athetoid cerebral palsy (recently examined at 12 months). Patient D showed truncal hypotonia and stereotyped movements at the age of 12 months.

DISCUSSION

In the present study, lactate resonances were demonstrated in the basal ganglia in 4 of the 5 term neonates with high bilirubin levels and abnormal brainstem auditory evoked responses. An abnormally high lactate resonance could be demonstrated only in patient E, who developed severe athetoid cerebral palsy. Serum lactate levels were measured only in patient E, being 1.2 mmol/L on the day of the magnetic resonance examination. This cerebral lactate may have been the result of changes in mitochondrial function, leading to a decreased aerobic metabolism.[2] Plasma base excess values in patients A, B, C, and D at the time of ^{1}H-MRS were within normal limits. Because ^{1}H-MRS was not repeated, we do not know if brain lactate levels have normalized. Normal ^{1}H-MRS, including a small amount of lactate, has been described recently in a term neonate with severe hyperbilirubinemia and a normal follow-up examination at 12 months.[14] The low NAA/Cho ratio in neonate E indicates neuronal loss corresponding with abnormal MRI findings and cerebral palsy, because NAA is considered a neuronal marker.[8] Disappearance of NAA within 24 to 48 hours after cerebral ischemia in adults has been reported.[15,16]

No abnormalities were seen with diffusion-weighted MRI, whereas definite changes were visible on T2- and inversion recovery-weighted images in patient E. ADC values of the globus pallidus were 1.013×10^{-3} (left) and 1.057×10^{-3} mm^2/second (right), which we consider a normal value for a term neonate.[17] In neonates with severe birth asphyxia, abnormalities can be detected by using diffusion-weighted MRI during the first week after birth.[13,18] Because the MRI was performed on patient E on the 5th day after birth, very soon after the hyperbilirubinemia and exchange transfusion, we expected diffusion-weighted MRI abnormalities as the result of cell swelling induced by energy depletion. Recently, Rodrigues et al[2] described bilirubin-induced apoptosis in isolated rat neurons. Changes in the mitochondrial membrane preceded cytochrome *c* release and activation of the apoptosis cascade. Apoptotic cells are not swollen and are not likely to cause changes in diffusion-weighted MRI. In contrast, a disturbed en-

TABLE 2. MRI, MRS, ABR, and Follow-up

Patient	MRI	MRS		ABR	Follow-up
		NAA/Cho	Lac/NAA		
A	Normal	0.65	0.06	Transient abnormalities	Normal
B	Normal	0.60	0.09	Transient abnormalities	Normal
C	Normal	0.67	0.07	Transient abnormalities	Normal
D	Normal	0.62	0	Transient abnormalities	Truncal hypotonia, stereotyped movements
E	Abnormal globus pallidus	0.58*	0.20*	Abnormal	Athetoid cerebral palsy

* NAA/Cho too low and lactate/NAA too high, according to reference values.[11]

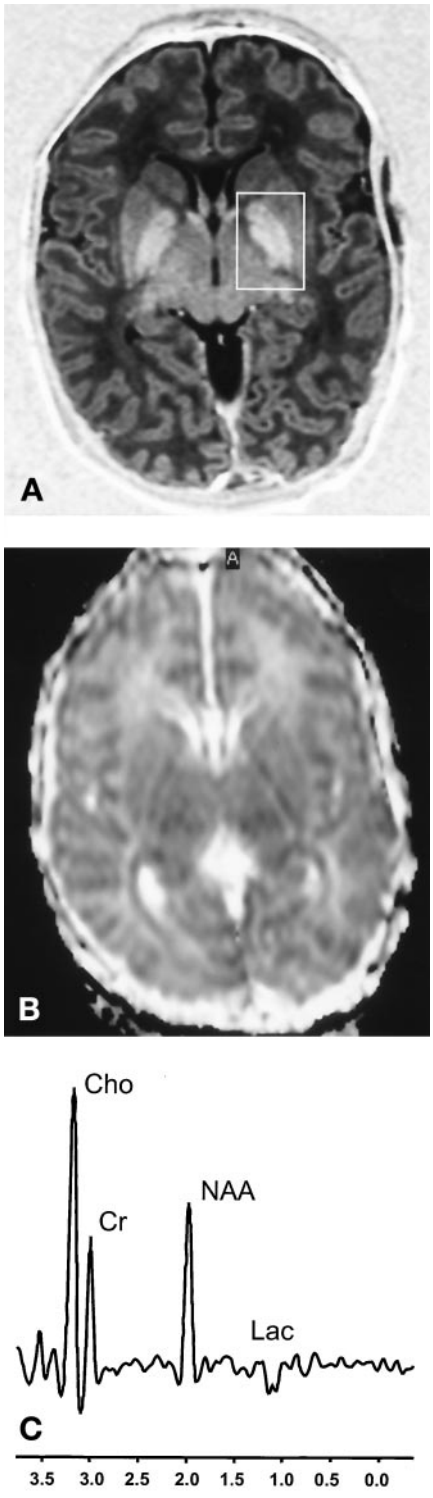

Fig 1. MRI and ^{1}H-MRS of patient E. A, MRI (inversion recovery image) showing an abnormal, bright signal in the globus pallidus. The posterior limb of the internal capsule does not show high signal suggestive of myelination. The cortex and putamen, as well as the unmyelinated white matter, have a normal appearance. B, magnetic resonance image showing a map of the ADC of water. Areas of restricted diffusion of water should appear dark. In this image, no abnormalities of the ADC are noted. ADC values of the left and right globus pallidus were 1.013×10^{-3} and 1.057×10^{-3} mm^2/second, respectively. C, ^{1}H-MRS of the left basal ganglia. NAA, Cho, (phospho-)creatine, and lactate resonances are indicated. Ratios of metabolites were calculated offline (see text for details).

ergy production of the cell will lead to failing of membrane pumps and cell swelling with concomitant changes of diffusion-weighted MRI, as is the case in neonatal hypoxic-ischemic brain injury.[13] Altered neurotransmitter metabolism and changes in the *N*-methyl-D-aspartate receptor have been described in experiments focusing on bilirubin toxicity.[5] These mechanisms may have played a role in the occurrence of seizures in patient E.

The normal appearance of diffusion-weighted MRI in patient E suggests that apoptosis caused by bilirubin might be more important than energy depletion and cell swelling in bilirubin encephalopathy. T1- and T2-weighted MRI abnormalities in the globus pallidus in patient E were obvious very early, ie, on the 5th day after birth. The posterior limb of the internal capsule did not appear normal. Because our patient was just <40 weeks' gestational age at the time of MRI, we do not consider this a sign of hypoxia-ischemia, as we do in neonates with a gestational age of 40 to 42 weeks.[19] Both normal and abnormal appearances of the internal capsule have been described in neonates with kernicterus.[20,21]

It is of interest that the 2 infants with glucose-6-phosphate dehydrogenase (G6PD) deficiency were affected most severely. The importance of G6PD deficiency in causing kernicterus has been stressed recently.[22]

In 4 neonates, the auditory abnormalities were transient. In 3 neonates (A, B, and C), the abnormalities normalized rapidly, and all 3 were normal at the age of 12 months. In patient D, recovery of the ABR was much slower, and his neuromotor development is suspect at 12 months. Recently, Hansen and coworkers[23] described the spectrum of outcome in 3 neonates with severe hyperbilirubinemia: normal in one patient, hearing loss in another, and cerebral palsy with deafness in the third. Future studies are needed to demonstrate if MRI and ^{1}H-MRS are better predictors of an adverse long-term outcome than clinical signs or ABR abnormalities persisting during the first week after birth in neonates with severe hyperbilirubinemia.

CONCLUSIONS

Changes in cerebral metabolism could be demonstrated in vivo in 1 neonate with severe hyperbilirubinemia using ^{1}H-MRS. These changes did not coincide with cell swelling, as demonstrated by using diffusion-weighted MRI.

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