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Magnetic Resonance Imaging of Bilirubin Encephalopathy: Current Limitations and Future Promise

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Abstract

Infants with chronic bilirubin encephalopathy often demonstrate abnormal bilateral, symmetric, high-signal intensity on T2-weighted magnetic resonance imaging of the globus pallidus and subthalamic nucleus, consistent with the neuropathology of kernicterus. Early magnetic resonance imaging of at risk infants, while frequently showing increased T1-signal in these regions, may give false positive findings due to the presence of myelin in these structures. Advanced magnetic resonance imaging including diffusion-weighted imaging, magnetic resonance spectroscopy, and diffusion tensor imaging with tractography may shed new insights into the pathogenesis of bilirubin-induced brain injury and the neural basis of long-term disability in infants and children with chronic bilirubin encephalopathy.

Introduction

Bilirubin-induced brain injury is distinctive, distinguished by remarkably selective involvement of the globus pallidus, subthalamic nucleus, selected brainstem nuclei, the CA2-CA3 sectors of the hippocampus, the reticular portion of the substantia nigra, and the dentate, roof nuclei and Purkinje cells of the cerebellum.^{1–4} The cerebral cortex, periventricular white matter, CA-1 sector of the hippocampus and putamen are spared.^{2,4}

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This regional distribution of neuropathological changes is very similar across term, preterm, previously well or septic neonates, and the rare adult reported with kernicterus and notably different from that of hypoxic, ischemic or hyperoxic induced brain injury in the newborn.¹⁻⁴ Magnetic resonance imaging (MRI) of infants with chronic bilirubin encephalopathy (kernicterus) often mirrors this pattern of involvement demonstrating abnormal bilateral, symmetric, high-signal intensity in the globus pallidus and less consistently in the subthalamic nucleus.⁵⁻¹² This review will detail our growing understanding of bilirubin-induced central nervous system (CNS) injury as reflected in MRI studies and suggest how future studies using MRI may help to shed new insights in the pathogenesis of acute and chronic bilirubin encephalopathy.

Terminology: ABE, CBE, and BIND

Before addressing the features of bilirubin-induced CNS injury on MRI it is important to define the clinical terms used to describe bilirubin effects on the brain and distinguish between acute bilirubin encephalopathy (ABE), chronic bilirubin encephalopathy (CBE) and bilirubin-induced neurologic dysfunction (BIND). ABE defines an encephalopathic state induced by hazardous hyperbilirubinemia during the first days of postnatal life and is characterized by a constellation of abnormal clinical signs typically progressive in their severity. In term (37^{0/7} weeks gestation) and late preterm (34^{0/7}–36^{6/7} weeks gestation) infants the initial phase of ABE is characterized by stupor (lethargy), hypotonia, and poor sucking.^{5,13} These non-specific signs are seen in numerous clinical contexts, but in a hyperbilirubinemic infant should raise the possibility of early ABE. Clinical signs of intermediate to advanced stages of ABE are increasingly more specific to bilirubin-induced neurotoxicity and herald a marked increased risk for permanent injury.^{5,13} These include hypertonia often manifested by retrocolis and opisthotonus, fever, and high pitched cry.^{5,13} Inability to feed and apnea may ensue.¹⁴ Infants <34 weeks gestation less frequently show these classic abnormal neuromotor signs.¹⁵ Recurrent apnea and desaturations may be the only clinical manifestations of ABE in preterm infants during the neonatal period, if any appear at all.¹⁶

In contrast, chronic bilirubin encephalopathy (CBE) defines the permanent clinical sequelae of bilirubin toxicity that become evident in the first year of life and is synonymous with the term kernicterus.^{5,13} The American Academy of Pediatrics (AAP) recommends the term kernicterus be reserved for the chronic and permanent neurologic sequelae of bilirubin toxicity.¹⁷ These include the extrapyramidal movement disorders of dystonia and/or choreoathetosis, hearing loss due to auditory neuropathy spectrum disorders, and the eye movement abnormality of paresis of upward gaze.^{5,13}

Bilirubin-induced neurologic dysfunction or BIND is a term whose meaning has evolved over time and thereby sown some confusion in the literature. In its current usage, BIND characterizes a constellation of more subtle neurodevelopmental disabilities without the classical findings of kernicterus that after careful clinical review appear to be due to bilirubin neurotoxicity.⁵ It includes disturbances of central auditory processing, coordination, muscle tone and sensorimotor integration.⁵ BIND and subtle kernicterus are synonymous.⁵ The concept of BIND as a clinical entity is an area of ongoing debate that

merits intense investigation. Indirect evidence suggests the cerebellum as a possible site of injury relevant to BIND particularly in premature neonates. The latter derives from the considerable overlap in the putative neurophenotype of BIND and other conditions associated with cerebellar injury in preterm neonates. Systematic MRI studies of subjects with BIND are as yet unavailable and urgently needed to help clarify the nature of this disorder. BIND will not be covered in this review.

Original Case Description of Kernicterus on MRI

The seminal MRI description of bilirubin encephalopathy was a case report of a term glucose-6-phosphate dehydrogenase deficient neonate who developed hazardous hyperbilirubinemia (peak TSB 50 mg/dl) associated with *E coli* sepsis.¹⁸ MRI studies on day of life 8 and 16 showed abnormal signal intensity in the globus pallidus, internal capsule, thalamus and hippocampus. These were most prominent as high signal on T1 weighted images, but also as subtle increased signal on T2-weighted images.¹⁸ A growing experience with neuroimaging of neonates with kernicterus has refined our understanding of MRI changes in bilirubin encephalopathy.

Conventional structural MRI (T1 and T2 weighted)

Although many studies have reported signal abnormalities in the globus pallidus and subthalamic nuclei, hippocampus and cerebellum in neonates with CBE, it is now well understood that the nature of these signal abnormalities varies with time. Chronically, bilateral, symmetrically increased T2- signal (or T2-FLAIR signal) in the globus pallidus and subthalamic nucleus of an infant with a history of hyperbilirubinemia and CBE remains the neuroimaging hallmark of kernicterus (Figure 1).^{5–12} Additionally, increased T2-signal may also be seen in the substantia nigra or in the dentate nucleus of the cerebellum, while T1-signal in all of these regions is variable in CBE.

By contrast, early MR studies of infants in the first days to weeks following ABE onset (i.e., subacute phase) who later meet criteria for CBE often demonstrate increased T1-signal in the globus pallidus and subthalamic nucleus, while T2-signal in these regions is most often unremarkable or subtly hyperintense (Figure 2).^{5,6,8,11,18–21} The anatomic loci of increased T1-signal identified in the subacute phase of bilirubin encephalopathy mirror the distinctive topographical pattern observed in CBE, and suggests that increased T1-signal could be used as a diagnostic marker of kernicterus. However, as we demonstrate below, the sensitivity and specificity of T1-signal changes during the subacute phase to predict kernicterus is hindered by both the evolving nature of the signal abnormalities and confounding signal changes associated with normal myelination.

In practice, conventional T1- and T2-weighted sequences are the most heavily utilized MR sequences because they visualize the underlying anatomy. In addition, T1-weighted MR sequences have a high sensitivity for hemorrhage and lipid content while T2-weighted sequences have a high sensitivity for water (CSF and/or tissue edema). As noted above, in infants who later show CBE, early MR images often demonstrate prominent, symmetric high T1-signal in the globus pallidus and subthalamic nucleus, and less so in the hippocampus and the dentate nucleus of the cerebellum. These T1 findings are not related to

hemorrhage but potentially reflect i) bilirubin-induced microglial activation as recently described *in vitro*^{22,23} and observed in animal models of kernicterus,^{24,25} although the neuropathology of kernicterus in human neonates provide conflicting evidence in this regard;^{4,26} or ii) calcium granules scattered within damaged neurons.⁴

Yet, because high T1-signal is not indicative of any specific pathology or indeed a pathological process at all, it is important to recognize that other phenomena, including normal maturational processes, can also lead to high T1-signal in these same regions at or near term-equivalency in human infants.²⁷ In the term neonate, a key differential is myelination. Due to its high lipid content, myelin is similarly visualized as high T1-signal, and although most often associated with “white matter,” myelin is present in both grey matter and white matter regions. In the human brain, myelination starts during the third trimester of fetal brain development. At term-equivalency, myelin is present in specific tracts in the brainstem and cerebral white matter (e.g., cortical spinal tract) as well as in specific grey matter nuclei, including the globus pallidus, subthalamic nucleus, and dentate nucleus of the cerebellum.²⁸ *Because the predominant early indication of kernicterus is also high signal in the subthalamic nuclei and globus pallidus, in clinical practice, it is possible to misattribute high signal associated with normal myelination as “kernicterus” in a neonate with a history of hazardous hyperbilirubinemia exposure or clinical signs of bilirubin encephalopathy.* Moreover, as shown in Figure 3, this likelihood is greater in modern, higher field (i.e., 3T) scanners even in healthy term neonates. Also of note, T1 contrast in the areas of the globus pallidus and subthalamic region can be variable in the neonatal brain depending on the type of pulse sequence employed (i.e. standard spin echo vs inversion recovery vs spoiled gradient echo recall), and in our experience, 3D MR sequences (e.g., “SPGR” or “MPRAGE”) demonstrate a particular propensity for high signal in these regions (Figure 3).

Given both the variability and transient nature of the signal abnormalities as well as the potential for confounding abnormal T1-signal with “normal” T1 signal reflecting myelination, it is not surprising therefore, that prospective studies examining the predictive validity of conventional early MRI demonstrate both false positives (i.e., cases in which T1-signal is elevated but neurologic outcome is normal) as shown in Figure 4 and false negatives (i.e., cases in which T1-signal does not appear abnormally elevated, but for whom neurologic outcome is abnormal).^{11,21} Although it is also possible that some of the transient, elevated T1-signal is “real” (i.e., reflecting an acute inflammatory reaction to bilirubin toxicity, which resolves without significant neuronal damage or loss), these results nonetheless demonstrate the need to establish additional biomarkers which together with the conventional MR imaging findings, or pathognomonic themselves, can predict CBE in neonates. It is also important to note that there are reports of infants with CBE who do not show any MRI abnormalities at all, either early on T1 or later on T2 weighted studies.⁵

These observations also highlight the importance of interpreting early MRI findings in a broader context including the infants clinical history, neurological exam and results of ancillary neurodiagnostic testing, most importantly the auditory brainstem evoked response (ABER).²⁹ In an infant with normal neurologic exam and ABER, a normal MRI is reassuring but not conclusive. By contrast, an abnormal early MRI coupled with abnormal

neurological exam and failed ABER would be very concerning. Because of our experience with the false positive potential of early MRI, we currently limit such studies to those at risk neonates with an abnormal exam or ABER, those who evidenced intermediate to advanced signs of ABE, or those whose TSB was extreme (>25 mg/dl).

Advanced imaging techniques in bilirubin encephalopathy: DWI and MRS

Advanced neuroimaging techniques include diffusion-weighted imaging (DWI) and MR Spectroscopy (MRS) and are widely available in most MR scanners. DWI is sensitive to the Brownian motion (i.e., diffusion) of water molecules, and has demonstrated remarkable sensitivity for intra- and extra-cellular edema. For example, after an acute stroke, diffusion is restricted in regions of cytotoxic (intracellular) edema, and as a result, these areas appear hyperintense (areas of high signal) on trace DWI or hypointense (areas of low signal) on apparent diffusion coefficient (ADC) images. Following stroke or other hypoxic-ischemic injuries, these changes are typically apparent less than 24 hours after injury and persist for approximately one week.³⁰

Although few acute DWI studies have been reported in neonates with ABE, there is very little to suggest that there is restricted diffusion in the globus pallidus during the acute period of bilirubin encephalopathy in neonates who later evidence CBE.^{10,21} In fact, a recent study suggested there may actually be increased diffusivity (ADC) in the vicinity of the globus pallidus in the subacute epoch;³¹ however, methodological concerns limit conclusions from that study.³² In contrast, there may be evidence of restricted diffusion in other brain regions, most prominently the ventrolateral and ventroanterior regions of the thalamus, in infants with ABE (Figure 2). Regardless, the absence of marked diffusion related changes in the globus pallidus is remarkable, and suggests that further research is needed to not only characterize the diffusion-weighted changes associated with acute and subacute ABE, but also to further understand the underlying pathogenesis of cellular injury (or death) in the setting of bilirubin toxicity.

In addition to diffusivity and cytotoxic edema, diffusion tensor imaging (DTI) with tractography may be useful for elucidating long-term changes along specific anatomical pathways. The subcortical regions most affected in CBE—globus pallidus, subthalamic nucleus, substantia nigra, dentate nucleus (cerebellum) and hippocampus—are interconnected with each other as well as other cortical and subcortical brain regions via numerous white matter pathways (e.g., cortico-ponto-cerebello-thalamo-cortical pathway, cortico-striato-thalamo-cortical pathways), and it is not known how these pathways are affected in the long-term in infants or children with CBE. DTI and tractography could be used to delineate long-term changes involving specific white matter pathways, further elucidating the neural basis of long-term disability in infants and children with CBE.

MR Spectroscopy (MRS) is another advanced imaging technique with great potential both in the early diagnosis and long term evaluation of neonates with CBE. In contrast to anatomical imaging, which detects the signal from protons associated with water molecules, MRS detects the signal from protons associated with other small molecules present in cerebral tissue (e.g., glutamate, glutamine, and lactate). Notably, large molecules, including albumin

and bilirubin are not detectable with MRS. Proton (¹H)-MRS studies of infants with ABE and/or severe hyperbilirubinemia have demonstrated decreases in the ratio of N-acetyl-aspartate (NAA) to choline and NAA to creatine as well as increases in the ratio of lactate to NAA in the vicinity of the basal ganglia in neonates who went on to develop CBE.^{10,21,33} NAA is synthesized in the mitochondria of mature neurons and axons, and is often used as a proxy for neuronal/axonal integrity. Accordingly, decreased NAA suggests neuronal/axonal dysfunction and/or loss, which is consistent with findings from neuropathological studies. By contrast, an additional study has suggested that elevations in the ratios of glutamate/glutamine (summed together and represented as “Glx”) relative to creatine and myo-inositol relative to creatine in the vicinity of the basal ganglia may be a signature for kernicterus.³⁴ However, in that study there was no relation between the metabolite ratios and clinical outcome, and indeed, the two infants with normal clinical outcomes at one year of age had two of the three highest Glx to creatine ratios, raising concerns about whether Glx concentrations in the basal ganglia can be an early signature for kernicterus. Thus, as for other advanced imaging techniques, much work remains to be done regarding MRS in neonates with bilirubin encephalopathy in regards to diagnosis, prognosis and furthering our understanding the pathogenesis of bilirubin neurotoxicity.

There are several areas where the application of MRS techniques in neonates with bilirubin encephalopathy could further elucidate the pathogenesis of cellular injury and ultimately CBE. The first area pertains to energy metabolism. It has been hypothesized that the pathogenesis of cell damage in kernicterus involves bilirubin acting directly upon mitochondria—uncoupling oxidative phosphorylation and inhibiting the ADP/inorganic phosphate reaction.³⁵ The uncoupling of oxidative phosphorylation in turn results in dramatic decrease in ATP concentrations and a concomitant increase in anaerobic metabolism, with a marked increase in lactate production. MRS is a well-established technique for demonstrating energy failure and anaerobic metabolism in neonates with perinatal brain injury. More than two decades ago, ¹H-MRS, coupled with ³¹P-Phosphorus (³¹P)-MRS, which allows for the direct quantitation of ATP, ADP, phosphocreatine as well as inorganic phosphates, established energy failure as a key mechanism in the pathogenesis of cell death in neonates with perinatal asphyxia.^{36–41} Although requiring additional hardware and software relative to conventional ¹H-MRS, ³¹P-MRS could provide a direct assessment of oxidative metabolism and ATP concentration in neonates with ABE. Moreover, even without the added value of ³¹P-MRS, conventional ¹H-MRS could still demonstrate much about energy metabolism in neonates with ABE. For example, the synthesis of NAA, as well as glutamate, is tightly coupled to oxidative metabolism and thus, monitoring NAA concentrations may provide valuable information regarding mitochondrial metabolism in neonates with ABE. Moreover, modern MR scanners with higher field strength (e.g., 3T) not only provide better signal to noise and better anatomical localization, but also, when coupled with advanced post-processing techniques (e.g., LCModel, Stephen Provencher) allow for the direct quantitation of many additional cerebral metabolites, including citrate, glutamate, aspartate, glutathione and phosphocreatine, as well as NAA, choline and lactate. Thus, in the present era of MRI, it is possible to carry out more detailed investigations of energy metabolism in the globus pallidus, as well as oxidative stress and excitotoxicity, in neonates with ABE and determine which metabolic features not only

herald the development of CBE, but also indicate the ongoing pathogenesis of injury and ultimately, indicate possible neuroprotective strategies as done in the past decades for HIE.

The second area where MRS techniques could make a substantial contribution to our understanding of CBE is in relation to γ -aminobutyric acid (GABA). GABA is the principal inhibitory neurotransmitter in the mammalian nervous system. [Note: in the developing nervous system, GABA may also function as an excitatory neurotransmitter.] It is remarkable that the primary brain regions affected in CBE are regions which contain, if not dominated by, populations of GABAergic neurons, including the globus pallidus, substantia nigra, hippocampus, cerebellum, and several brainstem auditory nuclei (i.e., ventral cochlear nucleus, superior olivary nucleus). Although GABA concentrations have been studied across a wide-range of populations using both conventional 1H-MRS as well as “GABA-edited MRS sequences”,^{42,43} to these authors’ knowledge, there have been no MRS studies of GABA concentrations in neonates with bilirubin encephalopathy. It is conceivable that acutely, changes in GABA concentration—coupled with altered energy metabolism—may be the most potent biomarker for early CBE, signifying ongoing damage to GABAergic neurons in the globus pallidus and related brain regions. In contrast, studying GABA concentrations chronically in infants with CBE in these regions as well as basal ganglia and cerebral cortex may provide new insights into the biological underpinnings of cerebral palsy and higher-order cognitive impairment in this population.

Neuroimaging of Gunn rat model of Kernicterus

The Gunn rat model has been used for decades to study kernicterus. Neonatal hyperbilirubinemia in homozygous j/j Gunn rat pups results from a deficiency of the bilirubin conjugating enzyme uridine-diphosphate-glucuronosyl transferase. When treated with sulfadimethoxine, bilirubin is acutely displaced from albumin resulting in increased brain bilirubin levels and ABE in neonatal j/j pups. These animals reproducibly show bilirubin-induced neuromotor disturbances (dystonia) and auditory brainstem evoked response abnormalities. The neuropathology of kernicterus in j/j Gunn rat pups mirrors that of preterm human neonates and is characterized by prominent cerebellar injury (hypoplasia) as well as globus pallidus damage.² Only recently has the Gunn rat model been used to explore bilirubin-induced MRI changes. A preliminary report focused on DTI to evaluate the globus pallidus and cerebellum in Gunn rat pups.⁴⁴ Fractional anisotropy (FA) was measured in the cerebellum (middle and inferior peduncles) and globus pallidus of j/j, j/j-sulfadimethoxine dosed, and wild type Gunn rat pups on postnatal day 15, the age at which hyperbilirubinemia characteristically peaks in this model. Cerebellar volumes were also determined. Decreased FA was observed in the i) GP of sulfa dosed j/j pups and ii) middle cerebellar peduncle of all hyperbilirubinemic j/j pups ($\pm$ sulfa). These findings suggest that DTI FA may serve as an in vivo marker for ABE.⁴⁴ Future MRI study of the Gunn rat and recently described neonatal mouse models of kernicterus^{24,45} is warranted.

Conclusion

In the last two decades, a growing experience with neuroimaging in infants with ABE and CBE has refined our understanding of bilirubin encephalopathy; however, much work

remains to be done. Although studies initially indicated that early T1-signal increases in the globus pallidus and subthalamic nuclei may be useful as diagnostic indicators of CBE, we now understand that these T1-signals alone have limited prognostic utility. At the same time, advanced imaging techniques continue to offer promise both as a means of establishing an early biomarker of injury and as a tool to better understand the pathogenesis of bilirubin-induced neurologic damage⁴⁶ and the adverse neurodevelopmental outcome of infants with CBE.

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Figure 1. Conventional MR imaging of a six-month old infant with CBE

Axial and coronal T2-weighted and coronal T1-weighted images from a 38 week gestation term male infant, who had a neonatal TSB of 27.0 mg/dl and dystonia, choreoathetosis and auditory neuropathy on neurodevelopmental follow up. The T2-weighted images demonstrate well-defined areas of increased signal in the globus pallidus, as well as more subtle increases in T2-signal in subthalamic nuclei and the substantia nigra (right). T1-weighted images obtained at the same time do not show marked increase in signal. GP = globus pallidus; STN = subthalamic nucleus; SN = substantia nigra; RN = red nucleus.

Figure 2. Conventional MRI performed on postnatal day five in infant who went on to develop CBE

Coronal T1-weighted, axial T2-weighted, and axial-ADC images from the same infant as in Figure 1. Increased T1-signal is readily apparent in the globus pallidus and subthalamic nucleus, with more subtle evidence of increased T1-signal in the substantia nigra and hippocampus (not labeled above). There is also subtle increased T2-signal in the globus pallidus. Remarkably, the ADC images did not demonstrate any restricted diffusion in the globus pallidus; however, there was subtle evidence of restricted diffusion in the thalamus (predominantly ventrolateral nucleus) and the posterior limb of the internal capsule as well as the hippocampus (not pictured).

Figure 3. Higher field strength and modern 3D-T1-weighted sequences provide greater signal-to-noise increasing sensitivity to pathology but also to normal developmental processes like myelination

Note that while high T1-signal is visualized in the globus pallidus and subthalamic nucleus on the 3D-T1-weighted (SPGR) sequence obtained at 3T in infant with ABE and later CBE (A), high T1-signal may be similarly visualized in these same regions in neonates without hyperbilirubinemia assessed using modern 3D-T1-weighted sequences, including MPRAGE (B: 3T, Siemens Healthcare, Erlanger, Germany), 3D-T1-TFE (C: 3T, Philips Medical Systems, Best, the Netherlands), and SPGR (D: 3T, GE Medical Systems, Milwaukee, WI, United States; E: 1.5T GE Medical Systems, acquired with a neonatal head coil, Resonance Technology, Inc., Northridge, CA, USA).

Figure 4. High signal in the globus pallidus and subthalamic nucleus on early MRI of at risk neonates has limited specificity for later CBE

T1-weighted images obtained subacutely in a five day old term infant with ABE who went onto develop CBE (A) as well as two different at risk hyperbilirubinemic infants who did not develop CBE (B, C). Neonate B is a term female with ABO hemolytic disease, a peak TSB of 19.6 mg/dl at 15 hours of age prior to double volume exchange transfusion who demonstrated signs of intermediate ABE on admission. Neonate C is a term female with Rh hemolytic disease and a peak TSB of 21.0 mg/dl without signs of ABE prior to double volume exchange transfusion. MR imaging was obtained on day of life 5 and 7 respectively in neonate B and C and both have displayed normal neuromotor exams and hearing on >18 months developmental follow-up by pediatric neurology.