Bilirubin-Induced Neurotoxicity in the Preterm Neonate

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KEYWORDS

• Kernicterus • Excitotoxicity • Low-bilirubin kernicterus • Auditory neuropathy
• Cerebellum • Hypoalbuminemia

KEY POINTS

- Bilirubin-induced neuronal injury likely reflects the adverse nature of hazardous unbound unconjugated bilirubin on plasma membranes and resultant excitotoxicity, neuroinflammation, oxidative stress, and perturbed cell cycle kinetics, including cell cycle arrest.
- Hazardous hyperbilirubinemia leading to acute bilirubin encephalopathy is increasingly recognized to adversely impact neural respiratory drive and manifest clinically as recurrent symptomatic central, mixed, and obstructive apnea events.
- Low-bilirubin kernicterus is a rare, but refractory cause of bilirubin-induced neurotoxicity in preterm neonates. Although low-bilirubin kernicterus is multifactorial in its pathogenesis, marked hypoalbuminemia is often a prominent clinical feature.

INTRODUCTION

The potential for bilirubin-induced neurotoxicity in the premature neonate (<37 weeks gestational age [GA]) remains a clinical concern. In addition to classic kernicterus, the preterm infant is at risk for auditory predominant chronic bilirubin encephalopathy (CBE) and low-bilirubin kernicterus.[1,2] Others suggest that bilirubin neurotoxicity in preterm neonates may be associated with less severe neurodevelopmental disabilities, a putative condition termed subtle kernicterus or bilirubin-induced neurologic dysfunction (BIND).[1] These conditions and postulated mechanisms of bilirubin-induced central nervous system (CNS) injury are highlighted in this review.

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NEUROPATHOLOGY OF KERNICTERUS

The neuropathology of bilirubin-induced brain damage is (i) remarkably similar across preterm and term neonates, and murine animal models; (ii) distinct from hypoxic-ischemic neonatal CNS injury; and (iii) notable in sparing the neocortex.[1,3] Classic kernicterus at postmortem in the preterm neonate is characterized by both (i) intense yellow staining of neurons in selected brainstem nuclear clusters and (ii) histopathologic evidence of neuronal damage in these stained regions. However, bilirubin staining in the *absence* of characteristic microscopic evidence of neuronal injury does not constitute kernicterus.[3] Brainstem regions typically affected in kernicterus include the following: the (i) globus pallidus; (ii) subthalamic nuclei; (iii) metabolic sector of the hippocampus; (iv) oculomotor nuclei; (v) ventral cochlear nuclei; (vi) Purkinje cells of the cerebellar cortex; and (vii) cerebellar dentate nuclei.[3]

NEUROIMAGING OF KERNICTERUS

MRI of the infant with kernicterus mirrors the distinct regional nature of bilirubin-induced neuropathology demonstrating abnormal bilateral, symmetric, high-intensity signals in the globus pallidus and subthalamic nuclei and on occasion the internal capsule and thalamus (**Fig. 1**).[4,5] Chronic bilateral, symmetrically increased T2-signal (or T2-FLAIR [fluid attenuated inversion recovery] signal) in the globus pallidus and subthalamic nuclei of an infant with a history of hyperbilirubinemia remains the neuroimaging hallmark of kernicterus. These structural MRI findings are equally apparent in affected preterm[6,7] and term neonates.

The subcortical regions affected in CBE are interconnected with each other as well as other cortical and subcortical brain regions via numerous white matter tracts (eg, cortico-ponto-cerebello-thalamo-cortical pathway, cortico-striato-thalamo-cortical pathways).[5] It is therefore expected that *advanced* MRI techniques, including diffusion-weighted imaging, apparent diffusion coefficient mapping, and diffusion tensor imaging with tractography, will shed insight into the abnormalities of structural and functional neural connectivity that underlie the long-term disability in infants and

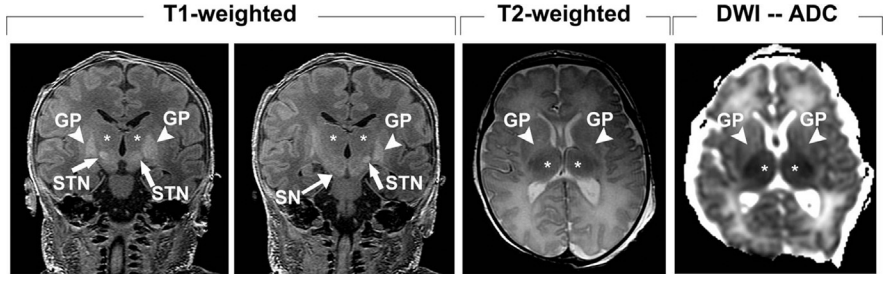

Fig. 1. Conventional MRI performed on postnatal day 5 in an infant who went on to develop CBE. Coronal T1-weighted, axial T2-weighted, and axial-apparent diffusion coefficient (ADC) images are shown. Increased T1-signal is readily apparent in the globus pallidus (GP) and subthalamic nucleus (STN), with more subtle evidence of increased T1-signal in the substantia nigra and hippocampus (not labeled). There is also subtle increased T2-signal in the GP. Remarkably, the ADC images did not demonstrate any restricted diffusion in the GP; however, there was subtle evidence of restricted diffusion in the ventrolateral nucleus of the thalamus, posterior limb of the internal capsule, and the hippocampus (not pictured). *, thalamus; DWI-ADC, diffusion-weighted image-apparent diffusion coefficient. (*From* Wisnowski JL, Panigrahy A, Painter M, et al. Magnetic resonance imaging of bilirubin encephalopathy: current limitations and future promise. Semin Perinatol 2014;38:424; with permission.)

children with CBE.[5] Use of magnetic resonance spectroscopy (MRS) of affected regions of interest may also shed new insights into the pathobiology of bilirubin-induced brain injury.[5]

MOLECULAR AND CELLULAR MECHANISMS OF BILIRUBIN NEUROTOXICITY

The complex cascades of molecular and cellular events that underlie bilirubin-induced neurotoxicity remain incompletely understood, but involve regional and cell-specific responses.[8] **Fig. 2** highlights the multiple reported effects of bilirubin on neurons and glia cells.[8] Which of these effects constitute the "core" processes or molecular triggers that ultimately lead to bilirubin neurotoxicity is unclear.[8–12] Nevertheless, the pathogenesis of bilirubin-induced neuronal cell injury likely reflects the adverse effects of hazardous unbound unconjugated bilirubin (UB) concentrations on plasma, mitochondrial, and/or endoplasmic reticulum membranes (**Fig. 3**).[8,12] These membrane perturbations, in turn, precipitate untoward cellular events that culminate in increased intracellular calcium concentrations (iCa^{2+}). Downstream events triggered by increased iCa^{2+} may include the activation of proteolytic enzymes, apoptosis, necrosis, as well as abnormalities of cell cycle progression, including cell cycle arrest.

Bilirubin and Membranes

The main isomer of bilirubin in humans is bilirubin-IXα (*Z*,*Z*), an amphipathic molecule, but one that has a high affinity for membrane lipids.[13,14] Several studies suggest that membranes are the primary or initiating targets of bilirubin toxicity (reviewed in Ref. [12]). However, there is surprisingly little information regarding the actual nature of bilirubin-membrane complexes under physiologic conditions and clinically relevant UB concentrations, nor information on why not all cell types or tissues bind bilirubin with equal affinity.[15] Zucker and colleagues[14] showed that the amphipathic nature of bilirubin facilitates interaction with phospholipid bilayers, localizing to the polar region near the membrane-water interface. Disruption of phospholipid asymmetry, inhibition of membrane-bound ATPases, lipid peroxidation, and other adverse sequelae may ensue.[16] Analogous bilirubin-induced adverse changes in mitochondrial membranes have also been reported.[17] Future studies further characterizing bilirubin-phospholipid membrane complexes and their localization are warranted.[15] In this regard, Ly and colleagues[18] recently shared preliminary findings that lipid raft microdomains may be a target of bilirubin toxicity and amenable to therapeutic intervention.

Excitotoxicity

Excitotoxicity has been proposed to contribute to bilirubin-induced CNS injury on the basis of compelling in vitro, in vivo, and MRI studies. Glutamate, the primary excitatory neurotransmitter in the CNS, and the *N*-methyl-D-aspartate (NMDA) glutamate receptor subtype are important in the pathogenesis of neonatal neuronal injury.[19] Once NMDA channels are open, downstream events of excitotoxicity become manifest, including (i) an early rapid glutamate receptor activation that leads to increased intracellular Na^+ and Cl^- and resultant cell swelling; followed by (ii) a delayed phase of Ca^{2+} influx and Ca^{2+} release from intracellular stores leading to activation of Ca^{2+}-dependent enzymes that activate apoptosis and/or necrosis.[20] Bilirubin-induced excitotoxicity may develop from a sustained period of mitochondrial energy failure with resultant neuronal depolarization and passive opening of NMDA glutamate channels.[19,21,22] Indeed, Novelli and colleagues[22] report that reduced intracellular energy levels are prerequisites for excitotoxicity; glutamate alone is necessary, but not sufficient.

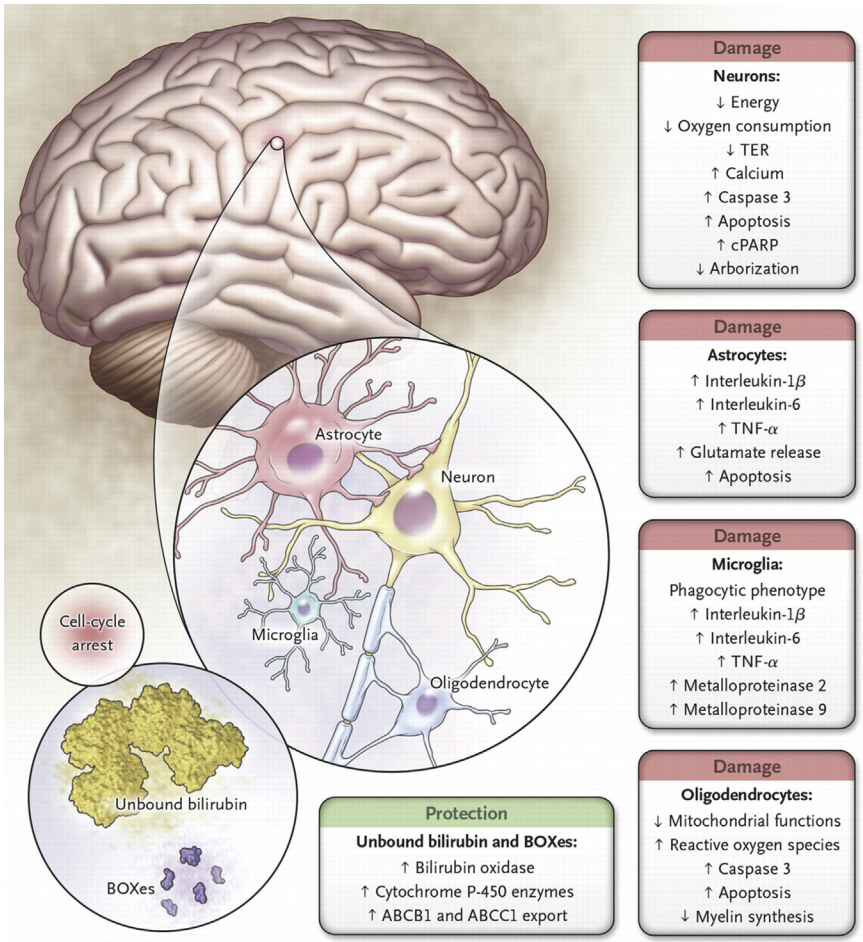

Fig. 2. Cell types and metabolic processes affected by bilirubin in the CNS. The main effects of bilirubin on neurons are decreased oxygen consumption and increased release of calcium and caspase 3, resulting in apoptosis. There is also decreased dendritic and axonal arborization. A similar pattern is observed in oligodendrocytes with increased apoptosis, impairment of the redox state (oxidative stress), and reduced synthesis of myelin. Microglia react to toxic injury associated with bilirubin by increased release of proinflammatory cytokines and metalloproteinase activity as cells manifest a phagocytic phenotype. A similar proinflammatory pattern is observed in astrocytes with enhanced release of glutamate and apoptosis. At the same time, cells may reduce the intracellular concentration of bilirubin either by extruding the pigment through the ATP-binding cassette transporters or by increasing the formation of the less toxic products through bilirubin oxidation products (BOXes) and/or cytochrome P-450 enzymes (1a1 and 1a2 in particular). These responses are protective, whereas all others result in cell damage; this suggests that once the intracellular concentration of bilirubin exceeds a toxic threshold (still to be defined), the polymorphic metabolic cascade leading to neurotoxicity ensues. cPARP, cleaved poly(adenosine diphosphate-ribose) polymerase; TER, transcellular resistance. (*From* Watchko JF, Tiribelli C. Bilirubin-induced neurologic damage—mechanism and management approaches. N Engl J Med 2013;369:2025; with permission from Massachusetts Medical Society.)

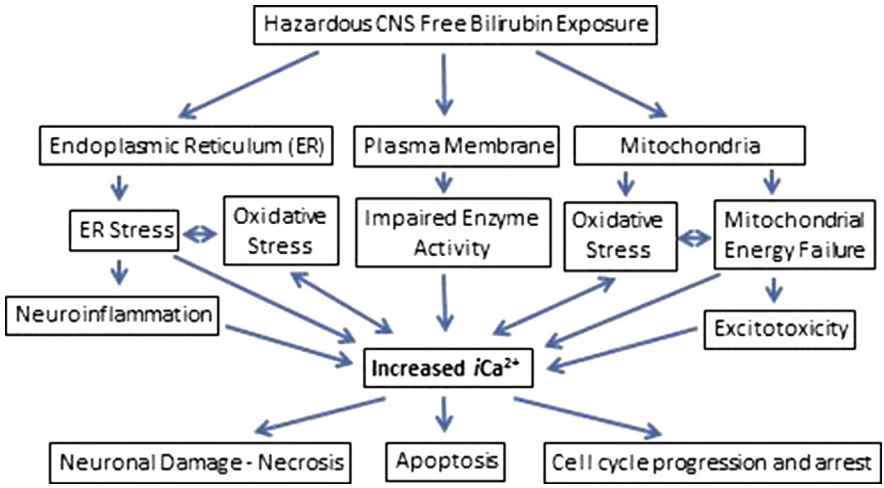

Fig. 3. Schematic of several hypothesized pathophysiological mechanisms in bilirubin-induced neuronal injury. Hazardous UB exposure in the CNS exerts direct effects at the level of the plasma membrane, mitochondria, and/or endoplasmic reticulum (ER), leading to ER stress, oxidative stress, impaired enzyme activity, and mitochondrial energy failure, culminating in neuroinflammation, excitotoxicity, and increased $i\mathrm{Ca}^{2+}$. If CNS UB exposure is of sufficient degree and/or duration than irreversible neuronal damage, that is, necrosis, and/or cell cycle arrest may ensue. (*Adapted from* Watchko JF. Kernicterus and the molecular mechanisms of bilirubin-induced CNS injury in newborns. Neuromolecular Med 2006;8(4):518; with permission.)

Excitotoxicity and mitochondrial energy failure suggest a possible explanation for the regional nature of bilirubin-induced CNS damage.[19,21] Neurons of the neonatal globus pallidus show a high resting (baseline) level of neuronal activity[21] and a high neuronal glutamate receptor density.[23] The former makes the region relatively more susceptible to subacute energy failure, whereas the latter would accentuate excitotoxicity injury. Consistent with this hypothesis are (i) evidence of excitotoxicity injury in the Gunn rat model of kernicterus[24]; and (ii) the reported cerebral metabolic signature on proton MRS of an elevated glutamate and glutamine:creatine ratio during acute severe hyperbilirubinemia.[25]

Neuroinflammation

Conditions associated with severe systemic inflammation, including sepsis, necrotizing enterocolitis, and the fetal inflammatory response syndrome (chorioamnionitis with funisitis) are each reported to potentiate bilirubin neurotoxicity.[2] This potentiating effect may be greater in less mature cells and in preterm neonates[26–28] and modulated by endoplasmic reticulum stress, the activation of nuclear factor-κB, and the unfolded protein response.[29] Hazardous levels of UB alone are also immunostimulatory and induce acute and chronic microglial activation in vivo, upregulate pro-inflammatory gene expression, and trigger the cellular (microglia and astrocyte) release of tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6 to produce a proinflammatory milieu.[2,8–10,26] Proinflammatory cytokines in turn enhance bilirubin-induced neuronal apoptosis and necrosis in monoculture.[9,10,26] These bilirubin-induced neuroinflammatory responses might contribute to neurologic damage.

Recent studies, however, suggest a more complex dynamic, including evidence that early bilirubin-induced proinflammatory responses can paradoxically

be neuroprotective.[30,31] The toll-like receptor 2 (TLR2) signaling pathway appears to be linked to hyperbilirubinemia-induced activation of microglia, astrocytes, resultant reactive gliosis, upregulation of TNF-α, IL-1β, IL-6 gene expression, and pronounced neuroinflammation in vivo.[30] Deletion of TLR2 in mice blocks the induction of these inflammatory cytokine genes yet is associated with increased cerebellar apoptosis and higher neonatal death rates.[30] These data suggest that TLR2 signaling and early neuroinflammation are neuroprotective.[30] Consistent with this complex dynamic, astrocytes in a coculture with neurons can protect or aggravate bilirubin-induced neurotoxicity depending on the duration of the cell-cell communication (preconditioning) and bilirubin exposure.[31]

In addition, chronic neuroinflammation is now recognized as an important risk factor for CNS injury in preterm neonates. In this regard, chronic bilirubin-induced neuroinflammation (microglial activation) is reported in the Gunn rat model of kernicterus and appears in association with abnormal brain development.[32,33] The possibility that bilirubin-induced CNS injury may extend beyond the initial insult via chronic inflammation is intriguing and suggests there may be a therapeutic window following acute bilirubin encephalopathy (ABE). This possibility merits clarification as does the study of possible neuroprotective intervention(s) targeted to inflammatory responses within the complex "yin and yang" of neuroinflammation.[34]

Oxidative Stress

Signs of oxidative stress are consistently associated with hazardous levels of bilirubin in vitro and in vivo and therefore feature prominently in many models of bilirubin-induced neurotoxicity. Nevertheless, it remains unclear whether bilirubin-induced oxidative stress is causally linked to bilirubin neurotoxicity. For example, although several equipotent antioxidants (minocycline; 12S-hydroxy-1,12-pyrazolinominocycline [PMIN]; taurourosdexoycholic acid) consistently and robustly reduce lipid peroxidation (4-hydroxynonenal) during ABE in the Gunn rat model of kernicterus, only minocycline prevents neurotoxicity.[35] This finding suggests that lipid peroxidation inhibition alone is not sufficient to prevent ABE and that the neuroprotective efficacy of minocycline involves action(s) independent of or in addition to its antioxidant effects. It also suggests that caution is warranted in inferring causal linkage between a bilirubin-induced effect and bilirubin neurotoxicity.

Bilirubin and Intracellular Calcium Homeostasis

Regardless of triggering mechanisms, it does appear that increased iCa^{2+} levels are critical to the development of bilirubin-induced cell injury (see **Fig. 3**).[10,12,16] Consistent with this assertion, PMIN, a noncalcium chelating derivative of minocycline, is not neuroprotective against ABE in the Gunn rat model of kernicterus, whereas calcium-chelating minocycline is.[35] Hazardous UB itself may further elevate iCa^{2+} levels by adversely impairing iCa^{2+} buffering via calmodulin-dependent protein kinase II,[36] calbindin, and parvalbumin.[37,38] Once developed, high iCa^{2+} levels via second messenger pathways may activate proteases, lipases, and endonucleases and trigger the generation of free radicals, a series of events that culminate in cell death via either apoptosis or necrosis.[10,12,16]

Perturbed Cell Cycle Kinetics, Cell Cycle Arrest, Altered Neurogenesis

In addition to cell death, hazardous bilirubin levels may adversely impact cell cycle kinetics. In this regard, the most consistent feature of bilirubin-induced neurotoxicity in both the Gunn rat and the *UGT1* knockout mouse models is cerebellar hypoplasia. Recent investigations suggest this may largely be a consequence of perturbed cell cycle

progression and apoptosis.[39] More specifically, Gunn rat cerebella show increased cell cycle arrest in late G0/G1, decreased cyclin D1, cyclin A/A1, cyclin-dependent kinase 2, and an increase in cyclin E, which augments apoptosis.[39] Bilirubin-impaired cell cycle progression, cell cycle arrest in G0/G1, and resultant marked reduction in number of proliferating cells are also observed in vitro (**Fig. 4**) and related to bilirubin concentration and exposure duration.[40] These cytostatic effects may be mediated by a variety of neuropeptides, neurotrophins, or growth factors, including among others fibroblast growth factor 1, insulin growth factor 1, sonic hedgehog (SHH), brain-derived neurotrophic factor, glial-derived neurotrophic factor, and neurotrophic factor 3. A preliminary study suggests that the SHH-smoothened signaling axis is not involved.[41] Cerebellar injury to Purkinje cells, the dentate nucleus, and cerebellar roof nuclei is a consistent feature of kernicterus neuropathology in human neonates.

Central Nervous System Immaturity

Few in vitro studies have explored developmental aspects of bilirubin effects on the CNS. Falcao and colleagues[42] studying rat cortical neurons at different duration of days in vitro (DIV) observed that "immature" neurons, that is, those with shorter DIV,

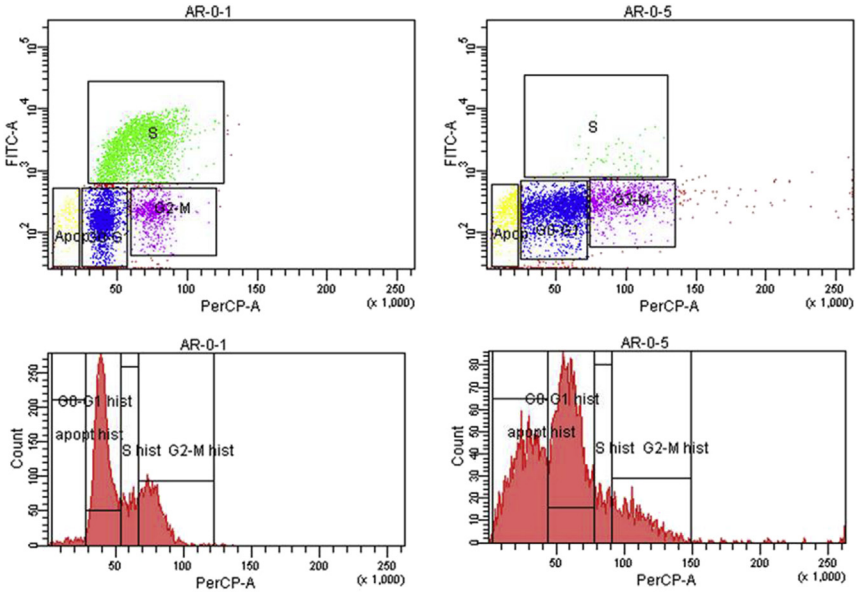

Fig. 4. Cell cycle kinetics determined by flow cytometry during hazardous UB exposure. Representative fluorescence activated cell sorting (FACS) dot plots (*top panels*) show marked changes in the distribution of human promyelocytic leukemia (HL-60) cells using fluorescein isothiocyanate (FITC) -labeled BrdU and correlative histograms (*bottom panels*) during 500-nM UB exposures of 24-hour duration (*right panels*) compared with control HL-60 cells (*left panels*). Notably, there is a marked increase in the number of cells in the G0/G1 phase, a decrease in the number of cells in the S phase, and an increase in the number of cells in apoptosis, consistent with bilirubin-induced cell cycle arrest in G0/G. AR-0-5, an internal shorthand for the parent HL-60 cell line used in this study at 500 nm bilirubin exposure; AR-0-1, parent cell HL-60 cell line under control conditions; PerCP-A, Peridinin-chlorophyll protein-A (the reagent used in the flow cytometry). (*From* Daood MJ, Azzuqa A, Watchko JF. Bilirubin-induced cytostasis, G0/G1 cell cycle arrest, and apoptosis as function of increasing concentration and duration of exposure in vitro. E-PAS 2014;4525.1.)

were more vulnerable to bilirubin-induced injury as manifest by apoptosis and reduction in neurite growth and branching. Notably, the Gunn rat and *UGT1* knockout mouse in vivo kernicterus models are representative of the preterm rather than the term neonate. CNS development in these murine species between birth and postnatal day 10 mirrors that seen in preterm human neonates between 24 and 38 weeks GA.[43] Thus, they offer a powerful experimental approach to study the effects of bilirubin on early postnatal preterm CNS injury.

Future Basic Research Efforts

Hazardous UB concentrations adversely affect many subcellular compartments and biochemical pathways. Despite the considerable efforts of numerous investigators in the field, a robust unifying hypothesis or consensus model of bilirubin neurotoxicity has yet to be firmly established: one that is parsimonious and consistent with the classic features of kernicterus neuropathology, neuroimaging, and neurologic outcomes. Basic research endeavors must strive to more fully integrate insights obtained in vitro (cell lines, coculture systems, and slice preparations) with those obtained in vivo (murine models) and ultimately those observed in human neonates (neuropathology and neuroimaging) to distinguish causal relationships from epiphenomenon. Investigators must also come full circle in their efforts to ensure that insights gained in basic research are relevant to human biology and ultimately translatable to the clinical arena.

Clinical Manifestations of Bilirubin-Induced Central Nervous System Injury in the Preterm Neonate

Acute bilirubin encephalopathy

ABE describes an altered neurologic state induced by hazardous hyperbilirubinemia during the first days of postnatal life characterized by a constellation of abnormal clinical signs typically progressive in their severity. In mature infants, the initial phase of ABE is characterized by stupor (lethargy), hypotonia, and poor sucking. These nonspecific 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 damage. These signs include hypertonia often manifested by retrocollis and opisthotonos, fever, and high-pitched cry. Apnea and inability to feed may ensue. Any infant with signs of intermediate to advanced ABE (hypertonia, arching, retrocollis, opisthotonos, fever, high-pitched cry) merits an immediate exchange transfusion in an attempt to avert CBE.[44,45]

Preterm neonates less frequently show these classic abnormal neuromotor signs, making it difficult to recognize ABE in the some preterm neonates. Recurrent apnea and desaturations may be the only clinical manifestations of ABE in preterm infants during the neonatal period, if any appear at all. Hazardous hyperbilirubinemia is increasingly recognized to adversely impact neural respiratory drive and lead to central, mixed, and obstructive apnea events.[46] Symptomatic apneic events, sometimes manifest as “cyanotic attacks,” are reported in premature infants.[46,47] Some suggest disordered control of breathing is a “distinctive picture” of ABE in preterm infants or at least a prominent clinical feature.[46,47] Therefore, recurrent symptomatic apneic events in a jaundiced neonate should prompt a total serum bilirubin (TB) measurement and evaluation for ABE.[46,48] Such events result from bilirubin-induced brainstem injury and blunted ventilatory responses to $Paco_2$ and or Pao_2.[46]

Chronic bilirubin encephalopathy

In contrast to ABE, 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.[1,45] The American Academy of Pediatrics recommends the term kernicterus be reserved for the chronic and permanent neurologic sequelae of bilirubin toxicity.[45] In classic kernicterus, these include the extrapyramidal movement disorders of dystonia and/or choreoathetosis, hearing loss due to auditory neuropathy spectrum disorder (ANSD), and the eye movement abnormality of paresis of upward gaze.[1] Dental enamel hypoplasia may also be seen in association with these neurologic findings. Classic kernicterus is well described in preterm neonates and possibly the most frequent manifestation of bilirubin-induced brain damage in this patient population.

Auditory-predominant kernicterus

The retrocochlear structures of the auditory system (brainstem auditory nuclei, inferior colliculi, and VIII cranial nerve) are vulnerable to bilirubin neurotoxicity. Auditory-predominant kernicterus can result, a subtype of CBE in which ANSD are the primary sequelae[1]; abnormalities in motor control and muscle tone, if present, are subtle.[1] Shapiro[1] suggests that auditory predominant kernicterus is more common in preterm neonates. Hearing screens in the neonatal intensive care unit must therefore include an auditory brainstem-evoked response in addition to otoacoustic emissions in order to detect affected infants with bilirubin-induced injury to the auditory pathway.

Low-Bilirubin Kernicterus

Low-bilirubin kernicterus is a rare, but refractory cause of bilirubin-induced neurotoxicity in preterm neonates.[2] The term refers to bilirubin-induced neuronal damage at TB levels generally thought to be nonhazardous, that is, those below double volume exchange transfusion (DVET) thresholds. The CNS bilirubin exposure, however, is neurotoxic, suggesting either (i) an albumin problem, that is, an abnormally low serum albumin and/or impaired albumin-bilirubin binding that results in a hazardous UB concentration; and/or (ii) a vulnerable neuronal pool resulting from cellular immaturity coupled with antecedent or concurrent insults that potentiate bilirubin neurotoxicity.[2] Oftentimes both an albumin problem and a vulnerable neuronal pool are evident in a given neonate with low-bilirubin kernicterus, suggesting this is a 2-hit or multihit phenomenon.[2] **Table 1** shows the frequency of adverse conditions in recent case reports.[2,6,7,49–53]

As highlighted in **Table 1**, one of the more common clinical conditions associated with low-bilirubin kernicterus is hypoalbuminemia, often at concentrations well below the standard critical serum albumin neurotoxicity risk factor criterion of less than 2.5 g/dL for preterm neonates.[44,54,55] Indeed, trough serum albumin levels in the low-bilirubin kernicterus case series of Govaert and colleagues[6] ranged from 1.3 to 1.9 g/dL. A modestly elevated TB, with this degree of hypoalbuminemia, holds the potential for neurotoxicity given the limited albumin-bilirubin binding capacity dictated by the very low serum albumin *alone*, independent of any adverse alteration in albumin-bilirubin binding affinity.

In this regard, it is notable that every neonate in the case series of Govaert and colleagues[6] of low-bilirubin kernicterus demonstrated elevated bilirubin/albumin ratios (BAR) that met or exceeded the DVET treatment thresholds set forth by Ahlfors[54] and used in the recent Bilirubin Albumin Ratio Trial (BARTrial)[56] (**Table 2**). In contrast, the TB treatment threshold for DVET was either not met or exceeded only after the BAR.

These findings suggest that although the BAR is an imperfect surrogate of free bilirubin and CNS bilirubin exposure, during extreme hypoalbuminemia the BAR may become a meaningful proxy of bilirubin neurotoxicity risk. Prior studies showing

Table 1
Adverse conditions in case reports of low-bilirubin kernicterus (2001–2013)

Reference	Serum Albumin <2.5 g/dL	BAR for DVET Exceeded	Infection/ Inflammation	Comorbid CNS Injury[a]	Preterm Gestation[d]	Two or More Risk Factors
Govaert et al,[6] 2003[b]	5/5	5/5	2/5	4/5	$25^{4/7}$–$29^{0/7}$	5/5
Odutolu & Emmerson,[49] 2013	1/1	1/1	1/1	0/1	$36^{6/7}$	1/1
Moll et al,[50] 2011	N/A	N/A	1/2	2/2	$24^{0/7}$–$26^{0/7}$	2/2
Okumura et al,[51] 2009[b]	N/A	N/A	N/A	1/5	$25^{0/7}$–$31^{0/7}$	1/5
Gkoltsiou et al,[7] 2008[c]	N/A	N/A	3/3	3/3	$27^{0/7}$–$35^{0/7}$	3/3
Sugama et al,[52] 2001	N/A	N/A	1/2	1/2	$31^{0/7}$–$34^{0/7}$	2/2
Kamei et al,[53] 2012	1/2	1/2	N/A	0/2	$24^{6/7}$–$27^{0/7}$	1/2
Adverse condition per cases	7/8	7/8	8/13	11/20	20/20	15/20

Numbers are likely an underestimate because conditions were not systematically screened across all the studies.

Abbreviations: IVH, intraventricular hemorrhage; N/A, not available; PVL, periventricular leukomalacia.

[a] PVL, IVH (grade II, III, and/or IV), hydrocephalus ex vacuo.

[b] Only 5 of 8 reported cases of kernicterus met the definition of low-bilirubin kernicterus.

[c] Only 3 infants in reported cases of kernicterus met the definition of low-bilirubin kernicterus.

[d] All infants were preterm (<$37^{0/7}$ wks GA), gestational age, or ranges as shown.

Adapted from Watchko JF, Maisels MJ. The enigma of low bilirubin kernicterus in premature infants: why does it still occur, and is it preventable? Semin Perinatol 2014;38:398; with permission.

Table 2
Bilirubin:albumin ratio trial phototherapy and exchange transfusion criteria

	Phototherapy				Exchange Transfusion			
	Standard Risk		High Risk		Standard Risk		High Risk	
Birth Weight (g)	TB	B/A	TB	B/A	TB	B/A	TB	B/A
<1000	5.8	2.3	5.8	2.3	9.9	3.9	9.9	3.9
1000–1250	8.7	3.5	5.8	2.3	12.8	5.1	9.9	3.9
1250–1500	11.1	3.7	8.7	2.9	15.2	6.1	12.8	5.1
1500–2000	12.8	4.2	11.1	3.7	16.9	6.8	15.2	6.1
2000–2500	14.0	4.6	12.8	4.2	18.1	7.2	16.9	6.8

At 48 h of postnatal age and older.

High risk: asphyxia, hypoxemia, acidosis, hemolysis, neurologic deterioration (sepsis, meningitis, intracranial hemorrhage > grade 2).

TB is expressed in milligrams per deciliter and bilirubin:albumin ratio (B/A) ratio is expressed as milligrams per gram.

From Hulzebos CV, Dijk PH, van Imhoff DE, et al. The bilirubin albumin ratio in the management of hyperbilirubinemia in preterm infants to improve neurodevelopmental outcome: a randomized controlled trial—the BARTrial. PLoS One 2014;9:e99466.

no difference in outcomes between infants managed using the BAR versus TB are constrained by their small number of subjects with marked hypoalbuminemia. Indeed, the BAR will not improve neurotoxicity risk prediction when the albumin level is normal[56] or when the TB concentration is exceedingly high.[57] Initiation of phototherapy at lower treatment thresholds when hypoalbuminemia is present as detailed using the BAR in the BARTrial (see **Table 2**)[56] or the TB level in a recently recommended preterm hyperbilirubinemia management guideline[44] holds promise to reduce the incidence of low-bilirubin kernicterus.[6]

Hypoalbuminemia

The underlying pathophysiologic mechanisms and clinical conditions associated with hypoalbuminemia in neonates are outlined in **Table 3**.[58,59] Those most frequently encountered mechanisms include albumin loss and altered albumin distribution.[58,59] The latter refers to leakage of albumin from the intravascular space into the extravascular, extracellular interstitial space reported in association with several conditions in sick preterm newborns (see **Table 3**). Significant albumin loss secondary to fetal perinatal hemorrhage is also an important contributor to marked hypoalbuminemia and is seen in (i) fetal maternal transfusion; (ii) the donor twin in twin-twin transfusion syndrome (TTTS)[60] and the twin anemia-polycythemia sequence (TAPS)[61]; and (iii) neonatal anemia associated with malformations of the placenta and cord.[62] For example, serum albumin levels of less than 2.5 g/dL were observed in half and less than 2.0 g/dL in almost one-quarter of donors in TTTS.[60] Whenever anemia is present in the immediate neonatal period, measurement of the albumin concentration is prudent.

Subtle kernicterus (bilirubin-induced neurologic dysfunction)

Although bilirubin-induced neurologic damage is most often thought of in terms of classic severe adverse neuromotor and auditory sequelae, it is postulated that bilirubin neurotoxicity may manifest as other less severe neurodevelopmental disabilities, a condition termed subtle kernicterus or BIND.[1,63–65] BIND is defined by a constellation of "subtle neurodevelopmental disabilities without the classical findings

Table 3
Mechanisms of hypoalbuminemia and associated clinical conditions in neonates

Mechanism	Clinical Conditions
1. Decreased synthesis	Hepatic failure, sepsis, inflammation
2. Increased catabolism	Critical illness
3. Abnormal loss	Hemorrhage • Fetal maternal hemorrhage • TTTS (donor) • TAPS (donor) • Surgical loss • Malformations of placenta and cord Nephrotic syndrome
4. Altered distribution between intravascular and extravascular space	Sepsis Necrotizing enterocolitis Inflammation Asphyxia Hydrops fetalis (nonimmune and immune) Isoimmune hemolytic disease of the newborn

Data from Uhing MR. The albumin controversy. Clin Perinatol 2004;31:475–88; and Nicholson JP, Wolmarans MR, Park GR. The role of albumin in critical illness. Br J Anaesth 2000;85:599–610.

of kernicterus that, after careful evaluation and exclusion of other possible etiologies, appear to be due to bilirubin neurotoxicity."[1] These purportedly include (i) mild to moderate disorders of movement (eg, incoordination, clumsiness, gait abnormalities, disturbances in static and dynamic balance, impaired fine motor skills, and ataxia); (ii) disturbances in muscle tone; and (iii) altered sensorimotor integration.[1,63–65] A putative association between postnatal hyperbilirubinemia and an increased risk of cognitive dysfunction (eg, lower intelligence quotient) and a range of neuropsychiatric syndromes including attention deficit-hyperactivity disorder, autism, and schizophrenia has also been alleged.[1,63–65] However, as with subtle kernicterus, the linkage between hyperbilirubinemia and their genesis remains uncertain and a source of continued study and debate.

The possible neuroanatomical basis for subtle kernicterus is unclear, but investigators have suggested a range of subcortical neuropathology, including the cerebellum and cerebellar projections.[66–68] The focus on the cerebellum derives from the following: (i) the cerebellum is vulnerable to bilirubin-induced injury, perhaps the most vulnerable region within the CNS; (ii) infants with cerebellar injury exhibit a neuromotor phenotype similar to BIND; and (iii) the cerebellum has extensive bidirectional circuitry projections to motor and nonmotor regions of the brainstem and cerebral cortex that impact a variety of neuromotor behaviors.[66,68]

Studies are needed to more precisely define the neural network abnormalities in infants with a BIND phenotype using advanced neuroimaging technology to shed light on its pathogenesis, including the putative role of bilirubin in the outcome.[5] Until these and other clinical investigations are completed, a causal linkage between bilirubin and subtle neurodevelopmental disabilities remains speculative.

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