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COMMENTARY

Control of Brain Intracellular Bilirubin Levels

Commentary on the article by Hankø et al. on page 441

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Neonatal hyperbilirubinemia is a transient state usually considered as benign except when high plasma levels associated with morbidity co-factors lead to bilirubin encephalopathy, or kernicterus. After an aggressive policy of treatment from the 1960s to the 1990s to prevent high plasma levels, it seemed that many infants were treated unnecessarily since kernicterus rarely developed. In addition, since newborn infants were being discharged earlier, the ability of physicians to diagnose jaundice at the time when the serum bilirubin concentration was likely to increase became very limited (1). Therefore, the American Academy of Pediatrics officially recommended in 1994 that the minimum bilirubin level requiring follow-up and treatment could be increased, in the absence of clinical risk factors for severe hyperbilirubinemia (2). However, the recent re-emergence of acute and chronic encephalopathy does require evaluation and revision of these recommendations (3).

At present, the main objective of therapy has been the prevention of high levels of plasma bilirubin that are linked to high brain bilirubin levels and kernicterus. However, one question remained unsolved: what level of plasma bilirubin is toxic? This question is actually difficult to answer since all infants with high plasma bilirubin levels do not develop bilirubin encephalopathy. Furthermore, recent studies suggest that even bilirubin levels lower than a "safe" threshold could be associated with neurodevelopmental consequences in apparently healthy newborns (4, 5). Finally, new techniques such as *in utero* transfusions or exchange transfusions have led to new neonatal clinical presentations and modifications in the postnatal management of jaundice related immunization (6). Therefore, optimal management of hyperbilirubinemia remains uncertain and the understanding of bilirubin toxicity mechanisms more and more confused.

Toxic effects of bilirubin are related to cellular injury and neurotoxicity. Bilirubin inhibits mitochondrial enzymes and can interfere with DNA and protein synthesis (7). It also inhibits *N*-methyl-D-aspartate-receptor ion channels function (8) and alters cerebral glucose metabolism (9). Thus, the concentration of bilirubin in the brain and the duration of exposure are major determinants of the neurotoxic effects of bilirubin. Hyperbilirubinemia may be due to excessive production, limited ability of elimination or hepatic conjugation, or uncoupling between production and excretion ability (1). However, correlation between serum bilirubin concentration and bilirubin encephalopathy is rather poor, except for very high levels of bilirubinemia, which suggests that factors other than blood levels must be involved (1). Since free bilirubin can cross the blood brain barrier, agents displacing bilirubin from albumin binding sites are responsible for increased toxicity. This leads to extra caution in the use of medications in the neonate at risk of hyperbilirubinemia. Other co-factors such as acidosis and hypoxia have been shown to increase brain bilirubin level and toxicity (10, 11).

The article by Hankø *et al.* in this issue introduces a new concept in the distribution of bilirubin. This concept might be a new clue in the understanding of bilirubin toxicity and its variability. It has been suggested that free bilirubin is a substrate for P-glycoprotein (P-Gp) (12). P-Gp is a member of the ATP-binding family of membrane transporters encoded by the human ABCB1 gene, also called MDR1 for *multidrug resistance* (12, 13). Its main function is the energy-dependent cellular efflux of lipophilic substrates (13). Watchko *et al.* (12) studied P-Gp deficient transgenic null mutant mice, 10 and 60 min after bilirubin transfusion. Although brain bilirubin clearance was unaffected, brain bilirubin concentrations significantly increased compared with controls. This suggested that limitation of P-Gp efflux resulted in elevated intracellular levels. In the study presented in the current issue of the Journal, Hankø *et al.* shows that several drugs given at therapeutic dosage, without significant bilirubin-albumin displacing properties, but known to inhibit P-Gp, have a direct impact on brain bilirubin level and distribution. This provides a functional aspect of the blood brain barrier with respect to bilirubin transport and metabolism.

A global cartoon from plasma bilirubin to cellular toxicity can then be suggested: unbalanced bilirubin production and excretion would lead to excessive free unbound bilirubin. This free bilirubin, whose level is influenced by pH and drugs with bilirubin-albumin displacing properties, would pass the blood brain barrier by diffusion but intracellular concentration would be affected by P-Gp function. This function might be altered by

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hypoxia or pharmacological drugs leading to toxic intracellular brain bilirubin levels (13).

This current report should lead to further research into the pathogenesis of hyperbilirubinemic encephalopathy. Since the authors studied adult animals it would be worthwhile to verify its validity in neonates with immature brains. In addition, sample sizes are small and differences in control values from two sets of experiments require further investigation. Finally the integrity of the blood brain barrier has not been checked in this study or the specificity of substrate between transporters as specified in the conclusion of the article. In any case, this study suggests a need for more caution in the newborn in the use of drugs that may impair P-Gp activity such as Ceftriaxone, Erythromycin or Rifampicin. Other drugs, used in Neonatology and known to be P-Gp substrates should also be investigated such as cardiac glycosides or glucocorticoids (14).

In addition, since genetic polymorphisms in the human P-Gp transporter gene has been demonstrated (14), this could explain the susceptibility of some newborns toward bilirubin toxicity. Furthermore, there is a possibility that discovery of a specific genetic marker could predict susceptibility to bilirubin toxicity; a similar potential role for genetic markers has been suggested as a means of improving anti-leukemic or anti-retroviral therapy (14).

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