

Review Article

Clinical Implications of Bilirubin-Associated Neuroprotection and Neurotoxicity

Nicholas V Mendez^{1#}, Jeffrey A Wharton^{1#}, Jenna L Leclerc¹, Spiros L Blackburn², Martha V Douglas-Escobar³, Michael D Weiss³, Christoph N Seubert¹ and Sylvain Doré^{1,4*}

¹Department of Anesthesiology, College of Medicine, University of Florida, USA

²Department of Neurosurgery, College of Medicine, University of Florida, USA

³Division of Neonatology, Department of Pediatrics, College of Medicine, University of Florida, USA

⁴Departments of Neurology, Psychiatry, and Neuroscience, College of Medicine, University of Florida, USA

[#]These authors contributed equally to this work

***Corresponding author**

Sylvain Doré, Department of Anesthesiology, Neurology, Psychiatry, and Neuroscience, University of Florida, 1275 Center Drive Gainesville, FL 32610-0254 USA, Tel: 352-273-9663; Email: sdore@anest.ufl.edu

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Abstract

Bilirubin is a primary product of heme catabolism and exhibits both neuroprotective and neurotoxic effects. When present at physiologic concentrations, bilirubin is a potent antioxidant and serves to protect brain tissue from oxidative stress insults. The use of the anesthetic propofol attenuates ischemic injury in rats by exploiting these neuroprotective properties. At pathologic levels, bilirubin has been implicated as a neurotoxic agent, demonstrating the ability to aggregate and adhere to cellular membranes, thereby disrupting normal cellular function. Bilirubin-associated toxicities are amplified by administering drugs such as anesthetics that compete with bilirubin for albumin binding sites, resulting in increased plasma bilirubin concentrations. As such, it is crucial that bilirubin is considered in the critical care management of patients with hemorrhagic stroke, cerebral ischemic damage, and critically ill newborns.

INTRODUCTION

Under physiologic conditions, the normal turnover of red blood cells releases hemoglobin, which is further broken down into heme and amino acids (from the globin part). Heme oxygenase (HO) is the rate limiting enzyme in the conversion of heme into unconjugated bilirubin, and it exists in the brain as two isoforms: HO-1 and HO-2 [1]. The production of HO-1 is inducible by heme and oxidative stress, both of which are present following pathologic events such as hemorrhagic stroke [2]. HO-2 is constitutively produced under physiologic conditions, where it facilitates the majority of heme catabolism [1]. Upon its production, bilirubin has been found to confer both neuroprotective antioxidant characteristics as well as neurotoxic properties [3]. A thorough understanding of the intricate interactions between bilirubin and the central nervous system is necessary since it may have profound implications on the treatment modalities used in the care of critically ill patients.

NEUROPROTECTIVE ROLE OF BILIRUBIN

Upon degradation of heme by HO, the product biliverdin is rapidly converted to unconjugated bilirubin by biliverdin reductase (BVR) [3]. Unconjugated bilirubin is prevented from crossing an intact blood brain barrier, and thus from accumulating in the central nervous system, because it is mostly bound to albumin in the plasma [4]. Our lab has previously shown that bilirubin exhibits potent antioxidant activity and is protective against H₂O₂-induced free radical damage to neuronal cells *in vitro* [5]. When bilirubin serves as an antioxidant, it is oxidized back to biliverdin, where it can then be acted upon once again by BVR to regenerate bilirubin [6]. This bilirubin-biliverdin redox loop might explain why bilirubin has such powerful antioxidant effects when it is present in low physiologic concentrations in neuronal cell cultures [5]. While HO-2 makes up most of the heme oxygenase activity in the brain, HO-1 has been identified in specific cell types in the nervous system including microglia and macrophages [6]. Recently, propofol post-treatment of rats

after middle cerebral artery occlusion was shown to attenuate ischemic injury in part by upregulating HO-1 [7]. Furthermore, experimental evidence in a mouse model of cerebral ischemia demonstrated greater neuronal damage in HO2^{-/-} knockout mice compared to their wild type counterparts, providing additional support for bilirubin's neuroprotective role in the brain [8]. Due to bilirubin's antioxidant capabilities, many have proposed that physiologic neonatal unconjugated hyperbilirubinemia evolutionarily developed as a protective mechanism [3].

BILIRUBIN-ASSOCIATED NEUROTOXICITY

In addition to serving a protective role, bilirubin has also been implicated in the progression of neurological dysfunction in many pathological states. Many of these conditions increase bilirubin concentrations above physiologic levels, where the toxic effects of bilirubin start to exceed the protective antioxidant benefits, resulting in damage to the nervous system [9]. Bilirubin is neurotoxic when it reaches micromolar concentrations, and this is the same level at which it was found to aggregate and adhere to cellular membranes, disrupting normal cellular function [10]. In the critical care setting, it is important that attention is given when selecting drugs that compete with bilirubin for albumin-binding sites since they effectively increase plasma bilirubin levels. For example, the fatty acid components of propofol displace bilirubin from albumin, amplifying bilirubin-associated neurotoxicity in susceptible patients [11].

CLINICAL IMPLICATIONS OF BILIRUBIN ACTIVITY

Neurotoxicity following hemorrhagic stroke

Hemorrhagic stroke occurs when a weakened blood vessel ruptures, resulting in bleeding into the brain and subsequent neuronal injury. Patients with hemorrhagic stroke can have additional complications that lead to secondary damage days after the initial insult, such as vasospasm and cerebral ischemia [12]. The presence of blood in the brain locally induces HO-1 causing an increase in production of unconjugated bilirubin [2]. Preclinical data has suggested that the environment immediately surrounding the hematoma is highly conducive to oxidative reactions, facilitating the conversion of bilirubin into bilirubin oxidation products (BOXes) [12]. BOXes in cerebrospinal fluid follow a similar time course as the onset of cerebral vasospasm, and have proven to be vasoactive in both *in vivo* and *in vitro* studies [2]. These results collectively suggest that BOXes either cause or contribute to cerebral vasospasm and the resulting delayed neurologic deterioration following hemorrhagic stroke.

Bilirubin as a negative prognostic biomarker in ischemic stroke

Ischemic stroke takes place when a blood vessel supplying the brain is obstructed, which produces downstream hypoxic-ischemic conditions and increased oxidative stress [13]. With these conditions, bilirubin formation is amplified significantly due to HO-1 induction. It has been proposed that the serum bilirubin level is a biomarker of the degree of ischemic damage following stroke [14]. Clinical studies have found that high serum bilirubin levels measured at the time of clinical presentation positively correlate with stroke severity and degree of disability three months post-ictus [13]. These data provide compelling

evidence for the use of bilirubin as an early clinical indicator in the management of patients following ischemic stroke.

Manifestations of bilirubin activity in the neonate

Newborns commonly develop a transient increase in unconjugated bilirubin levels, commonly referred to as "physiologic jaundice," which often resolves without any consequences [15]. By virtue of the intrinsic antioxidant effects of bilirubin, physiologic jaundice confers protection to neonatal tissue that otherwise would be more susceptible to damage by oxidative stress [10]. Additionally, breastfed neonates have higher unconjugated bilirubin levels than formula fed infants, suggesting that physiologic jaundice is well tolerated and maybe confers an evolutionary advantage [16]. However, additional sources of hemolysis may increase the production of unconjugated bilirubin concentrations above this physiologic range [3]. These conditions include hemolysis due to ABO or Rh blood incompatibilities between the mother and fetus, G6PD deficiency, and trauma during birth [17]. Pathological levels of bilirubin can be neurotoxic leading to kernicterus or neonatal bilirubin encephalopathy [9]. In the brain, high bilirubin concentrations can inhibit mitochondrial enzymes, disrupt DNA synthesis, and attenuate protein production [3]. Acute bilirubin encephalopathy results in injury to the basal ganglia and various brain stem nuclei [18]. Universal neonatal hyperbilirubinemia screening programs have been implemented to detect and treat pathologic jaundice and prevent kernicterus [18]. There is a paucity of publications about common anesthetic medications used in neonates (such as propofol used during neonatal surgery) and their interactions with bilirubin levels. Research in neurotoxicity and neonatal anesthesia should consider the interplay between anesthetics and levels of unconjugated bilirubin.

CONCLUSION

Preclinical and clinical evidence has shown that bilirubin can function as both a neuroprotective and neurotoxic agent. The neuroprotective mechanisms conferred by bilirubin warrant further exploration in an effort to exploit their therapeutic potential. Likewise, bilirubin has been found to play an important role in the progression of various neuropathologic conditions, such as hemorrhagic stroke, cerebral ischemia, and kernicterus of the newborn. Ultimately, it is apparent that bilirubin plays a significant role in both the development and prevention of neurological dysfunction and therefore should be considered in management of the critical care patient.

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REFERENCES

1. Wang J, Doré S. Heme oxygenase-1 exacerbates early brain injury after intracerebral haemorrhage. *Brain*. 2007; 130: 1643-1652.
2. Pyne-Geithman GJ, Morgan CJ, Wagner K, Dulaney EM, Carrozzella J, Kanter DS, et al. Bilirubin production and oxidation in CSF of patients

- with cerebral vasospasm after subarachnoid hemorrhage. *J Cereb Blood Flow Metab.* 2005; 25: 1070-1077.
3. Dennery PA. Evaluating the beneficial and detrimental effects of bile pigments in early and later life. *Front Pharmacol.* 2012; 3: 115.
 4. Palmela I, Sasaki H, Cardoso FL, Moutinho M, Kim KS, Brites D, et al. Time-dependent dual effects of high levels of unconjugated bilirubin on the human blood-brain barrier lining. *Front Cell Neurosci.* 2012; 6: 22.
 5. Doré S, Goto S, Sampei K, Blackshaw S, Hester LD, Ingi T, et al. Heme oxygenase-2 acts to prevent neuronal death in brain cultures and following transient cerebral ischemia. *Neuroscience.* 2000; 99: 587-592.
 6. Chang EF, Claus CP, Vreman HJ, Wong RJ, Noble-Haeusslein LJ. Heme regulation in traumatic brain injury: relevance to the adult and developing brain. *J Cereb Blood Flow Metab.* 2005; 25: 1401-1417.
 7. Liang C, Cang J, Wang H, Xue Z. Propofol attenuates cerebral ischemia/reperfusion injury partially using heme oxygenase-1. *J Neurosurg Anesthesiol.* 2013; 25: 311-316.
 8. Doré S, Sampei K, Goto S, Alkayed NJ, Guastella D, Blackshaw S, et al. Heme oxygenase-2 is neuroprotective in cerebral ischemia. *Mol Med.* 1999; 5: 656-663.
 9. Shapiro SM. Definition of the clinical spectrum of kernicterus and bilirubin-induced neurologic dysfunction (BIND). *J Perinatol.* 2005; 25: 54-59.
 10. Doré S. Decreased activity of the antioxidant heme oxygenase enzyme: implications in ischemia and in Alzheimer's disease. *Free Radic Biol Med.* 2002; 32: 1276-1282.
 11. Robards C, Brull SJ. The anesthetic implications of Crigler-Najjar syndrome. *Anesth Analg.* 2007; 104: 435-436.
 12. Clark JF, Loftspring M, Wurster WL, Beiler S, Beiler C, Wagner KR, et al. Bilirubin oxidation products, oxidative stress, and intracerebral hemorrhage. *Acta Neurochir Suppl.* 2008; 105: 7-12.
 13. Kurzepa J, Bielewicz J, Stelmasiak Z, Bartosik-Psujek H. Serum bilirubin and uric acid levels as the bad prognostic factors in the ischemic stroke. *Int J Neurosci.* 2009; 119: 2243-2249.
 14. Pineda S, Bang OY, Saver JL, Starkman S, Yun SW, Liebeskind DS, et al. Association of serum bilirubin with ischemic stroke outcomes. *J Stroke Cerebrovasc Dis.* 2008; 17: 147-152.
 15. Boyd S. Treatment of physiological and pathological neonatal jaundice. *Nurs Times.* 2004; 100: 40-43.
 16. Schneider AP 2nd. Breast milk jaundice in the newborn. A real entity. *JAMA.* 1986; 255: 3270-3274.
 17. Stevenson DK, Vreman HJ, Wong RJ. Bilirubin production and the risk of bilirubin neurotoxicity. *Semin Perinatol.* 2011; 35: 121-126.
 18. American Academy of Pediatrics Subcommittee on Hyperbilirubinemia. Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics.* 2004; 114: 297-316.

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