Clinical Implications of Bilirubin-Associated Neuroprotection and Neurotoxicity

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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 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.

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 neurotoxic 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 may confer 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 bilirubinencephalopathy [9]. In the brain, high bilirubin concentrations can inhibit mitochondrial enzymes, disrupt DNA synthesis, and attenuate protein production [3]. Acute bilirubinencephalopathy 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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