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Review Article

New Concept of Bilirubin-Induced Neurologic Dysfunction: Neonatal Brain Insults of Metal (Copper) Dyshomeostasis

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Summary

Excessive metal accumulation in the nervous system may be toxic, inducing oxidative stress, disrupting mitochondrial function, and impairing the activity of numerous enzymes. Damage caused by metal excess may result in permanent injuries, including severe neurological disorders. The immature and strikingly vulnerable neurons play an important role in the pathogenesis of bilirubin-induced neurologic dysfunction (BIND) as well. The increased vulnerability of neonates, especially the premature infants, to brain damage may be due to a proneness of immature nerve cells to toxic stimulus. The developing neurons undergo programmed cell death, a necessary phenomenon for proper nervous system development. Following this period, neurons mature and restrict the apoptotic pathway to permit long-term survival. On the basis of the above described abundant research data and hypotheses, according to our concept, the BIND is a neurodegenerative disease (ND) of immature brain caused by accumulation of free metals and unconjugated bilirubin (UCB), and UCB-Cu complex (as prooxidant), respectively, in the basal ganglia (BG) and other parts of the central nervous system (CNS) relevant to BIND. The main comorbidity is the hemolysis of neonatal red blood cells. During this process a great amount of heavy metals (mainly iron and copper) are liberated and producing reactive oxygen species (ROS). These elements may circulate in the bloodstream, and can pass through the immature blood-brain-barrier (BBB), finding entrance into the CNS. In addition, ROS contribute to increased BBB permeability creating a dangerous vitious circle in the neonatal brain, especially in the basal ganglia (BG).

Keywords: *Copper Toxicity; Bilirubin-Induced Neurologic Dysfunction; Oxidative Stress; Copper-Bilirubin Complex; D-Penicillamine in the neonatal period*

Abbreviations

AD: Alzheimer's Disease; BG: Basal Ganglia; BBB: Blood Brain Barrier; BIND: Bilirubin-Induced Neurologic Dysfunction; CNS: Central Nervous System; D-PA: D-Penicillamine; NHBI: Neonatal Hyperbilirubinemia; ND: Neurodegenerative Disease; PD: Parkinson Disease; ROP: Retinopathy of Prematurity; RBC: Red Blood Cells; ROS: Reactive Oxygen Species; TB: Total Bilirubin Level; UCB: Unconjugated Bilirubin; WD: Wilson Disease

Introduction

UCB at excessively high concentrations causes permanent neural damage in newborn infants, i.e. "chronic bilirubin encephalopathy" or kernicterus [1]. The precise threshold, however, at which UCB may be neurotoxic in a given infant is unknown. A better understanding of the pathophysiology as it is related to the rate of hemolysis and bilirubin-albumin binding may be help but not enough to resolve the uncertainty [2]. Copper is an essential trace element which plays an important role as cofactor in various enzymes, which connect copper with metabolic pathways such as mitochondrial respiration, antioxidative defense and neurotransmitter synthesis [3]. However, the high redox activity of copper can also be harmful to cells by inducing oxidative stress, which will damage cellular components and will

ultimately lead to cell death. Epidemiological and clinical studies have shown a strong correlation between aberrant metal exposure and a number of neurological diseases, including aceruloplasminemia, Alzheimer's disease, amyotrophic lateral sclerosis, autism spectrum disorders, Guillain–Barré disease, Gulf War syndrome, occipital horn syndrome, Huntington's disease, multiple sclerosis, Parkinson's (PD) and Wilson's diseases (WD) [4]. The neonatal brain, with its high concentrations of unsaturated fatty acids, high rate of oxygen consumption, low concentrations of antioxidants, and availability of redox-active iron and copper, is particularly vulnerable to oxidative damage [5]. Moreover, the copper metabolism in Wilson's disease and in the newborn infants is strikingly similar. Both have large quantities of copper in the liver and in the brain (mainly in the BG) which is contrasted by an unusually low ceruloplasmin level in the blood [6]. The molecular pathogenesis of bilirubin-induced neuronal cell injury, although incompletely understood, likely reflects the untoward effects of hazardous unconjugated bilirubin- or increased intracellular calcium- and copper concentrations. Diseases in the neonatal period such as bronchopulmonary dysplasia, ROP, necrotizing enterocolitis, periventricular leukomalacia, and according to our concept, bilirubin-induced neurologic dysfunction (BIND) are related to free radical damage. The incidence rates of Learning Disabilities and Attention Deficit Disorder are likely to continue to increase as excess copper is transmitted from one generation to the next. Aggressive and violent behavior also will tend to increase with the build-up of excess tissue copper levels. Addictive cravings and behavior, and depression or panic attack are likely to increase in frequency [7,8].

Acutely, copper-induced cell death occurred independently from an increase of intracellular free calcium $(Ca^{++})i$, but could be prevented by addition of agents interfering with ROS production [9]. D-Penicillamine (D-PA) by its ability to alleviates oxidative stress and chelates copper may have significant neuroprotective effects in cases jeopardized by BIND or retinopathy of prematurity (ROP). We conclude that treatment with D-PA might result in a wide range of health benefits, improved quality of life and reduced healthcare costs and may help reduce complications in the neonatal period [10]. It is also remarkable that copper chelators prevent both copper uptake and copper-induced damage. These data suggest that predominantly the cellular content of copper determines copper-induced toxicity in the brain [3,11].

Neonatal copper metabolism

Fetal liver copper levels are increased four to ten times those found in the normal adult. Copper is a very stimulating mineral to the nerves and central nervous system (CNS). Its effects on neurotransmitters can give rise to many psychological imbalances such as mood swings, depression, mental agitation, feeling overstimulated, restlessness, anxiety and insomnia. A significant portion of the toxicity of copper comes from its ability to accept and donate single electrons as it changes oxidation state. This increase in unmediated ROS is generally termed oxidative stress. When women become pregnant, their estrogen levels rise, greatly increasing the retention of copper in the body. This metal will pass through the placenta into the unborn child. So many children are being born with toxic levels of copper and other heavy metals which were stored in the mother's body [12].

Here, it is noteworthy, how prominent similarities exist between Wilson's disease (WD) and neonates with kernicterus concerning the copper metabolism, the neuropsychiatric manifestations and the histopathological findings (BOX 1).

High Copper in the Basal Ganglia Neuropsychiatric Manifestations
WD: Movement disorders, tremors, involuntary movements, choreoathetosis, dysarthria, dystonia, personality changes, uncontrolled emotional outbursts [13-16]. KERNICTERUS: generalized dystonia, athetoid cerebral palsy, paralysis of upward gaze, sensorineural hearing loss. BIND: impairment of audiologic, speech, and language processing as well as disturbances in visual-motor and cognitive functions associated with failure of fine neuromotor control (extrapyramidal signs) [1]. At AUTOPSY (both in WD and KERNICTERUS): marked neuronal loss with demyelination and astrocytic replacement [1,17-19].

BOX 1: *Common neuropsychiatric manifestations and pathological findings in WD and bilirubin encephalopathy.*

In the neonatal period the ability of the liver to synthesize ceruloplasmin is not fully developed and adult levels of the protein are not found in the blood till about three months of age. It is interesting therefore that the infant liver has a very much higher copper content than is found in the adult and a fall in concentration does not takes place until the ability to synthesize ceruloplasmin has fully developed [20].

Copper as a ligand of albumin

Albumin is best known for its ability to bind smaller molecules of many types. Albumin interacts with a broad spectrum of compounds. Most strongly bound are hydrophobic organic anions of medium size, 100 to 600 Da–long-chain fatty acids, hematin, and bilirubin. A large proportion of copper in serum is also bound to albumin. Copper atoms can undergo univalent redox reactions and catalyze the formation of free radicals. This feature makes copper toxic to cells. *In vivo*, the potential toxicity of extracellular copper is mitigated when it is bound to albumin. There is one high affinity site for copper per albumin molecule. When copper is bound to this site, it does not participate in the redox reactions associated with free radicals [21].

Formation of Bilirubin–metal complex

Very wide-ranging studies have long been made on the possible biochemical transformations of UCB, which is formed during the decomposition of hemoglobin. Particular attention has been paid to its photochemical and redox reactions [22] but the relevant publications comprise only a very small proportion of those dealing with the molecular biochemistry of UCB and metals interactions. Bilirubin has a special affinity for the globus pallidus, the hippocampus, and the subthalamic nucleus because they are also target brain regions for divalent metal (Cu, Fe, Zn et cet.) accumulation [23]. At least 3 loci exist where UCB and copper can be "fusioned" in the neonatal period: (1) during hemolysis high UCB and copper level can be developed in the blood; (2) one albumin can bind one Cu^{++} in the primary binding site. At higher concentration of copper (if possible under certain conditions), loosely bound atoms, and can be very easily taken out by UCB [24]. UCB itself can displace loosely bound copper ions, which are electrostatically attached due to high negative charge on the surface of albumin [25]; (3) in the basal ganglia (BG) [6].

To better understand of the BG's characteristics, a brief review of the physiology and pathophysiology is provided. They form the core of the extrapyramidal system and regulate motor activity. To produce adenosine triphosphate (ATP) serving chemical energy, the BG require a rich blood supply typically provides oxygen, glucose, and a high concentration of minerals. ATP is produced through aerobic respiration and oxidative phosphorylation within the mitochondria and this process requires a rich vascular supply to the BG to remove metabolic by-products that may be harmful if left in situ. The BG contain inherently high concentrations of trace minerals including heavy metals (Fe, Cu, Zn etc.) [26] many of which are important cofactors for metabolic activity.

Although little is known about copper distribution in this region and among various cell types or cell-specific regulation of copper homeostasis, Cu undertakes a complex journey crossing the extracellular and intracellular membranes and staying firmly on course while traveling in a cytosol [27,28]. Moreover, many neurodegenerative diseases are related to copper although the effects on brain microvascular endothelial cells are poorly understood [29].

Oxidative stress

Oxidative stress is caused by an imbalance between the production of free radicals and the ability of antioxidant system to detoxify them. Under certain circumstances the production of oxygen-free radicals overcomes antioxidant defense causing oxidative stress [30]. Too much reactive oxygen species (ROS) damage different organs (lung, brain, retina, and gastrointestinal tract), leading to severe acute and chronic conditions that will affect not only the survival but also the quality of life of these infants.

Involvement of ROS in blood-brain-barrier (BBB) dysfunction

The BBB forms a protective barrier around the brain, with the important function of maintaining brain homeostasis. Pathways thought to initiate BBB dysfunction converge on the same point: reactive oxygen species (ROS). Consequently, ROS contribute to increased BBB

permeability. These observations suggest that ROS are key mediators of BBB breakdown and implicate antioxidants as potential neuroprotectants in conditions like stroke and traumatic brain injury, and may be BIND [31]. Several studies have shown that elevated levels of UCB are responsible for neuronal oxidative stress [32-34]. At the same time maunting evidence suggests that the exact UCB concentration associated with kernicterus in the healthy term infant is unpredictable. In a Danish population-based study, the neonates with total serum bilirubin (TSB) levels of ≥ 25 mg/dL didn't show any neurologic dysfunctions at 5 years of follow up [35]. Toxicity levels may vary among ethnic groups, with maturation of an infant, and mainly in the presence of hemolytic disease. Bilirubin, which is derived from its metabolic precursor biliverdin, is the end product of heme catabolism. It has been proposed that UCB is an excellent endogen antioxidant present in human extracellular fluids. UCB can suppress oxidation of lysosomes at oxygen concentrations that are physiologically relevant. It can act as an important cytoprotector of tissues that are poorly equipped with antioxidant defense systems, including myocardium and nervous tissue. In addition, there is a strong body of evidence suggesting that bilirubin may have a beneficial role in preventing oxidative changes in a number of diseases including atherosclerosis and cancer, as well as a number of inflammatory, autoimmune and degenerative diseases. Moreover, UCB is now a predictor of oxidative stress-mediated diseases [39]. Bilirubin inhibits chronic disease development and kidney disease, and ultimately reduces the incidence of all-cause mortality [36-41]. The TSB in jaundiced and non-jaundiced pups exposed to 95% O2 shows a negative correlation with lipid hydroperoxides at 3 days of exposure. Higher UCB concentrations resulted in lower lipid hydroperoxide levels. Therefore, we think that UCB in itself is actually our friend, that is: "Bilirubin, The Gold Within" [42-45]. (BILIRUBIN-FRIEND OR FOE??: Function as natural antioxidants in newborns. Attenuates graft rejection in cardiac transplant models. Inverse relation between bilirubin and coronary artery disease. Inverse relation between bilirubin and colorectal cancer. – 2005 PowerPoint Presentation www.sfrbm.org/frs/FrielBilirubin.pdf).

Neurodegeneration: a return to immaturity? [46]

This question certainly arouses the attention of neonatologists as the immature and strikingly vulnerable neurons play important role in the pathogenesis of BIND. The increased vulnerability of premature infants to brain damage may be due to a proneness of immature nerve cells to toxic stimulus. The developing neurons undergo programmed cell death, a necessary phenomenon for proper nervous system development. Following the developmental period, neurons mature and restrict the apoptotic pathway to permit long-term survival. On the basis of above described abundant research data and hypotheses, according to our concept, the BIND is a neurodegenerative disorder of immature brain caused by accumulation of free metals and UCB-Cu complex (as prooxidant) in the BG and other parts of CNS relevant to BIND. The main comorbidity is the hemolysis of neonatal blood red cells. During this process a great amount of heavy metals (mainly iron and copper) and Cu-UCB complex may circulate in the bloodstream. They can pass through the immature BBB which shows ROS-induced increased permeability, and finding entrance into the CNS. Understanding the differences between neonatal and adult erythrocytes is critical in the evaluation of perinatal erythrocyte disorders. The reason for the reduced RBC survival observed in newborns is not known, although there are many biochemical differences between adult and neonatal RBCs [47-49]. Increased oxidant sensitivity of newborn red cells and relative instability of fetal hemoglobin have been considered as possible causes for this shortened lifespan. In a Chinese study [50] the erythrocyte's copper content was significantly lower in the maternal blood than in the newborn cord blood. The compounds to be bound and transported by albumin are quite diverse and include bilirubin, fatty acids, metal ions and therapeutic agents. The copper atoms in high concentration loosely bound to albumin, and can be very easily taken out by UCB. Free or loosely bound, redox active transition metal ions are potentially extremely pro-oxidant, having the ability to catalyze the formation of damaging and aggressive ROS from much more innocuous organic and inorganic species. In strictly biological terms the two most important such metals are iron and copper [24,25]. In fact, oxidative stress has been demonstrated to be a common link between several conditions such as PD, AD, stroke, prion diseases and UCB encephalopathy, where it is involved in neuronal injury [4].

Conclusions

The basic role of metal ions in neurological pathologies is generally accepted, -except for the case of BIND. Free copper ion in itself or binding to UCB and forming metal-bilirubin complex(es) involved in neurologic dysfunction, therefore they are important factors for

whole brain damage processes in BIND. Figure 1. demonstrates our concept about the chronic bilirubin encephalopathy based on the above described hypothesis.

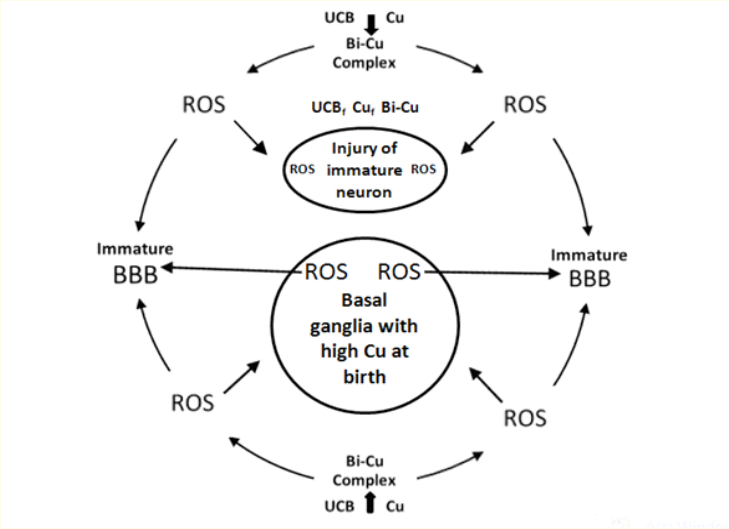

Figure 1: *Vitious circle leading to BIND. Explanation: in the text. (Abbreviations: UCB = unconjugated bilirubin, ROS = reactive oxygen species, BBB = blood-brain-barrier, UCBf = free UCB, Cuf = free Cu).*

D-Penicillamine in the neonatal period

D-penicillamine (D-PA) was first recognized as a potential benefit for neonatal hyperbilirubinemia (NHBI). [51] During this time there was a remarkably low incidence of retinopathy of prematurity (ROP) in the infants treated with D-PA [52]. Later, our studies were replicated in other institutes in Hungary, Poland, the USA, India and Mexico. It is important to note that there was no intolerance or short- or long-term toxicity of the medication, in spite of the fact that in the newborn period D-PA was used 10-20 times higher doses than those in adult. In our Letter to the Editor [53] and in a book just published [54] we have discussed the potential neuroprotective effects of D-PA in BIND and ROP. We hope that our theory will help answer some of the unsolved questions and concerns occurred in the etiology and pathomechanisms of BIND. The beneficial neuropharmacological actions of metal-targeted (chelating) agents most likely arise from local metal redistribution rather than from massive metal removal [55]. The chelation therapy for non-metal overload indications continues to be investigated. Our present chapter address the medical necessity of the use of a chelating agent (D-PA) in the treatment of neonatal hyperbilirubinemia and ROP.

Dedication and acknowledgements

The authors dedicate this article to † WILLIAM A. SILVERMAN on the occasion of his approaching centenary birthday. In his lecture held in Hamilton, Canada at 1998, Bill spoke about the "Subversion of Authority in Medicine". The following golden saying is one of brilliant examples of his witticisms: "Learn from mistakes of others! You can't live long enough to make them all yourself".

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