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D-PENICILLAMINE AS A NEONATAL NEUROPROTECTANT II: EFFECTS ON GASOTRANSMITTERS AND ENDOGENOUS NEUROMODULATORS

[1]*Lajos Lakatos and [2]György Balla

[1]Member of the Hungarian Academy of Science, University Debrecen, Faculty of Medicine, Department of Pediatrics, 4012 Debrecen, Nagyerdei Krt. 98.

[2]Kenezy Teaching Hospital, Department of Pediatrics, 4031 Debrecen, Bartók B, str. 2-26.

***Correspondence for Author: Lajos Lakatos**

Member of the Hungarian Academy of Science, University Debrecen, Faculty of Medicine, Department of Pediatrics, 4012 Debrecen, Nagyerdei Krt. 98.

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ABSTRACT

AIM: The aim of this review was to demonstrate a new concept in the etiology of bilirubin-induced neurologic dysfunction (BIND) and highlight the role of D-Penicillamine (D-PA). **METHOD:** We conducted a review searching the literature of bilirubin metabolism and of metal dyshomeostasis causing encephalopathy in the neonatal period. **RESULTS:** Unconjugated bilirubin has a special affinity for the globus pallidus, the hippocampus and the subthalamic nucleus (basal ganglia). Furthermore, immaturity of the blood-brain barrier also contributes to the development of kernicterus. Homeostasis of metal ions usually involves a huge set of proteins which regulate the proper metal biology. Metal ions, especially copper and iron play very important roles in the pathogenesis of neurodegenerative diseases including BIND, having impact on both protein structure (misfolding) and oxidative stress. **INTERPRETATION:** Our present research article address the medical necessity of the use of a chelating agent (D-PA) in the treatment of neonatal hyperbilirubinemia and in the prevention of retinopathy of prematurity.

KEYWORDS: Bilirubin-induced neurologic dysfunction; Reactive oxygen species; Copper dyshomeostasis;; Neurodegeneration; D-Penicillamine in the neonatal period.

ABBREVATIONS

AD - Alzheimer disease; ASD - Autism spectrum disease; BBB - Blood brain barrier; BG - Basal ganglia; BIND - Bilirubin-induced neurologic dysfunction; CNS- Central nervous system; Cp - Ceruloplasmin; CPR - Cardiopulmonary resuscitation; Cu_{Fr} - Free copper ion; D-PA - D-Penicillamine; ET – Exchange transfusion; MD - Metal dyshomeostasis; MRI - Magnetic resonance imaging; MT - Metallothionein; NHBI - Neonatal hyperbilirubinemia; OH^- - Hydroxyl radical; NDs - Neurodegenerative and neurodevelopmental diseases; PD - Parkinson disease; ROS - Reactive oxygen species; UCB - Unconjugated bilirubin; UCB_{Fr} - free UCB; WD - Wilson disease.

INTRODUCTION

There is a tremendous variability in babies' vulnerability toward unconjugated bilirubin (UCB) for reasons not yet explained, but preterm birth, sepsis, hypoxia, hypoperfusion, hyperosmolality, acidosis, hypalbuminemia and *hemolytic disease et cet.* (underlying diseases or comorbidities) are comprised as risk factors, so, the UCB levels and neurological abnormalities are not strictly correlated. Kernicterus[1] may only be the "tip of the iceberg." Subtle UCB damage may account for many more cases of: learning disabilities, central auditory processing disorders, dyslexia, oculomotor dyspraxia, movement disorders and autism spectrum disease (ASD) and may even predispose to Parkinson's disease (PD) or schizophrenia in adulthood.[2] The pathomechanisms of BIND have not been fully understood yet. The mechanisms of UCB neurotoxicity are still also unclear and little is known about lasting sequelae attributable to neonatal hyperbilirubinemia (NHBI). Our hypothesis addresses the medical necessity of chelation therapy (with D-PA) in the neonatal period[3,4] as it is feasible that UCB molecule reviels particular affinity to copper stored in basal ganglia (BG) of the neonatal brain, where copper-bilirubin complex can be formed together with the production of hydroxyl radical (OH^-). In addition, various amount of free metal ions can be found in the intravascular space and in the tissues (especially in BG) during *hemolytic processes*.

D-PENICILLAMINE: PHARMACOLOGY AND CLINICAL USES

D-PENICILLAMINE (D-PA) was first isolated as the amine, from the degradation products of penicillin by Abraham et al. *in 1942.*[5,6] Cornforth perceived the similarity to the naturally occurring amino acid L-cysteine and postulated the formulation as β-β-dimethylcysteine.[7] Unlike its precursor penicillin, D-PA does not have antibiotic activity and so initially,

interesting the compound arose only out of its position in the processes of degradation and synthesis of penicillin. Nevertheless, it subsequently has found an extensive use in medicine: in 1956 by Walshe' in the treatment of Wilson disease. It has since been used or suggested for use in the treatment of cystinuria, Rheumatoid arthritis (RA), juvenile RA, palindromic rheumatism, scleroderma, primary biliary cirrhosis, alcohol detoxification, heavy metal removal, chronic active hepatitis, morphea, keloid, keratosis follicularis, and hyperviscosity syndrome. In addition, it has been used as a ligand in the preparation of radiopharmaceuticals for liver and kidney imaging.[8] Only D-PA is used in medicine, since the L isomer and the DL isomer (or racemate), are toxic.

The chemical behavior of D-PA **(Figure 1.)** which is of importance in medicine can be discussed in terms of three types of reaction: (1) formation of disulfide links, (2) formation of thiazolidine rings and (3) formation of metal complexes and chelates.[92] The third type of reaction is the binding to metals. Metal ions accumulate in the brain with aging and in several neurodegenerative diseases (NDs). Aside from the copper storage disease, Wilson's disease (WD), recent attention has focused on the accumulation of zinc, copper and iron in the Alzheimer's disease (AD) brain and the accumulation of iron in Parkinson's disease (PD).[10] In the neonatal period the chelating effects play also important role: (i) in attenuating the oxidative stress, (ii) in the transient inhibition of heme oxygenase (HO) resulting in a decrease of UCB production and (iii) the age-relating effects of D-PA.[11] Chelation therapy is generally only recommmended when high levels of metal are present in the blood, since it does not seem to benefit those with lower levels. Currently, four drugs are used for chelation therapy: edetate calcium disodium (calcium EDTA), dimercaprol (BAL), succimer and D-PA. Succimer and D-PA are given only in pill form.

Transition biometals now are in the focus of the etiopathogenesis of NDs, including the bilirubin-induced neurodevelopmental dysfunction (BIND) in the neonatal period. Indeed, while studying the molecular basis for this heterogeneous group of diseases, it has become increasingly evident that biometals are often involved in pathology onset and progression, either by affecting the conformation of specific proteins or by exacerbating local oxidative stress. The neuropharmacological actions of metal-targeted agents most likely arise from *local metal redistribution rather than from massive metal removal.* This goal may be achieved by abolishing abnormal metal/protein interactions, by contrasting localized metal excesses, by normalizing intra/extra cellular metal ratios or by restoring the correct balance among the main biometals: Cu, Fe and Zn.[12]

CASE REPORTS

There were some very impressive cases in our practise in neonatology deserved to show them individually.[13] **The first patient** received D-PA treatment in the neonatal period was an AB0-incompatible preterm infant with birth weight of 2000 g. At an extremely high UCB (32.5 mg/dL) intravenous administration of D-PA was begun. The first dose caused a spectacular fall of 6.5 mg/dL in the level in 4 hours and under the influence of such treatment we were able to witness a gradual disappearance of the NHBI. She is now a member of a famous operhouse in Germany as an opera singer **(Figure 2.).**[14] This case is all the more remarcable because this baby showed typical symptoms of acute bilirubin encephalopathy at 3-6 days of age: somnolence, hypotonia, and loss of the Moro reflex and sometimes opisthotonus. In addition, she was in need of CPR (cardiopulmonary resuscitation) just at the beginning of the exchange transfusion (ET) because of a cardiac arrest, so, the ET was not performed and it proved to be unsuccessful concerning the high UCB level. Surviving of the neonatal period, however, she did not demonstrate any of the chronic manifestations of bilirubin encephalopathy, including the most common sequelae of sensorineural hearing impairment.[15,16] The lack of chronic (residual) symptomes may be due to the neuroprotective effects of D-PA in the neonatal period.

In 1999 we published a case of an AB0 incompatible term infant girl born to parents who were **Jehovah's Witnesses.**[17] The infant was admitted to our neonatal unit with a high SEBI necessitating ET. The parents signed a request that blood should not be administered under any circumstances. However, they authorised us to use of alternative treatments: orally administered D-PA, phototherapy, intravenous fluids and recombinant human erythropoietin (200 U/kg bw. subcutaneously on every second day for two weeks). This infant was discharged from our unit in good health. Her physical growth and motor milestones at 3 years of age revealed no red flags for neurodevelopmental maturation. In addition, the follow up audiometric tests performed on this infant were normal. She was the first baby in the world who received such a combined alternative (and "bloodless") treatment for serious AB0-HDN.

We recently cared for a term infant boy blood group B, Rh-positive who was born at 37. gestation to a 33-year old, blood group B, Rh-negativ mother.[18] The baby was born as an 11. offspring of his mother and appeared jaundice at 10 hours of life and had moderate anaemia. The direct Coombs test was strongly positive (++++) in the cord blood. The clinical characteristics of the infant with Rh-HDN are shown in the **BOX 1.**

Our case reports and other healthy and highly educated patients of the long-term (28-42 years) follow-up suggest that D-PA therapy of newborn infants may have significant neuroprotective effects in cases jeopardized by BIND or ROP. In addition, it was our privilage to follow a number of children who are now adults, including sons and daughters of our relatives, colleaques, close friends. They are now highly educated persons

working in health care (mostly as physicians), bank, computer and building industry, et cet.

PATHOLOGICAL BASAL GANGLIA ACTIVITY[19]

The BG is a collection of large subcortical nuclear masses. It is agreed that core components comprise the caudate nucleus, the nucleus accumbens, the putamen and the globus pallidus. The caudate nucleus and putamen together are sometimes called the striatum and the putamen and globus pallidus are together sometimes described as the lentiform nucleus.[20,21] Functionally, the BG has considerable connections to the cerebral cortex, thalamus and brain stem; so, anatomists consider portions of the thalamus as components of the BG.[22] A literature review was aimed at assisting us (as pediatricians) to provide further understanding with bilateral symmetrical BG and thalamic lesions on magnetic resonance imaging (MRI). The high-signal-intesity lesions on T_2-weighted images can be caused by edema, gliosis, demyelination, neuronal necrosis, or cystic degeneration both in WD and BIND.[23]

ROLE OF METALS AND OXIDATIVE STRESS IN THE HUMAN NEURODEGENERATIVE AND NEURODEVELOPMENTAL DISORDERS

The brain (mostly BG) accumulates among the highest levels of transition metals in the body for normal function, including redox-active copper. This high-redox metal load, in combination with the brain disproportionately active oxygen metabolism, makes this organ particularly susceptible to oxidative stress.[24-27] Metal ions such as calcium, zinc, iron and copper are key players in brain neurobiology; their homeostasis is altered in most ND conditions. The **metal dyshomeostasis (MD)** in the brain and related organs and **loss of the strict regulation** is implicated in neurotoxic stress[28-30] and in a variety of NDs including BIND and prion-mediated encephalopathies and other diseases.[31-33] The pathologic changes are presumed to result from an increased amount of extracellular copper, which causes oxidative stress and results in cell destruction.[34] Many diseases of the BG have some *disorder of movement* as their primary symptom, ranging from an excess of (abnormal) involuntary movements such as in chorea to a poverty and slowness of movement as in PD, Alzheimer disease (AD) and WD as illustrated in several clinical cases[35] and **UCB encephalopathy**[36] where a characteristic yellow staining can be observed in fresh or frozen sections of the brain obtained within 7-10 days after the initial bilirubin insult. If the affected infant survives the neonatal period and subsequently dies, the yellow staining may no longer be present, but the BG will display microscopic evidence of cell injury, neuronal loss and glial replacement. Newborns, especially preterm infants, are particularly vulnerable to reactive oxygen species (ROS) because they exhibit accelerated production of free radical and limited antioxidant protection, which increases the susceptibility of rapidly growing tissues to damage. „Free radical-related diseases" of neonates promote cellular, tissue and organ impairments. In 1988, Saugstad coined the phrase „oxygen radical disease in neonatology" to highlight the crucial role of ROS in a wide range of neonatal disorders.[37] There is now a large body of literature demonstrating that free or weakly bound iron and copper ions may exert their toxic action on BG. In a way, metals may provide the link between protein misfold and aggregation, oxidative stress and the cascade of biochemical alterations, eventually leading to neuronal cell death. Predominantly the cellular content of copper determines copper-induced toxicity in brain astrocytes.[38]

POTENTIAL MOLECULAR MECHANISMS OF BILIRUBIN-INDUCED NEUROLOGIC DYSFUNCTION

The "classic" interpretation of bilirubin neurotoxicity does not give sufficient answers to the following questions: (1) How to call bilirubin: friend or foe? (2) If the bilirubin is really an „enemy", how does it induce its dangerous effects?

Ad (1)

The exact UCB concentration associated with kernicterus in the healthy term infant is unpredictable. In a Danish popolation-based study, the neonates with total serum bilirubin levels of ≥ 25 mg/dL didn't show any neurologic dysfunctions at 5 years of follow up.[39] 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.[40] Bilirubin 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.[41-43] The UCB level in jaundiced and non-jaundiced pups exposed to 95% O_2 shows a negative correlation with lipid hydroperoxides at 3 days of exposure. Higher UCB concentrations resulted in lower lipid hydroperoxide levels.[44] Therefore, we think that UCB in itself is actually our friend, that is: Bilirubin, The Gold Within.[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).

Ad (2) Toxic Side of Bilirubin

Erythrocyte morphological changes have been seen with incubation of cells with different molar ratios of UCB. These changes occur as the bilirubin/human serum albumin molar ratio increases. This indicates that

bilirubin can illicit toxicity in the erythrocyte membrane in a concentration and temperature-dependent manner, causing *hemolysis*.[46] Several studies have found that NHBI is associated with higher risk of movement disorder and even more, developmental delay. The management dilemma for a clinician is that UCB is a beneficial antioxidant at low (and may be at moderatly higher) levels, but a neurotoxin at >20 mg/dL levels ("vigintophobia"[47]), where it can impair the normal developmental maturation of the neonatal brain.

Among the 23 elements with known physiological functions, 12 are metals (sodium, magnesium, potassium, calcium, vanadium, chromium, **manganese**, **iron**, cobalt, copper, zinc and molybdenum).[48, 49] Copper is essential for the normal growth and development of human fetuses, infants and children and it is crucial for the normal development of the brain.[50] which has among the highest levels of copper, as well as iron and zinc, in the body. *Copper* is an interesting essential micronutrient. *Deficiency and excess intake both induce a variety of clinical manifestations* affecting mainly the hematopoietic system, the skeleton, the liver and the brain. Although copper transport to the fetus is high and liver storage is efficient, copper export from the hepatocytes to the bile and to blood Cp are reduced during this stage of life because of liver function immaturity. This leads to a high copper accumulation in the liver and brain, in a magnitude *similar to that observed in WD*. In fact, an obvious analogy can be observed between the *newborns and patients with WD* in the field of the copper „(dys)homeostasis" (**Table 1**). The increased liver and brain copper storage of the fetus may have a selective evolutionary advantage since it may prevent copper deficiency during the first months of life when the child receives a relatively low copper supply from breast milk.[51]

METAL REGULATORY PROTEINS IN THE NEONATAL PERIOD

A variety of **proteins** are involved in the regulation of metal metabolism and the oxidative response and many are involved in iron or copper metabolism due to the redox activity of those metals. Protein misfolding and conformational changes are also a cornerstone of NDs. All metals with known physiological functions are bound by **albumin.**[52,53] A decrease in metal binding of albumin means *more free metal available to produce oxidative stress* and other physiological effects such as influence of calcium (Ca^{++}) homeostasis by altering the conformational structure of the pumps, enzymes, binding proteins, and channels that regulate Ca^{++} flow. Often, this results in elevating free intracellular Ca^{++} levels which may produce depletion of glutathione/GSH with a downstream induction of DNA damage and eventual cell death.[54] Therefore, the bilirubin-mediated neurotoxicity is partly due to increased rate of cell apoptosis and *higher levels of intracellular free Ca^{++} ion* level (as analogy, see Fahr's disease).[55]

Ceruloplasmin (Cp) is a large blood protein synthesized by the liver with the primary role of transporting copper. If a disease process (e.g. hepatic failure) or *insufficient synthesis in the neonatal period* lowers the production of Cp, the free copper would increase and copper mediated oxidative stress would be enhanced. In addition, there is some evidence that under oxidative stress conditions, Cp may induce further oxidative stress in a manner akin to a positive feedback mechanism. Also, when this protein is exposed to ROS, its ability to bind copper is reduced, releasing free copper, producing further oxidative stress.[56]

Copper transporter 1 (Ctr1) has a high affinity for copper and serves to transport copper into the interior of the cell. It is not highly expressed in the brain, where the choroid plexus may contain the greater proportion. The lower levels of expression in the brain, however, should not be taken as a sign that Cu metabolism is not important in the brain as several neural pathologies (Alzheimer's disease, spongiform encephalopathies) have been linked to disordered copper metabolism.[57]

Metallothionein (MT) is a cysteine rich protein involved in the regulation of zinc and other metals (mainly copper, and selenium). This protein is found in a variety of forms (I-IV) in mammals and MT_{II} are the most abundant in the CNS where MT is found mostly in astrocytes. MT plays an important role in cell signaling. Neonatal brain has lower MT concentrations than adult brain, increasing to adult levels by Day 21.[58] Elucidating the role of the **metallochaperone Atox1,**[59,60] it is obvious that Atox1-deficient cells accumulates high levels of intracellular copper, and metabolic studies indicate: this defect originated from the impaired cellular copper efflux. These data reveal a direct role for Atox1 in trafficking of intracellular copper to the secretory pathway of mammalian cells and demonstrate that metallochaperone plays a critical role in perinatal copper homeostasis. To sum up, the number of proteins involved in metal oxidative stress is large and fall into two groups: those involved in iron or copper metabolism, and those involved with the rest of the metals. **Bilirubin-metal complexes** have been made *in vitro* producing OH^- and there are no reasons not to believe that such complexes can also exist *in vivo*, - especially since UCB can take on a ring-like configuration with one of the end pyrrol-rings in a lactim form and the other in a lactam form.[61, 62] To clarify how hyperbilirubinemia influences neurodevelopmental outcome, more sophisticated consideration is needed both of how to assess bilirubin exposure leading to neurotoxicity and of those comorbid conditions which may lower the threshold for brain injury.[63] A decrease in metal binding means of course more free metal available to produce oxidative stress and other physiological effects. Six substances are transported with albumin: long chain fatty acids, *bilirubin*, steroids, thyroid hormones, drugs and *copper* (also other metals eg. Zn, Pb.).[64] 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. The equilibrium constant of UCB is about $3.8 \pm 2.0 \times 10^7$ M^{-1} and the calculated Cu ion binding constant is 1.50×10^6 M^{-1}. In comparison, albumin is interacting selectively and non-covalently with Cu ions.[65-67] Neonatal blood has low content of glutathione peroxidase, superoxide dismutase, β-carotene, riboflavin, α-proteinase, vitamin E, selenium, copper, zinc, Cp and other plasma factors. The premature brain is rich in polyunsaturated fatty acids and easily oxidized compared to monounsaturated fatty acids.[68]

COPPER AND OTHER METAL TOXICITY SYNDROME[69]

Copper toxicity is a condition that is increasingly common in this day and age, due to the widespread occurrence of copper in our food, copper fungicides, e-cigs, copper IUD's, hot water pipes, along with the common nutritional deficiencies in zinc, manganese and other trace minerals that help keep levels of copper in balance. Copper is a very stimulating mineral to the nerves and CNS. Its effects on neurotransmitter levels can give rise to many psychological imbalances such as mood swings, depression, mental agitation, feeling over-stimulated, 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 **(BOX 2.).**[70] Studies have also found heavy metals to deplete glutathione and bind to protein-bound sulfhydryl groups, resulting in inhibiting SH-containing enzymes and production of reactive oxygen species such as superoxide ion, hydrogen peroxide, and hydroxyl radical. In addition, toxic metals exert part of their toxic effects by replacing essential metals such as zinc at their sites in enzymes.

Here, it is noteworthy, how prominent similarities exist between WD and neonates with kernicterus concerning *the copper metabolism, the neuropsychiatric manifestations and the hystopathological findings* **(BOX 3**.). 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 caeruloplasmin has fully developed.[72-77]

NEW CONCEPT FOR DEVELOPMENT OF BILIRUBIN-INDUCED NEUROLOGICAL DYSFUNCTION

Very wide-ranging studies have long been made on the possible biochemical transformations of UCB, which is formed during the decomposition of haemoglobin. Particular attention has been paid to its photochemical and redox reactions[77] 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.[78]

Neurodegeneration: a return to immaturity?[79] 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 an *ND 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 rate of formation of UCB-Cu complex when bilirubin extracts copper from copper–albumin complex, as obtained in a very exciting experiment, is $34.98\ l\ mol^{-1}\ s^{-1}$.[80] The main comorbidity is the ***hemolysis*** of neonatal blood red cells. During this process a great amount of heavy metals (mainly iron and copper) may circulate in free form in the bloodstream, and can pass through the BBB, finding entrance into the CNS as well. 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.[81-83] 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,[84] 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. Bilirubin itself can displace metals (copper) from the albumin binding because UCB binds stronger to albumin than copper, in other words, **copper loosely bound to albumin.** 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.[85,86] 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.

8. 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 3**. demonstrates our concept about the chronic bilirubin encephalopathy based on the above described hypothesis.

We hope that our theory will help answer some of the unsolved questions and concerns ocurred 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.[87] The chelation therapy for non-metal overload indications continues to be investigated. Our present article address the medical necessity of the use of a chelating agent (D-PA) in the treatment of NHBI and ROP.

Conflict of interest: The authors declare no conflict of interest.

Figures and Boxes

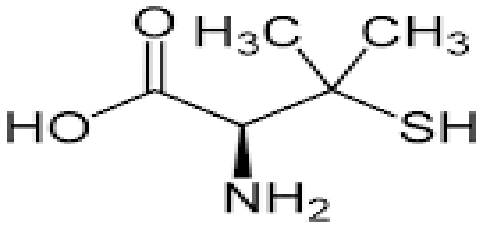

Fig. 1. Structural formula of D-Penicillamine

Fig 2. The first patient with her daughter of eighteen (with permission).

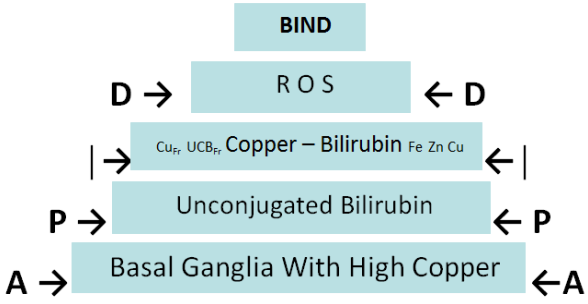

Fig. 3. Potential pathomechanisms of metal mediated BIND, and effects of D-PA in the NHBI. Interpretation of D-PA written sideways: D = direct antioxidant; | = scavenge of ROS and chelation of metals; P = inhibition of HO (a decreased production of UCB) only in neonates (age-related effect); A = chelation of metals.

BOX 1. Treatment of an infant with Rhesus-HDN without ET

Term infant boy was born as an 11. offspring of his mother at 37. gestation with 3100 g bw. Cord blood: direct Coombs test strongly positive, bilirubin level: 4.2 mg/dL	
Serum bilirubin	**Hemoglobin**
at 12 hs: 12.2	**119 g/L**
at 58 hs: 19.4	**108**
at 9 days: 2,8	**67 (50 ml PRBC)**
Th.: photherapy + DPA for 5 days	

BOX 2. Many children are being born with toxic levels of heavy metals

BOX 3. Common neuropsychiatric manifestations and pathologicalfindings in WD and bilirubin encephalopathy

BOX 3

- **HIGH COPPER IN THE BASAL GANGLIA**
- **NEUROPSYCHIATRIC MANIFESTATIONS**
  WD: Movement disorders, tremors, involuntary movements, choreoathetosis, dysarthria, dystonia, personality changes, uncontrolled emotional outbursts.[73, 74]
- **Kernicterus: generalized dystonia, athetoid cerebral palsy, paralysis of upward gaze, sensori-neural 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).[75]**
- **At AUTOPSY (both in WD and kernicterus):marked neuronal loss with demyelination and astrocytic replacement.[76, 77]**

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