European Journal of Biology and Medical Science Research

Vol.4, No.2, pp.11-30, May 2016

Published by European Centre for Research Training and Development UK (www.eajournals.org)

METAL DYSHOMEOSTASIS IN THE ETIOLOGY OF BILIRUBIN-INDUCED NEUROLOGIC DYSFUNCTION (D-PENICILLAMINE AS AN ANTIRETROVIRAL DRUG IN HIV OR EBOLA INFECTION DUE TO VERTICAL TRANSMISSION)

György Balla[1], Lajos Lakatos[2*], Zsuzsanna Vekerdy-Nagy[3]

[1]Department of Pediatrics, Clinical Centre, University of Debrecen, Debrecen, Hungary.
[2]Kenézy Teaching Hospital, Debrecen, Hungary.
[3]Department of Physical and Rehabilitation Medicine, Clinical Centre, University of Debrecen Debrecen, Hungary.

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) in the treatment of HIV or EBOLA infection due to vertical transmission. METHOD We conducted a review searching the literature of bilirubin metabolism and of metal-homeostasis, furthermore of neonatal HIV and EBOLA infection. RESULTS Over the past two decades there have been significant advances in our understanding of copper homeostasis and of neurodegenerative and neurodevelopmental diseases (NDs), and the pathological consequences of copper dysregulation. Thus, comprehension of metal homeostasis, details of transport and interactions with biomolecules, such as unconjugated bilirubin (UCB) or albumin, is essential for understanding the normal and pathological processes occurring in the neonatal period. UCB has a special affinity for the globus pallidus, the hippocampus, and the subthalamic nucleus. Furthermore, immaturity of the blood-brain barrier (BBB) also plays a role in 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 NDs including BIND, having impact on both protein structure (misfolding) and oxidative stress. INTERPRETATION 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 central nervous system (CNS) damage processes in BIND by the production of free radicals. Our present research article address the medical necessity of the use of a chelating agent (D-PA) in the treatment of neonatal hyperbilirubinemia (NHBI). Finally, the authors highlight that D-PA may have a huge impact on HIV or EBOLA infection caused by vertical transmission where NHBI is a very common symptom.*

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

INTRODUCTION

The classic form of chronic bilirubin encephalopathy (kernicterus) is a well-described clinical tetrad of ➤ abnormal movements and muscle tone, ➤ an auditory processing disturbance with or without hearing loss, ➤ oculomotor impairments, especially impairment of upward vertical gaze, and ➤ dysplasia of the enamel of deciduous teeth. Hervieux first described the condition in 1847, and Schmorl first used the term kernicterus as early as 1903. [1, 2] NHBI is a common condition in the first week of postnatal life. Although generally harmless, some neonates may

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

develop very high levels of UCB. Subtle encephalopathy or BIND refers to individuals with subtle NDs. [3-5] In the past decades, interest in bilirubin damage of the brain has been reawakened by an increase in its prevalence, owing to failure to closely observe infants discharged from the hospital well before the peak of NHBI. [6] There is a tremendous variability in babies' vulnerability toward 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 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. [7] 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 NHBI. Our hypothesis addresses the medical necessity of chelation therapy (with D-Penicillamine – D-PA) in the neonatal period [8, 9] 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*.

Pathological Basal Ganglia Activity[10]

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. [11, 12] 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. [13] 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, demyelinization, neuronal necrosis, or cystic degeneration both in Wilson disease (WD) and BIND. [14]

Role of metals and oxidative stress in the human neurodegenerative and neurodevelopmental disorders

The brain (mostly the 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. [15-18] 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 [19-21] and in a variety of NDs including BIND and prion-mediated encephalopathies and other diseases [22-24] which can be seen in the **Table 1.** [25-47] Parts emphasized with **bold-faces** show associations between the ND illnesses and BIND. Pathologic changes to the CNS in these disorders are always associated with a significant dyshomeostasis of tissue metals (particularly *copper*). Excess copper may combine with sulfhydryl, carboxyl, or amine groups, resulting in improper enzymatic activity or damage to cellular structure. Despite the ubiquitous presence of toxic copper within the brain,

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

pathologic findings are limited primarily to the BG, thalamus, and brain stem. Histopathologic studies have shown abnormalities throughout this system in patients suffering from MD. These abnormalities include atrophy, spongy softening, cavitation, a general reduction of neurons, increased cellularity, and the presence of characteristic cells (Opalski, Lewy bodies). The pathologic changes are presumed to result from an increased amount of extracellular copper, which causes oxidative stress and results in cell destruction. [48] 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 [49] and **UCB encephalopathy** [50] 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. [51] 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. [52]

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. [53] 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 [54]. 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. [55, 56] Serum bilirubin 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. [57] Therefore, we think that UCB in itself is actually our friend, that is: Bilirubin, The Gold Within.[58] (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

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

between bilirubin and colorectal cancer. – 2005 Powerpoint Presentation www.sfrbm.org/frs/Friel**Bilirubin**.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.* [59] 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" [60]), 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). [61, 62] 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 [63] 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 ceruloplasmin (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 Wilson disease.* In fact, an obvious analogy can be observed between the *newborns and patients with Wilson disease* in the field of the copper „(dys)homeostasis" (see: **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.[64]

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.** [65, 66] 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. [67] 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 **Table 1.**: Fahr's disease). [42]

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

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

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. [68]

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. [69]

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. [70] Elucidating the role of the **metallochaperone Atox1,** [71, 72] 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 hydroxyl radical ($OH^{\cdot}$) 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. [73, 74] 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 braininjury.[46] 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…).[75] 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 $1.50 \times 10^6\ M^{-1}$. In comparison, albumin is interacting selectively and non-covalently with Cu ions. [76-79] Neonatal blood has low content of glutathione peroxidase, superoxide dismutase, β-carotene, riboflavin, α-proteinase, vitamin E, selenium, copper, zinc, ceruloplasmin and other plasma factors. The premature brain is rich in polyunsaturated fatty acids, and easily oxidized compared to monounsaturated fatty acids. [80]

New concept for development of bilirubin-induced neurologic dysfunction

Table 1. illustrates well that a remarkable similarity exists between Wilson disease and BIND. 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 [81] but the relevant publications comprise only a very small proportion of those dealing with the molecular biochemistry of *UCB and*

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

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.[82]

Neurodegeneration: a return to immaturity? [83] 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} **[84].** The main comorbidity is the ***hemolysis*** of neonatal blood red cells. [85] 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.[86] 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, [87] 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.** [88] 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.[89] 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.

D-penicillamine as neonatal neuroprotectant [90, 91]

Our recently published case reports [92] 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. The first patient (42 ys) is now a member of a famous operahouse in Germany as an opera singer, the second one (16 ys) is excellent in music and matematics. These cases are all the more remarkable as the most common sequelae of NHBI is the sensorineural hearing impairment. These unexpected effects may be related to DPA capability to alter the most important gasotransmitters **(**nitric oxide /NO/ system, carbon monoxide /CO/, hydrogen sulfide /H2S/ biosynthesis, and Cu^{++} homeostasis in the brain**)**. According to our hypothesis DPA can modulate the function of these neurotransmitters and can protect the brain (especially the BG and retina) from injury, such as BIND and ROP. D-PA not only chelates copper from tissue, but also detoxifies tissue copper by promoting the synthesis of metallothionein, which forms a non-toxic combination

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

with copper. [36] There are very important **age-related effects of D-PA in the neonatal period.** In the **Table 2**. we demonstrate the results of our animal experiments regarding the age related differences in effects of D-PA. [93] The high activity of heme oxygenase in the newborn could reflect the enzyme-inducing action of metals: Cu and Fe derived from the breakdown of fetal erythrocytes. Chelation therapy in neonates restores the normal activity of enzymes participating in heme metabolism. Briefly, chelating agents facilitate heme synthesis and inhibit heme degradation. In other words, DPA as a chelating agent, boost or inhibit the immature enzyme systems to the adult levels The main comorbidity is the ***hemolysis*** of neonatal blood red cells. [85] 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.[94]

CONCLUSION

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. 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. [95] The chelation therapy for non-metal overload indications continues to be investigated. Our present research article address the medical necessity of the use of a chelating agent (D-PA) in the treatment of NHBI. [96-106]

Possible beneficial effects of D-PA on the lethality of HIV or EBOLA infection due to vertical transmission

West Africa is currently in the midst of the largest EBOLA outbreak in the history and HIV prevalence in sub saharan Africa is also very high.

The structural and functional properties of DPA make it suitable for exerting antiviral activity. This drug caused a marked inhibition of polyo-virus-specific RNA and the protein synthesis.[108] Searching the pertinent literature, several publications relating to the beneficial effects of D-PA-therapy in the treatment of AIDS-patients were found. The high doses resulted in good outcomes, but adult patients did not tolerate this therapy. In addition to this, it has been determined that the selective inhibition of replication of HIV type 1 (further: HIV) by this drug was concentration dependent, that is, at 40 microgram/ml concentration D-PA completely inhibited HIV replication in H9 cells in vitro [109] ***(a single 100 mg/kg bw. IVadministered DPA resulted in more multiple plasma concentration in premature infants [110])***. This study has a promising idea wondering whether or not it is true that DPA has possible beneficial effects on the AIDS or EBOLA associated infant mortality rates because of its prolonged antiviral activity. Abundant experimental evidence and clinical observations exist to suggest that early viremia and immune responses in vertical HIV infection are different from those of adults. The developing immune system might allow for more efficient viral replication and less efficient immune containment of viral replication. In this respect, D-PA-therapy may be a potent early regime to control HIV (or EBOLA) replication and offers the

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

golden opportunity to prevent or reverse the rapid progression of these diseases. The potential mechanism of antiretroviral actions of D-PA in infections caused by vertical transmission are as follows:

- It is presumed that antioxidant treatment (D-PA is a well-known direct antioxidant) may provide a promising and cost-effective therapeutic aproach in treating neonatal HIV or
- EBOLA infection. The newborn infants, especially the prematures, are suffering in an oxidative stress condition [111]
- It acts as a potent protease inhibitor in animal model [112.]
- The copper metabolism in Wilson's disease and in newborn infants is strikingly similar: they both have large quantities of copper in the liver and low ceruloplasmine in the blood. It was previously found that cupric chloride, in the presence of a chelating agent, could inhibit the HIV-1 protease [113].
- Extra cystein given in the form of DPA (β-β-dimethylcysteine) can cause an increase in intracellular cysteine and glutation content which play an important role as HIV inhibitors, at least in part because they facilitate the intracellular transport of Zn and Cu ions [114].
- The HIV-1 nucleocapsid p7 protein contains two retrovirus-type zinc finger domains that are required for multiple phases of viral replication. Considering the chelating properties of DPA and its disulfide reaction with cysteine, one can conclude that HIVor
- EBOLA- replication could be inhibited by this drug [115-120].

It would be very exciting to be involved in this work, especially that D-PA will have a huge impact on HIV or EBOLA infection caused by vertical transmission ***where NHBI is a very common symptom.***

ABBREVIATIONS

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; Cu_{Fr} - Free copper ion; D-PA - D-Penicillamine; 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

Conflict of Interest Statement The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Hansen TWR: Pioneers in the scientific study of neonatal jaundice and kernicterus. *Pediatrics* 2000; **106:** e15 http://www.pediatrics.org/cgi/content/full/106/2/e15;

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

2. Shapiro SM. Chronic bilirubin encephalopathy: diagnosis and outcome. *Semin Fetal Neonatal Med* 2010; **15:** 157–163.
3. Stevenson DK, Vreman HJ, Wong RJ. Bilirubin production and the risk of bilirubin neurotoxicity. *Semin Perinatol* 2011; **35:** 121-126.
4. Brites D. The evolving landscape of neurotoxicity by unconjugated bilirubin: role of glial cells and inflammation. *Front Pharmacol* 2012; **3:** 136.doi:10.3389/fphar.2012
5. Lunsing RJ. Subtle bilirubin-induced neurodevelopmental dysfunction (BIND) in the term and late preterm infant: Does it exist? *Semin Perinatol* 2014 **38:** 465–471.
6. Shapiro SM. Kernicterus; Neonatal Hyperbilirubinemia, Neurotoxicity and Bilirubin-Induced Neurological Disorders (BIND). Clinical Advences in Pediatrics November 16, 2012 Children's Mercy HOSPITALS & CLINICS. Kansas City
7. Maisels MJ, Newman TB. Jaundice in full-term and near-term babies who leave the hospital within 36 hours. The pediatrician' nemesis. *Clin Perinatol* 1998; **25**: 295–302.
8. Lakatos L, Kövér B, Oroszlán G, et al. D-Penicillamine Therapy in AB0 Hemolytic Disease of the Newborn Infant. *Europ J Pediat* 1976; **123:** 133-137.
9. Balla G, Lakatos L, Vekerdy-Nagy Z. Chelation therapy in the neonatal period: D-Penicillamine can exert neuroprotective effects in kernicterus and retinopathy of prematurity. *IJPSR* 2015; **6:** 4269-4276.
10. Wichmann T, Dostrovsky JO. Pathological basal ganglia activity in movement disorders. *Neuroscience* 2011; **198:** 232–244.
11. Russmann H, Vingerhoets F, Ghika J, et al. Acute Infarction Limited to the Lenticular Nucleus, Clinical, Etiologic, and Topographic Features. *Arch Neurol* 2003; **60:** 351-355.
12. Abbruzzese G, Berardelli A. Sensorimotor integration in movement disorders. *Mov Disord* 2003; **18:** 231-240.
13. Zuccoli G, Yannes MP, Nardone R et al. Bilateral symmetrical basal ganglia and thalamic lesions in children: an update. *Neuroradiology* 2015; DOI 10.1007/s00234-015-1568-7.
14. Bekiesinska-Figatowska M, Mierzewska H, Jurkiewicz E: Basal ganglia lesions in children and adults. Europ J Radiol 2013; **82:** 837–849.
15. Dodani SC, Firl A, Chan J, et al. Copper is an endogenous modulator of neural circuit spontaneous activity. *PNAS* 2014; **111:** 16280–16285.
16. Dickinson BC, Chang CJ. Chemistry and biology of reactive oxygen species in signaling or stress responses. *Nat Chem Biol* 2011; ***7:*** 504–511.
17. Barnham KJ, Masters CL, Bush AI.Neurodegenerative diseases and oxidative stress. *Nat Rev Drug Discov* 2004; 3: 205–214.
18. Savelieff MG, Lee S, Liu Y et al. Untangling amyloid-β, tau, and metals in Alzheimer's disease. *ACS Chem Biol* 2013; **8:** 856–865.
19. Schlief ML, Craigand AM, Gitlin JD. NMDA receptor activation mediates copper homeostasis in hippocampal neurons. *J Neurosci* 2005; **25:** 239–246.
20. Schlief ML, Wes T, Craig AM, et al. Role of the Menkes copper-transporting ATPase in NMDA receptor-mediated neuronal toxicity. *PNAS* 2006; **10:** 14919–14924.
21. Sparks DL, Schreurs BG. Trace amounts of copper in water induce beta-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. *PNAS* 2003; **100:** 11065–11069.
22. Singh I, Sagare AP, Coma M, et al. Low levels of copper disrupt brain amyloid-β homeostasis by altering its production and clearance. PNAS 2013; **110:** 14771–14776.
23. You H, Tsutsui S, Hameed S, et al. A beta neurotoxicity depends on interaction between copper ions, prion protein, and N-methyl-d-asparate receptors. *PNAS* 2012; **109:** 1737–1742.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

24. Barnham KJ, Bush AI. Biological metals and metal-targeting compounds in major neurodegenerative diseases**.** *Chem Soc Rev* 2014; **43**: 6727-6749.
25. Gonzalez-Cuyar LF, Perry G, Miyajima H, et al. Redox active iron accumulation in aceruloplasminemia. *Neuropathology* 2008; **28:** 466–471.
26. López OL, DeKosky ST. Clinical symptoms in Alzheimer's disease. *Handbook of Clinical Neurology* 2008; **89:** 207–216.
27. Henneman WJ, Sluimer JD, Barnes J, et al. Hippocampal atrophy rates in Alzheimer disease: added value over whole brain volume measures.*Neurology* 2009; **72:** 999-1007.
28. Squitti R, Polimanti R. Copper phenotype in Alzheimer's disease: dissecting the pathway. *Am J Neurodegener Dis* 2013; **21:** 46-56.
29. Kiernan MC, Vucic S, Cheah BC, et al. Amyotrophic lateral sclerosis. *The Lancet* 2011; **377:** 942–955.
30. Kaler SG, Holmes CS, Goldstein DS, et al. Neonatal Diagnosis and Treatment of Menkes Disease. *N Engl J Med* 2008; **358:** 605-614.
31. Liddell JR. Targeting mitochondrial metal dyshomeostasis for the treatment of neurodegeneration. *Neurodegener Dis Manag* 2015; **5:** 345-364.
32. David RB, Bentley DD, Bayliss SJ, et al: Cutis laxa. A review. *J Amer Acad Dermatol* 2012; **66:** 842.e1–842. e17.
33. Dexter DT, Jenner P, Schapira AHV, et al. Role of Iron and Oxidative Stress in the Pathogenesis of Parkinson's Disease.Alterations in levels of iron, ferritin, and other trace metals in neurodegenerative diseases affecting the basal ganglia. *Ann Neurol (Supplement)* 1992; **32:** S94-S100.
34. Salazar J, Mena N, Núñez MT. Iron dyshomeostasis in Parkinson's disease. *J Neural Transm Suppl* 2006; **71:** 205-213.
35. Meijer FJ, Goraj B. Brain MRI in Parkinson's disease. *Front Biosci (Elite Ed)* 2014; **6:** 360-369.
36. Das SK, Ray K. Wilson's Disease: An Update. *Nat Clin Pract Neurol* 2006; *2:* 482-493.
37. Rosencrantz R, Schilsky M. Wilson Disease. Pathogenesis and Clinical Considerations in Diagnosis and Treatment. *Semin Liver Dis* 2011; 31: 245-259.
38. Becker G, Berg D, Francis M, et al. Evidence for disturbances of copper metabolism in dystonia. From the image towards a new concept. *Neurology* 2001; **57:** 2290-2294.
39. Draganski B, Martino D, Cavanna AE, et al. Multispectral brain morphometry in Tourette syndrome persisting into adulthood. *Brain* 2010; **133:** 3661–3675.
40. Hume-Pratuch J. American Psychiatric Association. Cautionary statement for forensic use of DSM-5. In *Diagnostic and statistical manual of mental disorders (5th ed.).* 2013 doi:10.1176/appi.book.9780890425596.744053.
41. Erbetta A, Bulgheroni S, Contarino VE, et al. Low-Functioning Autism and Nonsyndromic Intellectual Disability: Magnetic Resonance Imaging (MRI) Findings. *J Child Neurol* 2015**; 30:** 1658-1663.
42. Hozumi I, Kohmura A, Kimura A, et al. High Levels of Copper, Zinc, Iron and Magnesium, but not Calcium, in the Cerebrospinal Fluid of Patients with Fahr's Disease. *Case Reports in Neurology* 2010; **2:** 46-51.
43. Szumowski J, Bas E, Gaarder K, et al. Measurement of brain iron distribution in Hallevorden-Spatz syndrome. *J Magn Reson Imaging* 2010; **31:** 482-489.
44. Ahdab-Barmada M, Moossy J. The Neuropathology of Kernicterus in the Premature Neonate: Diagnostic Problems. *J Neuropathol Exper Neurol* 1984; **43:** 45-56.
45. Watchko JF, Tiribelli C. Bilirubin-Induced Neurologic Damage: Mechanisms and Management Approaches. *N Engl J Med* 2013; **369:** 2021-2030.
46. Wusthoff CJ, Loe IM. Impact of bilirubin-induced neurologic dysfunction

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

on neurodevelopmental outcomes. *Semin Fetal Neonatal Med* 2015; **20:** 52-57.
47. Shapiro SM. Bilirubin Toxicity in the Developing Nervous System. *Pediatr Neurol* 2003; **29:** 410-421.
48. Harperand C, Butterworth R. Nutritional deficiencies and metabolic disorders. In: Greenfield JG, Hume Adams J, Duchen LW, eds. *Greenfield's Neuropathology.* 5th ed. London, UK: Edward Arnold; 1992; 838–844.
49. Rivlin-Etzion M, Marmor O, Saban G, et al. Low-pass filter properties of basal ganglia cortical muscle loops in the normal and MPTP primate model of parkinsonism. *J Neurosci* 2008; **28:** 633–649.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

50. Marseglia L, D'Angelo G, Manti GS, et al: Oxidative Stress-Mediated Aging during the Fetal and Perinatal Periods. *Oxid Med and Cell Long* 2014; Article ID 358375, 8 pages, doi:10.1155/2014/358375.
51. Saugstad OD. Hypoxanthine as an indicator of hypoxia: its role in health and disease through free radical production. *Pediatric Research* 1988; **23:** 143–150.
52. Bulcke F, Santofimia-Castaño P, Gonzalez-Mateoset A, et al. Modulation of copper accumulation and copper-induced toxicity by antioxidants and copper chelators in cultured primary brain astrocytes. *J Trace Elem Med Biol* 2015; **32:** 168-176.
53. Vandborg PK, Hansen BM, Greisen G, et al: Follow-up of neonates with total serum bilirubin levels ≥ 25 mg/dL: a Danish population-based study. *Pediatrics* 2012; **130:** 61-66.
54. Stocker R, Yamamoto Y, McDonagh AF, et al. Bilirubin is a an antioxidant of possible physiological importance. *Science* 1987; **235:** 1043-1046.
55. Boon A-C, Bulmer AC, Coombes JS, et al: Circulating bilirubin and defense against kidney disease and cardiovascular mortality: mechanisms contributing to protection in clinical investigations. *Am J Physiol Renal Physiol* 2014; **307:** F123–F136.
56. Friel JK, Friesen RW: Virtual Free Radical School. Bilirubin: Friend or Foe? Bilirubin 1/2003 – Updated 6/2006, *Soc Free Rad Biol* Med PPT Presentation **http://www.sfrbm.org/frs/FrielBilirubin.pdf**
57. Dennery PA, McDonagh AF, Spitz DR, et al. Hyperbilirubinemia results in reduced oxidative injury in neonatal Gunn rats exposed to hyperoxia. *Free Rad Biol Med* 1995; **19:** 395-404.
58. Seppen J, Bosma P: Bilirubin: The Gold Within. Circulation 2012; **126:** 2547-49.
59. Bhutani V, Wong R: Bilirubin-induced neurologic dysfunction. *Sem Fetal Neonat Med* 2015; **20:** 1-64.
60. Watchko JF, Oski FA: Bilirubin 20 mg/dL = Vigintiphobia. *Pediatrics* 1983; **71:** 660-663.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

61. Asad SF, Singh S, Ahmad A, et al. Prooxidant and antioxidant activities of bilirubin and its metabolic precursor biliverdin: a structure–activity study. *Chemico-Biolog Interact* 2001; **137:** 59–74.
62. Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. *Mol Aspects Med* 2005; **26:** 235–244.
63. de Romaña DL, Olivares M, Uauy R, et al. Risks and benefits of copper in light of new insights of copper homeostasis. *J Trace Elem Med Biol* 2011; **25:** 3-13.
64. Manuel O, Araya M, Uauy R: Copper Homeostasis in Infant Nutrition: Deficit and Excess. *J Pediatr Gastroenter Nutr* 2000; **31:** 102-111.
65. Leal SS, Botelho HM, Gomes CM. Metal ions as modulators of protein conformation and misfolding in neurodegeneration. *Coord Chem Rev* 2012; **256:** 2253–2270.
66. Oettl K, Stauber RE: Physiological and pathological changes in the redox state of human serum albumin critically influence its binding properties. *Br J Pharmacol* 2007; **151:** 580–590.
67. Simms BA, Zamponi GW. Trafficking and stability of voltage-gated calcium channels. *Cell Mol Life Sciences* 2012; **69:** 843–856.
68. Paradis M, Gagn´e J, Mateescu MA, et al. The effects of nitric oxide-oxidase and putative glutathione peroxidase activities of ceruloplasmin on the viability of cardiomyocytes exposed to hydrogen peroxide. *Free Rad Biol Med* 2010; **49:** 2019–2027.
69. Nose Y, Kim BE, Thiele DJ. Ctr1 drives intestinal copper absorption and is essential for growth, iron metabolism, and neonatal cardiac function. *Cell Metabolism* 2006; **4:** 235–244.
70. Waalkes MP, Klaassen CD: Postnatal ontogeny of metallothionein in various organs of the rat. *Tox Appl Pharm* 1984; **74:** 314–320.
71. Hamza I, A. Faisst, J. Prohaska, et al. The metallochaperone Atox1 plays a critical role in perinatal copper homeostasis. *Proc Natl Acad Sci U S A.* 2001; **98:** 6848–6852.
72. Lenartowicz M, Kennedy C, Hayes H, et al: Transcriptional regulation of copper metabolism genes in the liver of fetal and neonatal control and iron-deficient rats. *Bio Metals* 2015; **28:** 51-59.
73. Leal SS, Botelho HM, Gomes CM. Metal ions as modulators of protein conformation and misfolding in neurodegeneration. *Coord Chem Rev* 2012; **256:** 2253–2270.
74. Bulcke F, Santofimia-Castaño P, Gonzalez-Mateos A, et al. Modulation of copper accumulation and copper-induced toxicity by antioxidants and copper chelators in cultured primary brain astrocytes. *J Trace Elem Med Biol* 2015; **32:** 168–176.
75. Amin SB, Lamola AA. Newborn Jaundice Technologies: Unbound Bilirubin and Bilirubin Binding Capacity In Neonates. *Semin Perinatol* 2011; **35:** 134–140.
76. All about Albumin. 3 – Ligand Binding by Albumin. *Biochemistry, Genetics, and Medical* Applications ISBN: 978-0-12-552110-9 1995; 76–132.
77. Ranjini AS, Das P, Balaram P. Binding Constant Measurement by Hyper-Rayleigh Scattering: Bilirubin−Human Serum Albumin Binding as a Case Study**.** *J Phys Chem B* 2005; **109:** 5950-5953.
78. Moriya M, Ho Y-H, Grana A, et al: Copper is taken up efficiently from albumin and alpha-macroglobulin by cultured human cells by more than one mechanism. *Am J Physiol Cell Physiol* 2008, **295:** C708–C721.
79. Bata M, Kar B. Kinetic mechanism of copper(II) transfer between the native sequence peptide representing the copper(II)-transport site of human serum albumin and L-histidine. *Can J Chem* 1985; **63:** 3111-3116.
80. Hammerman C, Goldstein R, Kaplan M, et al. Bilirubin in the premature: Toxic waste or natural defense? *Clinic Chem* 1998; **44:** 2551-2553

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

81. Ryter SW, Tyrrell RM. The Heme synthesis and degradation pathways: role in oxidant sensitivity - Heme oxygenase has both pro- and antioxidant properties. *Free Rad Biol Med* 2000; **28:** 289-309
82. Zheng W, Monnot AD. Regulation of brain iron and copper homeostasis by brain barrier systems: Implication in neurodegenerative diseases. *Pharmacol Therap* 2011; **133:** 177-188.
83. Kole AJ, Annis RP, Deshmukh M. Mature neurons: equipped for survival. *Cell Death and Disease* 4: e689; doi:10.1038/cddis.2013.220. 2013
84. Adhikari S, Joshi R, Gopinathan C. Bilirubin as an anti precipitant against copper mediated denaturation of bovine serum albumin: formation of copper–bilirubin complex. *Biochim Biophys Acta* 1998; **1380:** 109–114.
85. Bracci R, Perrone S, Buonocore G. Oxidant injury in neonatal erythrocytes during the perinatal period. *Acta paediatrica (Oslo, Norway) Suppl.* 1992; 91, **438:** 130-134.
86. Park HJ, Kim K, Kook S-Y et al: Three dimensional refractive index tomograms and deformity of individual human red blood cells from cord blood of newborn infants and maternal blood. *J Biomed Opt* 20: 111208 doi:10.1117/1. JBO.20.11.111208. 2015
87. Shuiqiang M, Mingzhen C, Deyan Z, et al. Determination of zinc and copper contents of erythrocytes in maternal and cord blood. *J Guangdong Med Coll* 1993; **3:** 117-123.
88. Jianjun KZ, Wanzh X, Yanbo W, et al. A kinetic model and estimation for the process of binding copper to human serum albumin by a voltammetric method. *Anal Bioanal Chem* 2005; **381:** 1552-1557.
89. Steiner LA, Gallagher PG: Erythrocyte Disorders in the Perinatal Period in Adverse Pregnancy Outcome and the Fetus/Neonate. *Semin Perinatol* 2007; **31:** 254–261.
90. Lakatos L, Balla G: Letter to the Editor, Comment on "New Antioxidant Drugs for Neonatal Brain Injury". *Oxid Med Cell Longevity* 2015; Article ID 384372, 2 pages http://dx.doi.org/10.1155/2015/384372.
91. Balla G, Lakatos L. D-penicillamine as a neonatal neuroprotectant: clinical and neurodevelopmental studies. *Internat J Curr Res* 2015; **7:** 21282-21286.
92. Lakatos L, Balla G, Pataki I, et al. D-Penicillamine in the Neonatal Period: Case Reports. *IJMPCR* 4: 59-63, 2015
93. Lakatos L, Oroszlán Gy, Dézsi Z, et al. Age-Related Difference in Radioprotective Effect of D-Penicillamine. *Dev Pharmacol Ther* 1982; **5:** 120-126.
94. Maines MD, Kappas A: Metals as regulators of heme metabolism. *Science* 1977; **198:** 1215-1221.
95. Mot AI, Wedd AG, Sinclair L, et al. Metal attenuating therapies in neurodegenerative disease. *Exp Rev of Neurotherap* 2011; **11:** 1717-1745.
96. Lakatos L. D-Penicillamine and Retinopathy of Prematurity. *Pediatrics* 1988; **82:** 951-952.
97. Lakatos L, Kövér B: Az újszülöttkori hyperbilirubinaemiák D-Penicillamin therapiája. **D-Penicillamine Therapy In Neonatal Hyperbilirubinaemias. A Preliminary Report.** *Orv Hetil (Hungarian J Med)* 1974; **115:** 307-311.
98. Dolinay T, Szombathy G: D-Penicillamin felhasználása az újszülöttkori hyperbilirubinaemiák terápiájában. *Orv Hetil* 1974; **115:** 1431-1432.
99. Korányi G, Kovács J, Vörös I. D-Penicillamine treatment of hyperbilirubinemias of preterm infants. *Acta Paediatr Acad Sci Hung* 1978; **19:** 9-14.
100. Szűts A, Lechner E. ABO haemolytikus betegség konzervatív kezelése. *Gyermekgyógyászat* ***(Hungarian Pediatrics)*** 1999; **50:** 67-69.
101. Nagy A, Felszeghi E. Per os D-Penicillaminnal és fototerápiával sikeresen kezelt

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

Rh-isoimmunisation. *Gyermekgyógyászat* 2000; **51:** 81-83.
102. Rokicki W: D-Penicylamina-nowy lek w profilaktyce I terapii noworodka? D-Penicillamine - A New Drug For Prevention And Treatment In Neonates *Polish - Przeg Ped* 1989; **19:** 229-233.
103. Aldana-Valenzuela C, Personal information in a lecture-tour in Mexico 2008
104. Christensen RD, Alder SC, Richards SC, et al. D-Penicillamine administration and the incidence of retinopathy of prematurity. *J Perinatol* 2007; **27:** 103-11.
105. Phelps DL, Lakatos L, Watts JL. D-Penicillamine for preventing retinopathy of Prematurity. *Cochrane Database Syst Rev* (1): CD001073 2001.
106. Qureshi MJ, Kumar M. D-Penicillamine for preventing retinopathy of prematurity in preterm infants. *Cochrane Database Syst Rev* 3; 9: CD001073 2013.
107. Bruckmann G, Zondek SG. Iron, copper and manganese in human organs at various ages. *Biochem J* 1938; **33,** 1845-1857.
108. Merryman P, Jaffe IA, Ehrenfeld E, *et al.* Effect of D-Penicillamine on Poliovirus Replication In HeLa Cells. *J Virol* 1974; **13**: 881-887.
109. Scheib RG, Parentí DM, Simon GI. Prolonged antiviral activity of D-Penicillamine in human immunodeficiency virus infected homosexual men. *Am J Med* 1987; **83**: 608-612.
110. Oroszlán G, Szabó T, Lakatos L, *et al.* The pharmatokinetics of D-penicillamine in neonates. *Acta Paeditr Acad Sci Hung* 1987; **28**: 143-146.
111. Davis DA, Branca AA, Pallenberg AJ. Inhibition of the human immunodeficiency virus-1 protease and human immunodeficiency virus-1 replication by bathocuproine disulfonic acid CU1+. *Arch Bichem Biophys* 1995; **322**: 127-134.
112. Sprietsma J E. Cysteine, glutathione (GSH) and zinc and copper ions together are effective, natural, intracellular inhibitors of (AIDS) viruses. *Med Hypotheses* 1999; **52**: 529-538.
113. Shi Y, Berg JM. A direct comparison of the properties of natural and designed zinc-fingerproteins. *Chem Biol* 1995; **2**: 83-89.
114. Turpin JA. Inhibitors of Human immunodeficiency virus type zinc fingers. *J Virol* 1996; **70**: 6180–6189.
115. Lakatos L. Chelation therapy in the neonatal period: D-Penicillamine has possible beneficial effects on the lethality of HIV or EBOLA infection due to vertical transmission. *Intern J Ebola, AIDS, HIV and Infect Dis and Immunity* 2015; **1:** 1-26
116. Jamieson DJ, Uyeki TM, Callaghan WM, et al. What obstetrician-gynecologists should know about ebola: a perspective from the centers for disease control and prevention. *Obstet Gynecol* 2014; **124**, 1005-1010.
117. Chandran K, Sullivan NJ, Felbor U, *et al.* Endosomal proteolysis of the Ebola virus glycoprotein is necessary for infection. *Science* 2005; **308**, 1643–1645.
118. Schornberg K, Matsuyama S, Kabsch K, *et al.* Role of endosomal cathepsins in entry mediated by the Ebola virus glycoprotein. *J Virol* 2006; **80**, 4174–4178.
119. Turpin, J A. (1996). Inhibitors of Human immunodeficiency virus type zinc fingers. *J Virol* 1996; **70**, 6180–6189.
120. Müller S, Möller P, Blick MJ, *et al*. Inhibition of filovirus replication by the zinc finger antiviral protein. *J Virol* 2007; **81**, 2391-400.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

APENDIX

Table 1. Neurodegenerative and neurodevelopment diseases involving metal dyshomeostasis (interpretation can be found in the text)

Disease	**Clinical symptoms**	**Histopathology** ***MRI***	**Metal dyshomeostasis**
Aceruloplasminemia [25]	**Ataxia, chorea,** blepharospasmus, dementia	**Nerve cell loss,** **abnormal mastocytes, glubular structure;** *Bilateral* ***low signal on T2*** *at the basal ganglia*	Redox active **iron accumulation**
Alzheimer disease [26-28]	Dementia and **movement disorders**	Neuronal and synaptic loss, amyloid plaques, **neurofibrillar degeneration**; *Hippocampal and whole brain atrophy*	**Abnormal accumulation** and distribution of reactive iron and **copper**
Amyotrophic lateral sclerosis [29]	Slowly progressive, **painless weakness**, with limb involvement or bulbar symptoms	Cerebral atrophy, **upper motor neuron dysfunction** ***Hyperintense corticospinal tracts***	**Copper mediated** neurological disorder
Menkes's disease [30]	Brittle, kinky hair Feeding difficulties Irritability **Lack of muscle tone, floppiness** (hypotonia)	Extensive **neurodegeneration** int he gray matter and arteries of the brain. *Cerebral and cerebellar atrophy, delayed myelination,* ***bilateral basal ganglia changes (thalamus)***	**Copper mediated** neurological disorder
Huntington disease [31]	**Impairments in voluntary movements** and cognitive function, behavioral and pszichiatric disorders	**The striatum is the area** most severely impacted; the outer cortical regions also shows damage ***T2 ypointensities in basal ganglia***	Mitochondrial **metal dyshomeostasis** and dysfunction

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

			Copper and iron concentrations are increased in the striata
Occipital horn syndrome [32]	Soft skin, **weak muscle tone,** stroke, internal bleeding, kyphoscoliosis	A rare disorder of elastic tissue resulting in loose, redundant, hypoelastic skin. *Irregularities consistent with periventricular leukomalacia*	Inborn errors of metal **(copper) metabolism**

Table 1. Neurodegenerative and neurodevelopment diseases involving metal dyshomeostasis (cont.)

Disease	**Clinical symptoms**	**Histopathology** ***MRI***	**Metal dyshomeostasis**
Parkinson disease [33-35]	**Tremor (shaking)** **Slowness of movement** Rigidity (stiffness) Sleep problems Anxiety, Dementia Depression	Loss of dopaminergic neurons from the **substantia nigra** associated with the presence of intraneuronal inclusions called Lewy bodies. *With new imaging techniques: diffusional changes in the orbital-frontal region in the pre-motor phase of PD*	**Iron** accumulation and **dyshomeostasis**
Wilson disease [36, 37]	Deterioration in school performance or handwriting, mild **tremors, dystonia, ataxia,** muscular rigidity, **dysarthria**	**The lenticular nuclei** macroscopically appear brown in color, **degeneration** occurs with disease progression, leading to necrosis, gliosis and cystic changes, and lesions can be seen in the brainstem, thalamus, cerebellum and cerebral cortex, presence of Opalski cells. ***Hyperintense signals in putamen and globus pallidus***	**Copper accumulation in the basal ganglia**
Distonias [38]	Dystonia is an undesirable, **involuntary muscular movement** of a part of the body.	Related to a problem **in the basal ganglia.** *In most cases, no abnormalities are visible using MRI or other diagnostic imaging.*	Detrimental effects of transition metal **(copper) dysregulation**
Tourette syndrome [39]	**Different types of tics**: arm thrusting, eye blinking,	Structural changes in frontal cortex and **striatum**	Genetic heavy metal toxicity, Free **copper**

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

	jumping, kicking, repeated throat clearing or sniffing, shoulder shrugging	*Structural changes in the basal ganglia, limbic structures and prefrontal cortex,* ***T2 relaxation time asymmetries***	may be **elevated**
Autism spectrum disorders (ASD) [40, 41]	Delayed speech development, reacting unusally negatively, having **repetitive movements**. Developmental disorder affecting people interact with the world.	The cerebellum have documented cellular and neurochemical alterations, cellular and gross anatomic changes of the vermis cerebelli *Abnormally increased total brain volume.* *Decreases in cortical thickness in the frontal, temporal, and occipital lobes.*	The Zn/Cu ratio could be considered as biomarker of ASD. Free **copper** may be **elevated**

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

Table 1. Neurodegenerative and neurodevelopment diseases involving metal dyshomeostasis (cont.)

Disease	**Clinical symptoms**	**Histopathology** ***MRI***	**Metal dyshomeostasis**
Fahr's disease [42] IBSPDC.	**Gait** and speech **disturbance**, limb and truncal **ataxia**, dementia, **bradykinesia, mild parkisonism**	Idiopathic bilateral **striatopallidodentate calcinosis** (IBSPDC) *A striking **high density area in the basal ganglia** and the dentate nuclei of the cerebellum.*	The **increased levels of Cu, Zn, Fe** and Mg reflect the involvement of metabolism of several metals and/or metal-binding proteins during the progression of IBSPDC.
Hallervorden-Spatz (HSD) disease [43]	Progressive dementia, **spasticity, rigidity, dystonia, and choreoathetosis**	Juvenile-onset generalized **neuroaxonal dystrophy** ***Bilaterally symmetrical, hyperintense signal changes*** *in the anterior medial globus pallidus, with surrounding hypointensity in the globus pallidus, on T2-weighted images.*	**Neurodegeneration with brain** iron accumulation
BIND [44- 47]	Neurological, learning and movement disorders, isolated hearing loss, and auditory dysfunction	The abnormalities result from damage to the basal ganglia. *High T2 signal in globus pallidus*	„Abnormal" accumulation and distribution of reactive iron, *copper* and zinc in the basal ganglia

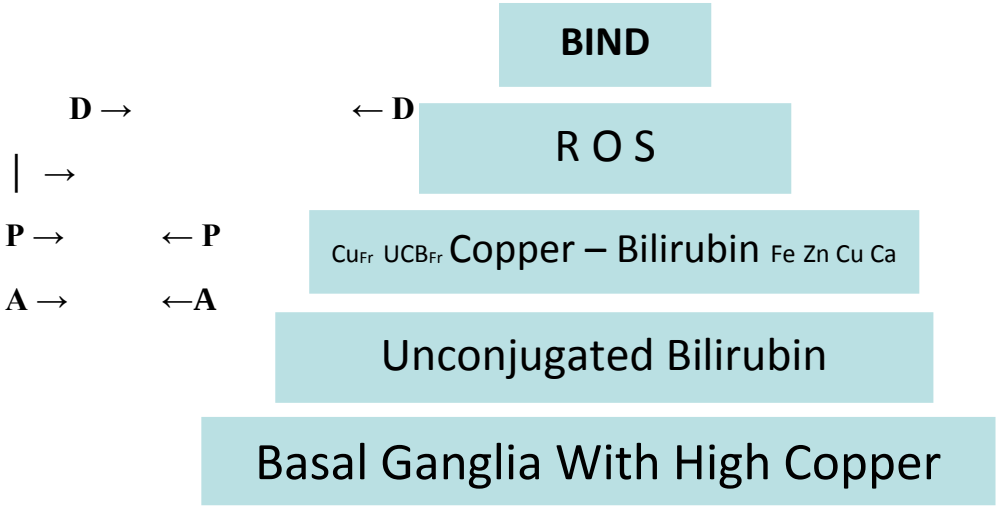
Table 2. Age-related differences in the effects of D-Penicillamine

	Neonates	**Adults**
Hexobarbital sleeping-time	**shortened**	**no effect**
Hem-oxygenase	**inhibited**	**no effect**
Cytochrom- P- 450	**increased**	**no effect**
Catalase	**increased**	**no effect**
Peroxidases	**increased**	**no effect**
LD_{50}	**> 4000 MG/KG (IP)**	**500 MG/KG (IV)**
Radioprotection	**significant**	**?**

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

Figure 1.

Potential pathomechanisms of bilirubin-induced (metal mediated) neurodevelopmental dysfunction (BIND), and effects of D-Penicillamine (D-PA) in the neonatal hyperbilirubinemia (ROS = reactive oxygen species; CuFr = free copper; UCBFr = free bilirubin). Interpretation of D-PA written sideways: D = direct antioxidant; | = scavenge of ROS and chelation of metals; P = inhibition of heme oxygenase (a decreased production of UCB) only in the newborn period (age related effect); A = copper chelation



ISSN 2053-406X(Print) ISSN 2053-4078(Online)