Potential Role of Penicillamine in the Early Diagnosis and Prevention of Autism Spectrum Disorder (ASD)

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Abstract

Our observations suggest that D-Penicillamine (D-PA)-therapy in the neonatal period may have significant neuroprotective effects in cases jeopardized by bilirubin-induced neurologic dysfunction (BIND), retinopathy of prematurity (ROP) and ASD. D-PA can be used in unusually high doses as a short-term therapy in the neonatal brain’s defence. It is recommended that all newborns should be screened for ASD, particularly the premature babies and infants suffering from hyperbilirubinemia. This would be a „great challenge” for neonatologists since the reference values and the quantitative judgement of copper metabolism for neonates are not established.

Although the 24-hour urine copper test is inconsistent in the neonatal period, the penicillamine challenge test has proved itself to be useful in the detection of high copper in the urine of newborn infants, too. The copper and bilirubin decreasing effects of D-PA play important role its neuroprotection in the newborn age.

Keywords

Autism Spectrum Disorders; Copper Toxicity; Bilirubin-Induced Neurologic Dysfunction; Oxidative/Nitrosative Stress; Excitotoxicity; D-Penicillamine In The Neonatal Period

Abbreviations

ASD Autism Spectrum Disorder
BG Basal Ganglia
BBB Blood-Brain-Barrier
BIND Bilirubin-Induced Neurologic Dysfunction
INOS Inducible Nitric Oxide Synthase
NO Nitric oxide
NHBI Neonatal Hyperbilirubinemia
LP Lipid Peroxidation
OS Oxidative Stress
NS Nitrosative Stress
WD Wilson’s Disease
RBC Red Blood Cells
RNS Reactive Nitrogen Species
ROS Reactive Oxygen Species
ROP Retinopathy of Prematurity
NICU Neonatal Intensive Care Unit
MT Metallotionein
UCB Unconjugated Bilirubin
ND Neurodegenerative Disease

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Introduction

ASD is a developmental behavioral disease characterized by troubles with social interaction and communication. Often there are also restricted and repetitive movements or misconducts. Parents usually discovered signs in the first two or three years of their child’s life. The definition of Autism and ASD is provided by the Diagnostic and Statistical manual of Mental Disorders (DSM-5). Children with ASD are less able to interact with their environment as other children do. Imaginative play (variable interests and behaviours) is typical of them [1, 2].

Causes and Theories

ASD is considered to be a neurodevelopmental disability with genetic origin. Countless informations, research data and hypotheses accumulated during the last 76 years [3] regarding problems that may interfere with normal brain development. These problems - from television watching, through environmental factors, till immune deficiency, food allergies and vaccines - can alter the usual patterns in genes. The National Institutes of Health, however, states that the evidence is not yet strong enough to show a causal relationship [4]. A quick index to the Theories of Autism can be found in a summary overview article by Mehl-Madrona [5]. Individuals have various characteristics on the "spectrum". So, the Autism is unique to each person and will be different for each one. Until we have more definitive answers on the causes of ASD, it is reasonable to focus on the early intervention, and to support researchers as they learn more about causes. This mental disorder occurs in every racial and ethnic groups, and across all socioeconomic levels. Boys are, however, significantly more likely to suffer ASD than girls. The latest data from the Centers for Disease Control and Prevention shows that 1 in 45 children has ASD [6].

The Aim of a New Approach

In this article there is a new theory (hypothesis or concept?) taking aim at earliest diagnosis of ASD as quick as possible, and the imaginable prevention of a neurodevelopment condition that threatens the growing children. Before we would give the basis idea in detail, it is necessary to outline the antecedents: (1) the use of D-PA in the neonatal period; (2) D-PA as a neuroprotective antioxidant; (3) long-term follow up of patients treated with D-PA in the neonatal period; (4) potential mechanisms of this drug in the prevention of brain injury acting in accordance with the presumed pathogenesis of ASD; (5) chelation and autism; (6) D-PA as a chelating and neuroprotectant agent in the neonatal period.

1. D-PA in the Prevention or Therapy of NHBI and ROP

D-PA was first recognized as a potential benefit for NHBI caused by hemolytic diseases of the newborn infant or immaturity of UDP-glucuronyltransferase enzyme. During the "penicillamine era", this drug was used intravenously (1973-1983) in the neonatal period, and there was a significantly lower incidence of ROP in the infants treated with D-PA than in the controls in our NICU. These studies were replicated in Hungary (other institutes), Poland, USA, India and Mexico. It is important to note that there was no intolerance or short- or long-term toxicity of the medication in the neonatal period. To our concept, the BIND and ROP are neurodegenerative and neurodevelopment diseases (NDs) of immature brain characterised by accumulation of free metals, unconjugated bilirubin (UCB) and UCB-Cu complex (= prooxidant) in the basal ganglia (BG) and other relevant parts of the central nervous system (CNS). The hemolysis of neonatal red blood cells is going with the induction of a great amount of heavy metals (mainly iron and copper) and producing reactive oxygen species (ROS). These elements are circulating in the bloodstream, and pass through the immature blood-brain-barrier (BBB), finding entrance into the CNS. In addition, ROS increases BBB permeability performing a dangerous vicious circle in the newborn brain (Figure 1) [7]. Recently, our hypothesis was reinforced by Božić B. et al. [8] with convincing chemistry experiments. It is also favourable for using D-PA in the neonatal period because its administration is possible in unusually high doses (300 mg/kg/bw. daily for 4-7 days). Although it is a low-cost drug, at the same time developed under the Orphan Drug Act of 1983 in the US which is a federal law concerning rare diseases (orphan diseases) [9, 10]. For example, the intravenous form of D-PA is nowadays not available in the market and the enteral preparation is produced by a few companies in the world. As far as the ROP cases concerned, the history of D-PA therapy in prematures under 1500 g birth weight can be divided into four periods [11], ending with the conduction of two strictly controlled, randomized prospective trial to investigate its presumably beneficial effects not only in the prevention of the cicatrical form of the disease but also in the reduction of the acute stages [12, 13]. Summarizing the results of these clinical trials: (i) D-PA treatment was
associated with elimination of all stages of ROP; (ii) in this single-centered comparison analysis, a 14-day course of D-PA administration resulted in no apparent short- and long-term toxicity. This drug may be regarded as causal agent in the prevention of ROP:

![Biochemical Mechanism of BIND](image)

### Figure 1: Biochemical Mechanism of BIND

- by its antioxidant effects [14],
- it can "liberate" nitric oxide (NO) by chalating divalent transition metals, including Cu, Fe, Hg et cet. which inhibits nitric oxide synthase (NOS) catalysis [15];
- by inhibition of human endothelial cell proliferation and neovascularization in vivo [16]; and
- has a low-molecular weight, active in CNS [17, 18].

### 2. D-PA as a neuroprotective drug in the neonatal period

First of all it is necessary to give the definition of **neuroprotection** which refers to the relative preservation of neuronal structure and/or function mainly in the CNS [19, 20]. Common mechanisms of neuronal damages include increased levels in oxidative stress (OS) and nitrosative stress (NS), mitochondrial dysfunction, excitotoxicity, inflammatory changes and heavy metal accumulation. Thus, the common neuroprotective treatments are glutamate antagonists and antioxidants as they limit excitotoxicity and OS/NS, respectively. In our earlier review we discussed the role of elevated copper in various molecular mechanisms according to item 1., focusing on the newborn infants [21]. D-PA is actually the drug most extensively used to treat copper overload [22, 23]. D-PA fulfills the criteria of a hybrid drug in the neonatal period: it modulates the OS and NO pathway, and it is a neuroprotective agent in the pathophysiology of neurologic dysfunction [24]. During this process we have to account with gasotransmitters. Along with NO and carbon monoxide (CO), hydrogen sulfide (H₂S) is also a potent endogenous neuromodulator and plays multiple roles in the CNS both in physiological and pathological conditions, especially in secondary neuronal injury. As a carbonyl scavenger [25], D-PA have been used with the aim of reducing the “aldehyde load” [26-29] and in several in vivo and in vitro studies have been investigated their effects on neuroprotection.

### 3. Long-Term Follow up of Patients Treated with D-PA in the Neonatal Period

The aim of these studies were to explore potential long-term effects of D-PA by measuring health state of children and adults treated with this drug in the newborn period. Evaluation of the data showed that D-PA has no side effects on either mortality or late development [30]. The same result was found in an onset cohort of children, ages 3-4 years who had NHBI and were treated with D-PA (31). Relative beneficial outcome were observed in children with birthweight 1000 g or less at 8-11 years of age (32). The aim of recent investigation was to measure the self perceived health and health related quality of life (HROQL) of adults (23-36 years of age) [33, 34]. During long-term follow up studies (3-36 years - N = 550) we found only one case of ASD in the children and adults who were treated with D-PA in their neonatal period (“New Prevalence Numbers for 2014: 1 in 45 US Children have autism” [6]).

The above-mentioned autistic male patient (he is now 30 years old) was born as a premature infant and suffered from a serious NHBI. He was treated with D-PA without success, because exchange transfusion was necessary to perform [35].


Autism is a complex developmental disorder with a seemingly complicating and uncertain pathogenesis and pathophysiology. The definitive mechanisms that promote autism are poorly understood and mostly unknown [36].
Numerous studies attribute the pathogenesis of autism specifically to various anomalies which have the potential to detrimentally effect CNS structure and function [37]. The "triple hit hypothesis - (i) a critical period of brain development, (ii) an underlying vulnerability, and (iii) exogenous stressor(s)" [38] easily understandable considering this multifactorial disorder during the continuous cerebral changes prevailing in the neonatal period and in the first year of life [39]. Thus, it is surprising that how precious little papers deal with ASD in the earliest period of life [40, 41].

Metal-Metabolism and Autism [42-45]

Metals (mainly copper and mercury) are implicated directly or indirectly in the pathogenesis of a large number of neurological diseases, including ASD, and we have found both direct and indirect evidence for the metal excess in the etiology of this disorder. From among the heavy metals most data refer to the role of "copper surplus" in the pathophysiology of ASD [46-52]. Several articles have it straight considered the heterozygotic manifestation of Wilson’s disease (WD) (low serum ceruloplasmin and copper level, and elevated copper concentration in the liver and brain - mainly in the basal ganglia and hippocampus) [53, 54]. Interesting that the same biochemical constellation is typical in the neonatal period as well [55, 56].

According to Walsh [57] the autism results from two factors: an inborn error and an environmental insult. A constitutional vulnerability causes metallothionein (MT) weakness for the neuronal development, and hypersensitivities to toxic metals, infections and viruses. Insults during gestation, infancy or early childhood, a virus or an infection, for example, can disable the weak MT protein system, and provoke the onset of autism. Glutathione and glutathione-related enzymes are strong antioxidants detoxifying the body to heavy metals such as cadmium, lead, iron and mercury. It’s active form is reduced 80% in kids with autism. Heavy metals have the potential to disrupt the biological activities of these proteins. Vice versa, glutathione can assist in the detoxification of heavy metals [58, 59]. D-PA not only chelates copper but also detoxifies tissue copper by promoting the synthesis of metallothionein, which forms a non-toxic combination with copper, and increases intracellular glutathione during the treatment for rheumatoid arthritis [60-62]. A possible central mechanisms of pathophysiology in ASD: interaction of activated microglia, excitotoxicity, formation of ROS and RNS, lipid peroxidation and the role of elevated androgen levels [63, 64].

Neuroglial Activation In The Brain of Patients With Autism [65].

An inflammatory process in the CNS plays an important role in the pathway leading to neuronal cell death in a number of neurodegenerative and neurodevelopmental diseases, including ASD. The inflammatory response is mediated by the activated microglia which is a hallmark of brain pathology. The chronic activation of microglia may cause neuronal damage through the liberation of potentially cytotoxic molecules such as cytokines, ROS, proteinases and complement proteins [66]. Therefore, suppression of microglia-mediated inflammation has been considered as an important strategy in the ASD therapy. Several environmental exposures may represent risk factors for ASD; for this reason administration of metal chelators and anti-inflammatory agents (such as D-PA) could affect disease outcomes [67].

Generation of ROS and OS [68]

The copper almost equally capable to generate ROS and RNS. However, under conditions of OS, ROS production is very high, resulting in damage of membrane lipids, proteins, and nucleic acids that may become irreversible, even cause cell death. The strategy which limits oxidant-induced tissue damage, called antioxidant defense mechanism, is a complex network of endogenous and exogenous systems for scavenging ROS. The copper is the strongest redox-active metal which can generate excessive amounts of free radicals. At the same time, the brain is more vulnerable to OS compared to other tissues. So, it seems reasonable that we need exogenous antioxidants which are effective in diminishing OS [69]. In the neonatal period excessive production of ROS and other highly reactive free radicals are capable of causing functional and structural damage to cell components. Consequently, they are prone to OS which may damage different organs (lung, brain, retina, and gastrointestinal tract) [70]. Figure 2, shows how D-PA is able to defense against free radicals produced various pathological conditions in the neonatal period.

Generation of RNS and NS [71]

Definition of this condition: "The reaction of body tissues to nitric oxide (NO), nitrous oxide or similar species’ at levels greater than can be neutralized". The NO
plays a pivotal role in plants and mammals, including the human organism, as a negative or positive regulator of cell apoptosis. Thus, the RNSs are fundamental regulators of oxidative metabolism in the cell [72-74]. NO reacts rapidly with O₂ and superoxide radical (O₂−) to generate a wide spectrum of RNSs that are highly damaging to cells. Studies indicate that mitochondrial permeability transition and NS represent major factors in copper-induced toxicity in astrocytes, and RNSs can cause neuronal injuries [75].

In the neonatal period several studies attribute an important role for inducible nitric oxide synthase (iNOS)-induced NS in periventricular leukomalacia and in necrotizing enterocolitis [76, 77].

D-PA alleviates OS and NS. These effects based on the capability of this drug to alter the NO system, and it is a strong antioxidant. Low molecular weight disulfsides are the major products of D-PA metabolism in humans [78, 79]. The oxidation of D-PA in vivo may also important in the mode of action of the drug through simultaneous reduction of the ROS and RNS. Consequently, D-PA fulfills the criteria of a hybrid drug in the neonatal period by its ability to modulate both oxidative stress and NO pathway, and can be a neuroprotective agent in the pathophysiology of neurologic dysfunction (Figure 3).

Figure 2: Penicillamine and the Neonatal Oxygen Toxicity

Figure 3: Molecular Mechanisms of Copper Induced Oxidative/Nitrosative Stress And Excitotoxicity in the Neonatal Period (MDA: Malondialdehyde; 4-HNE: 4-Hyroxynonenal; Pufas: Polyunsaturated Fatty Acids)

Lipid Peroxidation (LP)

LP occurs during the oxidative degradation of lipids. Initiation begins with ROS-induced hydrogen atom abstraction from polyunsaturated fatty acids (arachidonic-, linoleic-, eicosapentaenoic-and docosahexaenoic acid). Two damaging products of LP are 4-hyroxynonenenal and acrolein. Overproduction of ROS is creating a continuous cycle of ion imbalance, Ca²⁺ buffering impairment, mitochondrial dysfunction, glutamate-induced excitotoxicity and microvascular disruption. NO, formed from mitochondrial NOS, in turn reacts with superoxid anion to produce the highly toxic peroxynitrite radical [80]. In the neonatal period there are several conditions (hypoxia, hyperoxia, resuscitation, mechanical ventilation, phototherapy and intracerebral hemorrhage etc.) which set going the cascade of LP of unsaturated fatty acids leading to the formation of aldehydes as secondary LP products. These aldehydes can also act as messengers of ROS/RNS [81-88]. The carbonyl scavenger D-PA binds primarily to aldehydes in an irreversible manner [90], consequently this drug inhibits their damaging effects and it also scavenges peroxynitrit [91, 92]. Paediatric patients display different pharmacokinetic and pharmacodynamic responses to drugs. This is why we can speak about developmental or age-related pharmacology [93]. In the Figure 4. we demonstrate the results of our animal experiments regarding the age related differences in effects of D-PA [94]. The high activity of HO in the newborn could reflect the enzyme-inducing
action of metals (primarily of Cu and Fe) derived from the breakdown of fetal erythrocytes [95]. A number of articles have suggested alterations in the activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase in autism [96]. Chelation therapy in neonates restores the normal activity of enzymes participating in heme metabolism. Briefly, chelating agents, including D-PA, boost or inhibit the immature enzyme systems to the adult level. Because those enzymes that play an important role in antioxidant defense and drug metabolism (peroxidases, catalase, cytochrome P-450) are heme proteins, it can be assumed that in preventing NHBI and OS/NS, the mechanism of action of D-PA is identical: the protection of biomembrans against LP [97].

**Figure 4: Age-Related Effects D-pencillamine**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>SOD</th>
<th>Heme Oxigenase-1</th>
<th>Cytochrome P-450</th>
<th>Catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>100</td>
<td>20</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Neonate</td>
<td>80</td>
<td>10</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>HST</td>
<td>60</td>
<td>8</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

**SOD: Superoxide Dismutase**

**Excitotoxicity (ET)**

ET is the pathological process by which nervous cells are damaged and killed by the overactivations of receptors for the excitatory neurotransmitter glutamate, such as N-methyl-D-aspartate (NMDA)- and α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) receptor [98]. Excitotoxins like NMDA and kainic acid, as well as pathologically high levels of glutamate, can cause excitotoxicity by allowing high levels of calcium ions to enter the cell. This process activates a number of enzymes, including phospholipases, endonucleases, and proteases such as calpain. Latter enzymes go on to damage cell structures such as components of the cytoskeleton, membrane, and DNA. In the neonatal period excitotoxicity is an important mechanism involved in perinatal brain injuries. Perinatal injuries caused by hypoxia-ischemia, stroke, hypoglycemia, kernicterus (or BIND), and trauma can disrupt synaptic function leading to accumulation of extracellular glutamate and excessive stimulation of these receptors [99, 100]. Molecular mechanisms of neurotoxicity caused by hyperactivation of the N-methyl-D-aspartate (NMDA) receptor can be ameliorated with chelation. No data was found, however, in the literature accessible by us, regarding the direct inhibitory effects of D-PA on ET. At the same time, it is well-known that ROS generation triggers glutamate-mediated excitotoxicity.

D-PA is used as a copper chelator and strong ROS/RNS inhibitor for the treatment of Wilson’s disease and rheumatoid arthritis and it is known to scavenge carboxyls. Previous literature has shown penicillamine scavenging other toxic aldehyde by forming a thiazolidine compound with the aldehyde moiety (Figure 2.) [101].

**Mitochondrial Dysfunction**

Mitochondria turn sugar and oxygen into energy that the cells need to work. Mitochondrial diseases result from failures of these specialized tiny compartments. A child with a mitochondrial disease may also have an autism spectrum disorder, may have some of the symptoms/signs of autism, or may not have any signs or symptoms related to autism. Mitochondrial copper accumulation plays a pivotal role in the pathogenesis of WD, and provides a rationale for a study of the use of antioxidants in the treatment [96, 102-105]. Acute D-PA administration has improved neurological recovery in an animal model of concussive head injury and has protected brain mitochondria [27] and has a protective effects as a peroxynitrite scavenger [92].

**Chelation Therapy**

According to the current literature heavy metal chelation is not an effective autism treatment, and it may be dangerous. Some pediatricians and parents have considered this therapy as a potential autism treatment. They believe that autism is caused by mercury exposure, such as from childhood vaccines [106]. Chelating with drugs is indicated primarily for acute poisonings by heavy metals. Though the drugs may have dangerous adverse effects, the risks are considered worthwhile in the face of toxicity which may be fatal or cause serious, even permanent injury. No
clinical trial was conducted to suggest that pharmaceutical chelation is an effective intervention for ASD. Given prior reports of serious adverse events, the risks of using chelation for ASD currently outweigh proven benefits. These opinions primarily refer to succimer (DMSA, 2,3-dimercaptosuccinic acid), dimercaprol (British Anti-Lewisite, BAL), edetate calcium disodium (CaNa2EDTA) and deferoxamine and they do not affect D-PA [107-109].

The use of D-PA in the neonatal period is a little bit "shocking" for a large number of neonatologists since instead of the cautious "go low - go slow" dosing (Jaffe [110], and more recently Meng [111] ) we insist on the principle: "go high for a while". The pharmacokinetics of D-PA have also been cleared in this age group [112].

5. The Basic Idea

It was recommended by us [35], that all newborns should be screened for ASD, particularly the premature babies and infants suffering from hyperbilirubinemia. Presumably, hypercupriuria is found in all neonates since they have similar metabolism of copper as the patients suffering from WD [55, 56]. Investigation of WD and obstructive liver disease using a 24-hour urine specimen and penicillamine challenge test to measure the excreted copper/day. Unfortunately, the reference values for neonates are not established yet [115, 116]. Although the 24-hour urine copper test is inconsistent in the neonatal period, the Penicillamine challenge test [115], has proved itself to be useful in the detection of high copper in the urine of newborn infants too. First of all it is important to know the normal copper excretion/24 hours in the 2-4 days of life. Soon after it is reasonable to performe the challenge detecting the expectable hypercupriuria. If the excretion of copper is significantly greater than the standard value, it is suspicious for ASD, and 4-6 days D-PA-treatment may be necessary, especially as this type of chelation therapy beneficial in the treatment and prevention of NHBI and ROP as well.

Conclusion

Our observations - together with other convincing cases participating in the long-term (23-40 years) follow-up suggest that D-PA-therapy in the neonatal period may have significant neuroprotective effects in cases jeopardized by BIND, ROP and ASD. According to our concept, D-PA can alter the function of NO, CO and H₂S and the copper homeostasis in the brain [24] so, it can protect the brain (especially the BG and retina) from various injury, such as BIND, ROP and ASD. During the last 40 years neonatologists working in Hungary and in the rest of the world [116, 117] treated a number of term and preterm infants with D-PA without any serious adverse effects. We hope that our concept will help answer some of the unsolved questions and concerns occurred in the etiology and pathomechanisms of BIND and of the other neurodevelopmental disorders. The beneficial neuropharmacological actions of metal-targeted (chelating) agents most likely arise from local metal redistribution rather than from massive metal removal [3, 118-120]. 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 prevention or treatment of neonatal brain injuries.

References


35. Lakatos L, Pataki I, Balla G et al. (2017) Penicillamine -Preventing or Curing Autism Spectrum Disorders in the


49. Copperheads Email from Dr. Wm Walsh, December 14 (2003).


52. Dr Mercola interviews Dr Walsh (2017) Copper Overload Linked to Autism, Schizophrenia and Postpartum Depression. Second Opinion Physician Posted on by admin.


90. Rud JS, Saugstad OD (1990) D-Penicillamine attenuates
oxygen radical induced pulmonary hypertension in pigs. Pediatric Research 28:


